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Diabetes, hypertension and cardiovascular diseases, volume II

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Published in
Frontiers in Cardiovascular Medicine
Frontiers in Physiology

ISSN 1664-8714
ISBN 978-2-83251-426-9
DOI 10.3389/978-2-83251-426-9

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Diabetes, hypertension and cardiovascular diseases, volume II

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Citation

Choi, S.-K., Galán, M., Kassan, M., eds. (2023). *Diabetes, hypertension and cardiovascular diseases, volume II*. Lausanne: Frontiers Media SA.
doi: 10.3389/978-2-83251-426-9

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frontiers in Cardiovascular Medicine

ORIGINAL RESEARCH
published: 01 September 2021
doi: 10.3389/fcvm.2021.736001

High Level of Uromodulin Increases the Risk of Hypertension: A Mendelian Randomization Study

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Background: The association of uromodulin and hypertension has been observed in clinical studies, but not proven by a causal relationship. We conducted a two-sample Mendelian randomization (MR) analysis to investigate the causal relationship between uromodulin and blood pressure.

Methods: We selected single nucleotide polymorphisms (SNPs) related to urinary uromodulin (uUMOD) and serum uromodulin (sUMOD) from a large Genome-Wide Association Studies (GWAS) meta-analysis study and research in PubMed. Six datasets based on the UK Biobank and the International Consortium for Blood Pressure (ICBP) served as outcomes with a large sample of hypertension (n = 46,188), systolic blood pressure (SBP, n = 1,194,020), and diastolic blood pressure (DBP, n = 1,194,020). The inverse variance weighted (IVW) method was performed in uUMOD MR analysis, while methods of IVW, MR-Egger, Weighted median, and Mendelian Randomization Pleiotropy RESidual Sum and Outlier (MR-PRESSO) were utilized on sUMOD MR analysis.

Results: MR analysis of IVM showed the odds ratio (OR) of the uUMOD to hypertension ("ukb-b-14057" and "ukb-b-14177") is 1.04 (95% Confidence Interval (CI), 1.03-1.04, $P < 0.001$); the effect sizes of the uUMOD to SBP are 1.10 (Standard error (SE) = 0.25, P = 8.92E-06) and 0.03 (SE = 0.01, P = 2.70E-04) in "ieu-b-38" and "ukb-b-20175", respectively. The β coefficient of the uUMOD to DBP is 0.88 (SE = 0.19, P = 4.38E-06) in "ieu-b-39" and 0.05 (SE = 0.01, P = 2.13E-10) in "ukb-b-7992". As for the sUMOD, the OR of hypertension ("ukb-b-14057" and "ukb-b-14177") is 1.01 (95% CI 1.01–1.02, all $P < 0.001$). The β coefficient of the SBP is 0.37 (SE = 0.07, P = 1.26E-07) in "ieu-b-38" and 0.01 (SE = 0.003, P = 1.04E-04) in "ukb-b-20175". The sUMOD is causally associated with elevated DBP ("ieu-b-39": β = 0.313, SE = 0.050, P = 3.43E-10; "ukb-b-7992": β = 0.018, SE = 0.003, P = 8.41E-09).

Conclusion: Our results indicated that high urinary and serum uromodulin levels are potentially detrimental in elevating blood pressure, and serve as a causal risk factor for hypertension.

Keywords: uromodulin, hypertension, systolic blood pressure, diastolic blood pressure, Mendelian randomization

OPEN ACCESS

Edited by:
Modar Kassan, University of Tennessee Health Science Center (UTHSC), United States

Reviewed by:
Hui Lu, Capital Medical University, China
Lu Liu, Shandong University, China

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Specialty section:
This article was submitted to Hypertension, a section of the journal Frontiers in Cardiovascular Medicine

Received: *04 July 2021*
Accepted: *09 August 2021*
Published: *01 September 2021*

Citation:
You R, Chen L, Xu L, Zhang D, Li H, Shi X, Zheng Y and Chen L (2021) High Level of Uromodulin Increases the Risk of Hypertension: A Mendelian Randomization Study. Front. Cardiovasc. Med. 8:736001. doi: 10.3389/fcvm.2021.736001

INTRODUCTION

As a leading cause of cardiovascular disease, hypertension is a complex chronic clinical syndrome with multiple risk factors such as smoking (1), alcohol use (2), obesity (3), and high salt intake (4). Incidence has been rising throughout the last decades (5). At present, we have not discovered all the driving factors of hypertension.

Uromodulin, also named Tamm-Horsfall protein (THP), was first described by Carlo Rovida in 1873. It is produced by the cells in the thick ascending limb (TAL) and the distal convoluted tubule (DCT) with daily secretion of 50–150 mg in urine (6). Uromodulin is physiologically secreted into the renal interstitium, enters the blood to form serum uromodulin (sUMOD), with a level < 0.001 of the level of urinary uromodulin (uUMOD) (7). uUMOD plays a crucial role in various biochemical processes, such as protection against urinary tract infection, immunomodulation, and regulating water and salt balance (8). sUMOD was significantly associated with many diseases, such as impaired glucose metabolism, kidney function, and risk for kidney allograft failure (9–11). The association of uUMOD level and salt-sensitive hypertension was observed, but the causal effect of uUMOD on hypertension has not been confirmed (12). Since traditional observational studies might be biased by many underlying confounders such as lifestyles and socioeconomic status (5), the cost of a large randomized controlled trial (RCT), or cohort studies is extremely expensive; as such few studies have focused on exploring the causal relationship between uromodulin and hypertension. Therefore the causal effect of uromodulin on hypertension requires a new strategy in order to be investigated.

"Mendelian randomization" (MR) is an emerging research method that can simulate randomized controlled trials using genetic variants (usually single nucleotide polymorphisms, SNPs) as instrumental variables. Because the gene is allocated randomly at conception (13), MR was designed as a natural randomization method that could minimize the effects of confounders. Nowadays, the MR method has been widely applied to estimate the causal effect of exposure on outcome, and successfully confirmed that lower low-density lipoprotein (LDL) cholesterol contributed to fewer cardiovascular events (14). Recently, many studies have tried to disentangle the risk factors for hypertension by MR methods. Besides some traditional risk factors for hypertension such as body weight index (BMI), adiposity, dietary dairy consumption, smoking, and alcohol intake, it also disclosed some further potential risk factors; namely uric acid, vitamin D levels, gamma-glutamyl transferase, total bilirubin, glycated hemoglobin, beta-2-microglobulin, and apolipoprotein E (15).

In this study, we tried to use two-sample MR methods to unveil the causal effect of uromodulin on hypertension, systolic blood pressure (SBP), and diastolic blood pressure (DBP) using increasingly available public genome-wide association studies (GWAS) datasets.

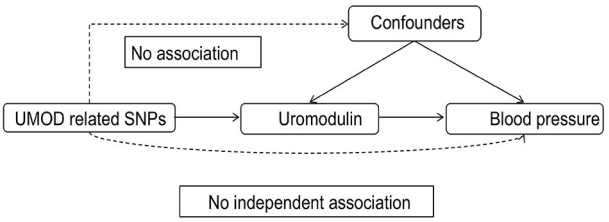

FIGURE 1 | Directed acyclic graph composed of the genetic instrument (UMOD-related SNPs), exposure (UMOD), and outcome (blood pressure). SNPs, Single nucleotide polymorphisms; UMOD, Uromodulin.

METHODS AND MATERIALS

Study Design

MR analysis is based on three assumptions: (1) The instrumental variable (IV) is closely associated with the exposure. (2) The IV is not associated with any potential confounders. (3) The IV can only influence the outcome via the exposure, and not by any other ways. We constructed a directed acyclic graph by using genetic instruments (UMOD-related SNPs), exposures (serum uromodulin and urinary uromodulin), and outcomes (hypertension, diastolic blood pressure, and systolic blood pressure, **Figure 1**).

IVs Selection

We initially extracted all five SNPs associated with uUMOD levels from the largest GWAS meta-analysis of uUMOD (16). This study was a fixed-effects meta-analysis combining results of 10,884 participants of European descent, consisting of three genetic isolates and three urban cohorts. The details of the SNPs are in **Supplementary Table 1**. We selected IVs with $P \leq 5 \times 10^{-8}$, minor allele frequency (MAF) >0.01, and low linkage disequilibrium (LD) ($r2 < 0.1$). Finally, two SNPs (rs12917707 and rs4494548) were valid for further MR analysis of uUMOD.

sUMOD-related SNPs were obtained from 4,147 participants in the Outcome Reduction with Initial Glargine Intervention (ORIGIN) trial (17), which selected SNPs within 300 kb of the UMOD gene significantly associated with sUMOD, and pruned the SNPs for LD at a threshold of $r2 > 0.1$ using 1,000 Genomes data (Europeans). Sixteen SNPs were selected as the IVs in the MR analysis of sUMOD, and rs12446494 was excluded for high LD to rs12917707 ($r2 = 0.16$) (**Supplementary Table 2**).

Outcome Data Sources

We extracted the outcome data (blood pressure) from the MR-base database (18) (https://gwas.mrcieu.ac.uk/), which is a curated database including a summary originated from 1,094 GWASs involving 889 traits of physiological characteristics and disease phenotypes. We searched the traits "hypertension", "high blood pressure", "systolic blood pressure", and "diastolic blood pressure" as keywords, filtered by the European population up to 2020 in the MR-base database. We chose the largest sample size study with available data in the different consortium as outcomes

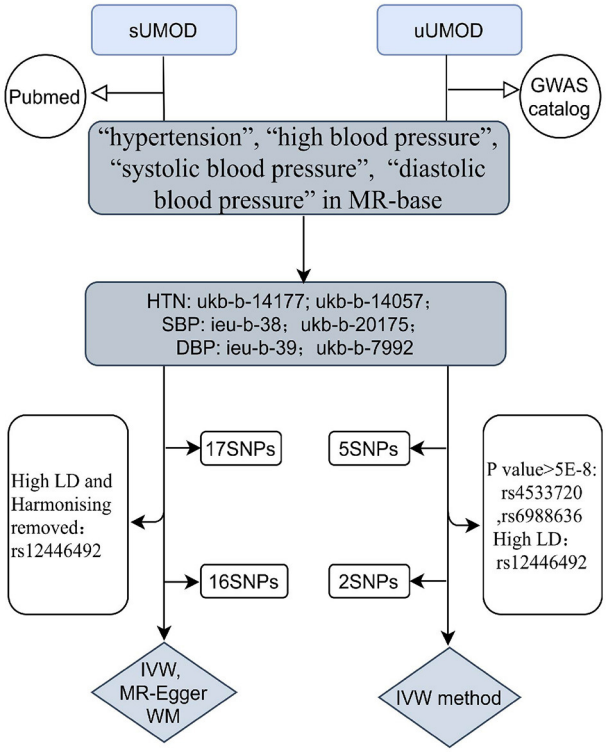

FIGURE 2 | The flow chart of the study design. For instrumental variants (IVs) of the uUMOD, we removed two SNPs (rs4533720 and rs6988636) because of their *p*-value (>5e-8), and one SNP (rs12446492) due to its high linkage disequilibrium (LD) to rs12917707 (r20 = 0.16). For IVs of the sUMOD, rs12446492 was excluded for the same reason as uUMOD being palindromic with intermediate allele frequencies. uUMOD, Urinary uromodulin; sUMOD, Serum uromodulin; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; HTN, Hypertension; IVW, Inverse variance weighted; WM, Weighted median; MR-Egger, Mendelian randomization-Egger; SNPs, Single Nucleotide Polymorphisms.

(**Figure 2**). Two summary datasets with IDs "ukb-b-14057" (non-cancer illness code, self-reported: Hypertension) and "ukb-b-14177" (vascular/heart problems diagnosed by doctor: High blood pressure) were selected as the outcome of hypertension and high blood pressure. They were originated from the MRC Integrative Epidemiology Unit (MRC-IEU) consortium (http://www.bristol.ac.uk/integrative-epidemiology/) based on the UK Biobank, which is a large and detailed genotyped biobank that has globally recruited over 500,000 participants (aged 40–69 years) between 2006 and 2010 (19). The "ukb-b-14057" ID contains 46,293 people while "ukb-b-14177" includes 46,188 participants. We selected two summary datasets "ukb-b-20175" (systolic blood pressure, automated reading) and "ieu-b-38" (systolic blood pressure) as the outcome of SBP. The IDs "ieu-b-39" (diastolic blood pressure) and "ukb-b-7992" (diastolic blood pressure, automated reading) were selected as the datasets of the DBP. The "ieu-b-38" (systolic blood pressure) and "ieu-b-39" IDs (diastolic blood pressure) included summary level data based on the International Consortium for Blood Pressure (ICBP) (20) (www.ncbi.nlm.nih.gov/projects/gap/cgibin/study.cgi?study_id=phs000585.v1.p1), which is a multi-stage GWAS study of systolic and diastolic blood pressure in 200,000 individuals of European descent (21, 22). While "ukb-b-20175" and "ukb-b-7992" included summary data of the UK biobank (**Supplementary Tables 3**, **4**).

MR Analysis

In two-sample MR, it is necessary to ensure that the effect allele of IVs in exposure and outcome between different databases correspond to the same allele. Thus, we tried to infer the forward strand alleles using allele frequency information to harmonize the data and discarded ambiguous IVs or not inferable palindromic ones.

For IVs with more than three SNPs, we performed MR analysis through several robust analytical methods based on different assumptions of two-sample MR analysis; namely inverse variance weighted (IVW), MR-Egger, and weighted median (WM). The IVW method utilizes a meta-analysis approach to pool Wald ratios for each SNP (i.e., the β coefficient of the SNP for UMOD is divided by the β coefficient of the SNP for outcomes) to get the combined estimates of the effect of uromodulin on outcomes (hypertension, DBP, SBP) (23). MR-Egger regression makes a weighted linear regression of the outcome coefficients on the exposure coefficients. It can provide unbiased estimates even when all genetic variants are invalid (24). The WM method calculates the median of the empirical distribution of MR association estimates weighted for their precision and offers consistent estimates. For IVs with less than three SNPs, we performed MR analysis by the IVW method.

We performed Cochran's Q statistic to assess heterogeneity between individual genetic variants in the IVW method. A random-effects model was used when the heterogeneity was high (25). We then conducted scatter plots and the leave-one-out method to evaluate the robustness of these findings. To confirm the influential outliers and horizontal pleiotropy, we adopted MR-PRESSO (Mendelian Randomization Pleiotropy RESidual Sum and Outlier) to detect and correct for potential outliers ($P < 0.05$). We also used MR-PRESSO to test the significant differences in the causal estimates before and after correction for outliers (26) and the intercept of MR-Egger to further test the horizontal pleiotropy ($P < 0.05$). The analysis was performed by packages "Two Sample MR" and "MR-PRESSO" in R 4.0.2 software.

Power Calculation and Weak Instrument Bias

We used the F statistic to evaluate the strength of the association between SNP and exposure. The formula to calculate the F statistic is $F = \frac{N-k-1}{k} \times \frac{R^2}{1-R^2}$ (27). Where N represents the sample size, k is the number of SNPs. The variance (R^2) represents the phenotype variance induced by the SNPs. When R^2 is not available, we use the formula $R^2 = 2 \times MAF \times (1–MAF) \times beta^2$ (where beta represents the effect value of the genetic variant in the exposure and MAF represents the effect allele frequency) (28). When the F statistic is >10, it reveals a strong correlation between SNP and exposure with sufficient statistical power. Combined F statistics were also conducted to further assess weak instrument

TABLE 1 | The outcomes of two-sample Mendelian randomization.

Trait	Id.outcome	Method	sUMOD			uUMOD		
			BETA	SE	*P*-value	BETA	SE	*P*-value
Hypertension	ukb-b-14057	IVW	0.013084182	0.001551856	8.06E-21	0.035563214	0.0033559	3.07E-26
		MR-Egger	0.015399292	0.002209318	3.18E-09			
		WM	0.014523675	0.00330377	3.67E-04			
High blood pressure	ukb-b-14177	IVW	0.01307694	0.002270396	8.42E-09	0.036032977	0.003402303	3.29E-26
		MR-Egger	0.016328943	0.003299669	2.14E-04			
		WM	0.01470498	0.001546514	1.93E-21			
Systolic blood pressure	ieu-b-38	IVW	0.370736243	0.070147745	1.26E-07	1.09960196	0.247554764	8.92E-06
		MR-Egger	0.579574617	0.074086021	2.85E-06			
		WM	0.496799596	0.055636561	4.28E-19			
	ukb-b-20175	IVW	0.010683065	0.002753179	1.04E-04	0.028258738	0.007757293	2.70E-04
		MR-Egger	0.014238981	0.004101904	3.74E-03			
		WM	0.012356676	0.003208704	1.18E-04			
Diastolic blood pressure	ieu-b-39	IVW	0.313093826	0.049871445	3.43E-10	0.881848889	0.192013206	4.38E-06
		MR-Egger	0.441395172	0.059915509	5.45E-06			
		WM	0.365472086	0.034446225	2.68E-26			
	ukb-b-7992	IVW	0.017532188	0.003043809	8.41E-09	0.049155426	0.007738922	2.13E-10
		MR-Egger	0.023557937	0.004161065	5.87E-05			
		WM	0.019209604	0.003139275	9.41E-10			

(MR) analysis with sUMOD and uUMOD. All methods of the MR analysis outcomes are significant ($p < 0.05$).
IVW, Inverse variance weighted; WM, Weighted median; SE, Standard error; uUMOD, Urinary uromodulin; sUMOD, Serum uromodulin; SNP, Single nucleotide polymorphisms; MR, Mendelian randomization.

bias. We recalculated the power using a web-based application (https://sb452.shinyapps.io/power/) (29).

RESULTS

uUMOD MR Analysis

For the outcome of hypertension, we observed that the elevated urinary uromodulin level could increase the risk of hypertension in dataset "ukb-b-14057" (Oodds ratio (OR) = 1.036, 95% CI, 1.029–1.043, $P = 3.07E{-}26$) and "ukb-b-14177" (OR = 1.036, 95% CI, 1.030–1.044, $P = 3.29E{-}26$) (**Table 1**). In the MR analysis of SBP, uUMOD is significantly causally associated with the SBP in "ieu-b-38" ($\beta = 1.100$, standard error (SE) = 0.25, $P = 8.92E{-}06$) and "ukb-b-20175" ($\beta = 0.03$, SE = 0.01, $P = 2.70E{-}04$). The causal relationship between uUMOD and DBP was significant, as the β coefficient of "ieu-b-39" is 0.88 (SE = 0.19, $P = 4.38E{-}06$) and 0.05 for "ukb-b-7992" (SE = 0.01, $P = 2.13E{-}10$).

sUMOD MR Analysis

The effect of sUMOD on hypertension is consistent with uUMOD as the IVW outcome of "ukb-b-14057" and "ukb-b-14177" is the same (OR = 1.013, 95% CI 1.009-1.0018, all $P < 0.001$). For the outcome of SBP, the β coefficient of IVW in "ieu-b-38" is 0.371 (SE = 0.070, $P = 1.26E{-}07$) and 0.011 in "ukb-b-20175" (SE = 0.003, $P = 1.04E{-}04$). In the MR analysis, the IVW outcome of the DBP in "ieu-b-39" and "ukb-b-7992" are both significant ($\beta = 0.313$ with SE = 0.050 and $\beta = 0.018$ with SE = 0.003, respectively, all $P < 0.001$). All outcomes of three-method MR analysis are shown in **Table 1** and **Figures 3**, **4**.

Heterogeneity and Pleiotropy Test

The Scatter plot shows the distribution of the single SNP's effect on the outcome (**Figure 3**). High heterogeneity was found in hypertension ("ukb-b-14177"), high blood pressure ("ukb-b-14057"), SBP ("ieu-b-38"), and DBP ("ieu-b-39"); while SBP ("ukb-b-20175") and DBP ("ukb-b-7992") possess low heterogeneity. The leave-one-out method suggested the outcome is robust except for SBP ("ukb-b-20175" and "ieu-b-38") (**Supplementary Figure 1**). The *p*-value of the MR-Egger intercept is more than 0.05 in hypertension, high blood pressure, and SBP (the dataset "ukb-20175"), indicating no evidence of genetic pleiotropy, while <0.05 in DBP and part of SBP (the dataset "ieu-b-38"). Further horizontal pleiotropy testing with MR-PRESSO showed there are no outliers in DBP ("ukb-b-7992") and SBP ("ukb-20175"). In the outcome of hypertension ("ukb-b-14177") and high blood pressure ("ukb-b-14057"), though outliers existed, the corrected outcomes are consistent with the global rate. Rs12917707 and rs12930599 are outliers in the DBP ("ieu-b-39") with the corrected outcome ($\beta = 0.190$, $P = 2.37E{-}02$). The MR-PRESSO outcome in SBP ("ieu-b-38") is not significant when rs12917707 was excluded as an outlier (**Table 2**).

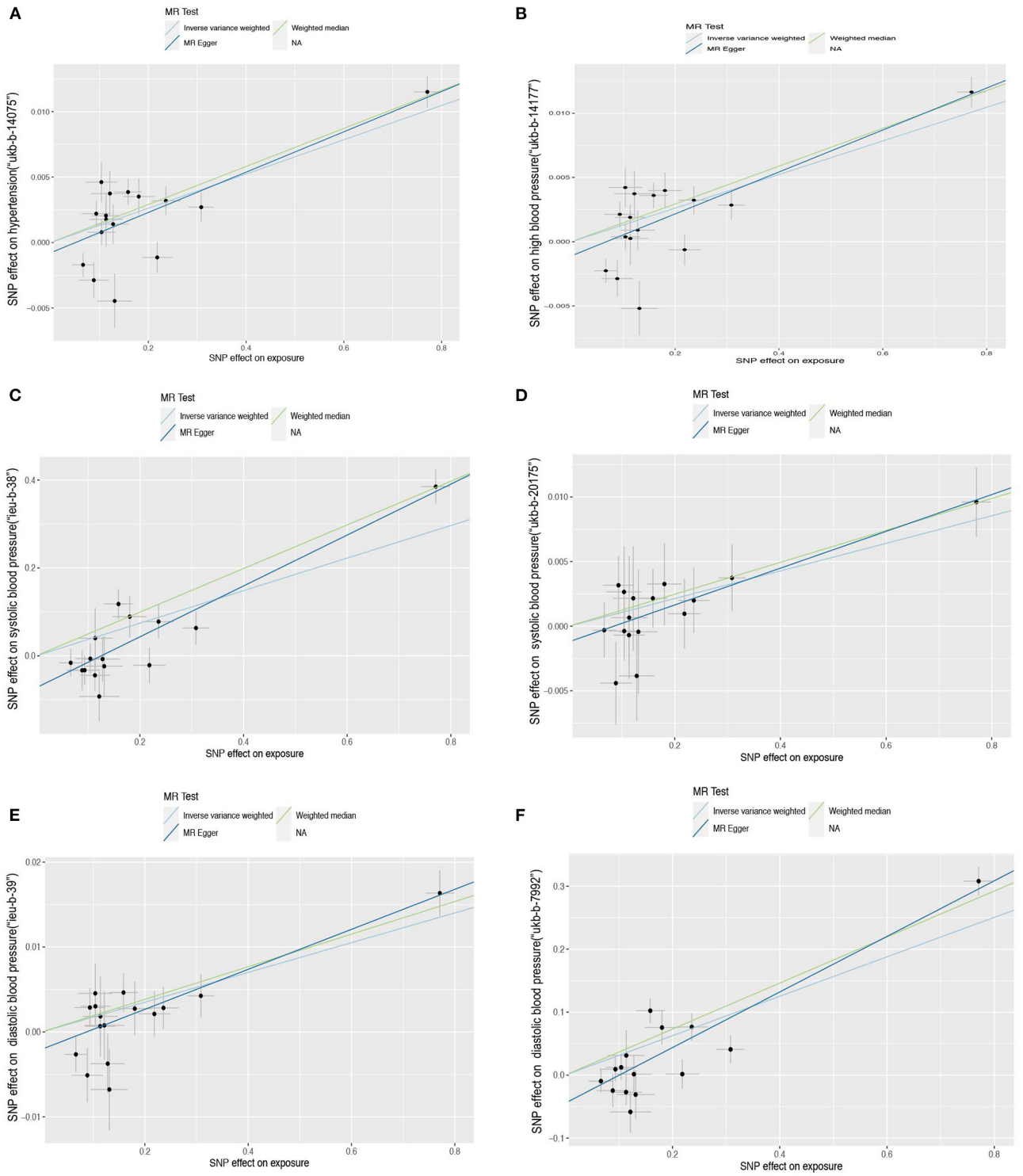

FIGURE 3 | Scatter plot of the Mendelian randomization (MR) outcome. Relationship between the effect size estimates on sUMOD (x-axis) and the effect size estimates on outcomes (y-axis): Hypertension **(A,B)**, systolic blood pressure **(C,D)**, diastolic blood pressure **(E,F)**. The slope of fitted lines represents the estimated causal effect of sUMOD obtained using the inverse variance weighted (IVW), MR-Egger, and weighted median (WM). Hypertension: **(A)** (ukb-b-14177), **(B)** (ukb-b-14-57); systolic blood pressure: **(C)** (ieu-b-38), **(D)** (ukb-b-20175); diastolic blood pressure: **(E)** (ieu-b-39), **(F)** (ukb-b-7992).

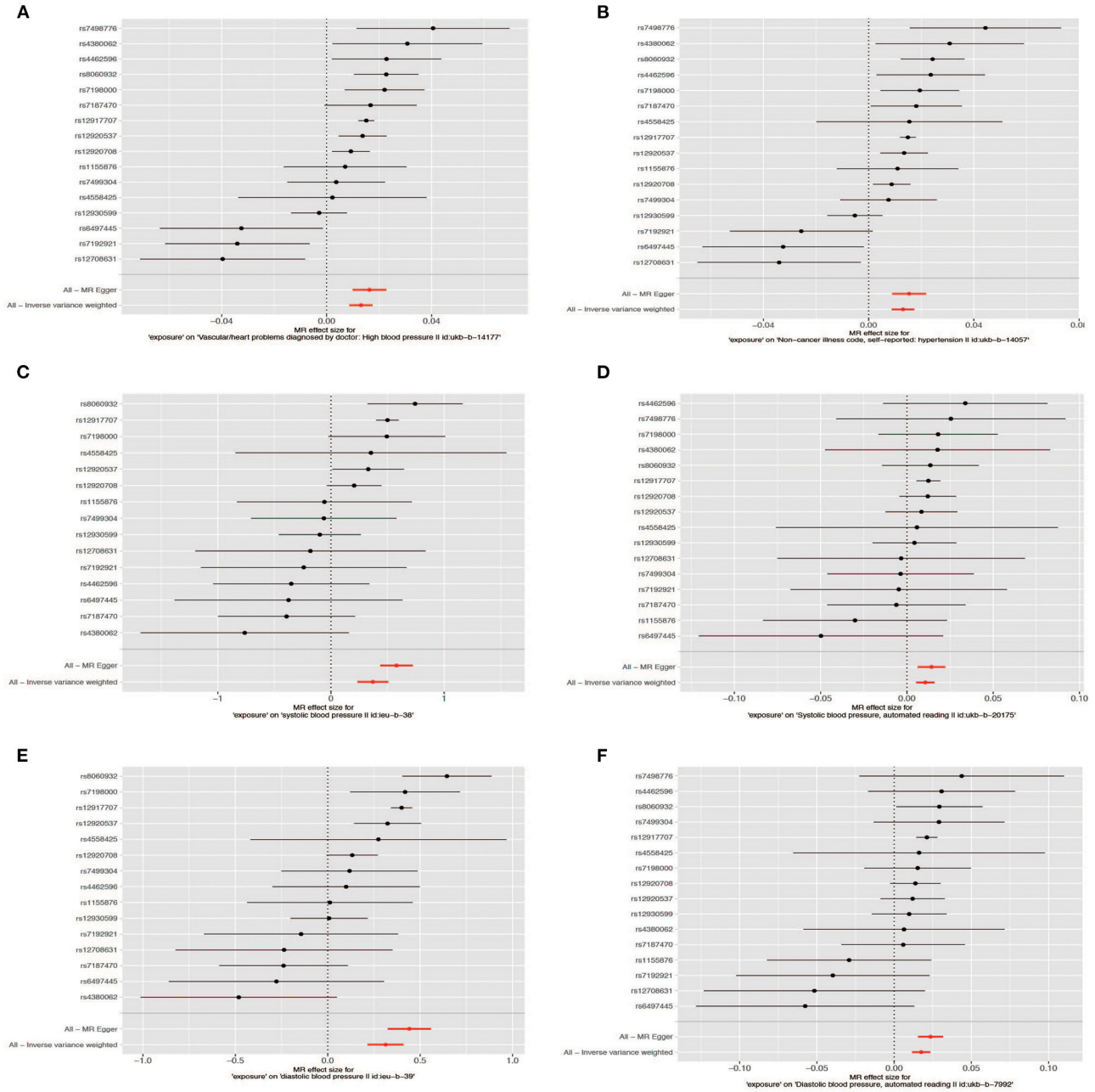

FIGURE 4 | Forest plot of the Mendelian randomization (MR) outcome. Hypertension: **(A)** (ukb-b-14177), **(B)** (ukb-b-14-57); systolic blood pressure: **(C)** (ieu-b-38), **(D)** (ukb-b-20175); diastolic blood pressure: **(E)** (ieu-b-39), **(F)** (ukb-b-7992).

Power Calculation

In the uUMOD MR analysis, the F statistics of rs12917707 and rs4494548 are 169.58 and 35.80, respectively. The mean F statistic of the sUMOD-related SNPs is 78.28. The high F statistic (empirically > 10) indicated a strong association between SNPs and urinary uromodulin and less weak instrument bias. The power of our MR analysis in different pairs was over 90% at an alpha rate of 5%, except the dataset "ukb-b-20175" (systolic blood pressure) which was 75.2% (**Supplementary Table 5**).

DISCUSSION

Our MR analysis unveiled the causal effect of both uUMOD and sUMOD on blood pressure by integrating publicly available GWAS datasets. High sUMOD and uUMOD could contribute to the risk of hypertension (the biggest OR is 1.036, 95% CI, 1.030–1.044). Both sUMOD and uUMOD are causally associated with both SBP (the largest causal estimate being a 0.10 mmHg per unit change in uromodulin) and DBP (the

TABLE 2 | Outcomes of Mendelian Randomization Pleiotropy RESidual Sum and Outlier (MR-PRESSO).

Datasets ID	Outliers	Corrected beta	*P* value
ukb-b-14177	rs12708631,rs12917707 rs12930599,rs7192921	0.014	6.93E−04
ukb-b-14057	rs12708631,rs12917707 rs2930599,rs7192921	0.012	4.00E−03
ieu-b-39	rs12917707,rs12930599	0.19	2.37E−02
ukb-b-7992	NA	NA	NA
ieu-b-38	rs12917707	0.148	0.13
ukb-b-20175	NA	NA	NA

largest causal estimate being a 0.88 mmHg per unit change in uromodulin).

To our limited knowledge, this is the first study designed to research the causal association of both serum and urinary uromodulin and hypertension by MR methods with potential confounders removed by genetic variants. One abstract using MR analysis to reveal the causal association of uUMOD of DBP and SBP only included the "ieu-b-39" and "ieu-b-38" datasets (30). We screened all the summary studies of DBP and SBP on the MR-base up to 2020 and added two datasets. Besides, we further assessed the causal effect of uromodulin on hypertension, utilizing the open data showing the association between SNPs and sUMOD.

We adopted three methods based on different assumptions to ensure our outcome. MR-Egger relies on the assumption that the SNP should affect the risk of the outcome through the exposure, not via other risk factors; namely Instrument Strength Independent of Direct Effect (InSIDE). The WM method does not require InSIDE to be taken into account. This method provides valid estimates when at least 50% of the weight comes from valid variants. It can improve the power of causal effect detection and decrease type I error with distinct superiorities over MR-Egger (31). MR-PRESSO enhances the detection of outliers by rigorously exploring whether the findings were biased due to pleiotropy. Although we cannot entirely rule out pleiotropy, we observed a consistent outcome between uromodulin levels and blood pressure in conventional MR analysis. Our results based on different methods and datasets strengthen the theoretical support for further well-designed prospective randomized clinical trials to verify the causal association of uromodulin and hypertension, larger than those that came before them. Furthermore, our results may further suggest that uromodulin might serve as a new therapeutic target for hypertension management.

The correlation between uromodulin and hypertension was first disclosed in 1998. Duława, J. reported that compared with healthy control, uUMOD excretion was significantly higher in hypertensive individuals (32); and could be normalized by angiotensin converting enzyme inhibitors (ACEI) (33). It is consistent in pre-eclampsia patients (34). RNA-seq data of wild-type (WT) mice treated by a high salt diet showed a significant upregulation of heat-shock proteins Hspa1b (Hsp70) and blood pressure that were both abolished in UMOD knockout mice (35–37). It indicated the potential causal relationship between UMOD and hypertension, but was difficult to confirm in a population study. Our study has shown that UMOD was a causal factor of hypertension by utilizing the Mendelian randomization method.

The underlying mechanism of uromodulin influencing blood pressure is due to its regulation of the ion channel's activity in TAL and DCT, including the renal outer medullary potassium channel (ROMK), epithelial sodium channel (ENaC), Na+-K+-2Cl- cotransporter (NKCC2), and Na+-Cl– cotransporter (NCC). Animal studies proved that UMOD could upregulate the ROMK and ENaC expression in TAL (38) and lead to salt-sensitive hypertension. UMOD knockout mice presented significantly lower systolic blood pressure compared to WT mice under basal conditions (39, 40). UMOD transgenic mice with increased expression and secretion of uromodulin showed higher BP and a significant increase of NKCC2 phosphorylation at activating sites (Thr96 and Thr101). In an *in vitro* study, co-expression of uromodulin in renal cells induced an obvious increase of NKCC2 phosphorylation and its activity (12). Uromodulin also facilitated NCC phosphorylation which was possible via SPS1-related proline/alanine-rich kinase/oxidative stress response kinase 1 (SPAK-OSR1) modulation (41). The upregulation activity of both NKCC2 and NCC contribute to NaCl reabsorption and retention, leading to salt-sensitive hypertension (42, 43).

Our study had some limitations. First, since all the data came from people of European origin, the results were not representative of a truly random population sample nor applicable to other ethnicities. In the uUMOD MR, we only included two SNPs, which meant we could not conduct MR-Egger, a median-based estimator, model-based estimators, and other analysis methods to examine the horizontal pleiotropy. Second, there was a likely overlap of uUMOD (population from the Framingham Heart Study: 24%) and ICBP (0.3%). However, the overlapping degree is small in ICBP and we included the UK Biobank to confirm the outcome. We assessed the effect of the overlap in the online app (https://sb452.shinyapps.io/overlap/), it showed that when the overlap is below 30%, the bias is <0.018 (44). Third, due to the presence of strong instruments, we consider this overlap not to introduce significant bias (45). Fourth, we could not perform the bidirectional Mendelian randomization owing to a lack of effect size data on hypertension-related SNPs in the exposure population. Fifth, due to the lack of individual data, all MR methods tested only the linear effect of uromodulin on blood pressure, and could not exclude a

modest or non-linear effect. Finally, as this was an MR analysis, we also could not overcome general limitations such as the possibility of population stratification, the pleiotropy of SNPs, and canalization (15).

CONCLUSION

In conclusion, our study based on open datasets suggests a potentially detrimental impact of high levels of uromodulin on the development of hypertension; which is the first time this has been shown to be consistent with the observational study and basic experimental study.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/**Supplementary Material**.

AUTHOR CONTRIBUTIONS

RY conceived and designed the study, performed the study, analyzed the data, wrote the paper, and prepared figures and/or tables. LC performed the MR analysis, analyzed the data, and reviewed drafts of the paper. LX and DZ analyzed the data, prepared figures and tables, and reviewed drafts of the paper. HL performed the study, analyzed the data, and prepared figures and tables. XS designed the study and reviewed drafts of the paper. YZ analyzed the data and reviewed drafts of the paper. LC conceived and designed the study, wrote the paper, and reviewed drafts of the paper. All authors contributed to the article and approved the submitted version.

FUNDING

This work was partially supported by grants from the National Natural Scientific Foundation of China (81970607, 81470937 to LC, 81860136 to YZ); the Key Research and Development Program of Ningxia Hui Autonomous Region (2018BFG02010 to LC); Capital's Funds for Health Improvement and Research (CFH 2020-2-4018 to LC); Beijing Natural Science Foundation (L202035 to LC); CAMS Innovation Fund for Medical Sciences (CIFMS 2020-I2M-C&T-A-001 to LC); Chinese Academy of Medical Sciences Innovation Fund for Medical Sciences (CIFMS 2016-I2M-2-004 to LC); and National Key-point Research Program Precision Medicine Grant (2016YFC0901500 to LC). The funders had no role in study design, data collection, and analysis, decision to publish, or preparation of the manuscript.

ACKNOWLEDGMENTS

We thank the GWAS Catalog and Doc. Matthias Olden et al. for making their data of uUMOD-related SNPs open. We also thank Doctor Jennifer Sjaarda's group for disclosing their study data, especially the effect sizes and standard error of the SNPs of serum uromodulin. Additionally, we are grateful to the MR-base, UK Biobank, ICBP, and MRC-IEU for making the GWAS summary data publicly available, and grateful to all the researchers and participants who contributed to those studies.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2021.736001/full#supplementary-material

REFERENCES

1. Talukder MA, Johnson WM, Varadharaj S, Lian J, Kearns PN, El-Mahdy MA, et al. Chronic cigarette smoking causes hypertension, increased oxidative stress, impaired NO bioavailability, endothelial dysfunction, and cardiac remodeling in mice. *Am J Physiol Heart Circ Physiol 300(1).* (2011) H388–396. doi: 10.1152/ajpheart.00868.2010
2. Klatsky AL. Alcohol-associated hypertension: when one drinks makes a difference. *Hypertension 44(6).* (2004) 805–6. doi: 10.1161/01.Hyp.0000146538.26193.60
3. AlWabel AH, Almufadhi MA, Alayed FM, Aloraini AY, Alobaysi HM, Alalwi RM. Assessment of hypertension and its associated risk factors among medical students in Qassim University. *Saudi J Kidney Dis Transpl 29(5).* (2018) 1100–8. doi: 10.4103/1319-2442.243959
4. He FJ, Li J, Macgregor GA. Effect of longer term modest salt reduction on blood pressure: Cochrane systematic review and meta-analysis of randomised trials. *Bmj.* (2013) 346:f1325. doi: 10.1136/bmj.f1325
5. Samadian F, Dalili N, Jamalian A. Lifestyle Modifications to Prevent and Control Hypertension. *Iran J Kidney Dis 10(5).* (2016) 237–63.
6. Goodall AA, Marshall RD. Effects of freezing on the estimated amounts of Tamm–Horsfall glycoprotein in urine, as determined by radioimmunoassay. *Biochem J 189(3).* (1980) 533–9. doi: 10.1042/bj1890533
7. Scherberich JE, Gruber R, Nockher WA, Christensen EI, Schmitt H, Herbst V, et al. Serum uromodulin-a marker of kidney function and renal parenchymal integrity. *Nephrol Dial Transplant 33(2).* (2018) 284–95. doi: 10.1093/ndt/gfw422
8. Devuyst O, Pattaro C. The UMOD locus: insights into the pathogenesis and prognosis of kidney disease. *J Am Soc Nephrol 29(3).* (2018) 713–26. doi: 10.1681/asn.2017070716
9. Leiherer A, Muendlein A, Saely CH, Kinz E, Brandtner EM, Fraunberger P, et al. Serum uromodulin is associated with impaired glucose metabolism. *Medicine (Baltimore) 96(5).* (2017) e5798. doi: 10.1097/md.0000000000005798
10. Bostom A, Steubl D, Garimella PS, Franceschini N, Roberts MB, Pasch A, et al. Serum uromodulin: a biomarker of long-term kidney allograft failure. *Am J Nephrol 47(4).* (2018) 275–82. doi: 10.1159/000489095
11. Usui R, Ogawa T, Takahashi H, Iwasaki C, Koike M, Morito T, et al. Serum uromodulin is a novel renal function marker in the Japanese population. *Clin Exp Nephrol 25(1).* (2021) 28–36. doi: 10.1007/s10157-020-01964-y
12. Trudu M, Janas S, Lanzani C, Debaix H, Schaeffer C, Ikehata M, et al. Common noncoding UMOD gene variants induce salt-sensitive hypertension and kidney damage by increasing uromodulin expression. *Nat Med 19(12).* (2013) 1655–60. doi: 10.1038/nm.3384
13. Emdin CA, Khera AV, Kathiresan S. Mendelian randomization. *Jama.* (2017) 318:1925–6. doi: 10.1001/jama.2017.17219

14. Preiss D, Tobert JA, Hovingh GK, Reith C. Lipid-modifying agents, from statins to PCSK9 inhibitors: JACC focus seminar. *J Am Coll Cardiol.* (2020) 75:1945–55. doi: 10.1016/j.jacc.2019.11.072
15. Grau-Perez M, Redon J. Unraveling new factors associated with hypertension: the Mendelian randomization approach. *J Hypertens.* (2020) 38:30–2. doi: 10.1097/hjh.0000000000002251
16. Olden M, Corre T, Hayward C, Toniolo D, Ulivi S, Gasparini P, et al. Common variants in UMOD associate with urinary uromodulin levels: a meta-analysis. *J Am Soc Nephrol.* (2014) 25:1869–82. doi: 10.1681/asn.2013070781
17. Sjaarda J, Gerstein HC, Yusuf S, Treleaven D, Walsh M, Mann JFE, et al. Blood HER2 and Uromodulin as Causal Mediators of CKD. *J Am Soc Nephrol.* (2018) 29:1326–35. doi: 10.1681/ASN.2017070812
18. Hemani G, Zheng J, Elsworth B, Wade KH, Haberland V, Baird D, et al. The MR-Base platform supports systematic causal inference across the human phenome. *Elife.* (2018) 7:e34408. doi: 10.7554/eLife.34408
19. Sudlow C, Gallacher J, Allen N, Beral V, Burton P, Danesh J, et al. UK biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. *PLoS Med.* (2015) 12:e1001779. doi: 10.1371/journal.pmed.1001779
20. Ehret GB, Munroe PB, Rice KM, Bochud M, Johnson AD, Chasman DI, et al. Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. *Nature.* (2011) 478:103–9. doi: 10.1038/nature10405
21. International Consortium for Blood Pressure Genome-Wide Association, Ehret S, Munroe GB, Rice PB, Bochud KM, Johnson MAD., et al. Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. *Nature.* (2011) 478:103–9.
22. Evangelou E, Warren HR, Mosen-Ansorena D, Mifsud B, Pazoki R, Gao H, et al. Genetic analysis of over 1 million people identifies 535 new loci associated with blood pressure traits. *Nat Genet.* (2018) 50:1412–25. doi: 10.1038/s41588-018-0205-x
23. Wu F, Huang Y, Hu J, Shao Z. Mendelian randomization study of inflammatory bowel disease and bone mineral density. *BMC Med.* (2020) 18:312. doi: 10.1186/s12916-020-01778-5
24. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. *Int J Epidemiol.* (2015) 44:512–25. doi: 10.1093/ije/dyv080
25. Bowden J, Spiller W, Del Greco MF, Sheehan N, Thompson J, Minelli C, et al. Improving the visualization, interpretation and analysis of two-sample summary data Mendelian randomization via the Radial plot and Radial regression. *Int J Epidemiol.* (2018) 47:1264–78. doi: 10.1093/ije/dyy101
26. Verbanck M, Chen CY, Neale B, Do R. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. *Nat Genet.* (2018) 50:693–8. doi: 10.1038/s41588-018-0099-7
27. Levin MG, Judy R, Gill D, Vujkovic M, Verma SS, Bradford Y, et al. Genetics of height and risk of atrial fibrillation: A Mendelian randomization study. *PLoS Med.* (2020) 17:e1003288. doi: 10.1371/journal.pmed.1003288
28. Palmer TM, Lawlor DA, Harbord RM, Sheehan NA, Tobias JH, Timpson NJ, et al. Using multiple genetic variants as instrumental variables for modifiable risk factors. *Stat Methods Med Res.* (2012) 21:223–42. doi: 10.1177/0962280210394459
29. Burgess S. Sample size and power calculations in Mendelian randomization with a single instrumental variable and a binary outcome. *Int J Epidemiol.* (2014) 43:922–9. doi: 10.1093/ije/dyu005
30. Tan LE, Aman A, McCallum L, Dominiczak AF, Padmanabhan S. Causal association of uromodulin with blood pressure independent of renal function. *J Hypertens.* (2018) 36:e345–6.
31. Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent estimation in Mendelian randomization with some invalid instruments using a weighted median estimator. *Genet Epidemiol.* (2016) 40:304–14. doi: 10.1002/gepi.21965
32. Duława J, Kokot F, Kokot M, Pander H. Urinary excretion of Tamm-Horsfall protein in normotensive and hypertensive elderly patients. *J Hum Hypertens.* (1998) 12:635–7. doi: 10.1038/sj.jhh.1000680
33. Guidi E, Giglioni A, Cozzi MG, Minetti EE. Which urinary proteins are decreased after angiotensin converting–enzyme inhibition? *Ren Fail.* (1998) 20:243–8. doi: 10.3109/08860229809045108
34. Carty DM, Siwy J, Brennand JE, Zürbig P, Mullen W, Franke J, et al. Urinary proteomics for prediction of preeclampsia. *Hypertension 57(3).* (2011) 561–9. doi: 10.1161/hypertensionaha.110.164285
35. Wolf MTF, Zhang J, Nie M. Uromodulin in mineral metabolism. *Curr Opin Nephrol Hypertens 28(5).* (2019) 481–9. doi: 10.1097/mnh.0000000000000522
36. Graham LA, Aman A, Campbell DD, Augley J, Graham D, McBride MW, et al. Salt stress in the renal tubules is linked to TAL-specific expression of uromodulin and an upregulation of heat shock genes. *Physiol Genomics 50(11).* (2018) 964–72. doi: 10.1152/physiolgenomics.00057.2018
37. Pockley AG, De Faire U, Kiessling R, Lemne C, Thulin T, Frostegård J. Circulating heat shock protein and heat shock protein antibody levels in established hypertension. *J Hypertens 20(9).* (2002) 1815–20. doi: 10.1097/00004872-200209000-00027
38. Bachmann S, Mutig K, Bates J, Welker P, Geist B, Gross V, et al. Renal effects of Tamm-Horsfall protein (uromodulin) deficiency in mice. *Am J Physiol Renal Physiol 288(3).* (2005) F559–567. doi: 10.1152/ajprenal.00143.2004
39. Arhatte M, Gunaratne GS, El Boustany C, Kuo IY, Moro C, Duprat F, et al. TMEM33 regulates intracellular calcium homeostasis in renal tubular epithelial cells. *Nat Commun.* (2019) 10:2024. doi: 10.1038/s41467-019-10045-y
40. Pitzer AL, Van Beusecum JP, Kleyman TR, Kirabo A. ENaC in Salt-sensitive hypertension: kidney and beyond. *Curr Hypertens Rep.* (2020) 22:69. doi: 10.1007/s11906-020-01067-9
41. Tokonami N, Takata T, Beyeler J, Ehrbar I, Yoshifuji A, Christensen EI, et al. Uromodulin is expressed in the distal convoluted tubule, where it is critical for regulation of the sodium chloride cotransporter NCC. *Kidney Int.* (2018) 94:701–15. doi: 10.1016/j.kint.2018.04.021 Epub 2018 Jul 12. 2018/07/17.
42. Caceres PS, Ortiz PA. Molecular regulation of NKCC2 in blood pressure control and hypertension. *Curr Opin Nephrol Hypertens.* (2019) 28:474–80. doi: 10.1097/mnh.0000000000000531
43. Thomson MN, Cuevas CA, Bewarder TM, Dittmayer C, Miller LN, Si J, et al. WNK bodies cluster WNK4 and SPAK/OSR1 to promote NCC activation in hypokalemia. *Am J Physiol Renal Physiol.* (2020) 318:F216–f228. doi: 10.1152/ajprenal.00232.2019
44. Burgess S, Davies NM, Thompson SG. Bias due to participant overlap in two-sample Mendelian randomization. *Genet Epidemiol.* (2016) 40:597–608. doi: 10.1002/gepi.21998
45. Luo S, Clarke SLN, Ramanan AV, Thompson SD, Langefeld CD, Marion MC, et al. Platelet glycoprotein Ib α-chain as a putative therapeutic target for juvenile idiopathic arthritis: a mendelian randomization study. *Arthritis Rheumatol.* (2021) 73:693–701. doi: 10.1002/art.41561

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frontiers in Cardiovascular Medicine

ORIGINAL RESEARCH
published: 21 September 2021
doi: 10.3389/fcvm.2021.735679

Baseline and Cumulative Blood Pressure in Predicting the Occurrence of Cardiovascular Events

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OPEN ACCESS

Edited by:
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Reviewed by:
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Specialty section:
This article was submitted to Hypertension, a section of the journal Frontiers in Cardiovascular Medicine

Received: *03 July 2021*
Accepted: *24 August 2021*
Published: *21 September 2021*

Citation:
Zuo Y, Zheng D, Chen S, Yang X, Yan Y, Liu F, Tian X, Wang M, Su X, Wen J, Zhai Q, Zhang Y, Gaisano HY, Wu S, Wang A and He Y (2021) Baseline and Cumulative Blood Pressure in Predicting the Occurrence of Cardiovascular Events. Front. Cardiovasc. Med. 8:735679. doi: 10.3389/fcvm.2021.735679

Background: Both baseline blood pressure (BP) and cumulative BP have been used to estimate cardiovascular event (CVE) risk of higher BP, but which one is more reliable for recommendation to routine clinical practice is unclear.

Methods: In this prospective study, conducted in the Kailuan community of Tanshan City, China, a total of 95,702 participants free of CVEs at baseline (2006–2007) were included and followed up until 2017. Time-weighted cumulative BP that expresses the extent of cumulative BP exposure is defined as the sum of the mean of two consecutive systolic or diastolic BP times the interval between the two determinations, then normalized by the total follow-up duration. Incident CVEs during 2006–2017 were confirmed by review of medical records. We performed a competing risk regression analysis to assess CVE risk of the different durations of higher BP exposure. ROC analysis was performed to assess the predictive value of higher BP on CVE occurrence.

Results: We found that when the risk of higher BP on CVE occurrence was estimated based on time-weighted cumulative BP, the hazard ratios (HRs) increased with the increase in duration of higher BP exposure in each of the four BP groups: <120/<80, 120–129/<80, 130–139/80–89, and ≥140/≥90 mmHg; this time trend also occurred across the four different BP groups, with the higher BP group exhibiting CVE risk earlier during the follow-up. These results were confirmed by the same analysis performed on participants without baseline hypertension. However, such reasonable time trends did not occur when a single baseline BP was used as the primary estimation. We also demonstrated that the predictive values of baseline systolic and diastolic BP that predict CVE occurrence were only 0.6–3.2 and 0.2–3.1% lower, respectively, than those of cumulative BP combined with baseline BP during follow-up.

Conclusions: Baseline BP remains a useful indicator for predicting future occurrence of CVEs. Nevertheless, time-weighted cumulative BP could more reliably estimate the CVE risk of higher BP exposure than baseline BP.

Keywords: hypertenion, baseline blood pressure, exposure, time-weighted cumulative blood pressure, cardiovasclar disease

INTRODUCTION

Hypertension is highly prevalent in the adult population, affecting about 1.13 billion adults worldwide, of which 226 million are in China (1). A previous study reported that ∼25% of cardiovascular events (CVEs) were attributable to hypertension (2). Therefore, a precise assessment of the influence of high blood pressure (BP) exposure on the occurrence of CVEs is critically important to gain a more complete understanding of CVE risk of hypertension.

For reasons of accessibility, the effect of a higher BP on CVEs has been frequently estimated based on baseline BP measurement (3–8). The presumption of this common practice is that future BP is better predicted by the current BP, but this presumption is actually misleading since BP changes particularly with the increase in age (9–11). More recently, the cumulative BP determination was developed, which includes several BP measurements that take into consideration of both how high the BP levels have been and the duration of the high BP exposure before risk estimation. Several studies have simultaneously used the cumulative BP and baseline BP determinations to assess the effect of high BP on CVE occurrence, which showed that the association of the cumulative BP determination with CVE was stronger than the baseline BP determination (12–15). Although cumulative BP seems to be superior than baseline BP in estimating CVE risk of high BP theoretically, no population studies have yet to verify this presumption. Thus, overall, it remains unclear whether cumulative BP vs. baseline BP determination is more reliable for recommendation to routine clinical practice.

Currently, baseline BP determination remains commonly used to predict the occurrence of CVEs. Two studies compared a single BP determination vs. cumulative BP determination in its predictive ability on the occurrence of cardiovascular disease (CVD), which found that cumulative BP determination had an improvement in disease risk prediction models for the improvement of net reclassification index (NRI) or C-statistics (15, 16). However, these studies did not demonstrate the individual predictive values (AUC) of cumulative BP and a single measurement of BP determinations; therefore, the absolute deviation between the two remains equivocal. This raises the unresolved issue that baseline BP determination could still be used as an indicator to predict the occurrence of CVEs.

To address the abovementioned issue, we have leveraged the Kailuan Study, a large prospective cohort study. We first aimed to compare the performance of baseline BP vs. cumulative BP determinations in estimating CVE risk of different durations of high BP measured during follow-up; and secondly, we compared the predictive value of baseline BP vs. cumulative BP determinations in predicting CVEs during each follow-up.

METHODS

Study Design and Participants

The data of this prospective study were derived from the Kailuan Study, which was a prospective cohort study that was conducted in the Kailuan community in Tanshan City, China (17). The participants in the Kailuan Study were employees and retirees of the Kailuan Group Company, which is the largest company of the coal mining industry in Tangshan. In brief, a total of 101,510 individuals (81,110 men and 20,400 women, aged 18–98 years) completed the first survey conducted between June 2006 and October 2007. We performed re-examinations at 2-year intervals up to the end of the last follow-up on December 31, 2017. The detailed design of the Kailuan Study has been described previously (18, 19).

In the present study, we excluded the following: 3,732 participants that had a history of cardiovascular disease, 1,159 participants with missing information on BP measurements, and 917 participants with hypotension at baseline. Therefore, a total of 95,702 participants were eligible to be included, of which 88,396 completed the 11 years of follow-up (**Supplementary Figure 1**). This study was approved by the Ethics Committees of Kailuan General Hospital and Beijing Tiantan Hospital and adhered to the principles of the Helsinki Declaration. All participants signed an informed consent.

Data Collection

In the Kailuan Study, all participants completed a questionnaire, which included information on age, sex, smoking, alcohol intake, physical activity, and medical history. Current smoking was defined as smoking for at least in the last year. Current alcohol consumption was defined as the average daily consumption of a strong spirit (alcohol content >50%) of 100 ml or more for at least in the previous year. Active physical activity was categorized as very active (≥80 min/week of moderate to vigorous intensity), moderately active (<80 min/week of moderate to vigorous intensity), and inactive (no exercise at all). Antihypertensive medication was defined as any self-reported use of antihypertensive drugs.

Anthropometric measurements included height and weight, and body mass index was calculated as weight in kilograms divided by height in meters squared. BP was measured with a manual sphygmomanometer in the period between 2006 and 2014 and with an electronic blood pressure meter (HEM-8102A; Omron Limited, Dalian, China) from 2014 onwards (19). Three readings of systolic blood pressure (SBP) and diastolic blood pressure (DBP) were taken at a 5-min interval after the participants had rested in a seated position for at least 5 min. The reading values were rounded to the nearest full figure. The average value of the three BP measures was used for further analysis.

Blood samples taken from the antecubital vein in the morning after an overnight fast (8–12 h) were assayed with an automatic analyzer (Hitachi 747; Hitachi, Tokyo, Japan) for fasting plasma glucose, total cholesterol, triglycerides, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol levels, and creatinine (20). The estimated glomerular filtration rate was calculated by the Chronic Kidney Disease Epidemiology Collaboration equation (21).

The diagnostic criteria for hypertension are as follows: a SBP ≥140 mmHg or DBP ≥90 mmHg, a diagnosis of hypertension by a previous physician, or the use of antihypertensive drugs (22). Hypotension was defined as SBP ≤90 mmHg or DBP

≤60 mmHg. Variability of SBP and DBP was estimated as the standard deviation (SD) of the measures during the whole follow-up periods. Diabetes was defined as a self-reported history of a specialist-made diagnosis of diabetes mellitus, the intake of hypoglycemic drugs, or a fasting plasma glucose ≥7.0 mmol/L (23).

Calculation of Time-Weighted Cumulative BP

To precisely determine the extent of BP exposure of an individual, a time-weighted cumulative BP determination using all available BP measurements from baseline to the end of this study or before any incident CVE was calculated (13). The time-weighted cumulative BP determination was defined as the sum of the mean of each two consecutive SBP or DBP times its corresponding interval and then normalized by the follow-up time of the individual. For example, if a participant was enrolled in 2006 and followed up in 2008, 2010, and 2012, then the time-weighted cumulative BP in 2012 was calculated as $[(BP_{2006} + BP_{2008})/2 * 2 + (BP_{2008} + BP_{2010})/2 * 2 + (BP_{2010} + BP_{2012})/2 * 2]/6$. Baseline and cumulative BP determinations were categorized into four mutually exclusive groups: (1) <120/<80 mmHg: SBP <120 mmHg and DBP <80 mmHg, (2) 120–129/<80 mmHg: SBP 120–129 mmHg and DBP <80 mmHg, (3) 130–139/80–89 mmHg: SBP 130–139 mmHg or DBP 80–89 mmHg, and (4) ≥140/≥90 mmHg: SBP ≥140 mmHg or DBP ≥90 mmHg.

Assessment of Incident CVEs

The participants of the present study were followed up from the baseline examination in 2006 up to the date of the occurrence of CVEs, or up to 2017 that is the very end of the follow-up period for this study, whichever came first. A CVE is the main outcome, which include stroke, myocardial infarction, and cardiovascular death (24). Stroke was diagnosed according to the World Health Organization criteria on the basis of clinical symptoms, images obtained by computed tomography or magnetic resonance imaging, and other diagnostic reports (25). Myocardial infarction was diagnosed according to the criteria of the World Health Organization based on clinical symptoms, electrocardiogram changes and cardiac enzyme levels, and various symptoms of chest pain (26). Cardiovascular death was defined as death from cardiovascular disease according to the tenth version of the International Classification of Disease (ICD-10). In this study, the outcomes information was obtained directly from the regular re-examinations conducted at 2-year intervals or from the hospital discharge summaries and medical records from the Hospital Discharge Register and the Municipal Social Insurance.

Statistical Analyses

Data were expressed as frequency and percentage, median with interquartile range, or mean ± standard deviation. Tests of differences in the characteristics across the categories for baseline BP determination were performed using the analysis of variance or the Kruskal-Wallis test for continuous variables according to distribution and the chi-square test for categorical variables. The incidence of CVEs was expressed as incidence density (per 1,000 person-years).

Univariate survival analysis was performed by the Kaplan–Meier method and log-rank test. To eliminate the bias from the competing risk of death, a Fine and Gray competing risk regression was constructed to examine the association of BP and the incidence of CVEs, whereas non-cardiovascular death was treated as a competing risk event (27). To assess the association of BP and the incident CVEs, we fitted three regression models. Model 1 was adjusted for age, sex, body mass index, smoking, alcohol consumption, physical activity, and SBP and DBP at baseline. Model 2 was adjusted for all the covariates in model 1 plus triglycerides, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, fasting plasma glucose, and estimated glomerular filtration rate at baseline. Model 3 was further adjusted for history of hypertension and antihypertensive drug intake at baseline and the variation of SBP and DBP during follow-up based on model 2.

Restricted cubic spline regression was established to examine the optimal range of BP by adjusted hazard ratios (HRs) and 95% confidence intervals (CIs), with 5 knots located at the 5th, 25th, 50th, 75th, and 95th percentiles of SBP or DBP (28). First, through the smooth curve and Cox regression model, we could find the point with the lowest risk of CVEs. Second, we used this lowest point as the reference to determine a non-significantly optimal range. The predictive values of baseline BP and time-weighted cumulative BP determinations for CVE occurrence were evaluated by the AUC, which was calculated from the receiver operating characteristic (ROC) curve analyses.

All analyses were conducted using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) or R version 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria). All reported *P*-values were based on a two-sided test of significance, and $P < 0.05$ was deemed statistically significant.

RESULTS

Baseline Characteristics of the Participants

The median age of the participants in the study was 51.6 years (IQR 43.5–58.8) and males accounted for 79.7% ($N = 76,310$). **Table 1** summarizes the baseline characteristics of the participants along the different baseline BP categories. Participants with a BP of ≥140/≥90 mmHg were older and had higher body mass index, fasting plasma glucose, low-density lipoprotein cholesterol, triglycerides, and total cholesterol and lower estimated glomerular filtration rate. The proportion of participants with diabetes and dyslipidemia was highest in the group with BP ≥140/≥90 mmHg.

Changes in BP and the Occurrence of CVEs

The mean SBP and DBP of the participants at baseline (year 2006–2007) were 131 and 84 mmHg, respectively; however, these BP levels changed to 139 and 83 mmHg after 10 years of follow-up (year 2016–2017), wherein 36,090 new incident

TABLE 1 | Characteristics of the participants according to the baseline BP categories.

Baseline characteristic	<120/<80 mmHg	120–129/<80 mmHg	130–139/80–89 mmHg	≥140/≥90 mmHg	*P*-value
No. (%)	18,999 (19.8)	5,781 (6.0)	43,847 (45.8)	27,075 (28.3)	
Age, years	46.4 (36.9–53.8)	51.7 (43.3–59.3)	51.7 (43.7–58.9)	54.3 (48.3–61.8)	<0.01
Male sex, *n* (%)	12,688 (66.8)	4,501 (77.9)	35,868 (81.8)	23,253 (85.9)	<0.01
Current smoker, *n* (%)	6,623 (35.4)	2,258 (40.1)	14,643 (34.4)	8,367 (31.8)	<0.01
Current alcohol, *n* (%)	7,479 (40.0)	2,405 (42.7)	16,014 (37.6)	8,998 (34.2)	<0.01
Physical activity, *n* (%)					
Inactive	1,834 (9.9)	506 (9.0)	3,721 (8.8)	2,008 (7.7)	<0.01
Moderately active	14,303 (77.4)	4,137 (74.0)	32,041 (76.1)	19,761 (75.7)	
Very active	2,333 (12.6)	950 (17.0)	6,328 (15.0)	4,321 (16.6)	
BMI, kg/m^2	23.4 (21.3–25.6)	24.3 (22.1–26.6)	24.9 (22.8–27.2)	26.0 (23.8–28.3)	<0.01
SBP, mmHg	110.0 (100.7–111.0)	120.7 (120.0–128.0)	130.0 (120.0–134.0)	150.0 (140.0–160.0)	<0.01
DBP, mmHg	70.0 (69.3–73.3)	75.0 (70.0–79.3)	80.0 (80.0–85.0)	96.0 (90.0–100.0)	<0.01
FPG, mmol/L	5.0 (4.6–5.4)	5.1 (4.6–5.6)	5.1 (4.7–5.7)	5.2 (4.8–6.0)	<0.01
LDL-C, mmol/L	2.2 (1.8–2.7)	2.3 (1.8–2.8)	2.4 (1.8–2.8)	2.4 (1.9–2.9)	<0.01
HDL-C, mmol/L	1.5 (1.3–1.7)	1.5 (1.3–1.8)	1.5 (1.3–1.8)	1.5 (1.3–1.8)	<0.01
TG, mmol/L	1.0 (0.7–1.6)	1.2 (0.8–1.7)	1.3 (0.9–2.0)	1.4 (1.0–2.2)	<0.01
TC, mmol/L	4.8 (4.1–5.4)	4.9 (4.3–5.6)	4.9 (4.3–5.6)	5.0 (4.4–5.7)	<0.01
eGFR (ml/min/1.73 m^2)	86.3 (73.8–99.7)	82.6 (70.4–95.4)	81.5 (68.0–96.1)	75.7 (63.3–90.8)	<0.01
History of hypertension, *n* (%)	398 (2.1)	346 (6.0)	3,947 (9.0)	6,289 (23.2)	<0.01
Diabetes mellitus, *n* (%)	874 (4.6)	469 (8.1)	3,878 (8.8)	3,437 (12.7)	<0.01
Dyslipidemia, *n* (%)	4,906 (25.8)	1,850 (32.0)	15,430 (35.2)	11,158 (41.2)	<0.01
Antihypertension medication, *n* (%)	330 (1.7)	310 (5.4)	3,395 (7.7)	5,426 (20.0)	<0.01

Data are median (IQR) or n (%) of the group.
BMI, body mass index; BP, blood pressure; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; FBG, fasting blood glucose; HDL-C, high-density lipoprotein cholesterol; IQR, interquartile range; LDL-C, low-density lipoprotein cholesterol; No., number; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides.

hypertension cases were observed. Among the four BP groups defined at baseline, three groups (except BP of ≥140/≥90 mmHg) experienced an increase in mean SBP during follow-up (all $P < 0.05$). Two groups with BP of <120/<80 and 120–129/<80 mmHg also experienced an increase in DBP, while the other two groups exhibited a decreasing trend (both $P < 0.05$) (**Supplementary Figure 2**). To precisely estimate the association of the different durations of high BP exposure and the occurrence of CVEs, time-weighted cumulative BP determinations between the baseline period and each follow-up interval were calculated. **Table 2** shows the distribution of participants in the different time-weighted cumulative BP groups and the corresponding CVEs in each follow-up.

During the whole follow-up period, we observed 6,301 incident CVEs, including 1,034 with MI, 3,810 with stroke, and 1,457 succumbing to cardiovascular death. The cumulative incident density rates of CVEs during the whole follow-up in the four time-weighted cumulative BP groups—BP <120/<80, 120–129/<80, 130–139/80–89, and ≥140/≥90 mmHg—were 2.16, 3.12, 5.55, and 13.30 per 1,000 person-years, respectively (**Table 2**). The incidence rates of CVEs during each follow-up according to the time-weighted cumulative BP groups and baseline BP group are shown in **Figures 1A,B**, respectively. The BP groups with ≥140/≥90 mmHg at baseline BP and time-weighted cumulative BP both exhibited the highest incidence density of CVEs (both $P < 0.05$).

Difference Between Baseline BP and Time-Weighted Cumulative BP in the Estimation of CVE Risk of Higher BP Exposure

To determine the difference between baseline BP and time-weighted cumulative BP in the estimation of CVE risk, we compared their association with the corresponding incident CVEs in each follow-up interval using the multiple variable adjusted Cox regression model. We found a time trend of HR when the estimation was based on the time-weighted cumulative BP. Firstly, HR increased with the increases of higher BP exposure duration, whereby in the BP group of ≥140/≥90 mmHg, the HRs from the first follow-up to the fifth follow-up were 1.05, 1.62, 1.73, 2.73, and 2.81, respectively, compared with the BP group of <120/<80 mmHg. The results from BP groups of 120–129/<80 and 130–139/80–89 mmHg were similar. We further found that the time trend of HR occurred across the different BP groups compared with the time-weighted cumulative BP of <120/<80 mmHg, whereby BP of ≥140/≥90 mmHg exhibited its CVE risk at the second follow-up (the 4th year since baseline) (HR = 1.62, 95% CI 1.20–2.20), BP of 130–139/80–89 mmHg at the fourth follow-up (the 6th year since baseline) (HR = 1.39, 95% CI 1.07–1.81), and a BP of 120–129/<80 mmHg exhibited unfavorable effects on CVEs at the sixth follow-up (the 8th year since baseline) (HR = 1.70,

TABLE 2 | Risk of occurrence of cardiovascular events estimated based on time-weighted cumulative BP.

BP categories, mmHg	Cases/total	Incidence density (per 1,000 person-years)	HR (95% CI)		
			Model 1	Model 2	Model 3
The whole follow-up period					
<120/<80	401/17,467	2.16	Reference	Reference	Reference
120–129/<80	291/8,795	3.12	0.94 (0.81–1.10)	0.96 (0.82–1.12)	0.99 (0.85–1.16)
130–139/80–89	2,759/47,564	5.55	1.29 (1.15–1.44)	1.31 (1.17–1.47)	1.34 (1.20–1.51)
≥140/≥90	2,850/21,876	13.30	2.03 (1.80–2.30)	2.06 (1.82–2.33)	2.06 (1.81–2.35)
The 2nd year since baseline (year 2008–2009)					
<120/<80	74/20,463	1.81	Reference	Reference	Reference
120–129/<80	38/6,354	3.01	0.86 (0.58–1.27)	0.85 (0.58–1.27)	0.85 (0.57–1.26)
130–139/80–89	368/44,984	4.12	0.90 (0.69–1.18)	0.91 (0.69–1.18)	0.87 (0.67–1.14)
≥140/≥90	479/23,901	10.16	1.13 (0.82–1.55)	1.14 (0.83–1.56)	1.05 (0.76–1.44)
The 4th year since baseline (year 2010–2011)					
<120/<80	80/20,119	2.00	Reference	Reference	Reference
120–129/<80	51/6,484	3.97	1.10 (0.78–1.57)	1.10 (0.77–1.57)	1.08 (0.76–1.54)
130–139/80–89	440/45,690	4.86	1.15 (0.89–1.49)	1.17 (0.90–1.51)	1.12 (0.87–1.49)
≥140/≥90	504/21,966	11.69	1.71 (1.26–2.32)	1.73 (1.28–2.35)	1.62 (1.20–2.20)
The 6th year since baseline (year 2012–2013)					
<120/<80	74/19,426	1.92	Reference	Reference	Reference
120–129/<80	50/6,555	3.86	1.18 (0.82–1.70)	1.17 (0.82–1.68)	1.15 (0.80–1.65)
130–139/80–89	505/45,843	5.58	1.46 (1.12–1.89)	1.46 (1.13–1.90)	1.39 (1.07–1.81)
≥140/≥90	432/20,305	10.90	1.86 (1.36–2.53)	1.86 (1.37–2.52)	1.73 (1.27–2.35)
The 8th year since baseline (year 2014–2015)					
<120/<80	52/18,216	1.44	Reference	Reference	Reference
120–129/<80	58/7,359	3.99	1.76 (1.20–2.56)	1.77 (1.21–2.58)	1.70 (1.16–2.48)
130–139/80–89	518/44,819	5.86	2.02 (1.50–2.74)	2.07 (1.53–2.80)	1.92 (1.42–2.59)
≥140/≥90	492/19,112	13.24	3.00 (2.14–4.20)	3.06 (2.19–4.28)	2.73 (1.96–3.80)
The 10th year since baseline (year 2016–2017)					
<120/<80	98/16,663	2.01	Reference	Reference	Reference
120–129/<80	122/8,297	4.98	1.70 (1.30–2.22)	1.69 (1.29–2.21)	1.62 (1.24–2.12)
130–139/80–89	1,015/43,655	7.86	2.16 (1.73–2.68)	2.14 (1.72–2.66)	2.02 (1.62–2.51)
≥140/≥90	851/18,196	16.08	3.11 (2.44–3.96)	3.07 (2.41–3.91)	2.81 (2.21–3.56)

Model 1: adjusted for age, gender, body mass index, smoking, alcohol consumption, physical activity, systolic blood pressure, and diastolic blood pressure at baseline. Model 2: model 1 plus total cholesterol levels, low-density lipoprotein cholesterol, fasting plasma glucose, and estimated glomerular filtration rate at baseline. Model 3: model 2 plus history of hypertension and antihypertensive drug intake at baseline, variability of systolic blood pressure, and diastolic blood pressure during follow-up.
CI, confidence interval; HR, hazard ratio.

95% CI 1.96–3.80) (**Table 2**). The trends of HR for each time-weighted cumulative BP group during these follow-up intervals are summarized in **Figure 2A**.

To eliminate the influence of higher BP on CVE occurrence before baseline, the same analysis was performed on the participants without baseline hypertension or a history of hypertension; the results were similar to the analysis performed on the whole participant population (**Figure 3**), and the trends of HRs over time are summarized in **Figure 2B**. However, when the estimation was performed based on baseline BP, the time trend observed with cumulative BP would no longer exist (**Supplementary Table 1**, **Figure 2C**).

We had also performed a multivariable adjusted restricted spline regression model to examine what was the most favorable level of BP at which participants had the lowest risk of CVE occurrence. We found a J-shaped association between time-weighted cumulative SBP (**Supplementary Figure 3A**) or DBP (**Supplementary Figure 3B**) and the risk of CVE occurrence, which demonstrated that the time-weighted cumulative SBP at 122 mmHg (range: 121–126 mmHg) and DBP at 78 mmHg (range: 76–80 mmHg), respectively, had the lowest risk of CVE occurrence.

The Predictive Value of Baseline BP and Time-Weighted Cumulative BP Determinations for the Incidence of CVEs

Since baseline BP is commonly used in the prediction of CVE occurrence currently, we examined the extent that baseline BP alone could precisely predict the occurrence of CVEs

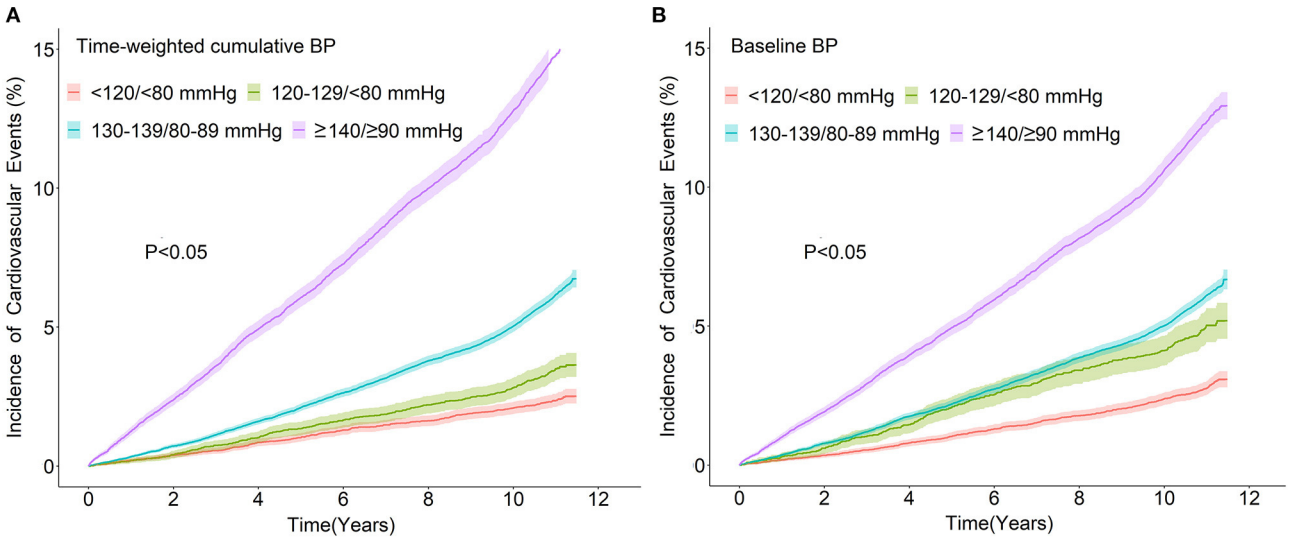

FIGURE 1 | Incidence of cardiovascular events over time according to different categories of time-weighted cumulative BP **(A)** or baseline BP **(B)**. BP, blood pressure.

by performing a ROC analysis. We compared the AUCs from the model that considered only the baseline BP with the "combined" model that considered both baseline BP and time-weighted cumulative BP determined at each follow-up. We found that although the combined model significantly improved the predictive ability compared with the baseline BP alone model (all $P < 0.05$) (**Supplementary Figures 4, 5**), the predictive ability (AUC) was increased only by 0.6–3.2% for SBP (**Supplementary Figure 4F**) and 0.2–3.1% for DBP (**Supplementary Figure 5F**), respectively, during the five intervals of follow-up.

DISCUSSION

In this prospective study, we had examined the difference between baseline BP and time-weighted cumulative BP determinations in the estimation of the effect of higher BP exposure on CVE occurrence. We demonstrated that when HR was estimated based on time-weighted cumulative BP determination, a reasonable time trend of HRs was clearly shown both in the same BP group and across the four BP groups; these results were confirmed by the same analysis performed on participants without baseline hypertension. However, these time trends did not exist when the estimation was based on baseline BP determination. We also demonstrated that the predictive value of baseline BP determination for the occurrence of CVEs was only slightly lower (<4%) compared with the time-weighted cumulative BP determination.

The current estimation of the effect of BP on CVE occurrence is mainly based on a single baseline BP measurement. However, there is a gap in time between baseline BP determination and risk estimation. Intuitively, this influence on CVEs would accumulate with a sustained duration of high BP exposure, and a time-weighted cumulative BP strategy was employed to assess the risk of CVE in this study. Indeed, we found that the HR of higher BP increased with the increase in duration of the higher BP (**Table 2**, **Figure 2A**), and this time trend also occurred across the four time-weighted BP groups, with the BP group of ≥140/≥90 mmHg being the earliest to exhibit its CVE risk, followed by BP groups of 130–139/80–89 and 120–129/<80 mmHg (**Table 2**, **Figure 2A**).

Although we had adjusted the baseline BP in the above analysis, to more precisely assess the influence of the different durations of high BP exposure on CVE occurrence, the influence of high BP before baseline should be completely eliminated. Therefore, we performed the same analysis on the participants without baseline hypertension, and the results were similar as the above analysis (**Figures 2B**, **3**). However, these time trends no longer existed when the time-weighted BP determination was substituted with baseline BP determination (**Supplementary Table 1**, **Figure 2C**). Currently, due to the lack of clear objective criteria, such as C-statistics or NRI in evaluating the improvement of predictive value (15, 16), to directly compare the superiority of baseline BP and cumulative BP determination in risk estimation, however, based on time-weighted cumulative BP determination, could more accurately assess the extent of BP exposure theoretically and could have a reasonable time trend of HR of risk estimation in the population. We conclude that the risk of higher BP on CVE occurrence estimated based on time-weighted BP determination could be more precise than that of baseline BP determination.

Although cumulative BP determination is shown in this study to be a better estimation of the risk of high BP on CVE occurrence, the greater accessibility and reduced resource utilization in performing baseline BP determination are good

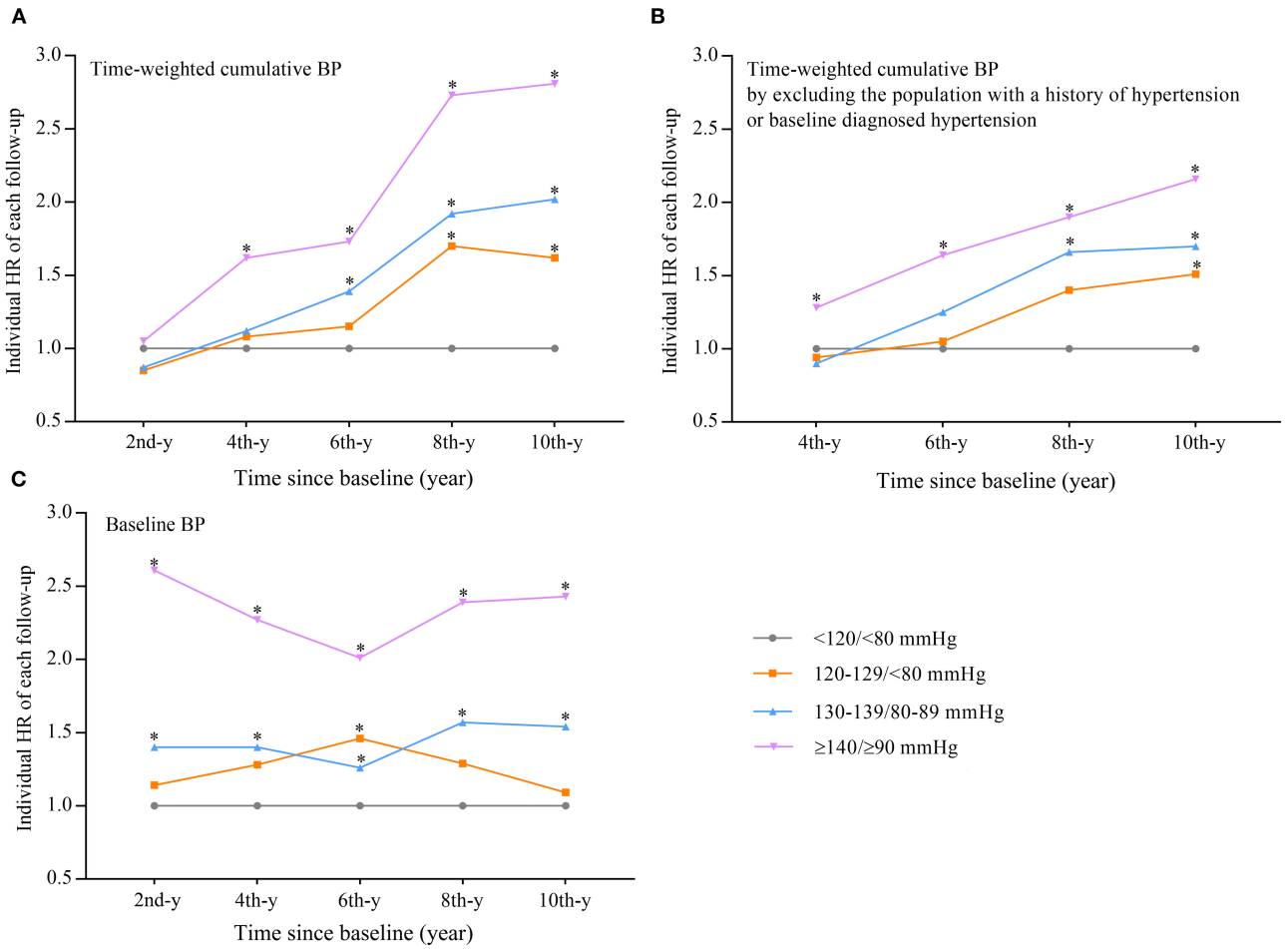

FIGURE 2 | The trend of HR influence of time-weighted cumulative BP **(A)** or time-weighted cumulative BP that excluded the participants with a history of hypertension or baseline diagnosed hypertension **(B)** or baseline BP **(C)** on new occurrence of cardiovascular events during follow-up. **(A,C)** Adjusted for the same variables indicated in model 3 in **Table 2** or **Supplementary Table 1**. **(B)** Adjusted for the same variables indicated in model 3 of **Table 2** at the second year since baseline. *Compared with BP of <120/<80 mmHg, the difference was significant ($P < 0.05$). The *x*-y: the *x*-year since baseline. BP, blood pressure; HR, hazard ratio.

reasons to continue using this to predict the risk of CVE occurrence; therefore, further assessment of its accuracy needs to be conducted. We compared the predictive values (AUC) of the baseline BP for CVE occurrence with the combined model of baseline BP plus time-weighted cumulative BP and found that the difference was statistically significant. This result was similar to the Lifetime Risk Pooling Project cohorts study (16). However, the AUC from the model that included baseline BP alone was only slightly lower (<4%) than that from the combined model (**Supplementary Figures 4, 5**), which suggests that baseline BP might still be a useful indicator for predicting the future occurrence of CVEs.

To assess for the optimal range of BP in which participants would be at relatively lower risk of the occurrence of CVEs, we performed a restricted cubic spline regression analysis and found that SBP at 122 mmHg and DBP at 78 mmHg exhibited the lowest risk for CVE occurrence, with their corresponding ranges being 121–126 and 76–80 mmHg, respectively. This result indicated that participants would benefit from the BP maintained at these ranges in reducing their risks for CVEs.

This study has several strengths. First, the data of this analysis were derived from a large-scale, community-based prospective study. Second, we had used the time-weighted cumulative BP determination to measure the extent of BP exposure since baseline; thus, both BP level and the corresponding duration of exposure were considered. Third, the results were confirmed in participants without baseline hypertension, which would eliminate the influence of hypertension before baseline, thus providing a precise estimate of the different durations of high BP on CVE occurrence. Fourth, since BP level varies over time, the duration of such BP levels would be expected to have an influence on CVE occurrence (29, 30); thus, in this study, we adjusted the

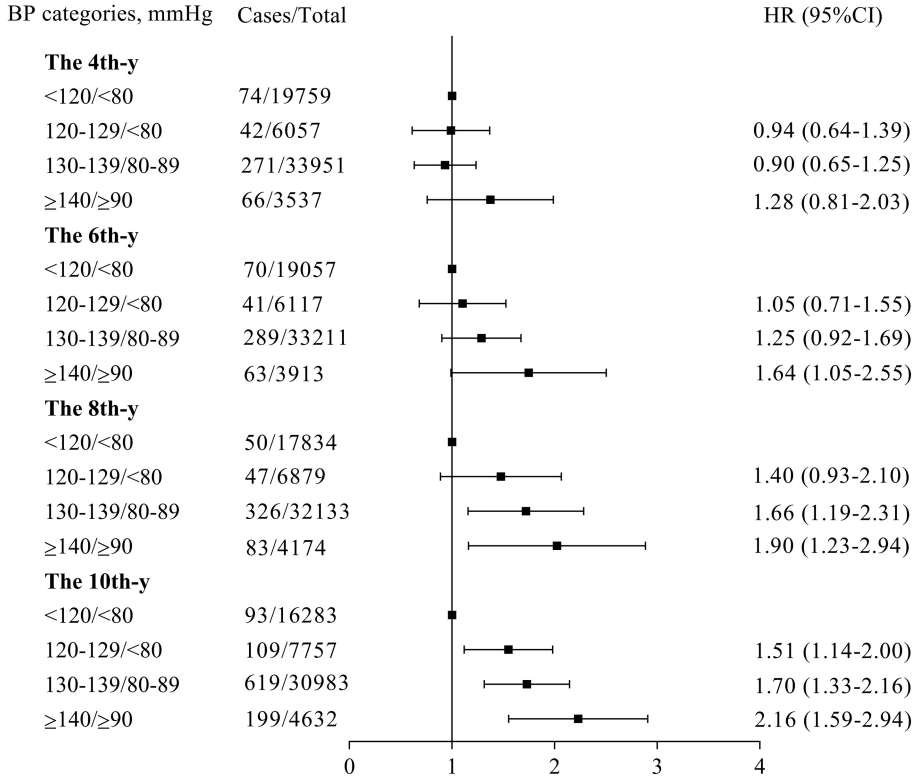

FIGURE 3 | Risks of new occurrence cardiovascular events in participants with different categories of time-weighted cumulative BP after excluding the participants with a history of hypertension or baseline diagnosed hypertension. This analysis was adjusted for the same variables indicated in **Figure 2B**. *The *x*-y: the *x*-year since baseline. BP, blood pressure; CI, confidence interval; HR, hazard ratio.

variation of BP level from five times of follow-up. In addition, we had also adjusted for other potential confounding factors, such as death competition risk to CVEs and history of antihypertension drug used.

There were also several limitations of this study. First, we used the cumulative BP exposure for each follow-up to estimate the corresponding risk, which poses an intrinsic limitation; such an estimation means we considered that high BP has the same influence on CVE occurrence in different stages of life: in elderly people, high BP may not be so significant as in relatively young people (4, 31). Second, in routine clinical practice, only baseline BP makes it possible to estimate CVE risk (such as the Framingham score), and that it remains necessary to develop risk estimators whose estimate of the risk evolves over time by taking into account the changes of different parameters. Third, considering the great advances in the management of hypertension over the past decade, the generalizability of the conclusion may be limited.

CONCLUSIONS

In this study, we first confirmed that baseline BP is a reliable indicator for predicting the occurrence of CVEs. Nevertheless, time-weighted cumulative BP could be a more reliable estimate of the risk of CVEs than baseline BP.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The study was performed according to the guidelines of the Helsinki Declaration and was approved by the Ethics Committee of Kailuan General Hospital (approval number: 2006-05) and Beijing Tiantan Hospital (approval number: 2010-014-01). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

SW and YH: had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. YZu and AW: concept and design. DZ, YY, XY, and FL: acquisition, analysis, or interpretation of data. YZu and AW: drafting of the manuscript. YH: critical revision of the manuscript for important intellectual content. SC, XT, MW, XS, JW, QZ, and YZh: statistical analysis. YH: obtained funding. SW and YH: supervision. HG: Other. All authors contributed to the article and approved the submitted version.

FUNDING

This study was funded by the National Natural Science Foundation of China (82073648 and 31672375), Beijing Municipal Administration of Hospitals Incubating Program (PX2020021), Beijing Excellent Talents Training Program (2018000021469G234), Young Elite Scientists Sponsorship Program by CAST (2018QNRC001), and National Key R&D Program of China (2018YFC1312400 and 2018YFC1312402).

ACKNOWLEDGMENTS

We thank all the staff and participants of the Kailuan Study for their invaluable contributions.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2021.735679/full#supplementary-material

REFERENCES

1. NCD Risk Factor Collaboration. Worldwide trends in blood pressure from 1975 to 2015: a pooled analysis of 1479 population-based measurement studies with 19·1 million participants. *Lancet.* (2017) 389:37–55. doi: 10.1016/S0140-6736(16)31919-5
2. Willey JZ, Moon YP, Kahn E, Rodriguez CJ, Rundek T, Cheung K, et al. Population attributable risks of hypertension and diabetes for cardiovascular disease and stroke in the northern Manhattan study. *J Am Heart Assoc.* (2014) 3:e001106. doi: 10.1161/JAHA.114.001106
3. Global Burden of Metabolic Risk Factors for Chronic Diseases Collaboration. Cardiovascular disease, chronic kidney disease, and diabetes mortality burden of cardiometabolic risk factors from 1980 to 2010: a comparative risk assessment. *Lancet Diabetes Endocrinol.* (2014) 2:634–47. doi: 10.1016/S2213-8587(14)70102-0
4. Qi Y, Han X, Zhao D, Wang W, Wang M, Sun J, et al. Long-term cardiovascular risk associated with stage 1 hypertension defined by the 2017 ACC/AHA hypertension guideline. *J Am Coll Cardiol.* (2018) 72:1201–10. doi: 10.1016/j.jacc.2018.06.056
5. Adler AI, Stratton IM, Neil HA, Yudkin JS, Matthews DR, Cull CA, et al. Association of systolic blood pressure with macrovascular and microvascular complications of type 2 diabetes (UKPDS 36): prospective observational study. *BMJ.* (2000) 321:412–9. doi: 10.1136/bmj.321.7258.412
6. Rapsomaniki E, Timmis A, George J, Pujades-Rodriguez M, Shah AD, Denaxas S, et al. Blood pressure and incidence of twelve cardiovascular diseases: lifetime risks, healthy life-years lost, and age-specific associations in 1·25 million people. *Lancet.* (2014) 383:1899–911. doi: 10.1016/S0140-6736(14)60685-1
7. Zhang Y, Jiang X, Bo J, Yin L, Chen H, Wang Y, et al. Risk of stroke and coronary heart disease among various levels of blood pressure in diabetic and nondiabetic Chinese patients. *J Hypertension.* (2018) 36:93–100. doi: 10.1097/HJH.0000000000001528
8. Lee H, Yano Y, Cho SMJ, Park JH, Park S, Lloyd-Jones DM, et al. Cardiovascular risk of isolated systolic or diastolic hypertension in young adults. *Circulation.* (2020) 141:1778–86. doi: 10.1161/CIRCULATIONAHA.119.044838
9. Zheng M, Xu X, Wang X, Huo Y, Xu X, Qin X, et al. Age, arterial stiffness, and components of blood pressure in Chinese adults. *Medicine.* (2014) 93:e262. doi: 10.1097/MD.0000000000000262
10. Levine DA, Lewis CE, Williams OD, Safford MM, Liu K, Calhoun DA, et al. Geographic and demographic variability in 20-year hypertension incidence: the CARDIA study. *Hypertension.* (2011) 57:39–47. doi: 10.1161/HYPERTENSIONAHA.110.160341
11. Zhao D, Liu J, Wang W, Zeng Z, Cheng J, Liu J, et al. Epidemiological transition of stroke in China: twenty-one-year observational study from the Sino-MONICA-Beijing Project. *Stroke.* (2008) 39:1668–74. doi: 10.1161/STROKEAHA.107.502807
12. Reges O, Ning H, Wilkins JT, Wu CO, Tian X, Domanski MJ, et al. Association of cumulative systolic blood pressure with long-term risk of cardiovascular disease and healthy longevity: findings from the lifetime risk pooling project cohorts. *Hypertension.* (2020) 77:347–56. doi: 10.1161/HYPERTENSIONAHA.120.15650
13. Wang YX, Song L, Xing AJ, Gao M, Zhao HY, Li CH, et al. Predictive value of cumulative blood pressure for all-cause mortality and cardiovascular events. *Sci Rep.* (2017) 7:41969. doi: 10.1038/srep41969
14. Cui X, Zhao Q, Yu J, Zhang R, Li W, Song L, et al. Cumulative mean arterial pressure and risks of adverse cardiac and cerebrovascular events: a prospective cohort study of 53,813 adults. *J Human Hypertens.* (2018) 32:585–93. doi: 10.1038/s41371-018-0075-9
15. Nwabuo CC, Appiah D, Moreira HT, Vasconcellos HD, Yano Y, Reis JP, et al. Long-term cumulative blood pressure in young adults and incident heart failure, coronary heart disease, stroke, and cardiovascular disease: the CARDIA study. *Europ J Preventive Cardiol.* (2020) 0:1–8. doi: 10.1177/2047487320915342
16. Pool LR, Ning H, Wilkins J, Lloyd-Jones DM, Allen NB. Use of long-term cumulative blood pressure in cardiovascular risk prediction models. *JAMA Cardiol.* (2018) 3:1096–100. doi: 10.1001/jamacardio.2018.2763
17. Wu S, Huang Z, Yang X, Zhou Y, Wang A, Chen L, et al. Prevalence of ideal cardiovascular health and its relationship with the 4-year cardiovascular events in a northern Chinese industrial city. *Circul Cardiovasc Quality Outcomes.* (2012) 5:487–93. doi: 10.1161/CIRCOUTCOMES.111.963694
18. Wu S, Li Y, Jin C, Yang P, Li D, Li H, et al. Intra-individual variability of high-sensitivity C-reactive protein in Chinese general population. *Int J Cardiol.* (2012) 157:75–9. doi: 10.1016/j.ijcard.2010.12.019
19. Li W, Jin C, Vaidya A, Wu Y, Rexrode K, Zheng X, et al. Blood pressure trajectories and the risk of intracerebral hemorrhage and cerebral infarction: a prospective study. *Hypertension.* (2017) 70:508–14. doi: 10.1161/HYPERTENSIONAHA.117.09479
20. Wang A, Sun Y, Liu X, Su Z, Li J, Luo Y, et al. Changes in proteinuria and the risk of myocardial infarction in people with diabetes or pre-diabetes: a prospective cohort study. *Cardiovasc Diabetol.* (2017) 16:104. doi: 10.1186/s12933-017-0586-7
21. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, 3rd, Feldman HI, et al. A new equation to estimate glomerular filtration rate. *Ann Internal Med.* (2009) 150:604–12. doi: 10.7326/0003-4819-150-9-200905050-00006
22. Unger T, Borghi C, Charchar F, Khan NA, Poulter NR, Prabhakaran D, et al. 2020 international society of hypertension global hypertension practice guidelines. *Hypertension.* (2020) 75:1334–57. doi: 10.1161/HYPERTENSIONAHA.120.15026
23. American Diabetes Association. Cardiovascular disease and risk management: standards of medical care in diabetes-2020. *Diabetes Care.* (2020) 43(Suppl. 1):S111–34. doi: 10.2337/dc20-S010
24. Zhang Q, Wang A, Zhang S, Li N, Chen S, Zhang Y, et al. Asymptomatic polyvascular disease and the risks of cardiovascular events and all-cause death. *Atherosclerosis.* (2017) 262:1–7. doi: 10.1016/j.atherosclerosis.2017.04.015
25. World Health Organization. Recommendations on stroke prevention, diagnosis, and therapy. report of the WHO task force on stroke and other cerebrovascular disorders. *Stroke.* (1989) 20:1407–31. doi: 10.1161/01.STR.20.10.1407
26. Tunstall-Pedoe H, Kuulasmaa K, Amouyel P, Arveiler D, Rajakangas AM, Pajak A. Myocardial infarction and coronary deaths in the World Health Organization MONICA Project. registration procedures, event rates, and case-fatality rates in 38 populations from 21 countries in four continents. *Circulation.* (1994) 90:583–612. doi: 10.1161/01.CIR.90.1.583

27. Austin PC, Fine JP. Practical recommendations for reporting Fine-Gray model analyses for competing risk data. *Statist Med.* (2017) 36:4391–400. doi: 10.1002/sim.7501
28. Durrleman S, Simon R. Flexible regression models with cubic splines. *Statist Med.* (1989) 8:551–61. doi: 10.1002/sim.4780080504
29. Lee SR, Choi YJ, Choi EK, Han KD, Lee E, Cha MJ, et al. blood pressure variability and incidence of new-onset atrial fibrillation: a nationwide population-based study. *Hypertension.* (2020) 75:309–15. doi: 10.1161/HYPERTENSIONAHA.119.13708
30. Muntner P, Whittle J, Lynch AI, Colantonio LD, Simpson LM, Einhorn PT, et al. Visit-to-visit variability of blood pressure and coronary heart disease, stroke, heart failure, and mortality: a cohort study. *Ann Internal Med.* (2015) 163:329–38. doi: 10.7326/M14-2803
31. Wang C, Yuan Y, Zheng M, Pan A, Wang M, Zhao M, et al. Association of age of onset of hypertension with cardiovascular diseases and mortality. *J Am Coll Cardiol.* (2020) 75:2921–30. doi: 10.1016/j.jacc.2020.04.038

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cardiovascular Medicine

ORIGINAL RESEARCH
published: 18 November 2021
doi: 10.3389/fcvm.2021.736073

Body Roundness Index Is a Superior Obesity Index in Predicting Diabetes Risk Among Hypertensive Patients: A Prospective Cohort Study in China

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Specialty section:
This article was submitted to Hypertension, a section of the journal Frontiers in Cardiovascular Medicine

Received: 04 July 2021
Accepted: 04 October 2021
Published: 18 November 2021

Citation:
Liu Y, Liu X, Guan H, Zhang S, Zhu Q, Fu X, Chen H, Tang S, Feng Y and Kuang J (2021) Body Roundness Index Is a Superior Obesity Index in Predicting Diabetes Risk Among Hypertensive Patients: A Prospective Cohort Study in China. Front. Cardiovasc. Med. 8:736073. doi: 10.3389/fcvm.2021.736073

Objective: Individuals with both hypertension and diabetes have been confirmed to significantly increase the risk of cardiovascular disease morbidity and mortality compared with those with only hypertension or diabetes. This study aimed to evaluate the potential of different anthropometric indices for predicting diabetes risk among hypertensive patients.

Methods: The study group consisted of 6,990 hypertensive adults without diabetes who were recruited in China. Demographic and clinical assessment, physical examinations, laboratory tests, and anthropometric measurements, including body mass index (BMI), waist circumference (WC), hip circumference (HC), waist-to-hip ratio (WHR), waist-to-height ratio (WHtR), and novel indices (ABSI, AVI, BAI, BRI, CI, WWI, and WHHR), were performed at baseline and during the (median) 3-year follow-up. Cox regression analyses were conducted to estimate effects from these indices for the onset of diabetes. Receiver operator characteristic (ROC) analyses were conducted to assess the predictive capacities of the anthropometric indices and determine the optimal cut-points.

Results: A total of 816 (11.7%) developed diabetes during our prospective study. Multivariate Cox regression analyses revealed weight, WC, WHR, WHtR, BAI, BRI, and WWI as the independent risk factor for diabetes among hypertensive patients, regardless of whether it was treated as a continuous or categorical variable ($P < 0.05$). Further Cox analyses combining BMI and different central obesity indices showed that elevated WC, WHR, WHtR, AVI, BRI, CI, regardless of the general obesity status, were found to be each independently associated with increased diabetes risk ($P < 0.05$). Dynamic increases of BRI < 5.24 to BRI ≥ 5.24 were associated with increased risk (HR = 1.29; 95% CI, 1.02, 1.64), and its reversal was associated with reduced risk (HR = 1.56; 95% CI, 1.23, 1.98) compared with the others (HR = 1.95; 95% CI, 1.63, 2.32). ROC analysis indicated that the areas under the ROC curves (AUC) of the anthropometric indices ranged from 0.531 to 0.63, with BRI (cut-off value = 4.62) and WHtR having the largest area.

Conclusions: Based on this novel study, BRI was the most superior predictor and independent determinant for diabetes onset among the hypertensive population. Hypertensive patients with BRI > 4.62, regardless of general obesity status, were at high risk of diabetes. Thus, the prompt screening and diagnosis of diabetes should be carried out among these patients for timely integrated intervention.

Keywords: hypertension, diabetes, cardiovascular disease, anthropometry, central obesity, body roundness index

INTRODUCTION

The global burden of diabetes and hypertension is tremendous and increasing continuously (1). Globally, around 422 million and 1.13 billion people are suffering from diabetes and hypertension, respectively. Both diabetes and hypertension are substantial risk factors for cardiovascular disease (CVD) morbidity and mortality. Diabetes and hypertension frequently coexist (2), suffering from both diseases significantly evaluate the morbidity and mortality of CVD compared with those with either condition alone (3). A 2-fold increase in CVD risk has been seen in individuals with both diabetes and hypertension compared with the hypertensive patients without diabetes. Hypertension could be easily identified by non-invasive BP measurements, yet diabetes often goes undetected until patients present with diabetic complications. Therefore, early recognition of hypertensive patients at high risk of diabetes may result in improved prevention and early detection.

Hypertension is characterized by increased peripheral vascular resistance and endothelial dysfunction, and diabetes is characterized by insulin resistance and β-cell dysfunction (4). These pathophysiological processes intercommunicate tightly in various ways, of which obesity act as an important confounder of the association between blood pressure and blood sugar since it is an established risk factor for both diabetes and hypertension (1, 5). More importantly, obesity is a reversible predisposing factor for these two conditions. There is considerable evidence to show that weight loss can reduce or delay the onset of diabetes among the high-risk population (6). Obesity mainly represents two main subtypes, general obesity, and central obesity. Recent studies revealed that BMI poorly performed in predicting diabetes, CVD, and death (7, 8). This may be explained by the characteristics of body composition in diabetes, including the increase in total fat mass (9, 10), and decrease of muscle mass or bone density, which could lead to a normal BMI even with an increase in fat mass. Moreover, mounting evidence has confirmed that central obesity is more closely correlated with insulin resistance, diabetes, and CVD than general obesity (11). Waist circumference (WC) is commonly used to define central obesity, which shows a good correlation with abdominal fat and CVD risk (12). However, WC is easily affected by the differences in height. Consequently, waist-to-hip ratio (WHR) and waist-to-height ratio (WHtR) have been developed and studied as alternatives to WC (13). Several recent studies have shown the superiority of WHtR and WHR, especially WHtR, over WC in predicting cardiometabolic diseases, while others have shown no obvious difference between them (14, 15). Additionally, some novel anthropometric indices, such as a body shape index (ABSI) (16), abdominal volume index (AVI) (17), body adiposity index (BAI) (18), body roundness index (BRI) (19), conicity index (CI) (20), weight-adjusted-waist-index (WWI) (21), and waist-hip-height ratio (WHHR) (22), have been applied as measures of adiposity. Anthropometry is a widely used, inexpensive, simple, and easy technique. Digging out the anthropometric index that is most strongly related to the occurrence of diabetes in hypertensive patients has significant clinical and public health significance. However, the relationships between different anthropometric indices with the occurrence of diabetes in Asian hypertensive patients are still scarce, and most of the available published clinical literature are cross-sectional designed and exhibit a lack of concern for the population of non-general obesity but with central obesity.

This study aimed to examine in detail the anthropometric indices in the assessment of diabetes among the Chinese hypertensive population. We compared the association of baseline and changing trends of different anthropometric indices, as well as the combinations of BMI and the indices of central obesity with diabetes risk. The predictive performances of these indices for pre-screening of diabetes were also examined.

METHODS

Study Design and Study Population

The present study was based on a prospective cohort design. All participants were recruited from Dongguan City, a medium-developed and representative urbanized area of China, from 2012 to 2015. Participants needed to meet the following inclusion criteria: (1) patients with a definitively diagnosed hypertension; (2) a minimum of 18 years of age; (3) willingness to do at least 1-year follow-up; (4) not currently pregnant; (5) without cancer or other serious diseases. Exclusion criteria were as follows: (1) lacking effective data of anthropometric indices or biochemical examinations; (2) length of follow-up of <6 months; (3) history of diabetes prior to the study start date (**Figure 1**). Finally, a total of 6,990 hypertensive patients were included. This study was performed in accordance with the Declaration of Helsinki and was approved by the Medical Research Ethics Committee of Guangdong Provincial People's Hospital. All participants provided written informed consent before voluntary participation.

Health Screening Questionnaire

All participants were required to complete a structured modified health screening questionnaire to determine their demographic

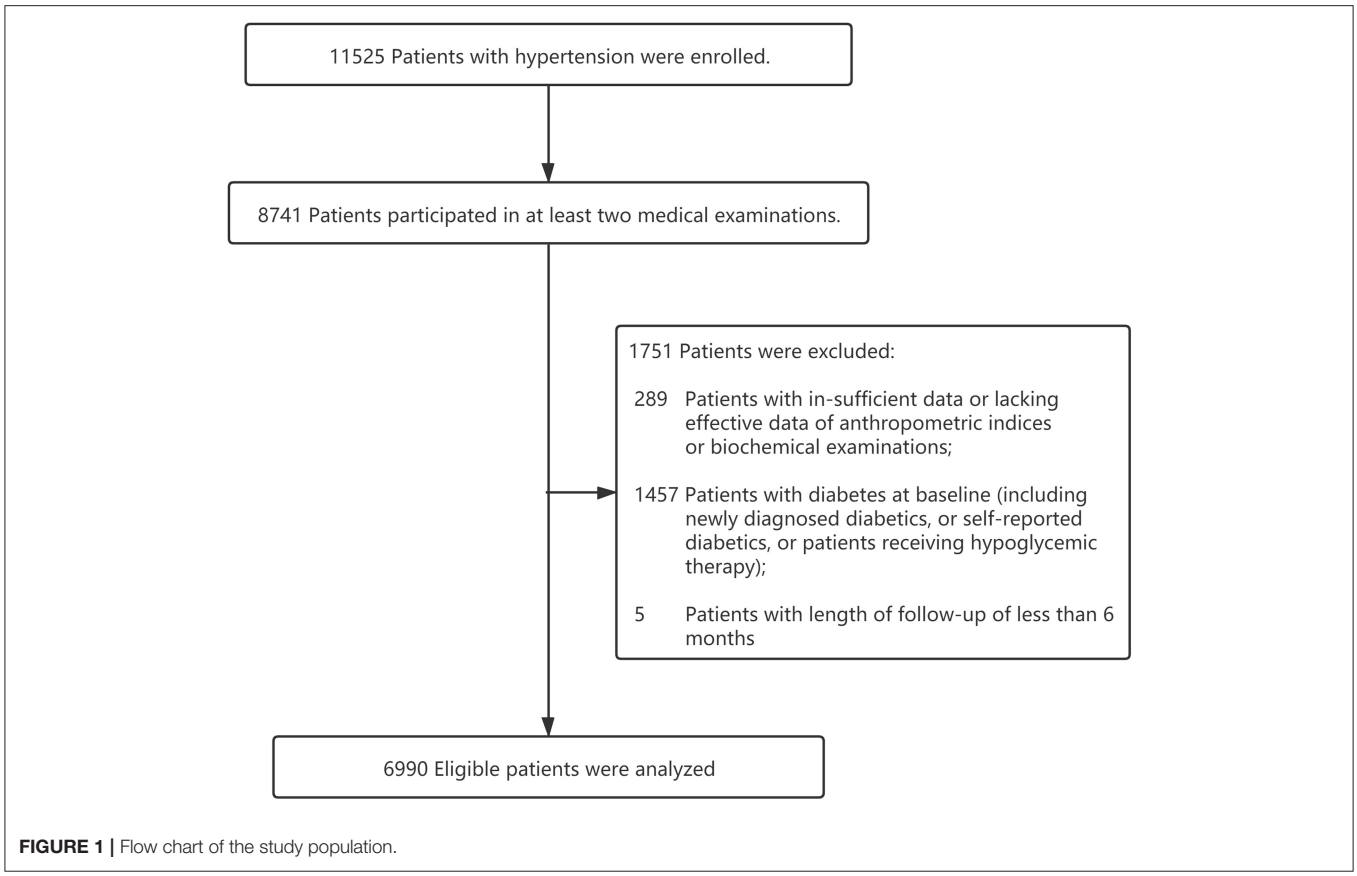


FIGURE 1 | Flow chart of the study population.

characteristics, including age, sex, ethnicities, the current medication use of hypertension, and lifestyles, including smoking status and drinking status.

Health Screening Measurements

Professional medical staff measured anthropometric indices, with participants wearing thin clothing with no footwear. Bodyweight, height, WC, and HC were measured according to standard protocols (23). Using these parameters, we evaluated other anthropometric indices, including BMI, WHR, WHtR, ABSI, AVI, BAI, BRI, CI, WWI, WHHR, according to the published formula (**Supplementary Table 1**).

Overweight and obesity were defined as BMI $\geq$ 24 kg/m^2 and $\geq$ 28 kg/m^2 according to BMI criteria established by the Working Group on Obesity in China (WGOC) (24), while abdominal obesity was defined as WC $\geq$ 90 cm in men or $\geq$ 85 cm in women according to Standards of care for type 2 diabetes in China, or WHR $\geq$0.90 in men or $\geq$0.85 in women according to WHO guidelines (25). The elevated WHtR was defined as $\geq$0.5 (26). Lacking uniform classification criteria, novel anthropometric indices (ABSI, AVI, BAI, BRI, CI, WWI, and WHHR) were divided into quartiles, and cut-points for these indices were initially selected at the level of 75% according to the distribution characteristics of BMI in the studied populations (**Supplementary Table 2**).

Blood pressure was measured after quiet sitting for 5 min. Hypertension was defined as systolic blood pressure (SBP) $\geq$ 140 mmHg or diastolic blood pressure (DBP) $\geq$ 90 mmHg, or with a self-reported history of hypertension, or use of antihypertensive medications (27).

The health screening measurements mentioned above would be measured at baseline and each annual follow-up.

Evaluation of Laboratory Parameters

Blood and urine samples were collected in the morning after an overnight fast for at least 8 h. Fasting plasma glucose (FPG), Serum triglycerides (TG), total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), uric acid (UA), creatinine (Cr), and urinary albumin excretion rate (UAER) were measured *via* a biochemical autonomic analyzer (OLYMPUS, Tokyo, Japan). The estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI creatinine equation (28).

The laboratory parameters mentioned above would be measured at baseline and each annual follow-up.

Clinical Outcome

Incident diabetes was the endpoint of the present study. Diabetes was diagnosed adopting World Health Organization (WHO) 1999 diagnosis criteria of diabetes (29), mainly defined as an elevated fasting plasma glucose (>7.0 mmol/L), self-reported

TABLE 1 | Baseline characteristics between subjects with and without diabetes.

	Total (n = 6,990)	New-onset diabetes (n = 816)	Non-diabetes (n = 6,174)	P-value
Age (years)	59.0 ± 13.9	61.1 ± 13.6	58.7 ± 14.0	<0.001***
Sex (male, n [%])	3,467 (49.6)	357 (43.8)	3,110 (50.4)	<0.001***
Smoking (n [%])	1,698 (24.3)	188 (23.1)	1,510 (24.5)	0.385
Drinking (n [%])	885 (12.7)	83 (10.2)	802 (13.0)	0.024*
SBP (mmHg)	139.25 ± 17.05	141.38 ± 17.56	138.97 ± 16.97	<0.001***
DBP (mmHg)	85.30 ± 11.10	85.12 ± 11.65	85.32 ± 11.03	0.641
Laboratory examination				
FPG (mmol/L)	4.90 ± 0.64	5.39 ± 0.73	4.83 ± 0.59	<0.001***
TG (mmol/L)	1.53 (1.09–2.21)	1.84 (1.32–2.75)	1.49 (1.06–2.16)	<0.001***
TC (mmol/L)	5.14 ± 1.12	5.18 ± 1.17	5.14 ± 1.11	0.369
HDL (mmol/L)	1.31 ± 0.35	1.30 ± 0.41	1.31 ± 0.35	0.290
LDL (mmol/L)	2.85 ± 0.81	2.88 ± 0.85	2.84 ± 0.80	0.197
eGFR (ml/[min·1.73 m²])	85.95 ± 21.84	84.06 ± 21.43	86.20 ± 21.88	0.009**
UAER (mg/24 h)	23.50 (10.90–71.40)	34.80 (16.25–126.70)	22.75 (10.60–67.31)	<0.001***
Anthropometric indices				
Weight (kg)	63.20 ± 11.94	65.85 ± 12.55	62.86 ± 11.82	<0.001***
BMI (kg/m²)	25.10 ± 3.65	26.47 ± 4.04	24.92 ± 3.55	<0.001***
WC (cm)	87.34 ± 9.19	90.72 ± 9.5	86.90 ± 9.04	<0.001***
HC (cm)	95.17 ± 7.69	97.29 ± 8.58	94.89 ± 7.52	<0.001***
WHtR	0.55 ± 0.06	0.58 ± 0.06	0.55 ± 0.06	<0.001***
WHR	0.92 ± 0.06	0.93 ± 0.08	0.92 ± 0.06	<0.001***
ABSI	0.081 ± 0.006	0.082 ± 0.006	0.081 ± 0.006	0.012*
AVI	15.49 ± 3.20	16.70 ± 3.45	15.33 ± 3.13	<0.001***
BAI	29.97 ± 4.92	31.47 ± 5.50	29.77 ± 4.81	<0.001***
BRI	4.47 ± 1.30	5.01 ± 1.43	4.40 ± 1.26	<0.001***
CI	1.27 ± 0.09	1.29 ± 0.09	1.27 ± 0.09	<0.001***
WWI	11.06 ± 0.89	11.25 ± 0.88	11.03 ± 0.89	<0.001***
WHHR	0.58 ± 0.05	0.59 ± 0.06	0.58 ± 0.05	<0.001***
Medication				
Hypotensive drugs (n [%])	4,726 (67.6)	4,175 (67.6)	551 (67.5)	0.955
ACEI/ARB (n [%])	3,518 (50.3)	3,113 (50.4)	405 (49.6)	0.672
Beta-receptor blocker (n [%])	637 (9.1)	551 (8.9)	86 (10.5)	0.132
CCB (n [%])	2,626 (37.6)	2,310 (37.4)	316 (38.7)	0.468
Diuretic (n [%])	562 (8.0)	502 (8.1)	60 (7.4)	0.442
Others (n [%])	107 (1.5)	90 (1.5)	17 (2.1)	0.171

Continuous data are shown as the mean ± SD or median (Q1–Q3), and categorical data as n (%).

FPG, fasting plasma glucose; TG, triglycerides; TC, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; UA, uric acid; Scr, serum creatinine; eGFR, estimated glomerular filtration rate; BMI, body mass index; WC, waist circumference; HC, hip circumference; WHtR, waist-to-height ratio; WHR, waist-to-hip ratio; ABSI, A body shape index; AVI, abdominal volume index; BAI, body adiposity index; BRI, body roundness index; CI, conicity index; WWI, weight-adjusted-waist index; WHHR, waist-hip-height ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; ACEI, angiotensin-converting enzyme inhibitors; ARB, Angiotensin Receptor Blocker; CCB, Calcium Channel Blockers.

$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

previous diagnosis of diabetes by the physician, and/or current use of hypoglycemic medication. All patients were followed until the earliest date of the following: the incident diabetes or the last follow-up date.

Statistical Analyses

As estimated in PASS software version 15 (IBM Corp, Armonk, NY, USA), 1,168 samples would be needed in a Cox regression of the log hazard ratio (HR) to provide 90% power at a.05 significance level to detect a regression coefficient equal to 0.20 under an overall event rate of 0.10. Data are presented as $M \pm SD$ (normal distribution) or median (first quartile and third quartile) (non-normal distribution) for continuous variables, and as frequency (percentages) for categorical variables. Differences among the groups were evaluated by the Student's t-test (normal distribution) and by the Kruskal-Wallis rank-sum test (non-normal distribution) for continuous variables, and the chi-square tests for categorical variables. Univariate Cox regression models were applied to evaluate the association of demographic, biochemical, and clinical characteristics, and

anthropometric indices with diabetes. The independent effect of baseline and dynamic changes of each anthropometric index on the risk of diabetes was estimated using multivariate Cox regression models. Two models with different sets of covariates were fitted. Stratified and interaction analyses were also conducted to evaluate the potential interactions between BRI and demographic, biochemical and clinical characteristics, and other anthropometric indices. The area under receiver operating characteristic (ROC) curves was calculated to evaluate the abilities of the anthropometric indices to predict diabetes to determine the optimal cut-off point of these indices. Subgroup ROC analyses were further performed for different gender and the menopausal status of women (49 years old was chosen as a cut-off to divide women into pre-menopausal and post-menopausal). All of the statistical analyses were conducted using the statistical software packages R version 4.0.3 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Baseline Characteristics of the Participants

Baseline characteristics of the participants were presented in **Table 1**. An overall 6,990 subjects (3,467 [49.6%] men, the average age of 59.0 ± 13.9 years) were studied. During the average follow-up of 3.1 years, a total of 816 hypertensive patients developed diabetes (the baseline characteristic stratified by sex are presented in **Supplementary Table 3**). The levels of the anthropometric indices, including weight, BMI, WC, HC, WHR, WHtR, ABSI, AVI, BAI, BRI, CI, WWI, and WHHR, were significantly higher in subjects with diabetes ($P < 0.05$). Besides, compared with subjects without diabetes, patients with diabetes were older, had a higher proportion of women, had higher values of FPG, TG, TC, UAER, and SBP, and with a lower eGFR and rate of drinking ($P < 0.05$).

Correlations Between Various Baseline Anthropometric Indices and Diabetes Among Hypertensive Patients

Correlations between various baseline clinical variables and diabetes are displayed in **Supplementary Table 4**. Univariate Cox regression analysis revealed that diabetes was positively correlated with age, TG, TC, LDL, UA, UAER, weight, BMI, WC, WHR, WHtR, WHR, ABSI, AVI, BAI, BRI, CI, WWI, and WHHR among the hypertensive patients ($P < 0.05$).

As shown in **Supplementary Table 5**, after fully adjusted for baseline age, sex, smoking status, drinking status, serum lipid levels, and blood pressure, multivariate Cox regression analysis revealed various anthropometric indices, weight (HR = 1.50; 95% CI, 1.38, 1.62), BMI (HR = 1.40; 95% CI, 1.31, 1.49), WC (HR = 1.42; 95% CI, 1.33, 1.52), HC (HR = 1.30; 95% CI, 1.22, 1.38), WHtR (HR = 1.39; 95% CI, 1.30, 1.49), WHR (HR = 1.16; 95% CI, 1.12, 1.21), AVI (HR = 1.40; 95% CI, 1.31, 1.49), BAI (HR = 1.27; 95% CI, 1.18, 1.37), BRI (HR = 1.36; 95% CI, 1.28, 1.45), CI (HR = 1.18; 95% CI, 1.10, 1.27), WWI (HR = 1.17; 95% CI, 1.08, 1.27), and WHHR (HR = 1.17; 95% CI, 1.10, 1.24), as the independent risk factor for diabetes among hypertensive patients (all $P < 0.001$). In addition, the risk of incident diabetes was found to be increased steadily with successively elevated levels of weight, BMI, WC, WHR, WHtR, BAI, BRI, and WWI (all $P < 0.05$; **Figure 2**). Namely, in the fully adjusted model, levels of weight, BMI, WC, WHR, WHtR, BAI, BRI, and WWI, were each associated with increased risk of diabetes, regardless of whether it was treated as a continuous or categorical variable.

To better examine the performance of the central obesity indices in predicting diabetes risk, we further assessed whether the combination of BMI and different central obesity indices could better stratify the hypertensive patients with a high risk of diabetes (**Supplementary Table 6** and **Figure 3**). The presence of elevated WC, WHR, WHtR, AVI, BRI, and CI at baseline, regardless of the general obesity status, were found to be each independently associated with increased diabetes onset risk in hypertensive patients (all $P < 0.05$). The HR (95% CI) of elevated BRI without general obesity group and elevated BRI with general obesity group were 1.74 (1.33, 2.28), 1.39 (1.13, 1.72), and 2.21 (1.85, 2.64), respectively (all $P < 0.01$). Additionally, similar to BRI, the highest risk of diabetes was all observed among the hypertensive patients with the elevated anthropometric indices mentioned above in obese states ($P < 0.001$).

Interaction and stratified analyses revealed no significant interaction between BRI and age, sex, serum lipid levels, blood pressure, smoking, and drinking status (**Supplementary Table 7**).

Correlations Between Dynamic Changes of Various Anthropometric Indices and Diabetes Among Hypertensive Patients

As shown in **Supplementary Table 8** and **Figure 4**, in the fully adjusted model, elevated BRI (BRI > 5.24) was associated with a higher risk of developing diabetes ($P < 0.05$) compared with the subjects whose BRI was <5.24 at baseline and follow-up. Diabetes risk increased significantly when patients with baseline BRI < 5.24 progressed to more than 5.24 during the follow-up (HR = 1.29; 95% CI, 1.02, 1.64; $P = 0.035$). There was also a decreasing trend toward diabetes risk when baseline BRI more than 5.24 reversed to <5.24 at follow-up (HR = 1.56; 95% CI, 1.23, 1.98; $P < 0.001$) compared with those whose BRI remained more than 5.24 at follow-up (HR = 1.95; 95% CI, 1.63, 2.32; $P < 0.001$). The highest risk of diabetes onset was observed when BRI was more than 5.24 both at baseline and follow-up. Similar patterns were also observed in BMI and AVI. BRI and AVI, as indicators of central obesity, were sensitive to diabetes risk and were capable of reflecting the risk condition of the patient on diabetes onset.

Predictive Capabilities of Various Anthropometric Indices for Diabetes Among Hypertensive Patients

The area under the ROC curve (AUC) was calculated to evaluate the capabilities of each anthropometric measure for the predicting of diabetes among hypertensive patients. As outlined in **Figure 5**, the AUC values of all the anthropometric indices ranged from 0.50 to 0.70, suggesting a moderate predictive

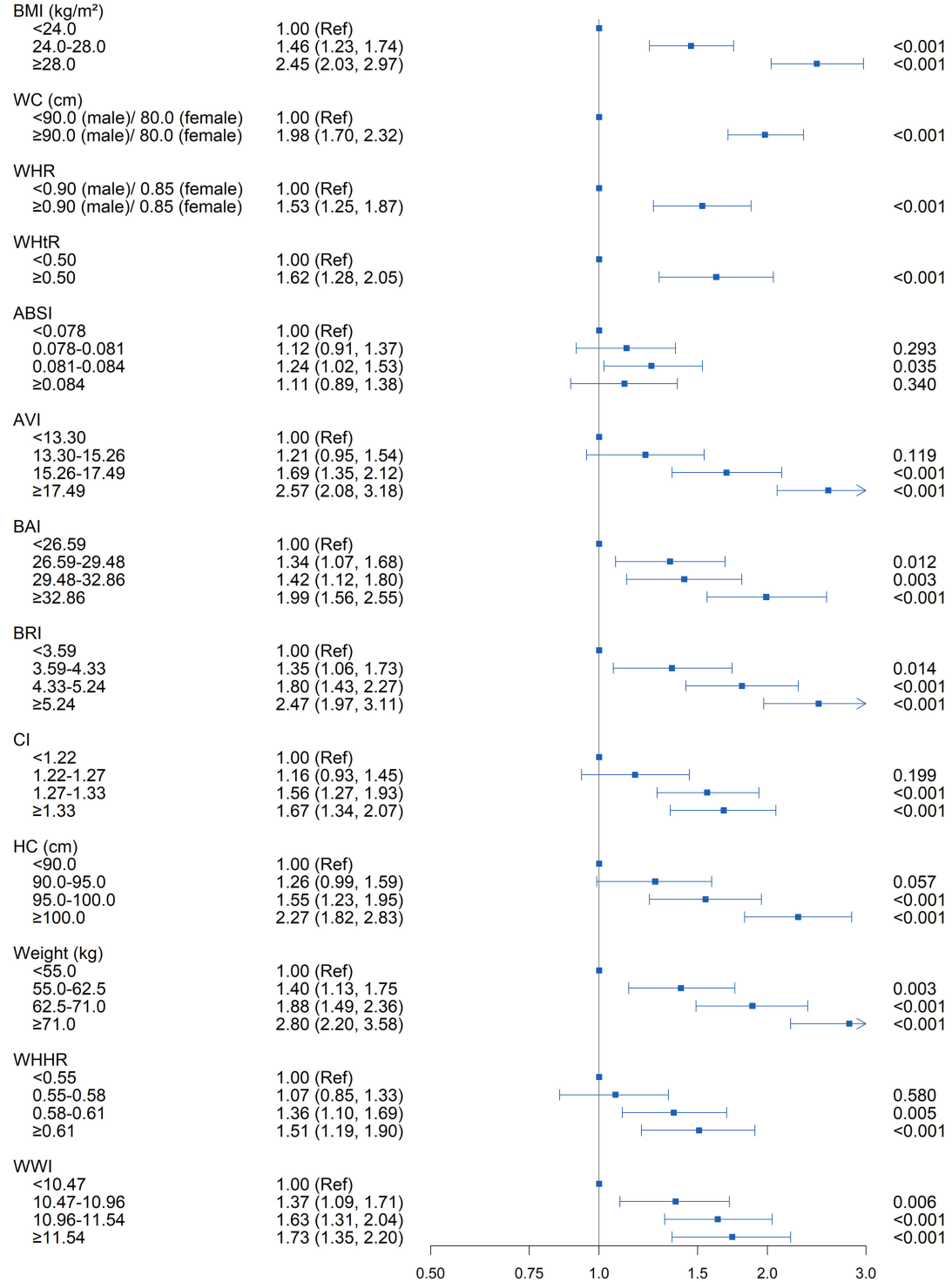


FIGURE 2 | Association between separate anthropometric indices with diabetes (body mass index [BMI], waist circumference [WC], waist-to-hip ratio [WHR], waist-to-height ratio [WHtR], a body shape index [ABSI], abdominal volume index [AVI], body adiposity index [BAI], body roundness index [BRI], conicity index [CI], hip circumference [HC], weight, waist-hip-height ratio [WHHR], weight-adjusted-waist index [WWI]). The correlation was assessed by multivariate cox regression analysis, adjusted by sex, age, smoking status, drinking status, fasting plasma glucose, serum triglycerides, total cholesterol, high-density lipoprotein, low-density lipoprotein, systolic blood pressure, and diastolic blood pressure at baseline. Hazard ratios (HRs) of the anthropometric indices were represented as the squares and 95% confidence intervals (CIs) by the lines through the squares.

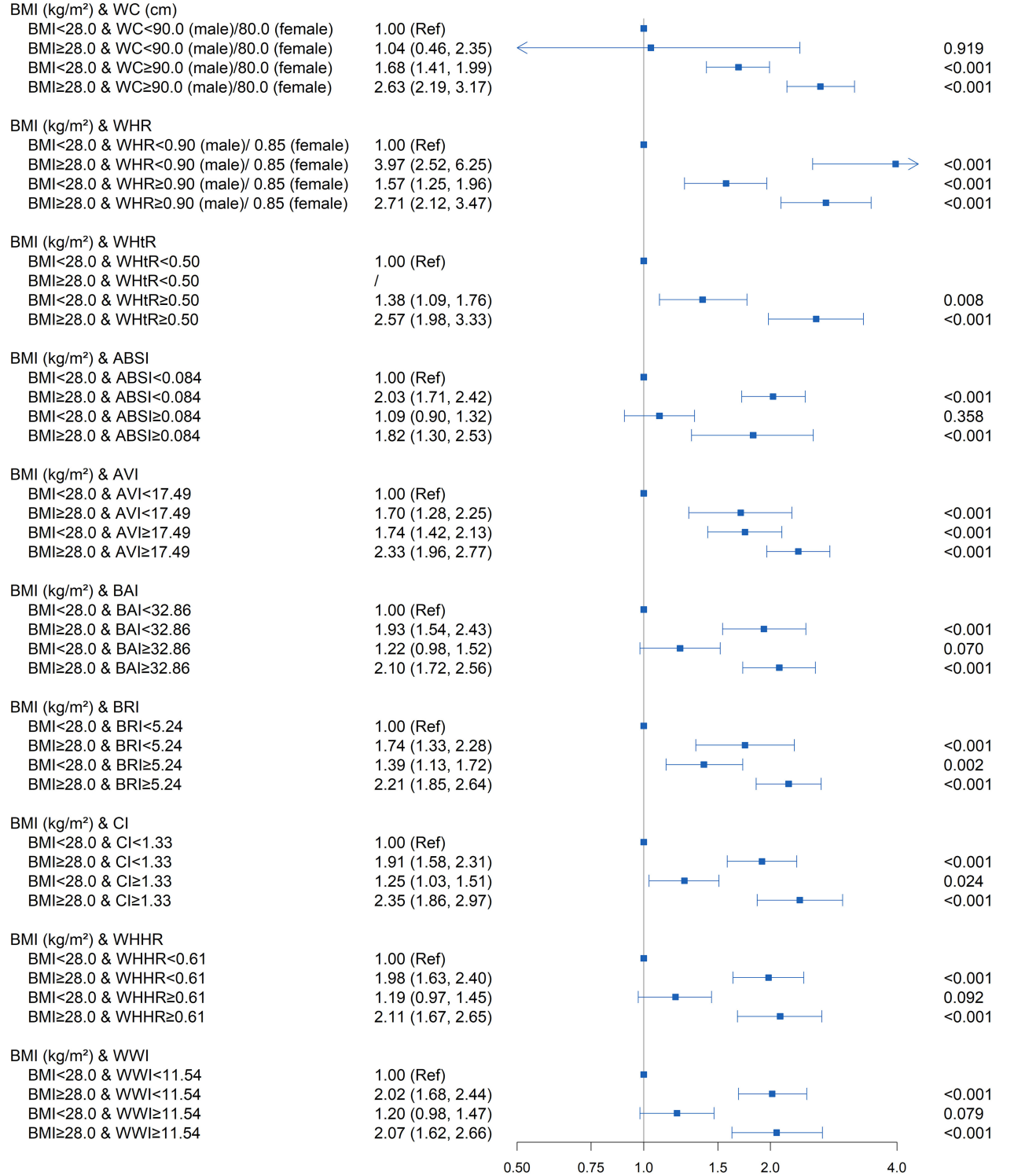


FIGURE 3 | Association between different combinations of body mass index (BMI) and anthropometric indices of central obesity (waist circumference [WC], waist-to-hip ratio [WHR], waist-to-height ratio [WHtR], a body shape index [ABSI], abdominal volume index [AVI], body adiposity index [BAI], body roundness index [BRI], conicity index [CI], waist-hip-height ratio [WHHR], weight-adjusted-waist index [WWI]) with diabetes. The correlation was assessed by multivariate cox regression analysis, adjusted by sex, age, smoking status, drinking status, fasting plasma glucose, serum triglycerides, total cholesterol, high-density lipoprotein, low-density lipoprotein, systolic blood pressure, and diastolic blood pressure at baseline. Hazard ratios (HRs) of the combined anthropometric indices were represented as the squares and 95% confidence intervals (CIs) by the lines through the squares.

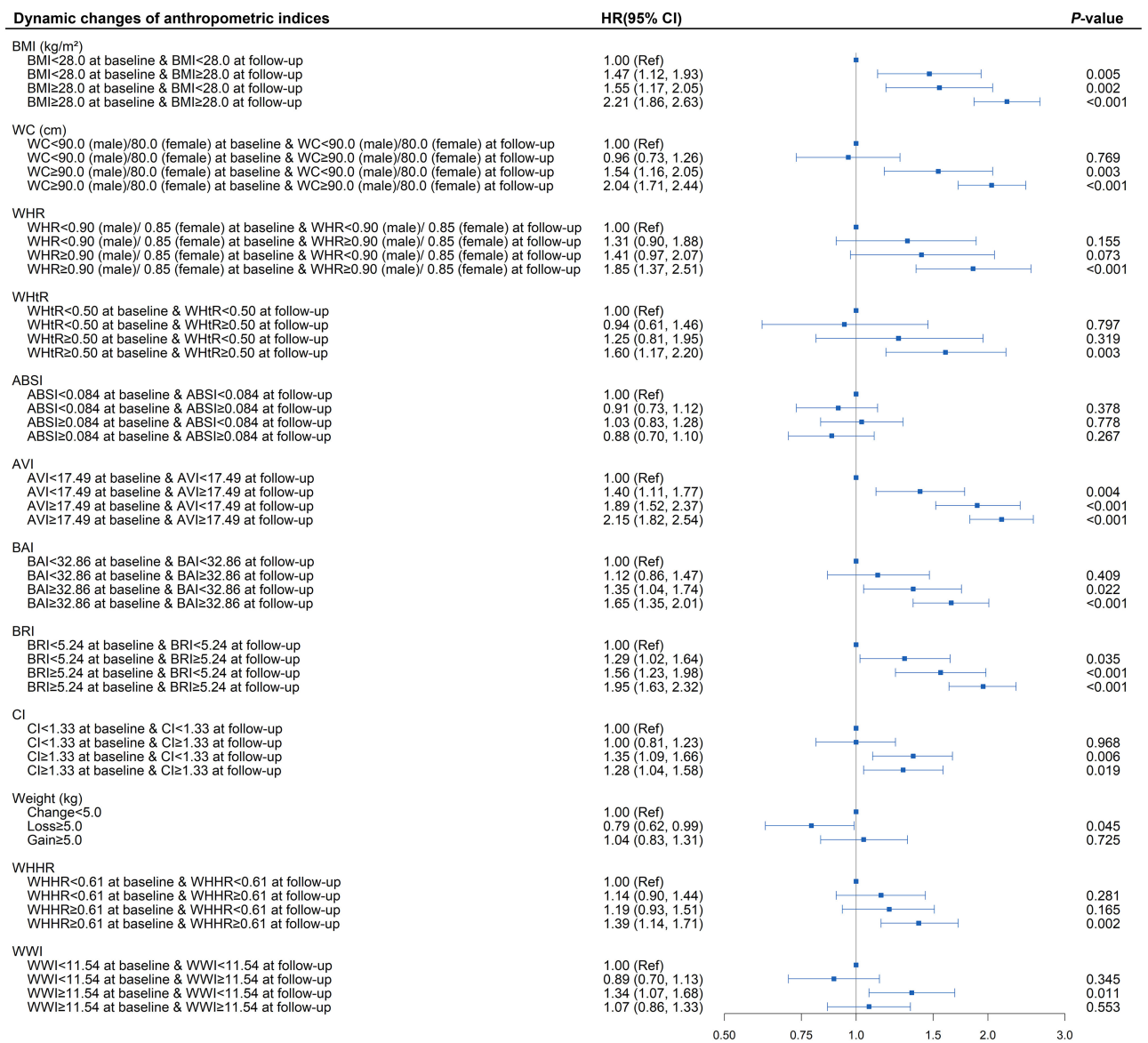


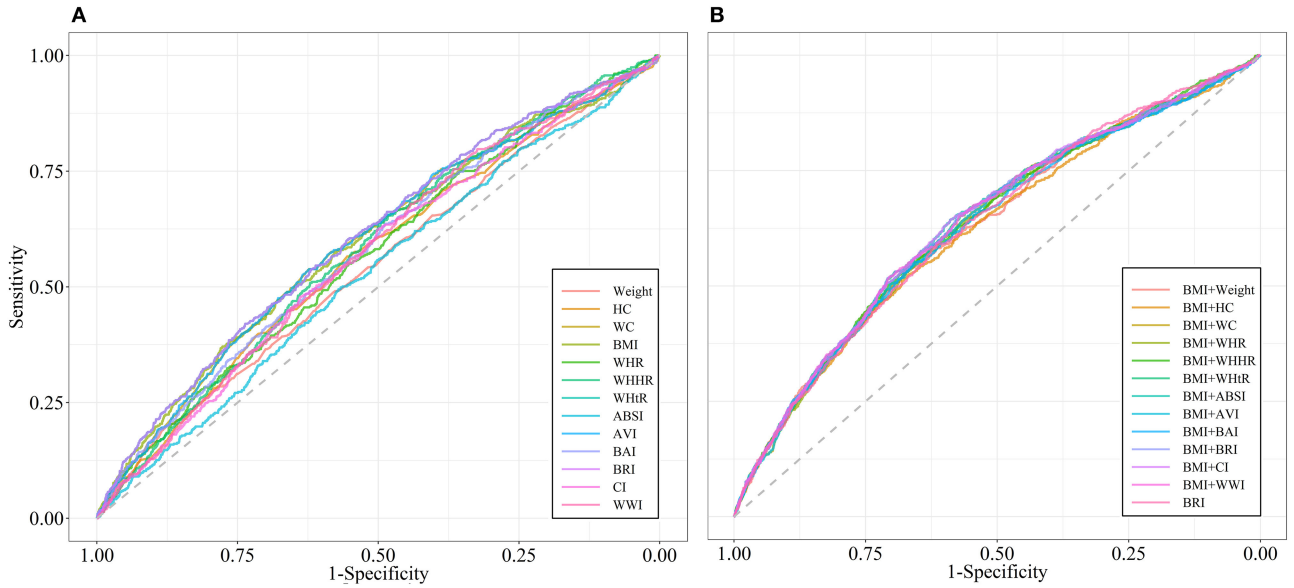
FIGURE 4 | Association between dynamic changes of separate anthropometric indices with diabetes (body mass index [BMI], waist circumference [WC], waist-to-hip ratio [WHR], waist-to-height ratio [WHtR], a body shape index [ABSI], abdominal volume index [AVI], body adiposity index [BAI], body roundness index [BRI], conicity index [CI], weight, waist-hip-height ratio [WHHR], weight-adjusted-waist index [WWI]). The correlation was assessed by multivariate cox regression analysis, adjusted by sex, age, smoking status, drinking status, fasting plasma glucose, serum triglycerides, total cholesterol, high-density lipoprotein, low-density lipoprotein, systolic blood pressure, and diastolic blood pressure at baseline. Hazard ratios (HRs) of the anthropometric indices were represented as the squares and 95% confidence intervals (CIs) by the lines through the squares.

significance for diabetes among hypertensive patients. BRI and WHtR exhibited the largest AUCs for predicting diabetes onset risk (both AUC = 0.63; 95% CI, 0.61, 0.65) among these anthropometric measures. The optimal cut-off value of BRI was determined at 4.62 among the overall hypertensive population, with 3.86 for men, 4.01 for pre-menopausal women, and 5.08 for post-menopausal women (**Supplementary Table 9**).

We further compared the AUCs of different models constituted by indicator for general obesity BMI and each of the indicators for central obesity. The models combined BMI with BRI or WHtR or WWI (AUC = 0.64; 95% CI, 0.62, 0.66), had a better predictive performance compared with BRI alone.

DISCUSSION

Individuals with both hypertension and diabetes have been confirmed to significantly increase the risk of CVD morbidity and mortality compared with those with either condition alone.



Anthropometric indices	Cut-off point	AUC (95% CI)
BRI	4.62	0.630 (0.610, 0.651)
WHtR	0.56	0.630 (0.610, 0.651)
AVI	15.74	0.623 (0.602, 0.644)
WC	88.95	0.623 (0.602, 0.643)
BMI	26.30	0.620 (0.599, 0.641)
BAI	30.90	0.597 (0.576, 0.618)
HC	98.95	0.595 (0.574, 0.616)
WHHR	0.57	0.582 (0.561, 0.602)
WWI	10.96	0.578 (0.557, 0.598)
WHR	0.92	0.574 (0.553, 0.595)
CI	1.27	0.574 (0.553, 0.595)
Weight	64.80	0.570 (0.549, 0.591)
ABSI	0.082	0.531 (0.510, 0.552)

* P<0.05; ** P<0.01; *** P<0.001.

Anthropometric indices	AUC (95% CI)
BRI	0.630 (0.610, 0.651)
BRI + BMI	0.638 (0.617, 0.659)
WHtR + BMI	0.638 (0.617, 0.659)
WWI + BMI	0.638 (0.617, 0.659)
CI + BMI	0.638 (0.617, 0.658)
ABSI + BMI	0.638 (0.617, 0.658)
WHHR + BMI	0.633 (0.612, 0.654)
WC + BMI	0.631 (0.610, 0.652)
AVI + BMI	0.631 (0.610, 0.652)
WHR + BMI	0.630 (0.610, 0.651)
BAI + BMI	0.627 (0.606, 0.648)
Weight + BMI	0.626 (0.605, 0.647)
HC + BMI	0.620 (0.599, 0.641)

* P<0.05; ** P<0.01; *** P<0.001.

FIGURE 5 | (A) Comparison of the receiver operating characteristic (ROC) curves for separate anthropometric indices (body mass index [BMI], waist circumference [WC], waist-to-hip ratio [WHR], waist-to-height ratio [WHtR], a body shape index [ABSI], abdominal volume index [AVI], body adiposity index [BAI], body roundness index [BRI], conicity index [CI], hip circumference [HC], weight, waist-hip-height ratio [WHHR], weight-adjusted-waist index [WWI]). **(B)** Comparison of the receiver operating characteristic (ROC) curves for the combinations of body mass index (BMI) and other anthropometric indices (Weight, WC, HC, WHR, WHtR, ABSI, AVI, BAI, BRI, CI, WHHR, and WWI).

Several anthropometric indices have been shown well to predict the progression of diabetes among general populations. However, less is known on the capabilities of the anthropometric indices in predicting the risk of diabetes among hypertensive patients. Hence, this study aimed to evaluate the potential of different anthropometric indices for predicting diabetes risk among hypertensive patients.

In this cohort study among hypertensive patients with the maximum follow-up of 6 years, the elevated overall and abdominal obesity indicators we examined were positively associated with the increased incident risk of diabetes. Among them, BRI, a novel central obesity index estimated with the use of height and WC whose baseline value and dynamic changes both sensitively reflect the occurrence and progression of diabetes among patients with hypertension, appeared to be the most superior predictor and independent determinant for incident diabetes in the hypertensive population. More importantly, hypertensive patients with elevated BRI, regardless of overall obesity status, were both at higher risk of diabetes. Our study indicated measuring measurements of central obesity, especially the BRI, in addition to BMI, could help to identify patients at a high risk of diabetes among the hypertensive population early.

Hypertensive individuals with a BMI over 25 kg/m^2 (23 kg/m^2 for Asian Americans) are recommended to undergo a test of risk for future diabetes according to the American Diabetes Association (30). Nevertheless, currently, the concept of diagnosing obesity using BMI has been challenged. Our findings indicated that BRI and WHtR should be considered the best anthropometric indices in predicting diabetes risk, which exhibited similar predictabilities (AUC = 0.63) in identify diabetes risk and slightly surpassed the performance of BMI (AUC = 0.62). Similarly, prior cross-sectional research on this

topic and the literature among the general population with the outcome of diabetes all showed a slight increase in AUC values for WHtR compared with BMI. The slight differences observed could be due to the insensitivity of AUC to the model improvement, performing as a small incremental change when adding a critical risk factor to the model (31). Even so, our results and the mentioned above studies all persuasively supported that some anthropometric measures of central obesity, such as WHtR and BRI, were more robust predictors of diabetes than BMI (14). It is also noteworthy that BRI and WHtR showed similar predictive capability for diabetes among hypertensive patients, which is possibly due to the reason that BRI is a one-to-one non-linear transformation of the WHtR, both based on WC and height. BMI is a measure of both fat and fat-free mass, while WC is an indicator for abdominal fat accumulation more closely correlated with insulin resistance than BMI (32), which might explain why BRI and WHtR could have better performances than BMI in predicting diabetes. From the initial analyses of the present study, although BRI and WHtR had the same AUC, it would seem that BRI was better than WHtR based on the HR values on the association between the dynamic changes in indices and diabetes risk; Among hypertensive patients, while those with BRI elevated during the follow-up was associated with a higher risk of developing diabetes, which was not found in patients with elevated WHtR during follow-up. The strength of the BRI over the WHtR is that the distribution of values of BRI could also be applied to estimate the body fat percentage and thus better reflect the physical health conditions. In addition, lower levels of WHtR and BRI during follow-up were both found to have a tendency toward association with decreased risk of diabetes, although the differences did not achieve statistical significance, which may have been because of the relatively short follow-up duration. Therefore, through long-term monitoring of these simple and non-invasive anthropometric measures and timely intervention, such as regular exercise, dietary control, and weight control, it was expected to promote a shift from abnormal toward normal levels of these indices, which was essential for the prevention of diabetes.

Epidemiologically, the prevalence of obesity in Asians is lower than the Caucasians, yet Asian populations are more easily susceptible to diabetes despite relatively low BMI. This could potentially be attributed to the fact that in general, obesity is defined by BMI, which does not consider central obesity in the clinical guidelines. Thus, people with normal BMI and central obesity are usually neglected (33). This viewpoint has been supported in our research. In this study, hypertensive patients with central obesity defined by WC, WHR, and WHtR had a significantly elevated incident risk of diabetes even in the absence of general obesity. There appear to be very few studies focusing on the diabetes incident risk among hypertensive patients with central obesity. In analogy to our findings, a cross-sectional study showed that central obesity including WC and WHtR were both independently related to pre-diabetes or diabetes after adjusting for BMI among the Asian hypertensive population (34). The potential mechanism of central obesity in promoting diabetes development could be via the role of abdominal fat as a marker of excess ectopic fat, which is key to metabolic abnormality and future development of diabetes (35, 36). In addition, the abdominal fat has more metabolically activity than subcutaneous fat, secreting a variety of lipoxins that have adverse effects on the body, and thus leads to hyperinsulinemia, increasing insulin resistance and enhancing inflammatory responses, which are established determinants of diabetes (37). Further, gratifyingly, BRI also performed similarly in reflecting the central obesity as WC, WHR, and WHtR in this study, showing a satisfactory identification ability of abdominal obesity. Equally important is that the combination of BMI and several anthropometric measures of central obesity could significantly increase predictive power than using a single index. Based on the above information, BMI should be used in conjunction with anthropometric measures of central obesity, of which BRI is a viable choice with a good performance.

BRI was a novel anthropometric index first developed by Thomas et al. (19), used for predicting the percentage of body fat, visceral fat, and provide an initial impression of physical health status. Up to date, BRI has been applied to predict metabolic syndrome in the general population, overweight and obese population, diabetic population, post-menopausal women, and all showed relatively good predictive performances (38, 39). BRI was also considered to be strongly correlated with diabetes and capable of identifying diabetes according to the previous cross-sectional studies (40, 41). The prior cohort study among the elderly population showed that BRI had a certain predictive capacity for diabetes (AUC: 0.609–0.629) (42), which was consistent with our result. Cut-off values of BRI ranged from 3.18 to 6.20 among different studies (42–46), which could be due to the differences in study populations, race, and diagnostic criteria. In the present study, the cut-off point for BRI was 4.62 among the overall hypertensive population, 3.86 in men, 4.01 in pre-menopausal women, and 5.08 in post-menopausal women, which were all within the range of BRI from the previous studies. No significant variation was detected in the cut-off points of BRI between men and pre-menopausal women, yet there was a difference between post-menopausal women and pre-menopausal women/men. This could be explained as prolonged estrogen deficiency among post-menopausal women. Estrogen regulates fat distribution and adipocyte differentiation, thus increasing the risk of weight gain and obesity in postmenopausal women, especially central obesity, suggesting that the optimum cut points for BRI should be selected based on gender and menstrual status of women. Therefore, hypertensive patients with BRI more than the corresponding cut-off value, regardless of the general obesity status, were at a high risk of diabetes, and thus, timely blood glucose monitoring and effective integrative intervention should be conducted for these patients.

The current study has important implications for clinical practice and public health. To begin with, BRI and WHtR were proposed to be the best indices in predicting diabetes among hypertensive in the present study, both of which could be simply calculated based on WC and height. However, WC is not routinely obtained in most clinical settings in China. Indeed, WC measurement could be easily implemented in different levels of the hospitals with only a tape used and simple standardized training of the healthcare personnel. Therefore, BRI could

emerge as the screening instrument to remind the healthcare professionals of the hypertensive patients at high risk of diabetes, providing additional benefits beyond BMI measurement. From the point of view of public health, using BRI as a non-invasive, simple predicting tool could help reduce the number of patients required for blood sampling to some degree and offer a practical approach of screening diabetes risk, especially for patients in areas with relatively poor medical resources. In addition, since comorbidities between diabetes and hypertension significantly increase the risk of CVD (5), applying a diabetes risk prediction tool among hypertensive patients appears beneficial for clinicians to better develop intervention strategies, leading to better prevention of CVD. More importantly, the dynamic changes of BRI could sensitively reflect the variation of diabetes onset risk. Since the height remained nearly unchanged, our findings emphasized that the decrease in WC is critical for public health preventive interventions for diabetes. For the above reasons, we thus recommend BRI as a pre-screening tool for diabetes and as a risk stratification tool for CVD among Chinese hypertensive patients.

The following limitations should be considered when interpreting our findings. First, the AUC values of all anthropometric measures in this study were <0.7, which implied modest discrimination performance. Secondly, this was a single-center study. Though Dongguan City is a very representative medium-developed urbanized rural area in China, considering the difference in lifestyles among the regions, our study might not represent the whole population. Third, some factors known to be associated with further development of diabetes, such as family history, dietary habits, and physical activity status, have not been accounted for in the present study. Fourth, the present study did not take hypertension-related target organ damage, such as left ventricular hypertrophy and carotid atherosclerosis, and the duration of hypertension into account, which was crucial to understanding the hypertensive status. Finally, due to a lack of uniform criteria for the novel anthropometric indices in the Chines population, the 75% value was initially selected as the cut-off point to explore the association with diabetes risk in this study. Therefore, further studies with a larger sample size from a multicenter population and a more rigorous experimental design were needed to validate our findings.

In conclusion, our results showed that anthropometric indices for both general obesity and central obesity studied in this study were closely associated with the diabetes onset risk among hypertensive patients. Compared with other anthropometric indices, BRI tends to perform optimally in predicting diabetes among the hypertensive population due to the superior sensitivity of both its baseline value and dynamic changes on reflecting the development of diabetes. Anthropometric indices for central obesity, especially BRI, could be measured in addition to the BMI, which could provide additional clinical and public health benefits in the pre-screening of hypertensive patients with a high risk of diabetes. Furthermore, our findings suggested that hypertensive patients with a BRI of more than 4.62, regardless of the general obesity status, are considered to be at high risk of diabetes. Therefore, interventions focusing on reducing WC were recommended being timely carried out among these patients to reduce the risk of diabetes.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, upon reasonable request.

ETHICS STATEMENT

The present study was approved by the Medical Research Ethics Committee of Guangdong Provincial People's Hospital. All participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JK, YF, and HG: conceptualization. HG, JK, YF, ST, and HC: methodology. YF and JK: validation, writing—review, editing, and supervision. YL and XL: formal analysis. XF: investigation. QZ: resources. SZ: data curation. YL: writing—original draft preparation. XL: visualization. JK, YF, and ST: project administration. JK and HC: funding acquisition. All authors have contributed to the creation of this manuscript for important intellectual content, contributed to the article, and approved the submitted version.

FUNDING

This work was supported by the National Key R&D Program of China under Grant No. 2018YFC1314100, the Key-Area Research and Development Program of Guangdong Province under Grant No. 2019B020230001, and the Science and Technology Plan of Guangzhou under Grant No. 201707010330.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2021.736073/full#supplementary-material

REFERENCES

1. Sarwar N, Gao P, Seshasai SR, Gobin R, Kaptoge S, Di Angelantonio E, et al. Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies. *Lancet.* (2010) 375:2215–22. doi: 10.1016/s0140-6736(10)60484-9
2. Bress AP, King JB, Kreider KE, Beddhu S, Simmons DL, Cheung AK, et al. Effect of intensive versus standard blood pressure treatment according to baseline prediabetes status: a post hoc analysis of a randomized trial. *Diabetes Care.* (2017) 40:1401–8. doi: 10.2337/dc17-0885
3. Strain WD, Paldánius PM. Diabetes, cardiovascular disease and the microcirculation. *Cardiovasc Diabetol.* (2018) 17:57. doi: 10.1186/s12933-018-0703-2

4. Ferrannini E, Cushman WC. Diabetes and hypertension: the bad companions. *Lancet.* (2012) 380:601–10. doi: 10.1016/s0140-6736(12)60987-8
5. Whelton PK, Carey RM, Aronow WS, Casey DE, Collins KJ, Dennison Himmelfarb C, et al. 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA guideline for the prevention, detection, evaluation, and management of high blood pressure in adults: a report of the American college of cardiology/American heart association task force on clinical practice guidelines. *Hypertension.* (2018) 71:e13–115. doi: 10.1161/hyp.0000000000000065
6. Kahn R, Davidson MB. The reality of type 2 diabetes prevention. *Diabetes Care.* (2014) 37:943–9. doi: 10.2337/dc13-1954
7. Flegal KM, Carroll MD, Ogden CL, Johnson CL. Prevalence and trends in obesity among US adults, 1999-2000. *JAMA.* (2002) 288:1723–7. doi: 10.1001/jama.288.14.1723
8. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive summary of the third report of the national cholesterol education program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). *JAMA.* (2001) 285:2486–97. doi: 10.1001/jama.285.19.2486
9. Choi SI, Chung D, Lim JS, Lee MY, Shin JY, Chung CH, et al. Relationship between regional body fat distribution and diabetes mellitus: 2008 to 2010 Korean national health and nutrition examination surveys. *Diabetes Metab J.* (2017) 41:51–9. doi: 10.4093/dmj.2017.41.1.51
10. Heshka S, Ruggiero A, Bray GA, Foreyt J, Kahn SE, Lewis CE, et al. Altered body composition in type 2 diabetes mellitus. *Int J Obes.* (2008) 32:780–7. doi: 10.1038/sj.ijo.0803802
11. American Diabetes Association. Standards of medical care in diabetes—2014. *Diabetes Care.* (2014) 37(suppl. 1):S14–80. doi: 10.2337/dc14-S014
12. Cornier MA, Després JP, Davis N, Grossniklaus DA, Klein S, Lamarche B, et al. Assessing adiposity: a scientific statement from the American Heart Association. *Circulation.* (2011) 124:1996–2019. doi: 10.1161/CIR.0b013e318233bc6a
13. Kodama S, Horikawa C, Fujihara K, Heianza Y, Hirasawa R, Yachi Y, et al. Comparisons of the strength of associations with future type 2 diabetes risk among anthropometric obesity indicators, including waist-to-height ratio: a meta-analysis. *Am J Epidemiol.* (2012) 176:959–69. doi: 10.1093/aje/kws172
14. Ashwell M, Gunn P, Gibson S. Waist-to-height ratio is a better screening tool than waist circumference and BMI for adult cardiometabolic risk factors: systematic review and meta-analysis. *Obes Rev.* (2012) 13:275–86. doi: 10.1111/j.1467-789X.2011.00952.x
15. Sommer I, Teufer B, Szelag M, Nussbaumer-Streit B, Titscher V, Klerings I, et al. The performance of anthropometric tools to determine obesity: a systematic review and meta-analysis. *Sci Rep.* (2020) 10:12699. doi: 10.1038/s41598-020-69498-7
16. Krakauer NY, Krakauer JC. A new body shape index predicts mortality hazard independently of body mass index. *PLoS ONE.* (2012) 7:e39504. doi: 10.1371/journal.pone.0039504
17. Perona JS, Schmidt Rio-Valle J, Ramírez-Vélez R, Correa-Rodríguez M, Fernández-Aparicio Á, González-Jiménez E. Waist circumference and abdominal volume index are the strongest anthropometric discriminators of metabolic syndrome in Spanish adolescents. *Eur J Clin Invest.* (2019) 49:e13060. doi: 10.1111/eci.13060
18. Bergman RN, Stefanovski D, Buchanan TA, Sumner AE, Reynolds JC, Sebring NG, et al. A better index of body adiposity. *Obesity.* (2011) 19:1083–9. doi: 10.1038/oby.2011.38
19. Thomas DM, Bredlau C, Bosy-Westphal A, Mueller M, Shen W, Gallagher D, et al. Relationships between body roundness with body fat and visceral adipose tissue emerging from a new geometrical model. *Obesity.* (2013) 21:2264–71. doi: 10.1002/oby.20408
20. Valdez R. A simple model-based index of abdominal adiposity. *J Clin Epidemiol.* (1991) 44:955–6. doi: 10.1016/0895-4356(91)90059-i
21. Park Y, Kim NH, Kwon TY, Kim SG. A novel adiposity index as an integrated predictor of cardiometabolic disease morbidity and mortality. *Sci Rep.* (2018) 8:16753. doi: 10.1038/s41598-018-35073-4
22. Carlsson AC, Risérus U, Engström G, Ärnlöv J, Melander O, Leander K, et al. Novel and established anthropometric measures and the prediction of incident cardiovascular disease: a cohort study. *Int J Obes.* (2013) 37:1579–85. doi: 10.1038/ijo.2013.46
23. McDowell MA, Fryar CD, Hirsch R, Ogden CL. Anthropometric reference data for children and adults: U.S. population, 1999-2002. *Adv Data.* (2005) 7:1–5.
24. Zhou BF. Coorperative meta-analysis group of China obesity task force. Predictive values of body mass index and waist circumference to risk factors of related diseases in Chinese adult population. *Chinese J. Epidemiol.* (2002) 23:5–10.
25. WHO. *World Health Organization Waist Circumference and Waist-Hip Ratio: Report of a WHO Expert Consultation*. Geneva: WHO (2008).
26. Jayawardana R, Ranasinghe P, Sheriff MH, Matthews DR, Katulanda P. Waist to height ratio: a better anthropometric marker of diabetes and cardiometabolic risks in South Asian adults. *Diabetes Res Clin Pract.* (2013) 99:292–9. doi: 10.1016/j.diabres.2012.12.013
27. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL, et al., et al. The seventh report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure: the JNC 7 report. *JAMA.* (2003) 289:2560–72. doi: 10.1001/jama.289.19.2560
28. Inker LA, Schmid CH, Tighiouart H, Eckfeldt JH, Feldman HI, Greene T, et al. Estimating glomerular filtration rate from serum creatinine and cystatin C. *N Engl J Med.* (2012) 367:20–9. doi: 10.1056/NEJMoa1114248
29. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med.* (1998) 15:539–53. doi: 10.1002/(sici)1096-9136(199807)15:7<539::Aid-dia668>3.0.Co;2-s
30. American Diabetes Association. 2. Classification and diagnosis of diabetes: standards of medical care in diabetes-2018. *Diabetes Care.* (2018) 41:S13–27. doi: 10.2337/dc18-S002
31. Pencina MJ, D'Agostino RB, D'Agostino RB, Vasan RS. Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond. *Stat Med.* (2008) 27:157–72. doi: 10.1002/sim.2929
32. Aleksandrova K, Jenab M, Leitzmann M, Bueno-de-Mesquita B, Kaaks R, Trichopoulou A, et al. Physical activity, mediating factors and risk of colon cancer: insights into adiposity and circulating biomarkers from the EPIC cohort. *Int J Epidemiol.* (2017) 46:1823–35. doi: 10.1093/ije/dyx174
33. Sun Y, Liu B, Snetselaar LG, Wallace RB, Caan BJ, Rohan TE, et al. Association of normal-weight central obesity with all-cause and cause-specific mortality among postmenopausal women. *JAMA Netw Open.* (2019) 2:e197337. doi: 10.1001/jamanetworkopen.2019.7337
34. Wang Y, Shirore RM, Ramakrishnan C, Tan NC, Jafar TH. Adiposity measures and pre-diabetes or diabetes in adults with hypertension in Singapore polyclinics. *J Clin Hypertens.* (2019) 21:953–62. doi: 10.1111/jch.13587
35. McQuaid SE, Hodson L, Neville MJ, Dennis AL, Cheeseman J, Humphreys SM, et al. Downregulation of adipose tissue fatty acid trafficking in obesity: a driver for ectopic fat deposition? *Diabetes.* (2011) 60:47–55. doi: 10.2337/db10-0867
36. Neeland IJ, Turer AT, Ayers CR, Powell-Wiley TM, Vega GL, Farzaneh-Far R, et al. Dysfunctional adiposity and the risk of prediabetes and type 2 diabetes in obese adults. *JAMA.* (2012) 308:1150–9. doi: 10.1001/2012.jama.11132
37. Hermsdorff HH, Monteiro JB. [Visceral, subcutaneous or intramuscular fat: where is the problem?]. *Arq Bras Endocrinol Metabol.* (2004) 48:803–11. doi: 10.1590/s0004-27302004000600005
38. Liu B, Liu B, Wu G, Yin F. Relationship between body-roundness index and metabolic syndrome in type 2 diabetes. *Diabetes Metab Syndr Obes.* (2019) 12:931–5. doi: 10.2147/dmso.S209964
39. Li G, Wu HK, Wu XW, Cao Z, Tu YC, Ma Y, et al. The feasibility of two anthropometric indices to identify metabolic syndrome, insulin resistance and inflammatory factors in obese and overweight adults. *Nutrition.* (2019) 57:194–201. doi: 10.1016/j.nut.2018.05.004
40. Chang Y, Guo X, Chen Y, Guo L, Li Z, Yu S, et al. A body shape index and body roundness index: two new body indices to identify diabetes mellitus among rural populations in northeast China. *BMC Public Health.* (2015) 15:794. doi: 10.1186/s12889-015-2150-2
41. Zhao Q, Zhang K, Li Y, Zhen Q, Shi J, Yu Y, et al. Capacity of a body shape index and body roundness index to identify diabetes mellitus in Han

Chinese people in Northeast China: a cross-sectional study. *Diabet Med.* (2018) 35:1580–7. doi: 10.1111/dme.13787
42. Yang J, Wang F, Wang J, Han X, Hu H, Yu C, et al. Using different anthropometric indices to assess prediction ability of type 2 diabetes in elderly population: a 5 year prospective study. *BMC Geriatr.* (2018) 18:218. doi: 10.1186/s12877-018-0912-2
43. Wang H, Liu A, Zhao T, Gong X, Pang T, Zhou Y, et al. Comparison of anthropometric indices for predicting the risk of metabolic syndrome and its components in Chinese adults: a prospective, longitudinal study. *BMJ Open.* (2017) 7:e016062. doi: 10.1136/bmjopen-2017-016062
44. Zhang J, Zhu W, Qiu L, Huang L, Fang L. Sex- and age-specific optimal anthropometric indices as screening tools for metabolic syndrome in Chinese adults. *Int J Endocrinol.* (2018) 2018:1067603. doi: 10.1155/2018/1067603
45. Suliga E, Ciesla E, Głuszek-Osuch M, Rogula T, Głuszek S, Kozieł D. The usefulness of anthropometric indices to identify the risk of metabolic syndrome. *Nutrients.* (2019) 11:2598. doi: 10.3390/nu11112598
46. Ramírez-Vélez R, Pérez-Sousa M, Izquierdo M, Cano-Gutierrez CA, González-Jiménez E, Schmidt-RioValle J, et al. Validation of surrogate anthropometric indices in older adults: what is the best indicator of high cardiometabolic risk factor clustering? *Nutrients.* (2019) 11:1701. doi: 10.3390/nu11081701

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frontiers in Cardiovascular Medicine

SYSTEMATIC REVIEW
published: 05 January 2022
doi: 10.3389/fcvm.2021.810105

Isolated Diastolic Hypertension and Risk of Cardiovascular Events: A Systematic Review and Meta-Analysis of Cohort Studies With 489,814 Participants

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OPEN ACCESS

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Specialty section:
This article was submitted to Hypertension, a section of the journal Frontiers in Cardiovascular Medicine

Received: *06 November 2021*
Accepted: *03 December 2021*
Published: *05 January 2022*

Citation:
Huang M, Long L, Tan L, Shen A, Deng M, Peng Y, Yang W, Li H, Wei Y, Li M, Liao F, Liu C, Lu A, Qu H, Fu C and Chen K (2022) Isolated Diastolic Hypertension and Risk of Cardiovascular Events: A Systematic Review and Meta-Analysis of Cohort Studies With 489,814 Participants. Front. Cardiovasc. Med. 8:810105. doi: 10.3389/fcvm.2021.810105

Background: The association between isolated diastolic hypertension (IDH) and cardiovascular events has been inconsistently reported. This meta-analysis of cohort studies was designed to investigate the effect of the 2018 European Society of Cardiology (ESC) definition of IDH on the risk of composite cardiovascular events, cardiovascular mortality, all-cause mortality, and all strokes including ischemic stroke (IS) and hemorrhagic stroke (HS).

Methods: PubMed, Embase, the Cochrane Library, and Web of Science were searched from inception to July 6, 2021. Cohort studies that investigated the association between IDH and cardiovascular events risk, compared to normotension, were included. Pooled hazard ratios (HRs) and 95% CIs were calculated using a random-effects models and heterogeneity was evaluated using *Q*-test and I^2 statistic. The robustness of the associations was identified using sensitivity analysis. The methodological quality of the studies was assessed using the Newcastle–Ottawa scale. Publication bias was assessed using funnel plot, trim-and-fill method, Begg's test, and Egger's test.

Results: A total of 15 cohort studies (13 articles) including 489,814 participants were included in this meta-analysis. The follow-up period ranged from 4.3 to 29 years. IDH was significantly associated with an increased risk of composite cardiovascular events (HR 1.28, 95% CI: 1.07–1.52, $p = 0.006$), cardiovascular mortality (HR 1.45, 95% CI: 1.07–1.95, $p = 0.015$), all strokes (HR 1.44, 95% CI: 1.04–2.01, $p = 0.03$), and HS (HR 1.64, 95% CI: 1.18–2.29, $p = 0.164$), but not associated with all-cause mortality (HR 1.20, 95% CI: 0.97–1.47, $p = 0.087$) and IS (HR 1.56, 95% CI: 0.87–2.81, $p = 0.137$). Subgroup analysis further indicated that IDH in the younger patients (mean age $\leq$ 55 years) and from Asia were significantly associated with an increased risk of composite cardiovascular events, while the elderly patients (mean age $\geq$ 55 years), Americans, and Europeans were not significantly associated with an increased risk of composite cardiovascular events.

Conclusion: This meta-analysis provides evidence that IDH defined using the 2018 ESC criterion is significantly associated with an increased risk of composite cardiovascular events, cardiovascular mortality, all strokes and HS, but not significantly associated with all-cause death and IS. These findings also emphasize the importance for patients with IDH to have their blood pressure within normal, especially in the young adults and Asians.

Trial Registration: PROSPERO, Identifier: CRD42021254108.

Keywords: isolated diastolic hypertension, cardiovascular events, cardiovascular mortality, stroke, meta-analysis, cohort study

INTRODUCTION

Isolated diastolic hypertension (IDH) is an important subtype of hypertension defined as a systolic blood pressure (SBP) of < 130 mm Hg and a diastolic blood pressure (DBP) of at least 80 mm Hg according to the 2017 American College of Cardiology (ACC)/American Heart Association (AHA) criterion (1) and an SBP of < 140 mm Hg with a DBP of at least 90 mm Hg according to the 2018 European Society of Cardiology (ESC) criterion (2). Compared with using the 2018 ESC guidelines, applying the 2017 ACC/AHA guidelines, it increased the prevalence of IDH from 1.3 to 6.5% in the United States (3), 7.79 to 24.72% in China (4), and 5.2 to 17.9% in Korea (5). However, IDH has usually been neglected and the treatment and awareness rates of this condition remain low. A previous study demonstrated that 86.1% of patients with IDH did not receive treatment and only 10.3% of untreated patients knew that they had hypertension (6). The number of deaths from cardiovascular events reached 17.7 million in 2017, accounting for approximately one-third of the total deaths (55 million) worldwide (7). Hypertension is the leading modifiable risk factor for cardiovascular events (8–10). The prognostic value of isolated systolic hypertension for cardiovascular events has been determined through a series of longitudinal clinical trials and meta-analysis studies (11–14), while there was only one meta-analysis reported that IDH diagnosed using the 2017 ACC/AHA criterion was not consistently associated with the cardiovascular disease (CVD) risk (15). However, whether IDH diagnosed using the 2018 ESC guidelines is associated with an increased risk of composite cardiovascular events remains controversial.

A recent prospective cohort study by Wu et al. indicated that IDH was associated with cerebral hemorrhage, myocardial infarction (MI), and total CVD compared to normotension (4). Conversely, McEvoy et al. demonstrated that IDH was not associated with the incidence of atherosclerotic CVD (3). Therefore, this systematic review and meta-analysis of published cohort studies were performed to further identify the association between IDH diagnosed using the 2018 ESC guideline and composite cardiovascular events, cardiovascular mortality, all-cause mortality, and all strokes including hemorrhagic stroke (HS) and ischemic stroke (IS).

METHODS

Data Sources and Searches

The meta-analysis was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) (16) and the Meta-analysis of Observational Studies in Epidemiology (MOOSE) guidelines (17), The protocol was registered in the International prospective register of systematic reviews (PROSPERO) (CRD42021254108). We conducted a meta-analysis of cohort studies that examined the association between IDH and the risk of cardiovascular events. Publications were identified by searching PubMed, Embase, the Cochrane Library, and Web of Science without language restrictions from inception to July 6, 2021. The following medical subject headings and free-text terms were searched (**eAppendix 1**): (hypertension or high blood pressure) and (diastole or isolated diastolic hypertension or IDH) and (cardiovascular diseases or cardiovascular events or cardiovascular deaths or cardiovascular or cardiac or myocardial ischemia or coronary artery disease or coronary heart disease or acute coronary syndrome or ischemic heart disease or myocardial infarction or heart failure or atrial fibrillation or stroke or cerebrovascular disorders or cerebrovascular accident or cerebrovascular disease or cerebrovascular or cerebral or complication or mortality or fatality or death) and (cohort studies or cohort or follow-up or observational or longitudinal or prospective). Additional articles were identified by manually searching the reference lists of pertinent articles.

Study Selection

Studies were included if they met the following inclusion criteria: (1) cohort study; (2) performed in the general adult population (age > 18 years); (3) reported the associations of IDH with the composite of cardiovascular events, cardiovascular mortality, all-cause mortality, all-strokes, IS, and HS; (4) defined IDH and normotension based on the 2018 ESC guidelines (SBP < 140 mm Hg/DBP ≥ 90 mm Hg vs. SBP < 140 mm Hg/DBP < 90 mm Hg); and (5) reported hazard ratios (HRs) with corresponding 95% CIs for the association between IDH and cardiovascular events or sufficient data for their calculation. We excluded studies if they: (1) involved pregnant, critically ill, or hospitalized participants or (2) were published as comments, conference abstracts, or letters to the editor. When republished

studies that included participants from the same cohort and reported similar outcome measures were found, articles reporting the most relevant data were selected. However, if duplicate studies provided information on different outcomes, they were included in the specific outcome analysis. Two investigators (MYH and HQ) independently screened all the titles or abstracts and reviewed the full texts to determine the eligibility of the identified studies and the validity of the extracted data. Any disagreements were resolved through a discussion or by a third reviewer (CGF).

Data Extraction and Quality Assessment

Two investigators (MYH and LZL) independently extracted data from each eligible publication using a standardized data collection form. Any disagreements were resolved by consulting a third investigator (CGF). We used HRs to measure the associations. The primary outcomes of interest in this study were the composite of cardiovascular events. The secondary outcomes of interest were cardiovascular mortality, all-cause mortality, all strokes, IS, and HS. When an article was unavailable or to obtain additional information for analyses, an e-mail requesting the article or information was sent to the corresponding author. We recorded the following study characteristics: first author, publication year, study design, country, age at entry, percentage of male participants, cohort sample size, key exclusion criteria, outcomes, follow-up duration, treatment status at baseline, methods of BP measurement, ascertainment of outcomes, HRs and 95% CIs, and confounding variables adjusted in the multivariate analysis.

Two investigators (MYH and HQ) assessed the study quality using the Newcastle–Ottawa quality assessment scale for cohort studies (18). This scale allocated a total of nine points for the following three aspects: study selection (0–4 points), comparability (0–2 points), and ascertainment of the outcome of interest (0–3 points). We assigned scores of 0–3, 4–6, and 7–9 for low-, moderate-, and high-quality studies, respectively. Disagreements on quality assessment were resolved through a discussion with a third investigator (CGF).

Statistical Analysis

Hazard ratios and 95% CIs were considered as the measure of the association between IDH and the cardiovascular event risk. All the studies included in the meta-analysis reported HRs and 95% CIs. We preferentially pooled the results from the multivariate-adjusted models with the most complete adjustment for underlying confounders. A random-effects model accounting for variation between studies was applied, as this can provide more conservative results than a fixed-effects model. We used Cochran's Q test ($p < 0.10$) to assess the heterogeneity among studies and I^2 statistic to quantify the percentage of the total variation due to that heterogeneity. Low, moderate, and high heterogeneity were defined as I^2 values of 0–25, 26–75, and $> 75\%$, respectively (19). We then conducted random-effects subgroup analyses and sensitivity analyses to identify the sources of heterogeneity among studies and evaluate the robustness of the associations. Subgroup analyses were stratified by mean age, study location, treatment status at baseline, body mass index (BMI), and method of BP measurement. In sensitivity analyses, we used a leave-one-out method to observe the influence of individual studies on the overall risk estimate of HR. Potential publication bias was evaluated using visual assessment of funnel plots, Begg's test, and Egger's test. Potential adjustment for missing studies was approached by Duval and Tweedie trim-and-fill method. All the statistical analyses were performed using the Stata (version 15.0; Stata Corporation, College Station, Texas, USA). All the tests were two-sided and statistical significance was set at $p < 0.05$.

RESULTS

Study Selection

Figure 1 shows the study selection process. We identified 1,459 articles from PubMed, 472 articles from Web of Science, 840 articles from Embase, and 290 articles from the Cochrane Library. After excluding 652 duplicates and 2,354 irrelevant articles based on titles and abstracts, 55 full articles remained for further examination. After a careful review of these records, 42 articles were excluded for the following reasons: IDH was not reported as the relevant exposure variable ($n = 9$), irrelevant IDH definition ($n = 3$), improper comparison ($n = 6$), irrelevant normotension definition (6), irrelevant study outcome ($n = 6$), republished studies ($n = 3$), studies not reporting HRs ($n = 2$), abstract-only articles ($n = 5$), cross-sectional studies ($n = 1$), and reviews ($n = 1$). Finally, 13 articles with 489,814 participants were included in the meta-analysis, as one publications included three independent cohort studies (3). Therefore, 15 studies from 13 articles were included in the meta-analysis.

Characteristics of Included Studies

Table 1 shows the characteristics of the included cohort studies published between 2000 and 2021. **Table 2** shows the characteristics of the participants and outcome ascertainment methods. Thirteen studies were prospective cohort studies and the other studies were retrospective cohort studies. The follow-up duration ranged from 4.3 to 29 years. Seven studies were conducted in Asia (four studies in China, two studies in Japan, and one study in Iran), four studies in the United States of America, and four studies in Europe (two studies in Finland, one study in the United Kingdom, and one study in Swedish). Overall, seven studies investigated the occurrence of composite cardiovascular events (coronary heart disease, strokes, and cardiovascular death in four studies; coronary heart disease and strokes in three studies; and coronary heart disease, strokes, heart failure, and cardiovascular death in one study), eight studies evaluated the risk of cardiovascular mortality, six studies assessed the risk of all-cause mortality, 4 studies evaluated the risk of all strokes, and 3 studies assessed the risk of IS and HS. All the studies performed adjustment for age. Most cohorts were controlled for some general risk factors including age ($n = 15$), smoking ($n = 14$), sex ($n = 13$), BMI ($n = 11$), diabetes mellitus ($n = 10$), alcohol consumption ($n = 9$), antihypertensive treatment ($n = 7$), hypercholesterolemia ($n = 7$), education (6), previous cardiovascular events ($n = 5$), SBP ($n = 5$), race ($n = 4$), physical exercise ($n = 3$), and other relative confounders. The results of the quality assessment based on the Newcastle–Ottawa Scale are

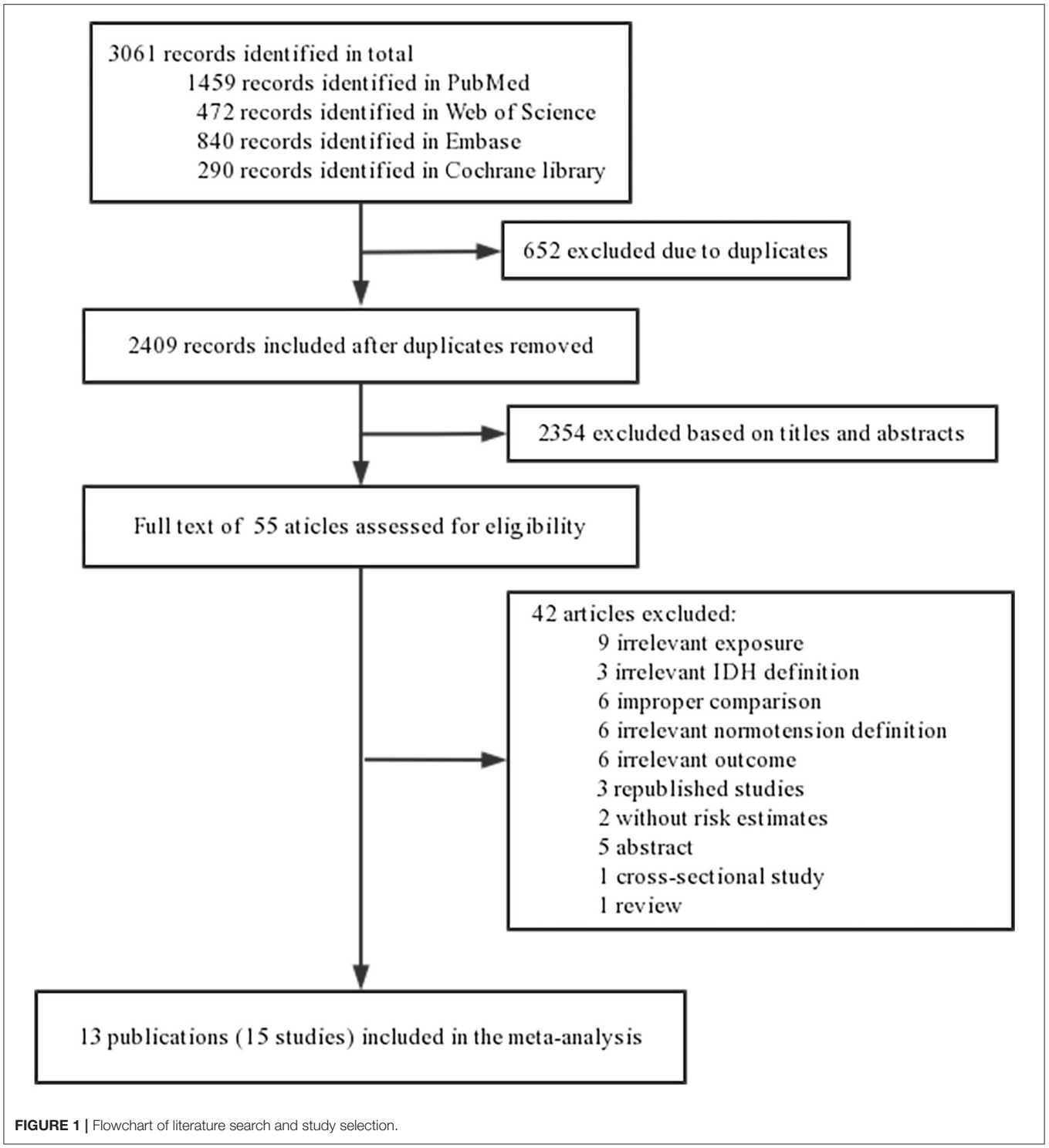

FIGURE 1 | Flowchart of literature search and study selection.

shown in **eTable 1**. The overall quality of the included studies was high, with a median score of 8 (range, 7–9).

Primary Outcomes

Composite Cardiovascular Events

Seven studies with 365,805 participants showed a significant association between IDH and composite cardiovascular events (HR 1.28, 95% CI: 1.07–1.52, $p = 0.006$) compared to normotension (**Figure 2**) with significant heterogeneity ($I^2 = 78.1\%$, $p < 0.001$). In sensitivity analyses, the summary HRs ranged from 1.17 (95% CI: 1.04–1.32) to 1.34 (95% CI: 1.12–1.60) when individual studies were excluded from the analysis (**eTable 2**). Therefore, no individual study had a significant impact on the overall results. Funnel plots did not exhibit a

TABLE 1 | Characteristics of studies included in the meta-analysis.

References	Study design (cohort)	Country	No of participants (n)	Age (years), range, mean	Male sex (%)	Follow-up duration (years)	Study outcomes
Hozawa et al. (20)	Prospective	Japan	1,492	≥40, 58.77	38.05	8.6	CV mortality
Fang et al. (21)	Prospective	China	18,787	≥35, 49.12	48.79	9.5	All strokes, IS, HS
Kelly et al. (22)	Prospective	China	128,752	≥40, 54.04	50.13	8.3	CV events, CV mortality
Barengo et al. (23)	Retrospective	Finland	13,537	25–64, 40.50	NA	16	CV mortality, all-cause mortality
Carlsson et al. (24)	Retrospective	Swedish	183	46–65	100	26	CV mortality
Carlsson et al. (24)	Retrospective	Swedish	173	46–65	0	26	CV mortality
Niiranen et al. (25)	Prospective	Finland	1,233	44–74, 53.8	41.6	11.2	CV events
Sun et al. (26)	Prospective	China	27,579	≥35, 48.25	50.73	4.3	All strokes, HS, IS
Lotfaliany et al. (27)	Prospective	Iran	5,959	30–64, 42.58	44.91	10.06	CV events, CV mortality, all-cause mortality
Lotfaliany et al. (27)	Prospective	Iran	425	≥65, 69.82	65.88	10.06	CV events, CV mortality, all-cause mortality
Hisamatsu et al. (28)	Prospective	Japan	1,474	30–49, 38.15	66	29	CV mortality
McEvoy et al. (3)	Prospective	USA	10,540	46–69, 54.54	43.24	25.2	CV events
McEvoy et al. (3)	Prospective	USA	43,097	≥20, 40	NA	9.8	CV mortality, all-cause mortality
McEvoy et al. (3)	Prospective	USA	17,687	≥20, 42	NA	28.7	CV mortality, all-cause mortality
McGrath et al. (29)	Prospective	UK	151,831	37–70, 54	40	9.8	CV events, all strokes
Jacobsen et al. (15)	Prospective	USA	5,104	45–84, 60.46	49	13	CV events, all-cause mortality
Wu et al. (4)	Prospective	China	61,961	18–98, 48.72	77	10.41	CV events, all-cause mortality, IS, HS

CV, cardiovascular; HS, hemorrhagic stroke; IS, ischemic stroke; UK, United Kingdom; USA, United States of America.

notable publication bias and no evidence of publication bias based on Egger's test ($p = 0.903$) or Begg's test ($p = 0.536$) was found (**eFigures 1A–C**).

Secondary Outcomes

Cardiovascular Mortality

The relationship between IDH and the cardiovascular mortality risk was evaluated in 8 studies with 212,779 participants. The pooled HR showed a significant association between IDH and cardiovascular mortality (HR 1.45, 95% CI: 1.07–1.95, $p = 0.015$), with moderate heterogeneity ($I^2 = 71.4\%$, $p < 0.001$) across the studies (**eFigure 2**). No evidence of publication bias was found using Egger's test ($p = 0.504$) or Begg's test ($p = 0.210$) and no asymmetry was observed in the funnel plots (**eFigure 3**).

All-Cause Mortality

Six studies with 147,770 participants were included in the meta-analysis of IDH and the risk of all-cause mortality (**eFigure 4**). However, the result showed that the association between IDH and the risk of all-cause mortality was not significant (HR 1.20; 95% CI: 0.97–1.47, $p = 0.087$), with moderate heterogeneity ($I^2 = 73.4\%$, $p = 0.001$). Visual inspection of the funnel plot indicated mild asymmetry. This was further confirmed by a significant Egger's test ($p = 0.048$), while the p-values of Begg's test were statistically non-significant ($p = 0.230$). Two missing studies were imputed in the contour-enhanced funnel plots and the application of the trim-and-fill method did not change the risk estimate (HR 1.07; 95% CI: 0.86–1.33, $p = 0.566$) (**eFigures 5A–D**).

Strokes

Four studies with 260,158 participants evaluated the association between IDH and all strokes (**eFigure 6**), demonstrating a significant association (HR 1.44, 95% CI: 1.04–2.01, $p = 0.03$). Three studies with 108,327 participants assessed the association between IDH and IS (**eFigure 7**), but not demonstrated a significant association (HR 1.56, 95% CI: 0.87–2.81, $p = 0.137$). Three studies with 108,327 participants evaluated the association between IDH and HS (**eFigure 8**), indicating a significant association (HR 1.64, 95% CI: 1.18–2.29, $p = 0.003$).

Subgroup Analyses

Subgroup analyses stratified by mean age at entry, location, treatment status at baseline, BMI, and method of BP measurement were performed. IDH was significantly associated with an increased risk of composite cardiovascular events in most subgroups (**Table 3**), except for the average age of participants ≥ 55 years (HR 1.27, 95% CI: 0.62–2.60, $I^2 = 0$, $P_{\text{heterogeneity}} = 0.362$), participants from America (HR 0.88, 95% CI: 0.62–1.24, $I^2 = 0$, $P_{\text{heterogeneity}} = 0.925$) and Europe (HR 1.48, 95% CI: 0.81–2.70, $I^2 = 80.1\%$, $P_{\text{heterogeneity}} = 0.025$). Meta-regression analysis showed significant correlations between methods of BP measurement ($p < 0.001$). The source of heterogeneity among studies on the composite cardiovascular events may be due to the methods of BP measurement. The heterogeneity disappeared in groups when BP was measured in mercury sphygmomanometers ($I^2 < 0.1\%$, $P_{\text{heterogeneity}} = 0.528$) and automatic digital BP monitor ($I^2 < 0.1\%$ $P_{\text{heterogeneity}} = 0.583$). Therefore, the source

TABLE 2 | Characteristics of included participants and outcome ascertainment.

References	Treatment status at baseline	BP measurement method	Key exclusion criteria	Outcome ascertainment	Adjusted covariates
Hozawa et al. (20)	Combined	The average of 2 readings taken by a nurse or technician with the subjects seated, after at least 2 min of rest, using a semiautomatic device	Dementia or bedridden status and out-of-town work	Defined as death from disease of the circulatory system based on ICD-10	Age, sex, smoking, obesity, antihypertensive treatment, previous CVD, hypercholesterolemia, and diabetes mellitus
Fang et al. (21)	Combined	Seated BP measured twice in the right arm with standard sphygmomanometer by clinic personnel	Previous stroke	Diagnosed by neurologists following the World Health Organization Monitoring Trends and Determinants in Cardiovascular Disease criteria	Age, BMI, smoking, drinking, and history of heart disease
Kelly et al. (22)	Untreated	The average of 2 readings taken by a trained observer with the subjects seated quietly for 5 min, using a standard mercury sphygmomanometer	Missing BP values and prevalent CVD, CHD, or stroke	Events investigated and validated using hospital records, death certificates, and interviews, and classified according to ICD-9	Age, sex, education, smoking, drinking, physical inactivity, BMI, geographic region, urbanization, and diabetes
Barengo et al. (23)	Treated	BP measured twice from the right arm of the participant in sitting position after at least a 5-min rest using a standard mercury sphygmomanometer	Previous CHD, HF, or cancer, or incomplete data at baseline	Record linkage to the nationwide death register of the Statistics of Finland coded according to ICD-10	Age, sex, region, study year, education, history of diabetes, smoking, cholesterol, BMI, and physical activity
Carlsson et al. (24)	NA	BP measured manually with the participant in a supine position after 20 min of rest	NA	Record linkage to the Swedish National Cause-of-Death Register and physician-issued certificates coded according to ICD-8/9	Age
Niiranen et al. (25)	Combined	The average of 2 office BP measured by a nurse using mercury sphygmomanometer from the sitting individual's right arm after a 10-min rest.	Previous CVD	Ascertained through linkage to the National Hospital Discharge Register and the nationwide Causes-of-Death Register coded according to ICD-10	Age, sex, smoking, antihypertensive treatment, previous CVD, hypercholesterolemia, and diabetes mellitus
Sun et al. (26)	Combined	Seated BP measured three times by a trained and certified observer using a standardized electronic sphygmomanometer after a 5-min rest.	Suffering stroke at baseline, a history of tumors, HF or pregnancy	Ascertained through home visits, hospital records, autopsy reports, death certificates coded according to ICD-9	Antihypertensive treatment, age, sex, BMI, smoking, drinking, diabetes, lipid disorder, CHD, and SBP
Lotfaliany et al. (27)	Untreated	Two measurements of BP were performed using a standardized mercury sphygmomanometer on the right arm after a 15-min rest in a sitting position.	Previous CVD	Followed up annually for any medical event and death certificate	Age, sex, smoking, diabetes status, hypercholesterolemia, low HDL, and BMI
Hisamatsu et al. (28)	Untreated	BP was measured by trained public health nurses using a standard mercury sphygmomanometer on the right arm of seated participants after at least 5 min of rest.	CVD, use of antihypertensive medications	Linkage to the National Vital Statistics database of Japan coded according to ICD-9/10	Age, sex, smoking, drinking, BMI, total cholesterol, and diabetes mellitus
McEvoy et al. (3)	Combined	BP was measured after 5 min of rest in the sitting position. We recorded BP as the mean of the last 2 of 3 measurements collected over 5-min intervals	Previous CVD	Confirmed using hospital discharge records and death certificates	Age, sex, race, education, smoking, drinking, HDL, LDL, triglycerides, eGFR, BMI, antihypertensive treatment, diabetes, SBP and pulse pressure

(Continued)

TABLE 2 | Continued

References	Treatment status at baseline	BP measurement method	Key exclusion criteria	Outcome ascertainment	Adjusted covariates
McEvoy et al. (3)	NA	NA	Missing data on relevant variables of interest or SBP	Follow-up of participants continued until any death or cardiovascular death coded according to ICD-10	Age, sex, race, smoking, drinking, BMI, total cholesterol, lipid-lowering medications, diabetes, and SBP
McEvoy et al. (3)	NA	BP was taken 3 times in the sitting position and the third reading was used for analyses.	Missing blood pressure measurements	Confirmed using the National Death Index, Maryland death certificates, local newspaper obituaries, and reports by next of kin	Age, sex, race, education, smoking, antihypertensive treatment, and SBP
McGrath et al. (29)	Combined	The average of two BP measurements were taken after 5 min in the seated position using an automatic digital BP monitor.	Systolic hypertension or baseline CVD	Linkage to national hospital records, death registrations, and primary care diagnoses	Age, sex, education, socioeconomic status, enrolment center, smoking, drinking, HDL, LDL, triglycerides, diabetes mellitus, BMI, antihypertensive treatment, eGFR, and SBP
Jacobsen et al. (15)	Combined	BP was measured three times after 5 min of seated rest using an automated oscillometric sphygmomanometer, and the mean of the last two measurements was used for analyses.	Systolic hypertension at baseline	Regular interview and self-reported diagnoses were verified using medical records and death certificates, and independently classified by two physicians	Age, sex, race, BMI, smoking, drinking, LDL, HDL, triglycerides, eGFR, antihypertensive medications, history of diabetes, and SBP
Wu et al. (4)	Untreated	The average of three BP measurements were taken, we obtained three readings at 5-min intervals after the participants had rested for at least 5 min.	Use of antihypertensive medications at baseline	Adjudicated through medical records from the Municipal, Social Insurance Institution and the Hospital Discharge Register by three experienced masked physicians	Age, sex, smoking, alcohol drinking, physical activity, education, income level, previous CVD, BMI, fasting serum glucose, TC, serum uric acid, CRP, and eGFR, and SBP

BMI, body mass index; BP, blood pressure; CHD, coronary heart disease; CRP, C-reactive protein; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; HF, heart failure; IDH, isolated diastolic hypertension; ICD, International Classification of Diseases; LDL, low-density lipoprotein; NHIS, National Health Insurance Service; SBP, systolic blood pressure; TC, total cholesterol.

of heterogeneity among studies on the composite cardiovascular events may be due to the methods of BP measurement.

DISCUSSION

To the best of our knowledge, this is the first quantitative meta-analysis investigating the associations between the 2018 ESC definition of IDH and composite cardiovascular events, cardiovascular mortality, all-cause mortality, all strokes, IS, and HS. In this meta-analysis with 15 cohort studies involving 489,814 participants, three main findings emerged. First, the pooled results indicated that IDH is associated with an increased risk of composite cardiovascular events, cardiovascular mortality, all strokes, and HS, but not for all-cause mortality and IS. Second, IDH in the younger patients (mean age $\leq$ 55 years) was associated with an increased risk of composite cardiovascular events, but not in the elderly patients (mean age $\geq$ 55 years). Third, patients with IDH in Asia were significantly associated with an increased risk of composite cardiovascular events, while Americans and Europeans were not significantly associated with an increased risk of composite cardiovascular events.

The previous meta-analysis showed that IDH defined according to the 2017 ACC/AHA criterion was not consistently associated with new-onset CVD and the relative size of any potential association was slight (15). While in our meta-analysis, we found that the 2018 ESC definition of IDH was significantly associated with an increased risk of composite cardiovascular events. The discrepancy may be due to the difference in the definitions of IDH and the age of population. As also pointed by Jacobsen et al., people with IDH were at higher risk of composite cardiovascular events only when the DBP $\geq$ 90 mm Hg (15). The Hypertension Optimal Treatment (HOT) trial supported the point that the DBP value between 80 and 90 mm Hg had no adverse prognostic clinical importance, if the SBP was within the normal range and reported that a strategy of reducing the DBP to 80 mmHg was irrelevant to significant help in reducing the end point events, compared with lowering DBP to 90 mm Hg (30). In addition, the previous meta-analysis

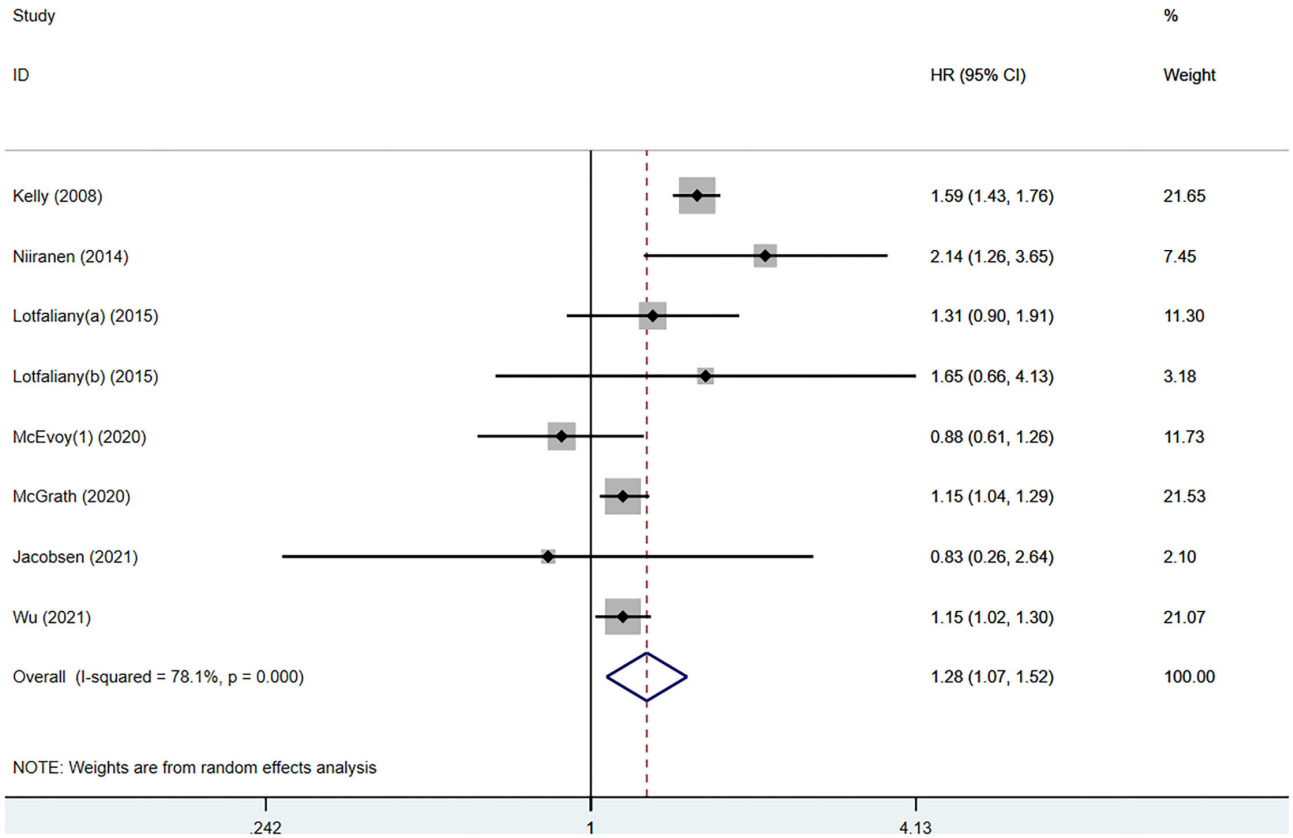

FIGURE 2 | Forest plot of hazard ratios (HRs) for the association between isolated diastolic hypertension and cardiovascular event risk.

mainly focused on middle-aged or elderly people and may not apply to adults younger than 40 years, while our meta-analysis population included the age span of 18–98 years. Clinical and observational studies have proved elevated SBP as a more powerful predictor of the adverse cardiovascular outcomes than DBP in the elderly patients (11). However, younger subjects should not be ignored, as DBP instead of SBP was associated with composite cardiovascular events (31).

Furthermore, we performed subgroup analyses stratified by age, BMI, geographic location, treatment status at baseline, and methods of BP measurement. Some significant findings were obtained. The relationship between IDH and the risk of cardiovascular events varies according to age, geographic location, and treatment status at baseline. In our subgroup analyses by location, a significant association between IDH and an increased risk of composite cardiovascular events was found in Asia, mainly in China. However, no statistically significant association was found between American and European populations. This was an interesting finding and the observed discrepancy in this study was partly related to lower awareness and treatment rate among patients with IDH in China. On account of the traditional concept that "SBP matters most" in association with cardiovascular events and hypertension mostly asymptomatic (32), the awareness and treatment rate of IDH is low. Data from the National Health and Nutrition Examination Survey (NHANES) III cohort in America demonstrated that awareness among patients with IDH (46.8%) was significantly lower than patients with isolated systolic hypertension (ISH) (58.4%) and combined systolic and diastolic hypertension (SDH) (67.2%) (33). Data from the China PEACE Million Persons Project indicated that awareness among patients with IDH was 10.3% and that 86.1% of these were untreated (6). Moreover, the observed discrepancy may also reflect the genetic susceptibility and lifestyle differences between different regions (34–37). Thus, these results provide some clues for future studies on the biological mechanism between IDH and composite cardiovascular events among different ethnic backgrounds.

Isolated diastolic hypertension results from an increase in peripheral vascular resistance and is more prevalent in young and middle-aged adults (6, 22, 38–40). Chrysant observed that IDH was associated with an adverse cardiovascular events in younger patients (32). Similarly, a significant association between IDH and an increased risk of composite cardiovascular events was found in younger people (mean age $<$ 55 years), but not in the elderly people (mean age $\geq$ 55 years). Fang et al. demonstrated that the prevalence of IDH was 8% in the 35–59 years age group and 4% in the elderly group (21). Similarly, Berney et al. confirmed that cardiovascular events are significantly related to SBP and pulse pressure in the elderly people, but are mainly related to DBP in younger people (41). Furthermore, our findings

TABLE 3 | Subgroup analyses of hazard ratios for the association between isolated diastolic hypertension (IDH) and composite cardiovascular events.

Variable	No of studies	HR (95% CI)	I^2 (%)	*P*-value for heterogeneity
Mean age (years)*				
<55	6	1.28 (1.06, 1.54)	83.9	<0.001
≥55	2	1.27 (0.62, 2.60)	0	0.362
Location				
Asia	3	1.36 (1.07, 1.73)	81.3	0.001
America	2	0.88 (0.62, 1.24)	0	0.925
Europe	2	1.48 (0.81, 2.70)	80.1	0.025
Treatment status at baseline				
untreated	3	1.36 (1.07, 1.73)	81.3	0.001
combined	4	1.18 (0.86, 1.62)	60.7	0.054
BMI (kg/m^2)				
<28	3	1.34 (1.04, 1.74)	87.4	<0.001
≥28	4	1.13 (1.02, 1.25)	0	0.415
Method of BP measurement				
Mercury sphygmomanometers	3	1.59 (1.44, 1.75)	0	0.528
Automatic digital BP monitor	2	1.15 (1.03, 1.28)	0	0.583
NA	2	1.06 (0.84, 1.35)	46.8	0.170

BMI, body mass index; BP, blood pressure; HR, hazard ratio.
**Study by Lotfaliany et al., reported HRs stratified by middle-aged and the elderly persons.*

were consistent with those of a previous study that indicated that the impact of IDH on cardiovascular events and mortality was stronger in younger adults (age < 60 years) (42). Therefore, more attention should be paid to younger patients with IDH.

Furthermore, whether IDH needs treatment is controversial and the treatment rate of IDH is low (6). In our subgroup analysis by baseline treatment status, studies including only untreated participants at baseline showed that IDH was associated with an increased risk of composite cardiovascular events, whereas studies incorporating participants with treatment at baseline did not show this association. The results of our meta-analysis indicate that active treatment of IDH is helpful in reducing the risk of long-term composite cardiovascular events. However, there is no evidence from clinical trials on the efficacy of antihypertensive medications on BP reduction and long-term cardiovascular events in IDH. Therefore, clinical trials of antihypertensive medications are warranted to determine the effects on IDH.

This systematic review and meta-analysis had a number of strengths. First, the meta-analysis included close to 500,000 participants, providing sufficient statistical power to detect associations between IDH and cardiovascular events. Second, this meta-analysis was based on several cohort studies from various populations such as Asian, American, and European, which strengthened the generalizability of the findings. Third, the inclusion of cohort studies ensured that the exposure preceded the outcome, reduced the potential selection bias, and avoided recall bias. Furthermore, all of the included studies were of high quality and sensitivity analyses, further ascertained the robustness of the results.

LIMITATIONS

This meta-analysis had several limitations. First, the definition of composite cardiovascular events was somewhat inconsistent in the included studies, which led to some bias; however, we defined the composite cardiovascular events as coronary heart disease, strokes, heart failure, and/or cardiovascular mortality; all data in this study were extracted according to the definition. Second, there was significant between-study heterogeneity as well. When performing subgroup analysis based on the measurement methods of BP for the composite cardiovascular events, the heterogeneity in the mercury sphygmomanometers group and the automatic digital BP monitor group disappeared and the results remained consistent. In consequence, the measurement methods of BP may be the main source of heterogeneity. Mercury sphygmomanometers and automatic digital BP monitors are two main methods of measuring DBP, while the mechanisms differ slightly for DBP (mercury sphygmomanometers being based on human auscultation and automatic digital BP monitors being based on algorithms that detect vibrations in the arterial wall). Previous study indicated that the automatic BP device may underestimate DBP by up to 3 mm Hg (43). Finally, although we extracted the maximum fully adjusted risk estimate, the adjusted confounders are not exactly the same in the included studies. Differential adjustment for confounders across different studies could potentially influence this study.

CONCLUSION

This meta-analysis provides evidence that IDH defined using the 2018 ESC criterion is significantly associated with an increased risk of composite cardiovascular events, cardiovascular mortality, all strokes, and HS, but not significantly associated with all-cause death and IS. Subgroup analysis further indicates that the correlation between IDH and composite cardiovascular events is significant in younger people and Asians. Therefore, further studies are needed to clarify the age-stratified associations of IDH with cardiovascular events and attach importance to the young population and Asians with IDH. Furthermore, the results of subgroup analysis stratified by treatment status at baseline indicate that active treatment of IDH is helpful in reducing the risk of long-term composite cardiovascular events. However, there is no evidence from clinical trials on the efficacy of antihypertensive medications on BP reduction and long-term cardiovascular events in IDH. In consequence, future studies are needed to assess the impacts and cost-effectiveness of non-pharmacological and pharmacological treatments of IDH for reducing the risk of cardiovascular events.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

FUNDING

This study was supported by the Youth Talent Promotion Project of China Association for Science and Technology (No.2020-QNRCI-02) and the Science and Technology Innovation Project of China Academy of Chinese Medical Sciences (NO.CI2021A05013).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2021.810105/full#supplementary-material

REFERENCES

1. Whelton PK, Carey RM, Aronow WS, Casey DE, Collins KJ, Dennison Himmelfarb C, et al. 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA guideline for the prevention, detection, evaluation, and management of high blood pressure in adults: executive summary: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *Circulation*. (2018) 138:e426–83. doi: 10.1161/cir.0000000000000597
2. Williams B, Mancia G, Spiering W, Agabiti Rosei E, Azizi M, Burnier M, et al. 2018 ESC/ESH Guidelines for the management of arterial hypertension. *Eur Heart J*. (2018) 39:3021–104. doi: 10.1093/eurheartj/ehy339
3. McEvoy J, Daya N, Rahman F, Hoogeveen R, Blumenthal R, Shah A, et al. Association of isolated diastolic hypertension as defined by the 2017 ACC/AHA blood pressure guideline with incident cardiovascular outcomes. *JAMA*. (2020) 323:329–38. doi: 10.1001/jama.2019.21402
4. Wu S, Ji C, Shi J, Chen S, Huang Z, Jonas J. Isolated diastolic hypertension as defined by the 2017 American College of Cardiology/American Heart Association blood pressure guideline and incident cardiovascular events in Chinese. *J Hyperten.* (2021) 39:519–25. doi: 10.1097/hjh.0000000000002659
5. Cho SMJ, Lee H, Kim HC. Differences in prevalence of hypertension subtypes according to the 2018 Korean Society of Hypertension and 2017 American College of Cardiology/American Heart Association guidelines: The Korean National Health and Nutrition Examination Survey, 2007-2017 (KNHANES IV-VII). *Clin Hypertens.* (2019) 25:26. doi: 10.1186/s40885-019-0129-5
6. Mahajan S, Zhang D, He S, Lu Y, Gupta A, Spatz ES, et al. Prevalence, awareness, and treatment of isolated diastolic hypertension: insights from the China PEACE million persons project. *J Am Heart Assoc.* (2019) 8:e012954. doi: 10.1161/jaha.119.012954
7. GBD 2017 Risk Factor Collaborators. Global, regional, and national comparative risk assessment of 84 behavioural, environmental and occupational, and metabolic risks or clusters of risks for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet*. (2018) 392:1923–94. doi: 10.1016/s0140-6736(18)32225-6
8. Lim SS, Vos T, Flaxman AD, Danaei G, Shibuya K, Adair-Rohani H, et al. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet*. (2012) 380:2224–60. doi: 10.1016/s0140-6736(12)61766-8
9. Yusuf S, Joseph P, Rangarajan S, Islam S, Mente A, Hystad P, et al. Modifiable risk factors, cardiovascular disease, and mortality in 155 722 individuals from 21 high-income, middle-income, and low-income countries (PURE): a prospective cohort study. *Lancet*. (2020) 395:795–808. doi: 10.1016/s0140-6736(19)32008-2
10. Forouzanfar MH, Liu P, Roth GA, Ng M, Biryukov S, Marczak L, et al. Global Burden of hypertension and systolic blood pressure of at least 110 to 115 mm Hg, 1990-2015. *JAMA*. (2017) 317:165–82. doi: 10.1001/jama.2016.19043
11. Bavishi C, Goel S, Messerli F. Isolated systolic hypertension: an update after SPRINT. *Am J Med.* (2016) 129:1251–58. doi: 10.1016/j.amjmed.2016.08.032
12. Grassi G, Seravalle G, Brambilla G, Dell'Oro R, Trevano F, Fici F, et al. Multicenter randomized double-blind comparison of nebivolol plus HCTZ and irbesartan plus HCTZ in the treatment of isolated systolic hypertension in elderly patients: results of the NEHIS Study. *Adv Ther.* (2017) 33:2173–87. doi: 10.1007/s12325-016-0427-1
13. Modesti P, Omboni S, Taddei S, Ghione S, Portaluppi F, Pozzilli P, et al. Zofenopril or irbesartan plus hydrochlorothiazide in elderly patients with isolated systolic hypertension untreated or uncontrolled by previous treatment: a double-blind, randomized study. *J Hyperten.* (2016) 34:576–87. doi: 10.1097/hjh.0000000000000805
14. Staessen JA, Gasowski J, Wang JG, Thijs L, Den Hond E, Boissel JP, et al. Risks of untreated and treated isolated systolic hypertension in the elderly: meta-analysis of outcome trials. *Lancet.* (2000) 355:865–72. doi: 10.1016/s0140-6736(99)07330-4
15. Jacobsen AP, Al Rifai M, Arps K, Whelton SP, Budoff MJ, Nasir K, et al. A cohort study and meta-analysis of isolated diastolic hypertension: searching for a threshold to guide treatment. *Eur Heart J.* (2021) 42:2119–29. doi: 10.1093/eurheartj/ehab111
16. Liberati A, Altman D, Tetzlaff J, Mulrow C, Gøtzsche P, Ioannidis J, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. *BMJ.* (2009) 339:b2700. doi: 10.1136/bmj.b2700
17. Stroup D, Berlin J, Morton S, Olkin I, Williamson G, Rennie D, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. *JAMA*. (2000) 283:2008–12. doi: 10.1001/jama.283.15.2008
18. Wells G SB, O'connell D, Peterson J, Welch V, Losos M, Tugwell P. *The Newcastle-Ottawa Scale (NOS) for Assessing the Quality of Nonrandomised Studies in Meta-Analyses*. (2021). Available online at: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp (accessed Oct 1, 2021).
19. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ.* (2003) 327:557–60. doi: 10.1136/bmj.327.7414.557
20. Hozawa A, Ohkubo T, Nagai K, Kikuya M, Matsubara M, Tsuji I, et al. Prognosis of isolated systolic and isolated diastolic hypertension as assessed by self-measurement of blood pressure at home: the Ohasama study. *Arch Intern Med.* (2000) 160:3301–6. doi: 10.1001/archinte.160.21.3301
21. Fang X, Zhang X, Yang Q, Dai X, Su F, Rao M, et al. Subtype hypertension and risk of stroke in middle-aged and older Chinese: a 10-year follow-up study. *Stroke.* (2006) 37:38–43. doi: 10.1161/01.Str.0000195005.65998.38
22. Kelly TN, Gu D, Chen J, Huang JF, Chen JC, Duan X, et al. Hypertension subtype and risk of cardiovascular disease in Chinese adults. *Circulation.* (2008) 118:1558–66. doi: 10.1161/circulationaha.107.723593
23. Barengo NC, Antikainen R, Kastarinen M, Laatikainen T, Tuomilehto J. The effects of control of systolic and diastolic hypertension on cardiovascular and all-cause mortality in a community-based population cohort. *J Hum Hypertens.* (2013) 27:693–7. doi: 10.1038/jhh.2013.22
24. Carlsson AC, Johansson SE, Theobald H, Wändell PE. Blood pressure measures and their predictive ability of cardiovascular mortality: a 26-year follow-up. *Blood Press Monit.* (2013) 18:72–7. doi: 10.1097/MBP.0b013e32835ea233
25. Niiranen T, Rissanen H, Johansson J, Jula A. Overall cardiovascular prognosis of isolated systolic hypertension, isolated diastolic hypertension and pulse pressure defined with home measurements: the Finn-home study. *J Hyperten.* (2014) 32:518–24. doi: 10.1097/hjh.0000000000000070
26. Sun Z, Han X, Zheng L, Zhang X, Li J, Hu D, et al. Subtypes of hypertension and risk of stroke in rural Chinese adults. *Am J Hyperten.* (2014) 27:193–8. doi: 10.1093/ajh/hpt197

27. Lotfaliany M, Akbarpour S, Mozafary A, Boloukat R, Azizi F, Hadaegh F. Hypertension phenotypes and incident cardiovascular disease and mortality events in a decade follow-up of a Middle East cohort. *J Hyperten.* (2015) 33:1153–61. doi: 10.1097/hjh.0000000000000540
28. Hisamatsu T, Miura K, Ohkubo T, Kadota A, Kondo K, Kita Y, et al. Isolated systolic hypertension and 29-year cardiovascular mortality risk in Japanese adults aged 30–49 years. *J Hyperten.* (2020) 38:2230–36. doi: 10.1097/hjh.0000000000002533
29. McGrath B, Kundu P, Daya N, Coresh J, Selvin E, McEvoy J, et al. Isolated diastolic hypertension in the UK Biobank: comparison of ACC/AHA and ESC/NICE Guideline Definitions. *Hypertension.* (2020) 76:699–706. doi: 10.1161/hypertensionaha.120.15286
30. Hansson L, Zanchetti A, Carruthers SG, Dahlöf B, Elmfeldt D, Julius S, et al. Effects of intensive blood-pressure lowering and low-dose aspirin in patients with hypertension: principal results of the Hypertension Optimal Treatment (HOT) randomised trial. HOT Study Group. *Lancet.* (1998) 351:1755–62. doi: 10.1016/s0140-6736(98)04311-6
31. Khattar RS, Swales JD, Dore C, Senior R, Lahiri A. Effect of aging on the prognostic significance of ambulatory systolic, diastolic, and pulse pressure in essential hypertension. *Circulation.* (2001) 104:783–9. doi: 10.1161/hc3201.094227
32. Chrysant S. The clinical significance of isolated diastolic hypertension. *Postgraduate Med.* (2020) 132:624–28. doi: 10.1080/00325481.2020.1788294
33. Franklin SS, Jacobs MJ, Wong ND, L'Italien GJ, Lapuerta P. Predominance of isolated systolic hypertension among middle-aged and elderly US hypertensives: analysis based on National Health and Nutrition Examination Survey (NHANES) III. *Hypertension.* (2001) 37:869–74. doi: 10.1161/01.hyp.37.3.869
34. Morris AA, Ko YA, Hutcheson SH, Quyyumi A. Race/Ethnic and sex differences in the association of atherosclerotic cardiovascular disease risk and healthy lifestyle behaviors. *J Am Heart Assoc.* (2018) 7:8250. doi: 10.1161/jaha.117.008250
35. Wang Y, Xing F, Liu R, Liu L, Zhu Y, Wen Y, et al. Isolated diastolic hypertension associated risk factors among Chinese in Anhui Province, China. *Int J Environ Res Public Health.* (2015) 12:4395–405. doi: 10.3390/ijerph120404395
36. Liu F, Adi D, Xie X, Li XM, Fu ZY, Shan CF, et al. Prevalence of isolated diastolic hypertension and associated risk factors among different ethnicity groups in Xinjiang, China. *PLoS ONE.* (2015) 10:e0145325. doi: 10.1371/journal.pone.0145325
37. Huang J, Wildman RP, Gu D, Muntner P, Su S, He J. Prevalence of isolated systolic and isolated diastolic hypertension subtypes in China. *Am J Hypertens.* (2004) 17:955–62. doi: 10.1016/j.amjhyper.2004.06.007
38. Anstadt G. Pulse pressure and isolated diastolic hypertension. *JAMA.* (2020) 323:2431. doi: 10.1001/jama.2020.5931
39. Fang J, Madhavan S, Cohen H, Alderman MH. Isolated diastolic hypertension. A favorable finding among young and middle-aged hypertensive subjects. *Hypertension.* (1995) 26:377–82. doi: 10.1161/01.hyp.26.3.377
40. Ekpo EB, Ashworth IN, Fernando MU, White AD, Shah IU. Prevalence of mixed hypertension, isolated systolic hypertension and isolated diastolic hypertension in the elderly population in the community. *J Hum Hypertens.* (1994) 8:39–43.
41. Berney M, Burnier M, Wuerzner G. [Isolated diastolic hypertension: do we still have to care about it?]. *Revue Med Suisse.* (2018) 14:1607–10.
42. Li F, He Y, Yang H, Liu H, Zhou R, Chen G, et al. Isolated systolic and diastolic hypertension by the 2017 American College of Cardiology/American Heart Association guidelines and risk of cardiovascular disease: a large prospective cohort study. *J Hyperten.* (2021) 391594–601. doi: 10.1097/hjh.0000000000002805
43. Ni H, Wu C, Prineas R, Shea S, Liu K, Kronmal R, et al. Comparison of Dinamap PRO-100 and mercury sphygmomanometer blood pressure measurements in a population-based study. *Am J Hypertens.* (2006) 19:353–60. doi: 10.1016/j.amjhyper.2005.10.020

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frontiers in Cardiovascular Medicine

REVIEW
published: 21 January 2022
doi: 10.3389/fcvm.2021.770975

A Mixed Comparisons of Aerobic Training With Different Volumes and Intensities of Physical Exercise in Patients With Hypertension: A Systematic Review and Network Meta-Analysis

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OPEN ACCESS

Edited by:
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Reviewed by:
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André L. Teixeira, University of Guelph, Canada

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Specialty section:
This article was submitted to Hypertension, a section of the journal Frontiers in Cardiovascular Medicine

Received: *05 September 2021*
Accepted: *16 December 2021*
Published: *21 January 2022*

Citation:
Lu Z, Song Y, Chen H, Li S, Teo E-C and Gu Y (2022) A Mixed Comparisons of Aerobic Training With Different Volumes and Intensities of Physical Exercise in Patients With Hypertension: A Systematic Review and Network Meta-Analysis. Front. Cardiovasc. Med. 8:770975. doi: 10.3389/fcvm.2021.770975

It is essential for patients with hypertension to effectively reduce and maintain appropriate blood pressure levels. As one of the non-pharmacological and invasive methods, physical exercise seems to improve blood pressure of the patients with hypertension. However, different volumes and intensities of physical exercise on the improvement of hypertension are different. To understand the effects of the type of exercise training on blood pressure and the other health status of patients with hypertension, a network meta-analysis was used to compare the mixed effects of different types of exercise training. This systematic review includes all eligible randomized controlled trials of PubMed, Medline, Cochrane Library, and CINAHL. Twelve studies met the inclusion criteria (n = 846 participants at the end of the study). The results show that a medium-intensity training (MIT) is best in improving the blood pressure of patients with hypertension, while a high-volume high-intensity interval training (HVHIIT) is better in reducing body mass and resting heart rate. In addition, the analysis of the exercise capacity shows that HVHIIT has a better effect on the improvement of patients with hypertension. Noticeably, long-term high-volume and appropriate intensity exercise can effectively improve the health status of patients with hypertension. In short, for patients with high blood pressure, MIT seems to be better at lowering blood pressure, while HVHIIT can better improve exercise ability and physical fitness. However, larger randomized controlled trials with a longer duration than those included in this meta-analysis are needed to confirm these results.

Keywords: hypertension, exercise, blood pressure, high intensity intermittent, moderate-intensity aerobic exercise, network meta-analysis

INTRODUCTION

The prevalence of hypertension is very high worldwide, and is the most common disease in primary care. It is commonly treated with chronic prescription drugs (1–4). Hypertension is a major risk factor for cardiovascular disease (5–8), and there is evidence that hypertension is a major cause of chronic kidney disease, dementia, and stroke (9–12). Obesity, unhealthy diet, lack of exercise, and

alcoholism are all possible factors leading to high blood pressure (7, 13). There is ample evidence that there is a nearly linear relationship between body mass index (BMI) and blood pressure (14–17). It is reported that obese people (BMI > 30 kg/m^2) account for more than 60% of the incidence of hypertension, and obese people are 3.5 times more likely to develop a hypertension (18, 19). Previous studies have shown that the prevalence of hypertension among adults has reached 40% (4, 20), and more than 7 million people die of high blood pressure each year (4, 14–16). A meta-analysis shows that when the systolic blood pressure (SBP) is > 115 mmHg or the diastolic blood pressure (DBP) is > 75 mmHg, the likelihood of cardiovascular events increases with the increase of blood pressure (21). For every increase in SBP (20 mmHg) or DBP (10 mmHg), the risk of fatal cardiovascular events doubled (22). Therefore, it is necessary to optimize the prevention and treatment of hypertension to reduce the morbidity and the mortality caused by related diseases.

Hypertension is either genetic or environmental factors related, or even both. Although the genetic susceptibility to hypertension cannot be changed, improving the lifestyle can significantly reduce the disease risk or improve hypertension (7). There is some controversy on the therapeutic effect of physical exercise (PE) as means in benefitting the health and improve chronic diseases (13, 23). As a non-drug treatment (24, 25), it has been proven by many studies that PE has a positive impact on the improvement of hypertension (4, 13, 23, 26–31). There is much evidence that both aerobic training and resistance training can improve systolic and diastolic blood pressure, and aerobic training seems to be superior to resistance training in reducing blood pressure (23, 32–34). However, some studies have shown that there may be differences between these two pieces of training (23). A review shows that aerobic and resistance training may have the same effect on blood pressure, but the potential physiological mechanisms are different (13). The effects of different intensities of exercise on people with hypertension may be different. Epidemiology shows that higher intensity of exercise can lower blood pressure (35), while too much high intensity of exercise may have adverse effects on the body (36). The different effects of exercise due to different capacities, types, frequencies, and times need further study.

In a recent meta-analysis (3), the effects of high-intensity interval training (HIIT) and medium-intensity training (MIT) on patients with hypertension were compared. The results showed that HIIT and MIT decreased SBP in patients with hypertension, and there was no significant difference between the two interventions in reducing SBP. The decrease of DBP was more significant in the HIIT group. In addition, the study also found that compared with the MIT group, the HIIT group also promoted the improvement of maximum oxygen uptake. It is worth noting that there were significant differences in the exercise capacity of the HIIT group included in the study in this meta-analysis. A review by Whitaker et al included 142 subjects in 7 studies (37). The results showed that compared with MIT, HIIT decreased the arterial blood flow velocity and response to CO_2, and it also decreased the dynamic self-regulation phase, while significantly increased the deoxyhemoglobin compared with rest. However, the subjects included in this review are all healthy people, and the evidence for hypertensive people is still not very rich. Some studies have found that the high-volume HIIT seems to be more beneficial to weight loss, but there seems to be no difference in lowering blood pressure among 46 HIIT with different volumes (38, 39). However, although several studies have analyzed the effects of different volumes of HIIT on patients with hypertension, most studies have not clearly shown which volume and which type of exercise can minimize the blood pressure. Accordingly, we assume that different volumes and intensities of physical exercise may affect the blood pressure and health status of people with hypertension, and there is an optimal intervention plan. This review will study the effects of different volumes of HIIT, MIT, general physical activity, and blank on people with hypertension.

Human blood pressure is affected by various stimuli such as breathing, temperature, body posture, emotion, or physical stress (40, 41). Studies have shown that blood pressure drops slowly and eventually slows down within 16 min of sitting in a chair before blood pressure measurements are taken, and 75% of the blood pressure drops occur in the first 10 min (42). In addition, heart rate (HR) is closely related to blood pressure. For patients with hypertension, the increase of HR further increases the risk of adverse outcomes. There is evidence that HR is an independent risk factor for cardiovascular disease morbidity and for the overall mortality in patients with hypertension (43). In this review, resting systolic blood pressure, resting diastolic blood pressure, and resting HR were selected as the main indicators to evaluate the condition of patients with hypertension, and BMI was selected as the simple and commonly used index to evaluate the degree of obesity.

To better understand the effect of exercise on the hypertension population, this review aims to make indirect and mixed comparisons of the interventions on rest SBP, rest DBP, rest HR, and BMI in patients with hypertension using a network meta-analysis method. So far, no studies have adjusted and mixed comparisons. Hence, we implemented the adjusted and mixed comparisons with network meta-analysis to provide better advice for the hypertension population.

METHOD

This review was conducted based on the Preferred Reporting Items for Systematic Reviews and Meta-Analysis guidelines (PRISMA). Literature collection, exclusion criteria, and retrieval strategies are jointly proposed and agreed upon by two authors and established a priori to minimize bias.

Data Acquisition

The study included randomized design studies published before August 2021. This study was reviewed by another peer. The population, interventions, comparisons, and outcomes of this review were as follows:

Participants/Population

The subjects in the study included were adults with high blood pressure. According to the hypertension guidelines of the American Heart Association, an SBP value between 130 and

139 mmHg and/or a DBP value between 80 and 89 mmHg is considered to be stage 1 arterial hypertension 43. In this review, 12 studies were included, with a total of 846 subjects aged between 31.8 and 78 years old.

Intervention(s)

The exercise interventions in this review include high-volume high-intensity interval training (HVHIIT), low-volume high-intensity interval training (LVHIIT), medium-intensity training (MIT), general physical activity, and blank. This review excluded studies that had only one intervention.

According to the classification of previous studies, in this review, the criteria for the variety of LVHIIT are as follows: (1) Exercise in which the heart rate is ≥80% maximum heart rate (max HR), and the total duration is not more than 30 min; (2) Exercise in which the oxygen uptake is ≥80% maximal oxygen consumption (Max VO2), and the total duration is not more than 30 min; (3) Exercise in which the power is ≥80% peak power, and the total duration does not exceed 30 min; and (4) The authors of the study classified it as LVHIIT. The high-volume high-intensity interval training (HVHIIT) classification criteria are as follows: (1) Exercise in which the heart rate is ≥80% maximum heart rate (max HR), and the total duration is more than 30 min; (2) Exercise in which the oxygen uptake is ≥80% maximum VO2, and the total duration is more than 30 min; (3) Exercise in which the power is ≥80% peak power, and the total duration is more than 30 min; and (4) The authors of the study classified it as HVHIIT. The medium intensity training (MIT) classification criteria are as follows: (1) Exercise with an average HR between 55 and 80% max HR; (2) Oxygen uptake during exercise is between 55 and 80% maximum VO2; (3) Exercise with an average power of 50–60% peak power; and (4) The authors of the study specified it as MIT.

In addition, among the 12 studies included, 9 used treadmills or power bicycles to test the maximum heart rate, maximum oxygen uptake, or maximum power (38, 39, 44–48) of the subjects, and 1 study used sub-maximum oxygen uptake to estimate maximum oxygen uptake (49), while the other two did not elaborate on it (50, 51).

Comparator(s)/Control

The indirect comparisons of the above interventions are feasible because the network meta-analysis is based upon the theorem of Bayes (51). The comparator(s)/control criteria were the same as the intervention(s) criteria.

Outcomes

The outcome indicators of this review are SBP, DBP, BMI, and rest HR.

Other indicators included in this review were too rare or had different detection methods to perform a reticular meta-analysis, so they were treated as secondary indicators for supplementary analysis. These indicators included the following: time to exhaustion, ventilatory thresholds, body fat, Max VO2, total cholesterol, max HR, and mean arterial pressure.

Information Sources

This review uses PubMed, Medline, Cochrane Library, and CINAHL to conduct a comprehensive and repeatable literature search before August 2021. If the data is insufficient, the author can be contacted to provide the exact data.

Search

(1) In PubMed, the search term was "(hypertension [Title/Abstract]) OR (blood pressure [Title/Abstract]) AND (HIIT [Title/Abstract]) OR (high intensity interval training [Title/Abstract]), OR (high intensity interval [Title/Abstract]), AND (randomized [Title/Abstract]) OR [(randomized [Title/Abstract])."

(2) In Medline, Cochrane Library, and CINAHL, the search term was "(hypertension OR blood pressure TI) OR (hypertension OR blood pressure AB), AND (HIIT OR high-intensity interval training OR high-intensity interval TI), AND (randomized OR randomized AB)."

The selection of the title, abstract, and full text is jointly completed by two independent authors. The differences will be judged by a third independent arbitrator.

Study Selection

The process of screening the abstract and the text is done by two independent authors. When no opinion can be reached, the disagreement will be judged by a third independent arbitrator.

Studies would be excluded if they meet the following conditions: (1) Studies with healthy subjects or minors; (2) Studies which only performed one-time exercise; (3) Studies using invasive interventions such as surgery and injections; and (4) Studies in which specific data of outcome indicators are not provided, or where the authors do not receive timely answers.

Data Collection Process

All potential studies were downloaded and imported into Endnote X9 (Thomson Reuters, Carlsbad, California, USA), and the duplicated tasks were deleted. The data collection is done by two independent authors. When the opinions cannot be reached, the third independent arbitrator will judge the differences. The information included demographic characteristics (average age and gender), clinical characteristics (Body mass index), details of experimental design (sample size, intervention method, and follow-up time), and outcome indicators.

Data Items

The funders of this study did not contribute to design or implementation. Therefore, the author is fully responsible for data collection, analysis, interpretation, and reporting. Corresponding authors have access to all data and are ultimately responsible for the submission of publications.

Risk of Bias in Assessment

Two evaluators evaluate the risk of bias using the Cochrane Collaboration Risk of Bias Assessment Tool. When no agreement can be reached, the disagreement will be judged by a third independent arbitrator.

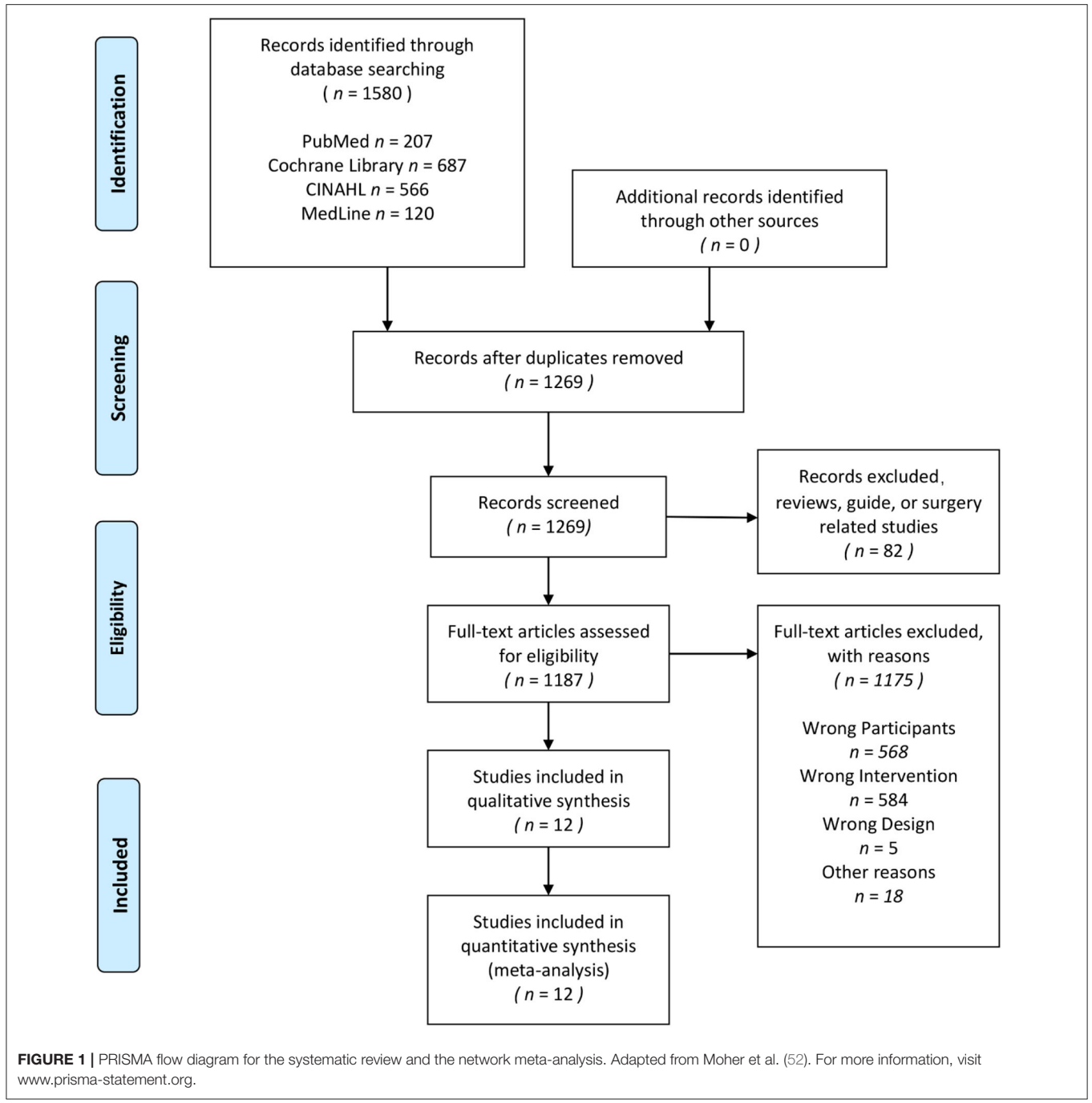

FIGURE 1 | PRISMA flow diagram for the systematic review and the network meta-analysis. Adapted from Moher et al. (52). For more information, visit www.prisma-statement.org.

Summary Measures

The data preprocessing and analysis were made by two independent investigators. Microsoft Excel (Version 16.0, Microsoft Corporation, Redmond, WA, USA) was used to pre-process the original data and convert the results into average and standard deviation (Mean ± SD).

The processed data are analyzed by the Aggregate Data Drug Information System (ADDIS V1.16.8 produced by Drugis.org, http://drugis.org/software/addis/index), calculated the effect size, the data are aggregated into the network meta-analysis, and all the graphs and results were the output. The results of the network meta-analysis are introduced in the following parts.

RESULTS

Search Strategy and Information Extraction

A total of 1,580 studies were searched for screening through the electronic search of four scientific databases of which 310 repetitive studies were deleted; after filtering by title and abstract, additional 1,258 articles were excluded. Finally, 12 studies with

all subjects age between 18 and 78; and all these subjects included in the analysis were patients that were hypertensive, with an SBP value of more than 130 mmHg and/or a DBP value of more than 80 mmHg.

Figure 1 and **Table 1** show the details of the article filtering process and the information of all included studies, respectively.

Risk of Bias

The risk of bias in the 12 included studies was assessed, and the consensus was reached after discussion. The overall result is shown in **Figure 2**. Randomization and concealment methods of the participants were well-reported in all studies. In percentage, 66.7% of studies did not adequately describe participant or staff blinding, and 25% of the studies made it clear that there was no double blindness. Proportionately, 58.3% of the studies did not describe whether the evaluator was blind. Two studies had incomplete results due to subjects dropping out. All the studies recorded their research plan and researched according to the program.

Network Meta-Analysis

Figures 3, **4** show the overall network structure of intervention for systolic blood pressure (SBP), diastolic blood pressure (DBP), body mass index (BMI), and rest heart rate (RHR) with mixed intervention comparison of various training activities, and their respective ranking of intervention probability by ranking 1 as the worst, and ranking 5 as the best, respectively.

Systolic Blood Pressure

Figure 3A shows the geometry of the SBP motion intervention network. It shows a mixed intervention comparison that developed from the traditional Meta-analysis. It expanded from the standard double-arm test Meta-analysis to a series of different treatment factors to analyze, compare each other and synthesize simultaneously. The mixed intervention comparison includes direct comparison and indirect comparison. As the evidence is a closed loop, the inconsistency of the evidence was evaluated.

In the mixed comparison of HVHIIT, LVHIIT, MIT, blank, and general physical activity, the random effect standard deviation of the consistency model showed 95% confidence interval of 3.18 (1.52, 6.39), while the random effect standard deviation of the inconsistency model showed 95% confidence interval of 3.22 (1.51, 6.51). The standard inconsistency deviation of the inconsistency model with 95% confidence interval was 5.90 (0.30, 11.61). There were no significant differences in the standard deviations of random effects between the congruent and incongruent models, suggesting there is no consistency difference. Hence, the consistency model should be used. **Table 2** shows the interventions for systolic blood pressure (SBP) based on the network geometry of **Figure 3A**.

From **Figure 4**, the results show the lower the SBP, the better the situation. So, for patients with hypertension, the best to worst means of reducing SBP are MIT, LVHIIT, HVHIIT, general physical activity (GA), and blank.

Diastolic Blood Pressure

Figure 3B shows the geometry of the DBP motion intervention network with a mixed intervention comparison. The mixed intervention comparison includes direct comparison and indirect comparison. Similarly, as the evidence is a closed loop, the inconsistency of the evidence should be evaluated.

In the mixed comparison of HVHIIT, LVHIIT, MIT, blank, and general physical activity, the random effect standard deviation of the consistency model showed 95% confidence interval of 1.54 (0.17, 3.99), while the random effect standard deviation of the inconsistency model showed 95% confidence interval of 1.55 (0.28, 3.99). The standard inconsistency deviation of the inconsistency model with 95% confidence interval was 3.39 (0.16, 6.60). There were no significant differences in the standard deviations of random effects between the congruent and incongruent models, hence, the consistency model was adopted. **Table 3** shows the intervention for systolic blood pressure (DBP) based on is a sorted table of the network geometry of **Figure 3B**.

Figure 4 shows the ranking of measurements and of probabilities. Noting that the lower the DBP in this study, the better the situation, thus, the best-to-worst ways to reduce DBP for patients with hypertension are MIT, HVHIIT, LVHIIT, general physical activity (GA), and blank.

Body Mass Index

Similarly, **Figure 3C** shows the geometry of the BMI motion intervention network with a mixed intervention comparison. The mixed intervention comparison includes direct comparison and indirect comparison. As there is evidence of a closed loop, the inconsistency of the evidence was evaluated.

In the mixed comparison of HVHIIT, LVHIIT, MIT, blank, and general physical activity, the random effect standard deviation of the consistency model showed a 95% confidence interval of 0.72 (0.02, 1.82), the random effect standard deviation of the inconsistency model showed 95% confidence interval of 0.71 (0.04, 1.82). The standard inconsistency deviation of the inconsistency model with 95% confidence interval was 0.96 (0.05, 1.88). There were no significant differences in the standard deviations of random effects between the congruent and incongruent models, indicating no consistency difference, and the consistency model should be adopted. **Table 4** showed the interventions for BMI based on the network geometry of **Figure 3C**.

Figure 4 shows the ranking of measurements and of probabilities. Noting that the lower the BMI in this study, the better the situation, thus, the best-to-worst ways to reduce BMI for patients with hypertension is HVHIIT, GA, LVHIIT, MIT, and blank. It is important to note that LVHIIT and MIT are very close in reducing the effectiveness of BMI.

Rest Heart Rate

Figure 3D shows the geometry of the rest HR motion intervention network with a mixed intervention comparison. As there is evidence of a closed loop for direct comparison and indirect comparison, the inconsistency of the evidence was again evaluated.

TABLE 1 | The study characteristics of included studies.

References	Title	Subjects		Intervention			Index
		Average age (n-n years)	Male/ Female	N	Type of training	Training frequency	Content
Dalal et al. (43)	Short-duration high-intensity interval exercise training is more effective than long duration for blood pressure and arterial stiffness but not for inflammatory markers and lipid profiles in patients with stage 1 hypertension	48.0 (43.0–53.8)	30/0	10	LVHIIT	3 times/week, 8 weeks	80%VO2max 27 × 30 s	SBP (↓), DBP (↓)
				10	LVHIIT		85%VO2max 4 × 4 min	SBP (↓), DBP (↓)
				10	Blank		Blank	SBP (⟷), DBP (⟷)
Bahmanbeglou et al. (44)	The benefits of high-intensity interval training on cognition and blood pressure in older adults with hypertension and subjective cognitive decline: results from the heart & mind study	71.1 (63.3–78.0)	67/61	65	LVHIIT	3 times/week, 24 weeks	85–95% HRmax 25 min	SBP (↓), Time to exhaustion (↑)
				63	MIT		60–80% HRmax 25 min	SBP (↓), Time to exhaustion (↑)
Lins-Filho et al. (49)	Effects of interval training on blood pressure and endothelial function in hypertensive patients	51.3 (40.9–61.1)	8/6	7	LVHIIT	5 times/week, 4 weeks	80% HRmax 5 × 3 min	SBP (↓), DBP (↓), BMI (⟷), Rest HR (↓)
				7	MIT		60% HRmax 35 min	SBP (↓), DBP (⟷), BMI (⟷), Rest HR (⟷)
Whitaker et al. (37)	Effects of different aerobic exercise programs with nutritional intervention in sedentary adults with overweight/obesity and hypertension: EXERDIET-HTA study	54.0 (44.4–63.5)	120/55	40	MIT	2 times/week, 16 weeks	65% VO2max 45 min	SBP (↓), DBP (↓), BMI (↓), Rest HR (↓), MBP (↓), Anaerobic thresholds (⟷)
				42	HVHIIT		95% VO2max 45 min	SBP (↓), DBP (↓), BMI (↓), Rest HR (↓), MBP (↓), Anaerobic thresholds (↑)
				41	LVHIIT		90% VO2max 20 min	SBP (↓), DBP (↓), BMI (↓), Rest HR (↓), MBP (↓), Anaerobic thresholds (↑)
				40	General physical activity		General physical activity	SBP (↓), DBP (↓), BMI (↓), Rest HR (↓), MBP (↓), Anaerobic thresholds (⟷)
Boa Sorte Silva et al. (45)	High-intensity interval training lowers blood pressure and improves apelin and NOx plasma levels in older treated hypertensive individuals	61.7 (51.1–69.3)	23/19	Unknown	LVHIIT	3 times/week, 6 weeks	85–90% HRmax 35 min	SBP (↓), DBP (↓), Rest HR (⟷), Max VO2 (↑), Time to exhaustion (↑)

(Continued)

TABLE 1 | Continued

References	Title	Subjects		Intervention				Index
		Average age (n-n years)	Male/ Female	N	Type of training	Training frequency	Content	
				Unknown	Blank		blank	SBP (⟷), DBP (⟷), Rest HR (⟷), Max VO2 (⟷), Time to exhaustion (⟷)
Izadi et al. (46)	Effects of high-intensity interval training vs. moderate-intensity continuous training on epicardial fat thickness and endothelial function in hypertensive metabolic syndrome	50.9 (42.6–60.3)	18/16	17	LVHIIT	3 times/week, 8 weeks	85% HRmax 5 × 3 min	SBP (↓), DBP (↓), BMI (↓), Rest HR (↓), Total cholesterol (↓)
				17	MIT		60 HRmax 35 min	SBP (↓), DBP (↓), BMI (↓), Rest HR (⟷), Total cholesterol (⟷)
Sosner et al. (48)	Affective responses to different prescriptions of high-intensity interval exercise in hypertensive patients	65.3 (61.1–69.5)	0/20	Unknown	LVHIIT	8 times	80–85% VO2max 5 × 2 min	SBP (⟷), DBP (⟷), Rest HR (⟷)
				Unknown	Blank		blank	SBP (⟷), DBP (⟷), Rest HR (↓)
Gorostegi-Anduaga et al. (38)	Effects of different aerobic exercise programs on cardiac autonomic modulation and hemodynamics in hypertension: data from EXERDIET-HTA randomized trial	53.7 (31.8–61.7)	158/91	61	HVHIIT	16 weeks	45 min	SBP (↓), DBP (↓), Rest HR (↓), Max VO2 (↑)
				62	LVHIIT		20 min	SBP (↓), DBP (↓), Rest HR (↓), Max VO2 (↑)
				60	MIT		45 min	SBP (↓), DBP (↓), Rest HR (↓), Max VO2 (↑)
				59	General physical activity		General physical activity	SBP (↓), DBP (↓), Rest HR (↓), Max VO2 (⟷)
Soltani et al. (53)	Effects of antihypertensive medication and high-intensity interval training in hypertensive metabolic syndrome individuals	58.7 (53.2–64.2)	Unknown	Unknown	LVHIIT (Take Placebo)	3 times/week, 16 weeks	90% HRmax 4 × 4 min/5 × 5 min (Take Placebo)	SBP (⟷), DBP (⟷), Rest HR (⟷), Mean arterial pressure (⟷)
				Unknown	LVHIIT (Take antihypertensive drug)		90% HRmax 4 × 4 min/5 × 5 min (Take antihypertensive drug)	SBP (⟷), DBP (⟷), Rest HR (↓), Mean arterial pressure (⟷)
Taha et al. (51)	High-intensity interval training irrespective of its intensity improves markers of blood fluidity in hypertensive patients	48.0 (43.0–53.8)	30/0	10	LVHIIT	3 times/week, 8 weeks	80–100%VO2max 2 × 30 s	SBP (↓), MAP (⟷), BMI (⟷)
				10	LVHIIT		75–90%VO2max 4 × 4 min	SBP (↓), MAP (↓), BMI (⟷)

(Continued)

TABLE 1 | Continued

References	Title	Subjects		Intervention				Index
		Average age (n-n years)	Male/ Female	N	Type of training	Training frequency	Content	
				10	Blank		Blank	SBP (⟷), MAP (⟷), BMI (⟷)
Jo et al. (47)	Ambulatory blood pressure reduction following 2 weeks of high-intensity interval training on an immersed ergo cycle	65.0 (54.0–72.0)	22/20	Unknown	LVHIIT	3 times/week, 2 weeks	100% Peak Power 2 × 15 s (Dryland)	SBP (⟷), BMI (⟷), Rest HR (⟷)
				Unknown	LVHIIT		100% Peak Power 2 × 15 s (Immersed)	SBP (↓), BMI (↓), Rest HR (↓)
				Unknown	MIT		50%PeakPower	SBP (⟷), BMI (⟷), Rest HR (⟷)
Jo et al. (50)	Effect of high-intensity interval training on endothelial function in postmenopausal hypertensive patients: a randomized controlled trial	48.0 (45.2–50.4)	0/46	23	LVHIIT	3 times/week, 10 weeks	80–85% HRmax 4 × 4 min	SBP (↓), DBP (↓), BMI (⟷)
				23	Blank		Blank	SBP (⟷), DBP (⟷), BMI (⟷)

Blank, Blank control; LVHIIT, low-volume high-intensity interval training; HVHIIT, high-volume high-intensity interval training; MIT, Medium intensity training; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; HR, heart rate; Max VO2, maximal oxygen consumption; ↑, Significantly rise; ↓, Significantly decrease; ⟷, No statistically significant change.

In the mixed comparison of HVHIIT, LVHIIT, MIT, blank, and GA, the random effect standard deviation of the consistency model showed 95% confidence interval of 1.17 (0.11, 4.75), the random effect standard deviation of the inconsistency model showed 95% confidence interval of 1.13 (0.09, 4.80). The standard inconsistency deviation of the inconsistency model with 95% confidence interval was 4.07 (0.20, 7.91). There were no significant differences in the standard deviations of random effects between the congruent and incongruent models, indicating no consistency difference, hence, the consistency model was used. **Table 5** showed the interventions for HR based on the network geometry of **Figure 3D**.

In the ranking of measurements and probabilities, as shown in **Figure 4**, with the lower rest HR as the better situation, the results show that for patients with hypertension, HVHIIT, GA, LVHIIT, MIT, and blank are the means to reduce the rest HR from the best to the worst.

DISCUSSION

In this review, the network meta-analysis method is used to mix and indirectly compare HIIT with different volumes and other types of sports. This study aimed to determine the effects of different volumes and types of exercise on the decrease of blood pressure and other health conditions in people with hypertension. According to the exercise intervention classification, 12 randomized controlled trials of five different interventions: HVHIIT, LVHIIT, MIT, general physical activity (GA), and blank were reviewed. All the subjects studied were people with hypertensive and with SBP >130 mmHg and/or DBP >80 mmHg.

All of the 12 studies reported on SBP and 11 of them showed that SBP levels decreased significantly compared with the baseline, after a period of exercise training ($p < 0.05$) (38, 39, 44–51, 53), only 1 study showed no significant change in SBP levels after exercise ($p > 0.05$) (54). Of the 9 studies reported on DBP (38, 39, 44, 46, 47, 49–51, 54), only 1 study showed no significant change in DBP after exercise ($p > 0.05$) (49), and the other 8 studies showed DBP decreased significantly after the exercise. Subsequently, 6 studies reported on BMI (38, 47, 48, 50, 51, 53), and only 2 studies showed a significant decrease in BMI after exercise (38, 47, 48). Based on 8 studies reported on rest HR (38, 39, 46–50, 54), 6 studies showed rest HR decreased after exercise (38, 39, 47, 48, 50, 54), and 2 studies showed rest HR did not change significantly after exercise (46, 49). However, in the study by Lins-Filho et al., rest HR decreased significantly in the blank control group (49).

The review shows that proper physical exercise is beneficial to hypertension patients with stable health. In the 12 studies included, almost all the different volumes and types of training positively impacted the health status of patients with hypertension, and no adverse effects of exercise as an intervention were reported. And some studies have shown the exercise ability of the subject improved to a certain extent after receiving exercise intervention for a period of time (38, 45, 46).

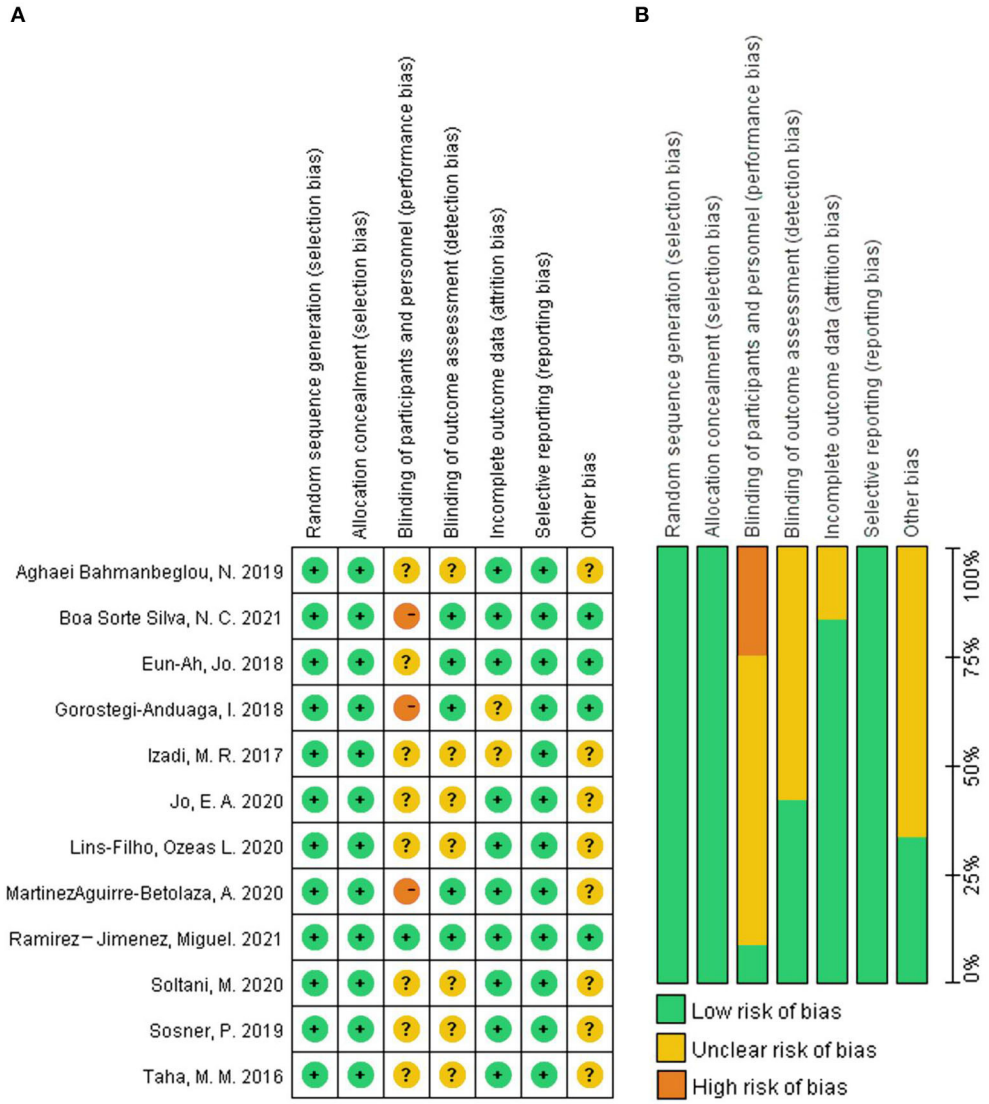

FIGURE 2 | The result of the risk of bias assessment. **(A)** Risk of bias graph; **(B)** Risk of bis summary.

According to our analysis, MIT is the best in reducing SBP, while the blank is the worst, suggesting exercise training can improve SBP in people with hypertension, which is agreed with the results of many previous studies (55–57). The effect of high-volume HIIT or low-volume HIIT on reducing SBP seems to be less than that of MIT, which may be related to the mechanism of lowering blood pressure on these two types of exercise. The antihypertensive effect of HIIT may be caused by multiple factors, such as the decrease of cardiac output, heart rate, and vascular resistance (55).

For DBP, MIT is still the best, and blank is the worst in reducing DBP. The effect of HIIT with different capacities on reducing DBP is similar, and it is not as good as MIT. In reducing BMI, the effect of HVHIIT is the best, and the list is blank.

In terms of reducing rest HR, HVHIIT is the best, and blank is the worst. A study by Lins-Filho et al. (49), reported that SBP did not change significantly in HIIT and blank groups, but with a slight increase in SBP when the measurement time was taken 60 min after exercise. The transient increase in blood pressure from the exercise did not fully recover after rest. In Ramirez-Jimenez et al. (54) investigating of both groups of subjects received LVHIIT intervention, with a group taking antihypertensive drugs and the other group receiving placebo, aiming to achieve an additional drop in blood pressure. However, the results showed only the placebo group had a significant decrease in resting heart rate ($p < 0.05$), and no statistically significant difference was found in other indicators. We hypothesize that MIT seems to have a significant advantage

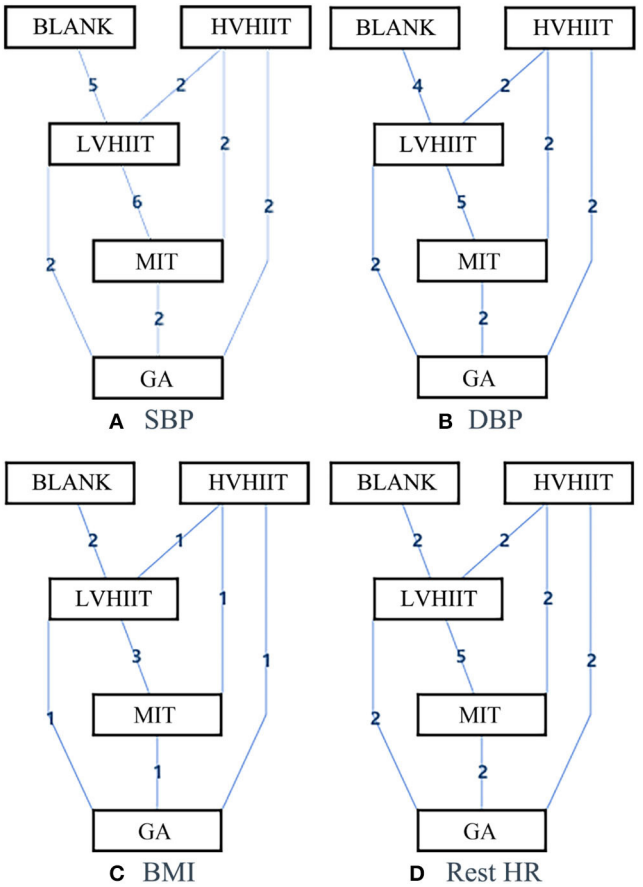

FIGURE 3 | The network structure of the intervention of systolic blood pressure (SBP), diastolic blood pressure (DBP), body mass index (BMI), and rest heart rate (HR). Blank, Blank control; LVHIIT, low-volume high-intensity interval training; HVHIIT, high-volume high-intensity interval training; MIT, Medium intensity training; GA, general physical activity.

in reducing blood pressure, while HVHIIT is effective in reducing BMI and rest HR.

Of the 12 studies included, only Martinez Aguirre-Betolaza et al. gave follow-up results (39). In this study, long-term follow-up showed that the daytime DBP of the exercise group was lower than that of the subjects with general physical activity, and the blood pressure variability (quantified as 24 h, day, and night average SD) of the exercise intervention subjects was <16 mmHg, while the nocturnal blood pressure variability of the subjects with general physical activity was greater than that of 16 mmHg. Hence, exercise intervention seems to reduce the occurrence of cardiovascular risk events.

Though there is evidence showing that exercise has a positive effect on blood pressure and health in people with high blood pressure, excessive strenuous exercise in the short term may increase the risk (36). Therefore, it is necessary to recommend appropriate exercise according to the severity of hypertension. In addition, the effects of different types and intensity of exercise on different subjects may be different. A study by Danielle et al., on the effects of age and gender on resting blood pressure changes caused by grip strength training, showed older women had the most significant decrease in SBP (58); patients who are obese and hypertensive showed improved insulin resistance during exercise-induced fat loss (59); exercise as an auxiliary means of lowering blood pressure combined with taking antihypertensive drugs seemed to achieve better blood pressure control (60). In the following sections, we further explore and discuss these various effects.

Age

It is evident that exercise improves blood pressure, but the effect may vary according to age. Compared with young patients with hypertension, elderly and frail patients are more likely to develop age-related diseases, and their adverse side effects and adverse results are often more worrying (61).

According to the definition of age defined by the United Nations World Health Organization, of the 12 studies evaluated, 8 studies were young hypertensive patients aged 31.8–63.5 years old (38, 39, 44, 47, 50, 51, 53, 54), and 7 of these studies showed that after a period of physical exercise, the blood pressure and/or other health conditions of the subjects improved significantly ($p < 0.05$). However, there was no statistically significant change in SBP and DBP after receiving HIIT exercise intervention (54). All the subjects who participated in HIIT exercise intervention were patients with metabolic syndrome in addition to high blood pressure, with one group of subjects also receiving antihypertensive drugs during the intervention, while the other group was receiving a placebo (54). Therefore, the insignificant change in blood pressure may be due to the heterogeneity of different diseases or the side effects of drug use.

In 3 of the four studies in subjects aged from 51.08 to 78 years old, their blood pressure and/or health status improved significantly after a period of physical exercise (45, 46, 48), and only one study had no statistically significant change in blood pressure after receiving HIIT intervention (49). For the study with subjects who received only eight HIIT interventions, the blood pressure was measured 60 min after each intervention, the insignificant change may be due to the short cycle of the intervention, whereby the blood pressure did not return to the resting level after exercise.

In the 12 studies that were analyzed, there was no difference in the improvement of blood pressure by exercise in different age groups of adult patients with hypertension. However, none of these studies are directly age-related, it is impossible to make a more accurate analysis of this topic. Further related studies needed to be carried out.

Gender

Of the 12 studies included, only 4 included patients with hypertension of a single sex (44, 49, 51, 53). In the Bahmanbeglou et al. (44) and Soltani et al. (53) study, a total of 60 male patients with hypertension received HVHIIT, LVHIIT, and blank control intervention, respectively, significant improvement in blood pressure was observed in 40 subjects who received exercise intervention. In the Taha MM study (51), 46 female patients with hypertension received LVHIIT and blank control intervention,

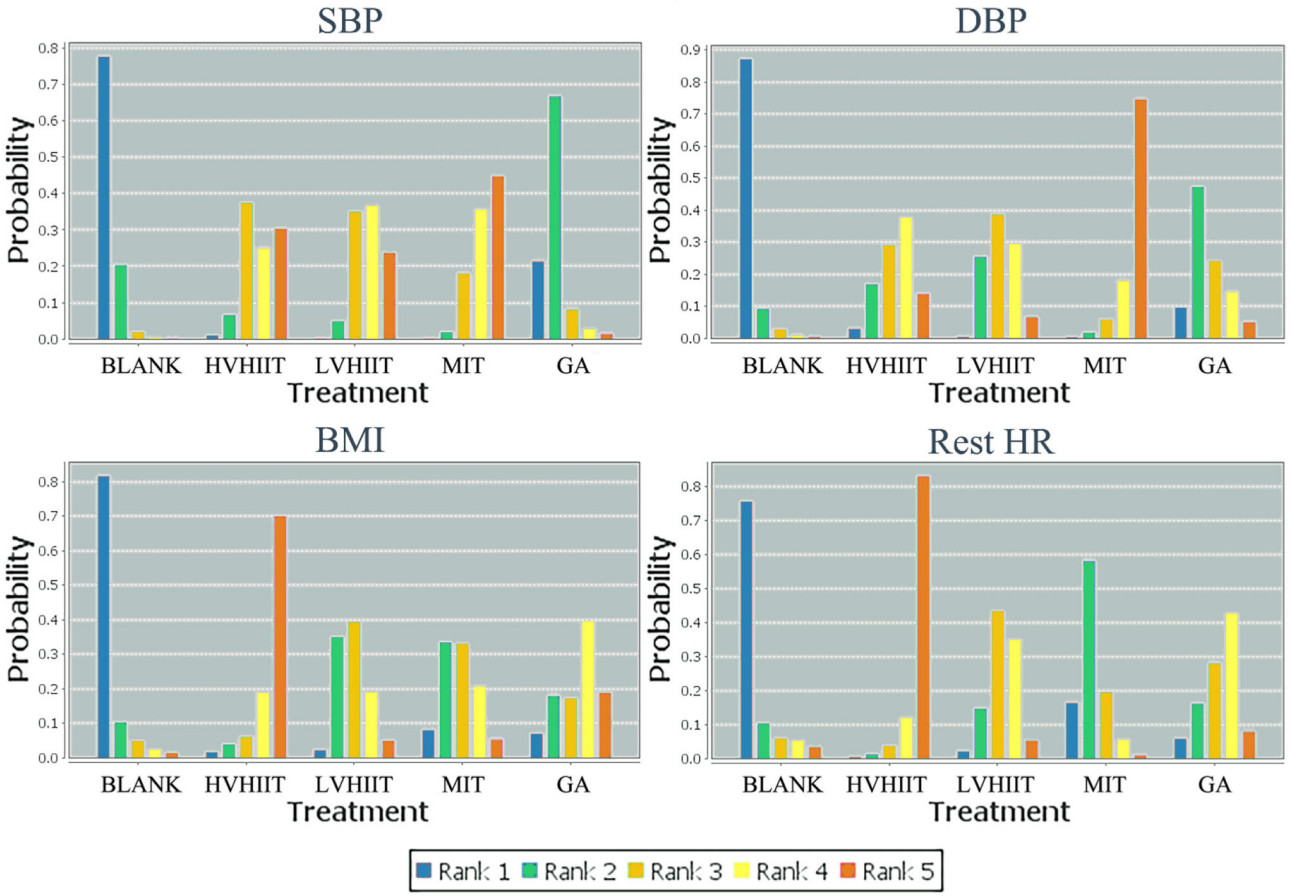

FIGURE 4 | Measurement of systolic blood pressure (SBP), diastolic blood pressure (DBP), body mass index (BMI), rest heart rate (HR), and the ranking of intervention probability. Blank, Blank control; LVHIIT, low-volume high-intensity interval training; HVHIIT, high-volume high-intensity interval training; MIT, Medium intensity training; GA, general physical activity.

TABLE 2 | The league table of the interventions for systolic blood pressure (SBP).

Blank				
7.21 (0.14, 14.41)	HVHIIT			
7.27 (2.51, 11.53)	0.04 (−5.88, 5.26)	LVHIIT		
7.96 (2.25, 14.09)	0.77 (−4.63, 6.19)	0.69 (−2.86, 5.03)	MIT	
2.78 (−4.49, 10.06)	−4.44 (−10.95, 1.90)	−4.49 (−10.34, 1.71)	−5.20 (−11.44, 0.60)	General physical activity

Blank, Blank control; LVHIIT, low-volume high-intensity interval training; HVHIIT, high-volume high-intensity interval training; MIT, Medium intensity training.

respectively. After 10 weeks of intervention with 80–85% max HR intensity HIIT, the blood pressure of the patients was significantly improved ($p < 0.05$). In Lins-Filho OL's study (49), it only reported that the resting heart rate of the blank control group has decreased significantly, which may be because the subjects in the exercise group received only 8 times interventions, with short rest time after exercise that their blood pressure levels had not fully recovered yet.

According to our results, the improvement of the blood pressure of male subjects after HIIT intervention is better than that of women, contrary to the results of previous studies (58). However, in the study of Danielle CB, subjects received grip resistance training, and different types of exercise led to different effects. In addition, the other 8 studies we revived did not indicate gender differences, and the relevant evidence is still too little to draw accurate conclusions on gender-related effects. More

TABLE 3 | The league table of the interventions for diastolic blood pressure (DBP).

Blank				
3.72 (−0.88, 7.67)	HVHIIT			
3.41 (0.40, 5.99)	−0.32 (−3.43, 2.95)	LVHIIT		
5.04 (0.94, 8.77)	1.37 (−1.75, 4.60)	1.66 (−1.15, 4.35)	MIT	
2.65 (−1.93, 6.86)	−0.97 (−4.51, 2.56)	−0.68 (−4.17, 2.69)	−2.35 (−5.75, 1.03)	General physical activity

Blank, Blank control; LVHIIT, low-volume high-intensity interval training; HVHIIT, high-volume high-intensity interval training; MIT, Medium intensity training.

TABLE 4 | The league table of the interventions for body mass index (BMI).

Blank				
2.76 (−0.42, 5.41)	HVHIIT			
1.40 (−0.52, 2.90)	−1.37 (−3.62, 1.00)	LVHIIT		
1.32 (−1.16, 3.48)	−1.38 (−3.70, 0.98)	−0.02 (−1.63, 1.60)	MIT	
1.83 (−1.32, 4.54)	−0.93 (−3.40, 1.59)	0.47 (−2.02, 2.86)	0.48 (−1.97, 2.88)	General physical activity

Blank, Blank control; LVHIIT, low-volume high-intensity interval training; HVHIIT, high-volume high-intensity interval training; MIT, Medium intensity training.

studies on the effects of exercise on patients with hypertension of different genders should be carried out in the future.

Obesity

Of the 12 studies reviewed, only 11 studies reported the BMI levels of the subjects (38, 39, 45, 48, 49, 51, 53, 54). According to the BMI established by the World Health Organization (Between 25 and 29.9 is overweight, and more than 30 is obese), the patients were divided into standard weight (47, 50), overweight (45, 46, 48, 49, 53) or obese (38, 39, 51, 54) hypertensive ones according to the BMI index.

In the study of Jo et al. (47, 50), 48 subjects of standard weight received LVHIIT and MIT intervention, respectively. The results showed that the blood pressure levels of these subjects who received exercise intervention were significantly improved ($p < 0.05$), and the resting heart rate of subjects who received HIIT intervention also decreased significantly ($p < 0.05$).

Of the five studies with subjects who had overweight hypertension, only 2 had no significant blood pressure and health status changes after exercise intervention (48, 49). In the study of Sosner et al. (48) and Lins-Filho et al. (49), there were only 8 and 6 interventions in total, so the reason for this insignificance may be due to the short intervention cycle. In several other studies (45, 46, 53), 200 patients who are overweight and hypertensive showed significant improvements in blood pressure and/or other health conditions after exercise intervention ($p < 0.05$).

In 4 studies of patients that are obese with hypertension (38, 39, 51, 54), 3 studies reported significantly improved in blood pressure after exercise intervention ($p < 0.05$) (38, 39, 51), while Ramirez-Jimenez M study (54) did not show any significant change, which may be due to the effects of metabolic syndrome and additional antihypertensive drugs.

Our results showed no differences in blood pressure, weight loss, or other indicators in patients with hypertension with different body weights. However, in this review, there are only two studies in which the subjects are non-overweight people with BMI close to 25, it is impossible to make an accurate analysis of obesity-related issues in patients with hypertension, further studies are needed.

Exercise

Neural activity may also affect cerebral blood flow during the exercise (62). Previous studies have shown that exercise training can reduce sympathetic excitation by reducing the activation of neurons in the cardiovascular region of the brain, thereby reducing the risk of cardiovascular disease (63). In addition, endurance training seems to reduce the intake of the brain of non-oxidizing carbohydrates and maintain brain oxygenation during sub-extreme exercise (62). However, in the 12 pieces of literatures included in this study, there is no mention of the movement of the sympathetic nervous system, and the effect of exercise on the activity of the sympathetic nervous system in people with hypertension needs to be further studied. Insulin resistance is closely related to arterial hypertension (64). Studies by Jelleyman et al. have shown that HIIT seems to improve metabolic health more effectively (65). However, studies on the effects of different intensity, volume, and types of exercise on patients with hypertension are still rare. Our previous studies have analyzed the effects of exercise on oxidative stress, and the results show that long-term high-intensity aerobic training has a better effect on the improvement of oxidative stress in people with chronic diseases (36). However, the effect of medium-intensity exercise on the improvement of oxidative stress is still controversial. A recent study showed that traditional Chinese exercise can also effectively improve the quality of life in patients with hypertension (66), however, no study has compared the effects of HIIT and traditional Chinese exercise on blood pressure control in patients with hypertension. However, for people with chronic diseases, exercise training programs are usually personalized to achieve better health results. Likewise, the effect of training is determined by many factors, including training

TABLE 5 | The league table of the interventions for rest heart rate (HR).

Blank				
6.05 (−0.88, 12.57)	HVHIIT			
3.60 (−2.87, 9.35)	−2.36 (−5.79, 1.21)	LVHIIT		
2.49 (−3.84, 8.53)	−3.51 (−6.83, −0.06)	−1.23 (−3.98, 1.68)	MIT	
3.90 (−3.12, 10.35)	−2.14 (−5.82, 1.44)	0.17 (−3.45, 3.89)	1.47 (−2.25, 4.81)	General physical activity

Blank, Blank control; LVHIIT, low-volume high-intensity interval training; HVHIIT, high-volume high-intensity interval training; MIT, Medium intensity training.

time, intensity, interval times and types, etc. (67); thus, it is necessary to classify and analyze.

Volume

Of the 12 studies that were included, subjects in 4 studies performed HIIT or MIT for more than 30 min (38, 39, 47, 50). The results showed that the blood pressure levels of the subjects who received higher-volume exercise have improved significantly.

All 12 studies involved in low-volume exercise showed improvement in blood pressure, health, and exercise ability. On the contrary, Sosner et al. (48) and Ramirez-Jimenez et al. (54) studied with subjects who received 8 and 6 exercise interventions, respectively, and their blood pressure did not change significantly.

Generally, high-volume exercise has a good effect on improving the health status of patients with hypertension. However, only four studies on high-volume training are included in this review, lacking studies on the effects of different volume training, especially HIIT, on patients with hypertension. More evidence is needed to justify this result.

Intensity

For a long time, high-intensity exercise has been considered to produce more robust physiological adaptability (55). However, it has been reported that high-intensity aerobic exercise does not produce a better antihypertensive effect (23).

In the 12 studies included in this review, all groups that used HIIT as an intervention reported a significant improvement in blood pressure levels, and health indicators also showed a good development trend (38, 39, 44–47, 50, 51, 53). On the contrary, the study by Lins-Filho et al. (49) did not show a significant change in blood pressure after eight times of the HIIT intervention which may be due to either the short training period or the subjects had not fully recovered when the indicators were measured. In the study of Ramirez-Jimenez et al. (54), the subjects received HIIT intervention for 16 weeks, and their blood pressure levels did not change significantly, these subjects had metabolic syndrome or took additional medication antihypertensive drugs. In Sosner P's study (48), the blood pressure of the subjects did not change significantly after receiving HIIT intervention for a total of 6 times in 2 weeks. These subjects exercised twice at the intensity of 100% peak power for 15 s, except for warm-up and rest, which may lead to a low volume of the exercise cycle.

For 5 studies using MIT intervention (38, 39, 45, 47, 50), the blood pressure levels of the subjects showed significant improvement.

In 2 control group studies, the subjects followed the physical activity guidelines for low-intensity general physical activity. The results showed that the blood pressure levels, and resting heart rate of these subjects were significantly improved ($p < 0.05$).

According to our results, MIT seems to be the best way to improve hypertension in patients. However, it is worth noting that HIIT is more effective than MIT in reducing BMI and resting HR in patients, and in the studies of Gorostegi-Anduaga et al. (38), Boa Sorte Silva et al. (45), and Izadi et al. (46), the exercise ability of the subjects was significantly improved after the intervention of HIIT, which was characterized by a longer time to reach exhaustion or an increase in anaerobic threshold.

Limitations

Due to a high degree of clinical heterogeneity and lack of data, our analysis cannot extrapolate to all the movements. However, similar trends in blood pressure and other health indicators were observed in patients with hypertension who received exercise intervention.

The limitations of this study are as follows: (1) The number of articles included in the reticular meta-analysis is limited, and the publication bias cannot be evaluated; (2) The patients with hypertension included in the study may also take antihypertensive drugs in addition to exercise intervention, but they are rarely mentioned in the articles; (3) Insufficient sample size may lead to overestimation of the intervention effect; (4) The mechanism of blood pressure reduction is a complex process, and our reticular meta-analysis is only a simplified method for this phenomenon; (5) In recent years, there are few studies on the effects of different volumes of HIIT on hypertensive patients, and there are few studies on resistance training, so, a more comprehensive analysis cannot be carried out; (6) There is almost no analysis of gender in the literature, so it is impossible to analyze the image of the effect of sports intervention from the perspective of gender; and (7) Only a few studies have conducted long-term follow-up on the effect of the intervention, and it is hard to analyze the long-term effect after the intervention.

CONCLUSION

In the network meta-analysis, magnitudes of SBP and DBP (main indicators of blood pressure), BMI (index of obesity), rest HR, time to exhaustion, ventilatory thresholds, body fat,

Max VO2, total cholesterol, max HR, and mean arterial pressure (index of another health status) of patients with hypertension are widely adopted to assess the health improvement. Taking the blood pressure level and the improvement of other health conditions after intervention as the main criteria, the effects of different volumes and intensity of physical exercise on the profile of people with hypertension were indirectly compared. The verified consistency model is applied to network meta-analysis. According to the results of our systematic review and web meta-analysis on patients with hypertension, MIT intervention is superior to other types of exercise in improving blood pressure, while HVHIIT is more effective in reducing BMI and rest HR. In addition, the effect of exercise on improving health status is different among different types of patients, suggesting that exercise with different volumes and intensity should be selected according to the severity of the disease. The use of antihypertensive drugs combined with the exercise intervention may lead to misjudgment. The results show that high-volume and appropriate-intensity exercise still has great potential in improving the health status of people with hypertension. However, there are few studies on the effects of different volume of HIIT and the other types of exercise on people with hypertension. More systematic well-planned studies are needed to evaluate the role of different volumes, intensity, and types of exercise training with long-term intervention in the improvement of the health of patients with hypertension.

AUTHOR CONTRIBUTIONS

ZL, YS, and YG: conceptualization. YG, HC, SL, and YS: methodology and validation. ZL and YG: writing-original draft preparation. YS, SL, and HC: writing-review and editing. E-CT and YG: supervision. All authors have read and agreed to the published version of the manuscript.

FUNDING

This study was sponsored by the Major Program of the National Social Science Foundation of China (Grant number: 19ZDA352), the National Key R&D Programs of China (Grant number: 2018YFF0300903), Philosophy and Social Sciences Project of Zhejiang Province of China (Grant number: 22NDQN223YB), Public Welfare Science and Technology Project of Ningbo of China (Grant number: 2021S134), and K. C. Wong Magna Fund in Ningbo University.

REFERENCES

1. Muntner P, Carey RM, Gidding S, Jones DW, Taler SJ, Wright JJT, et al. Potential US population impact of the 2017 ACC/AHA high blood pressure guideline. *Circulation.* (2018) 137:109–18. doi: 10.1161/CIRCULATIONAHA.117.032582
2. Yoon SS, Gu Q, Nwankwo T, Wright JD, Hong Y, Burt V. Trends in blood pressure among adults with hypertension: United States, 2003 to 2012. *Hypertension.* (2015) 65:54–61. doi: 10.1161/HYPERTENSIONAHA.114.04012
3. Leal JM, Galliano LM, Del Vecchio FB. Effectiveness of high-intensity interval training versus moderate-intensity continuous training in hypertensive patients: a systematic review and meta-analysis. *Curr Hypertens Rep.* (2020) 22:1–13. doi: 10.1007/s11906-020-1030-z
4. Costa EC, Hay JL, Kehler DS, Boreskie KF, Arora RC, Umpierre D, et al. Effects of high-intensity interval training versus moderate-intensity continuous training on blood pressure in adults with pre-to established hypertension: a systematic review and meta-analysis of randomized trials. *Sports Med.* (2018) 48:2127–42. doi: 10.1007/s40279-018-0944-y
5. Forouzanfar MH, Liu P, Roth GA, Ng M, Biryukov S, Marczak L, et al. Global burden of hypertension and systolic blood pressure of at least 110 to 115 mm Hg, 1990-2015. *JAMA.* (2017) 317:165–82. doi: 10.1001/jama.2016.19043
6. Gakidou E, Afshin A, Abajobir AA, Abate KH, Abbafati C, Abbas KM, et al. Global, regional, and national comparative risk assessment of 84 behavioural, environmental and occupational, and metabolic risks or clusters of risks, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet.* (2017) 390:1345–422. doi: 10.1016/S0140-6736(17)32366-8
7. Carey RM, Muntner P, Bosworth HB, Whelton PK. Prevention and control of hypertension: JACC health promotion series. *J Am Coll Cardiol.* (2018) 72:1278–93. doi: 10.1016/j.jacc.2018.07.008
8. Whelton PK, Carey R, Aronow WJ, Casey DE, Collins KJ, Dennison Himmelfarb C, et al. 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA guideline for the prevention, detection, evaluation, and management of high blood pressure in adults: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *J Am Coll Cardiol.* (2018) 71:e127–248. doi: 10.1161/HYP.0000000000000076
9. Fox CS, Larson MG, Leip EP, Culleton B, Wilson PW, Levy DJ. Predictors of new-onset kidney disease in a community-based population. *JAMA.* (2004) 291:844–50. doi: 10.1001/jama.291.7.844
10. Klag MJ, Whelton PK, Randall BL, Neaton JD, Brancati FL, Ford CE, et al. Blood pressure and end-stage renal disease in men. *N Engl J Med.* (1996) 334:13–8. doi: 10.1056/NEJM199601043340103
11. Hsu C-y, McCulloch CE, Darbinian J, Go AS, Iribarren C. Elevated blood pressure and risk of end-stage renal disease in subjects without baseline kidney disease. *Arch Intern Med.* (2005) 165:923–8. doi: 10.1001/archinte.165.8.923
12. Hwang S, Jayadevappa R, Zee J, Zivin K, Bogner HR, Raue PJ, et al. Concordance between clinical diagnosis and Medicare claims of depression among older primary care patients. *Am J Geriat Psychiat.* (2015) 23:726–34. doi: 10.1016/j.jagp.2014.08.009
13. Sabbahi A, Arena R, Elokda A, Phillips SA. Exercise and hypertension: uncovering the mechanisms of vascular control. *Prog Cardiovasc Dis.* (2016) 59:226–34. doi: 10.1016/j.pcad.2016.09.006
14. Hall JE, do Carmo JM, da Silva AA, Wang Z, Hall ME. Obesity, kidney dysfunction and hypertension: mechanistic links. *Nat Rev Nephrol.* (2019) 15:367–85. doi: 10.1038/s41581-019-0145-4
15. Kotchen TA. Obesity-related hypertension: epidemiology, pathophysiology, and clinical management. *Am J Hypertens.* (2010) 23:1170–8. doi: 10.1038/ajh.2010.172
16. Kotsis V, Stabouli S, Papakatsika S, Rizos Z, Parati G. Mechanisms of obesity-induced hypertension. *Hypertens Res.* (2010) 33:386–93. doi: 10.1038/hr.2010.9
17. Hall JE. The kidney, hypertension, and obesity. *Hypertension.* (2003) 41:625–33. doi: 10.1161/01.HYP.0000052314.95497.78
18. Mokdad AH, Ford ES, Bowman BA, Dietz WH, Vinicor F, Bales VS, et al. Prevalence of obesity, diabetes, and obesity-related health risk factors, 2001. *JAMA.* (2003) 289:76–9. doi: 10.1001/jama.289.1.76
19. Must A, Spadano J, Coakley EH, Field AE, Colditz G, Dietz WH. The disease burden associated with overweight and obesity. *Jama.* (1999) 282:1523–9. doi: 10.1001/jama.282.16.1523

20. Keating SE, Coombes JS, Stowasser M, Bailey TG. The role of exercise in patients with obesity and hypertension. *Curr Hypertens Rep.* (2020) 22:1–21. doi: 10.1007/s11906-020-01087-5
21. Lewington S, Clarke R, Qizilbash N, Peto R, Collins R. Age-specific relevance of usual blood pressure to vascular mortality. *Lancet.* (2003) 361:1391–2. doi: 10.1016/S0140-6736(03)13064-4
22. Lewington S. Prospective studies collaboration. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. *Lancet.* (2002) 360:1903–13. doi: 10.1016/S0140-6736(02)11911-8
23. Cornelissen VA, Smart NA. Exercise training for blood pressure: a systematic review and meta-analysis. *JAHA.* (2013) 2:e004473. doi: 10.1161/JAHA.112.004473
24. Pedersen BK, Saltin B. Exercise as medicine-evidence for prescribing exercise as therapy in 26 different chronic diseases. *Scand J Med Sci Sports.* (2015) 25:1–72. doi: 10.1111/sms.12581
25. Fiuza-Luces C, Garatachea N, Berger NA, Lucia A. Exercise is the real polypill. *Physiology.* (2013) 28:330–58. doi: 10.1152/physiol.00019.2013
26. FitzGerald W. Labile hypertension, jogging, and diet: clues to the etiology of essential hypertension? *Med Hypotheses.* (1984) 14:321–33. doi: 10.1016/0306-9877(87)90136-8
27. Eicher JD, Maresh CM, Tsongalis GJ, Thompson PD, Pescatello LS. The additive blood pressure lowering effects of exercise intensity on post-exercise hypotension. *Am Heart J.* (2010) 160:513–20. doi: 10.1016/j.ahj.2010.06.005
28. Cornelissen VA, Fagard RH, Coeckelberghs E, Vanhees L. Impact of resistance training on blood pressure and other cardiovascular risk factors: a meta-analysis of randomized, controlled trials. *Hypertension.* (2011) 58:950–8. doi: 10.1161/HYPERTENSIONAHA.111.177071
29. Fagard RH, Cornelissen VA. Effect of exercise on blood pressure control in hypertensive patients. *Eur J Cardiovasc Prev Rehabil.* (2007) 14:12–7. doi: 10.1097/HJR.0b013e3280128bbb
30. Kelley GA, Kelley KA, Vu Tran Z. Aerobic exercise and resting blood pressure: a meta-analytic review of randomized, controlled trials. *Prev Cardiol.* (2001) 4:73–80. doi: 10.1111/j.1520-037X.2001.00529.x
31. Carlson DJ, Dieberg G, Hess NC, Millar PJ, Smart NA. Isometric exercise training for blood pressure management: a systematic review and meta-analysis. *Mayo Clin Proc.* (2014) 89:327–34. doi: 10.1016/j.mayocp.2013.10.030
32. Huang G, Shi X, Gibson CA, Huang SC, Coudret NA, Ehlman MC. Controlled aerobic exercise training reduces resting blood pressure in sedentary older adults. *Blood Press Monit.* (2013) 22:386–94. doi: 10.3109/08037051.2013.778003
33. Kelley GA, Kelley KS. Progressive resistance exercise and resting blood pressure: a meta-analysis of randomized controlled trials. *Hypertension.* (2000) 35:838–43. doi: 10.1161/01.HYP.35.3.838
34. Pescatello LS, MacDonald HV, Lamberti L, Johnson BT. Exercise for hypertension: a prescription update integrating existing recommendations with emerging research. *Curr Hypertens Rep.* (2015) 17:1–10. doi: 10.1007/s11906-015-0600-y
35. Cornelissen VA, Fagard RH. Effects of endurance training on blood pressure, blood pressure-regulating mechanisms, and cardiovascular risk factors. *Hypertension.* (2005) 46:667–75. doi: 10.1161/01.HYP.0000184225.05629.51
36. Lu Z, Xu Y, Song Y, Bíró I, Gu Y. A mixed comparisons of different intensities and types of physical exercise in patients with diseases related to oxidative stress: a systematic review and network meta-analysis. *Front Physiol.* (2021) 12:700055. doi: 10.3389/fphys.2021.700055
37. Whitaker AA, Alwatban M, Freemyer A, Perales-Puchalt J, Billinger SA. Effects of high intensity interval exercise on cerebrovascular function: A systematic review. *PLoS ONE.* (2020) 15:e0241248. doi: 10.1371/journal.pone.0241248
38. Gorostegi-Anduaga I, Corres P, MartinezAguirre-Betolaza A, Pérez-Asenjo J, Aispuru GR, Fryer SM, et al. Effects of different aerobic exercise programmes with nutritional intervention in sedentary adults with overweight/obesity and hypertension: EXERDIET-HTA study. *Eur J Prev Cardiolog.* (2018) 25:343–53. doi: 10.1177/2047487317749956
39. MartinezAguirre-Betolaza A, Mujika I, Fryer SM, Corres P, Gorostegi-Anduaga I, Arratibel-Imaz I, et al. Effects of different aerobic exercise programs on cardiac autonomic modulation and hemodynamics in hypertension: data from EXERDIET-HTA randomized trial. *J Hum Hypertens.* (2020) 34:709–18. doi: 10.1038/s41371-020-0298-4
40. Pickering TG, Harshfield GA, Kleinert HD, Blank S, Laragh JH. Blood pressure during normal daily activities, sleep, and exercise: comparison of values in normal and hypertensive subjects. *JAMA.* (1982) 247:992–6. doi: 10.1001/jama.247.7.992
41. Parati G, Saul JP, Di Rienzo M, Mancia G. Spectral analysis of blood pressure and heart rate variability in evaluating cardiovascular regulation: a critical appraisal. *Hypertension.* (1995) 25:1276–86. doi: 10.1161/01.HYP.25.6.1276
42. Sala C, Santin E, Rescaldani M, Magrini F. How long shall the patient rest before clinic blood pressure measurement? *Am J Hypertens.* (2006) 19:713–7. doi: 10.1016/j.amjhyper.2005.08.021
43. Dalal J, Dasbiswas A, Sathyamurthy I, Maddury SR, Kerkar P, Bansal S, et al. Heart rate in hypertension: review and expert opinion. *Int J Hypertens.* (2019) 2019:2087064. doi: 10.1155/2019/2087064
44. Bahmanbeglou NA, Ebrahim K, Maleki M, Nikpajouh A, Ahmadizad S. Short-duration high-intensity interval exercise training is more effective than long duration for blood pressure and arterial stiffness but not for inflammatory markers and lipid profiles in patients with stage 1 hypertension. *J Cardiopulm Rehabil Prev.* (2019) 39:50–5. doi: 10.1097/HCR.0000000000000377
45. Boa Sorte Silva NC, Petrella AF, Christopher N, Marriott CF, Gill DP, Owen AM, et al. The benefits of high-intensity interval training on cognition and blood pressure in older adults with hypertension and subjective cognitive decline: Results from the heart & mind study. *Front Aging Neurosci.* (2021) 13:643809. doi: 10.3389/fnagi.2021.643809
46. Izadi MR, Afousi AG, Fard MA, Bigi MAB. High-intensity interval training lowers blood pressure and improves apelin and NOx plasma levels in older treated hypertensive individuals. *J Physiol Biochem.* (2018) 74:47–55. doi: 10.1007/s13105-017-0602-0
47. Jo E-A, Cho K-I, Park J-J, Im D-S, Choi J-H, Kim B-J, et al. Effects of high-intensity interval training versus moderate-intensity continuous training on epicardial fat thickness and endothelial function in hypertensive metabolic syndrome. *Metab Syndr Relat Disord.* (2020) 18:96–102. doi: 10.1089/met.2018.0128
48. Sosner P, Gayda M, Dupuy O, Garzon M, Gremeaux V, Lalonge J, et al. Ambulatory blood pressure reduction following 2 weeks of high-intensity interval training on an immersed ergocycle. *Arch Cardiovasc Dis.* (2019) 112:680–90. doi: 10.1016/j.acvd.2019.07.005
49. Lins-Filho OL, Ritti-Dias RM, Santos TM, Silva JF, Leite GF, Gusmao LS, et al. Affective responses to different prescriptions of high-intensity interval exercise in hypertensive patients. *J Sports Med Phys Fitness.* (2020) 60:308–13. doi: 10.23736/S0022-4707.19.10155-7
50. Jo E-A, Cho K-I, Lim D-S, Choi J-H, Park J-JJ. Effects of interval training on blood pressure and endothelial function in hypertensive patients. *IJASS.* (2018) 30:50–61. doi: 10.24985/ijass.2018.30.1.50
51. Taha MM, El-Rahman Mohamed MA, Hasanin ME. Effect of high intensity interval training on endothelial function in postmenopausal hypertensive patients: randomized controlled trial. *IJPHY.* (2016) 3:39–44. doi: 10.15621/ijphy/2016/v3i1/88908
52. Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA Statement. *PLos Med. (2019)* 6:e1000097. doi: 10.1371/journal.pmed1000097
53. Soltani M, Aghaei Bahmanbeglou N, Ahmadizad SJC, Hypertension E. High-intensity interval training irrespective of its intensity improves markers of blood fluidity in hypertensive patients. *Clin Exp Hypertens.* (2020) 42:309–14. doi: 10.1080/10641963.2019.1649687
54. Ramirez-Jimenez M, Morales-Palomo F, Moreno-Cabañas A, Alvarez-Jimenez L, Ortega JF, Mora-Rodriguez R, et al. Effects of antihypertensive medication and high-intensity interval training in hypertensive metabolic syndrome individuals. *Scand J Med Sci Sports.* (2021) 31:1411–19. doi: 10.1111/sms.13949
55. Boutcher YN, Boutcher SH. Exercise intensity and hypertension: what's new? *J Hum Hypertens.* (2017) 31:157–64. doi: 10.1038/jhh.2016.62

56. Nascimento L, Santos A, Lucena J, Silva L, Almeida A, Brasileiro-Santos M. Acute and chronic effects of aerobic exercise on blood pressure in resistant hypertension: study protocol for a randomized controlled trial. *Trials.* (2017) 18:1–8. doi: 10.1186/s13063-017-1985-5
57. Xiao J. *Physical Exercise for Human Health*. Singapore: Springer Nature (2020).
58. Bentley DC, Nguyen CH, Thomas SG. Resting blood pressure reductions following handgrip exercise training and the impact of age and sex: a systematic review and narrative synthesis. *Syst Rev.* (2018) 7:1–17. doi: 10.1186/s13643-018-0876-5
59. Son W-M, Sung K-D, Bharath LP, Choi K-J, Park S-Y, Hypertension E. Combined exercise training reduces blood pressure, arterial stiffness, and insulin resistance in obese pre-hypertensive adolescent girls. *Clin Exp Hypertens.* (2017) 39:546–52. doi: 10.1080/10641963.2017.1288742
60. Maruf FA, Salako BL, Akinpelu AOJ. Can aerobic exercise complement antihypertensive drugs to achieve blood pressure control in individuals with essential hypertension? *J Cardiovasc Med.* (2014) 15:456–62. doi: 10.2459/JCM.0b013e32836263b2
61. Setters B, Holmes HM. Hypertension in the older adult. *Prim Care.* (2017) 44:529. doi: 10.1016/j.pop.2017.05.002
62. Gibala MJ, Little JP, MacDonald MJ, Hawley JA. Physiological adaptations to low-volume, high-intensity interval training in health and disease. *J Physiol.* (2012) 590:1077–84. doi: 10.1113/jphysiol.2011.224725
63. Seifert T, Secher NH. Sympathetic influence on cerebral blood flow and metabolism during exercise in humans. *Prog Neurobiol.* (2011) 95:406–26. doi: 10.1016/j.pneurobio.2011.09.008
64. Mueller PJ. Exercise training and sympathetic nervous system activity: evidence for physical activity dependent neural plasticity. *Clin Exp Pharmacol P.* (2007) 34:377–84. doi: 10.1111/j.1440-1681.2007.04590.x
65. Lamounier-Zepter V, Ehrhart-Bornstein M, Bornstein SR. Insulin resistance in hypertension and cardiovascular disease. *Best Pract Res Clin Endocrinol Metab.* (2006) 20:355–67. doi: 10.1016/j.beem.2006.07.002
66. Song Y, Li J, István B, Xuan R, Wei S, Zhong G, et al. Current evidence on traditional Chinese exercises for quality of life in patients with essential hypertension: a systematic review and meta-analysis. *Front Cardiovasc Med.* (2021) 7:394. doi: 10.3389/fcvm.2020.627518
67. Jelleyman C, Yates T, O'Donovan G, Gray LJ, King JA, Khunti K, et al. The effects of high-intensity interval training on glucose regulation and insulin resistance: a meta-analysis. *Obes Rev.* (2015) 16:942–61. doi: 10.1111/obr.12317

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frontiers in Cardiovascular Medicine

CLINICAL TRIAL
published: 24 January 2022
doi: 10.3389/fcvm.2021.808071

Risk Prediction Model for Uncontrolled Hypertension in Chinese Community

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Specialty section:
This article was submitted to Hypertension, a section of the journal Frontiers in Cardiovascular Medicine

Received: 03 November 2021
Accepted: 23 December 2021
Published: 24 January 2022

Citation:
Gao Z, Chen S, Huang X, Ye J, Liu J, Huang Z, Chen J, Li L, Liu Y and Lin S (2022) Risk Prediction Model for Uncontrolled Hypertension in Chinese Community. Front. Cardiovasc. Med. 8:808071. doi: 10.3389/fcvm.2021.808071

Background: Uncontrolled hypertension rate was still high across China. This study develops and validates an index to help quantify the combination of socio-behavioral aspects to screen high-risk patients in uncontrolled hypertension in Chinese primary care.

Methods: A cross-sectional study included 1,039 of patients with hypertension in the Chinese community. We assessed independent risk factors of uncontrolled blood pressure (defined as having a blood pressure ≥140/90 mmHg, even with antihypertensive therapy) and develop a risk prediction model.

Results: Among the 1,039 patients (53.9% male, the average age was 61 ± 13 years), 452 (43.5%) were uncontrolled hypertensive. Multivariable analysis showed that worker (odds ratio, OR: 1.98, 95% CI: 1.46–2.69), no health insurance (OR: 3.47, 95% CI: 2.08–5.80), non-marital status (OR: 2.01, 95% CI: 1.35–3.27), and other socio-behavioral aspects were independent risk factors of uncontrolled hypertension, which were included the final prediction model (C-static: 0.781). With internal validation by the bootstrap method, the risk score showed good discriminating ability and predicting ability for the incidence of uncontrolled hypertension (C-static: 0.771).

Conclusions: This study showed that nearly half of the patients suffered from uncontrolled hypertension in the Chinese community. We established a prediction model with good predictability to help quantify the combination of socio-behavioral aspects and screen high-risk patients with uncontrolled hypertension.

Keywords: uncontrolled hypertension, screen, risk factor, prediction model, community

INTRODUCTION

Hypertension is an important health challenge worldwide because of its high prevalence, leading to cardiovascular disease, premature death, and disability (1, 2). National reports have indicated that the unawareness and uncontrol of hypertension improved substantially in high-income countries, while there has been little improvement in low- and middle-income countries (3). In China, the prevalence of hypertension in adults is gradually increasing, reaching 27.9%, but the control rate is only 15.3% (4).

Previous studies have shown that China cardiovascular outpatient clinics of comprehensive second- and third-level hospitals show that the protective factors of blood pressure control rate include older, retirees, medical care, physical activity, and isolated hypertension patients (5). Patients in economically developed areas have a high accuracy rate of self-reported hypertension. The American Health Nutrition Survey shows that elderly, obesity, and diabetes are independent risk factors for controlled hypertension. The characteristics and rates of controlled hypertension in general hospitals and communities are different (6).

The risk assessment of uncontrolled hypertension in community hypertension patients can help identify high-risk patients and carry out active blood pressure control measures for target factors. The recent systematic review showed a moderate to large effect of patients' education (healthy knowledge and behavior) on adherence to lifestyle modifications and blood pressure control (7). However, there was a lack of an index to help quantify the combination of socio-behavioral aspects, such as knowledge of hypertension and medicine adherence, which can screen high-risk patients in uncontrolled hypertension in Chinese community clinics.

Therefore, we aimed to develop and validate a useful risk prediction for uncontrolled hypertension among patients receiving antihypertensive therapy in Chinese community primary care.

METHODS

Data Sources and Study Population

A total of 1,089 patients with a history of hypertension and receiving antihypertensive medicines who went to the community hypertension clinic were consecutively enrolled from 5 communities within a limited time window (1 week) in Guangzhou and Dongguan, China, between February 2018 and March 2018, participated in this survey. We exclude patients $<$18 years old; patients with blood pressure $\geq$180/120 mmHg or antihypertensive medicines $\geq$4 (definitely recommend they seek treatment from a specialist) ($n = 48$); Patients unable to complete the questionnaire due to mental problems ($n = 2$). Finally, 1,039 patients were included in the final analysis (**Figure 1**). Our study obtained written informed consent from all patients in compliance with the Declaration of Helsinki (approved by the Ethics Research Committee of Guangdong Provincial People's Hospital) (8).

Data Collection

We collected the social-demographic information, disease awareness, hypertension management, and the use and demand of mobile health tools in patients with hypertension in the community primary care in person, which was previously described (9). All patients completed the survey, and the average time it took participants to complete the survey was 20 min. Sex, age, height, weight, education (International Standard Classification of Education, ISCED), occupation, and medical care were evaluated by standard survey items (**Supplementary Materials**).

Definitions

Uncontrolled blood pressure is defined as having a blood pressure $\geq$140/90 mmHg, even with antihypertensive therapy. The blood pressure was measured after the patient sat still for a while, with at least two measurements, with an interval of 1–2 min (if the previous two measurements are very different, additional measurements will be taken). The patient's marital status of either unmarried, divorced, or widowed is defined as non-marital status. Working full-time was defined as the total daily working hours of the patient of more than 8 h. Patients with a clear definition of hypertension and understanding of the risks of hypertension were defined as having the correct knowledge of hypertension treatment. Patients who take medication regularly, monitor blood pressure regularly, and spend more than 0.5 h of daily activity in total are defined as right knowledge of hypertension treatment. Good medication compliance was defined as missed medication less than once a week. The willingness to remind blood pressure measurement information is defined as having been using or willing to accept health software or SMS reminders as an auxiliary tool to regularly measure blood pressure. Further detailed definitions are provided in **Supplementary Materials**.

Statistical Analyses

We estimated that 10 risk factors would be included in the multivariate regression model. In regression analysis, the sample size used for prognostic risk factor analysis requires at least 20 individuals uncontrolled hypertension (events) for each prognostic factor, so we need 200 total events. The rate of uncontrolled hypertension in patients with hypertension is not $<$20%. Accordingly, a total of 100 samples are needed.

We compared patients with and without uncontrolled blood pressure. Continuous variables were reported as the mean and SD, categorical variables were described as frequencies and percentages and compared using the χ^2 test. Clinical potential confounders, the baseline variables with differences, and value of $p < 0.05$ in the univariate logistic analyses were regarded as candidate covariates. The variables with $P < 0.05$ in logistic regression multivariate analysis, including candidate covariates, are independent risk factors that affect uncontrolled blood pressure. The risk factors that are important in univariate analysis can be used to select the final prediction model for uncontrolled hypertension. Full-time work, no medical care insurance, non-marital status, poor cognition of hypertension diagnosis, poor drug compliance, poor cognition of hypertension treatment, and unwillingness to accept informational intervention were identified as independent predictors of blood pressure in the community with hypertension. The modeling data set of 906 community hypertension patients was used, and the final prediction model was assessed using the area under the receiver operating characteristic (ROC) curve and concordance c-statistic for discriminative ability, and the Hosmer–Lemeshow goodness-of-fit statistic for calibration using fifths of the fitted risk values (10). Moreover, the final model was tested by the bootstrapping method (1,000 times) to evaluate the stability of the c-statistics. The statistical analysis was conducted using SAS V.9.4.

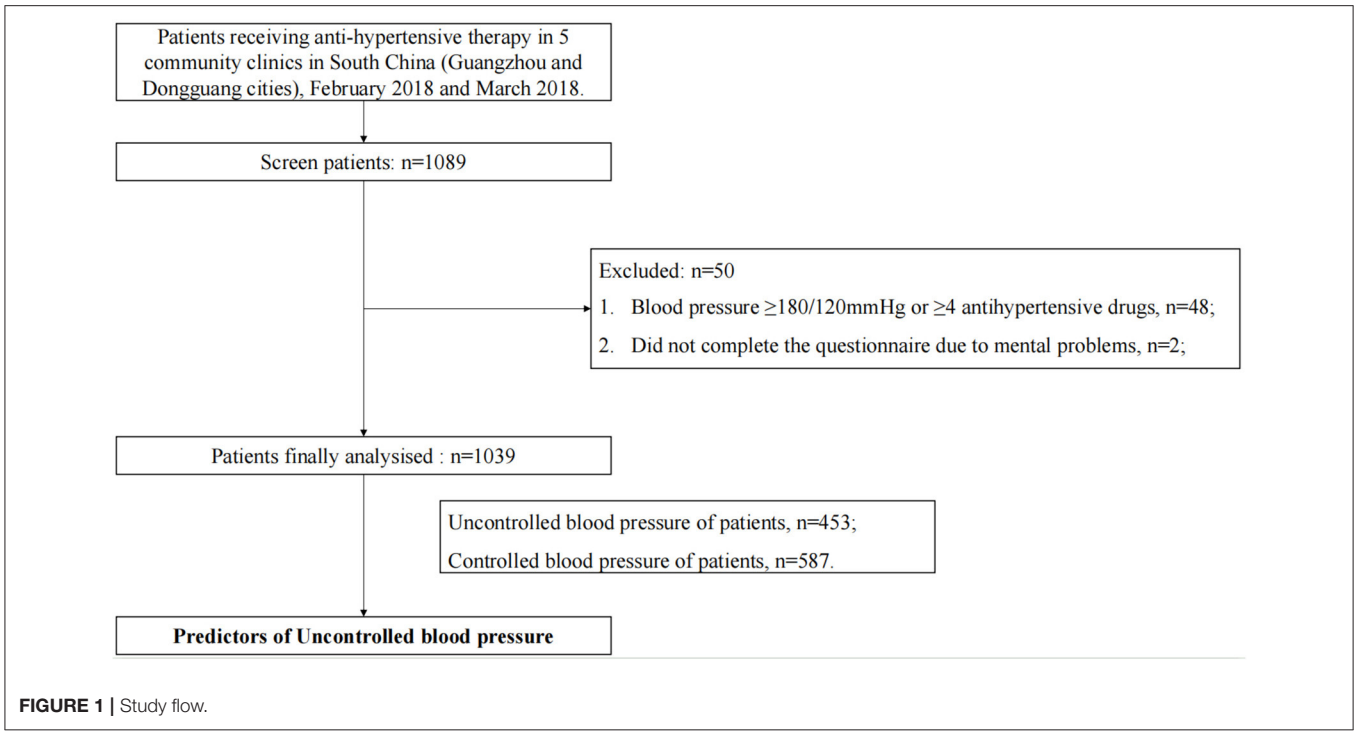

FIGURE 1 | Study flow.

TABLE 1 | Sample characteristics of uncontrolled hypertension patients.

Item	Missing	Total sample *N* = 1,039	Controlled hypertension *N* = 587 (56.5)	Uncontrolled hypertension *N* = 452 (43.5)	*P*
Age (SD)	22	61 (13)	62 (13)	59 (12)	<0.001
Age > 75 years, *n* (%)	22	161 (15.83)	106 (18.47)	55 (12.42)	0.009
Male, *n* (%)	21	549 (53.9)	303 (53.06)	246 (55.03)	0.53
BMI (SD)	51	24.3 (3.2)	24.4 (3.1)	24.1 (3.4)	0.092
Non-marital status, *n* (%)	7	153 (14.7)	72 (12.3)	88 (19.5)	<0.001
Employment status, not working, *n* (%)	7	451 (43.41)	220 (37.5)	231 (51.1)	<0.001
No medical insurance, *n* (%)	15	108 (10.5)	29 (5.0)	79 (17.6)	<0.001
Good knowledge of hypertension diagnosis, *n* (%)	5	613 (59)	427 (72.7)	186 (41.2)	<0.001
Good knowledge of hypertension treatment, *n* (%)	16	494 (47.6)	352 (60.0)	142 (31.4)	<0.001
Good knowledge of anti-hypertension medicine, *n* (%)	21	712 (68.5)	455 (77.5)	257 (56.9)	<0.001
Good BP monitoring, *n* (%)	19	387 (37.25)	223 (38.0)	164 (36.3)	0.57
Good medicine adherence, *n* (%)	4	325 (31.4)	222 (38.0)	103 (22.9)	<0.001
Willing to remind medication information, *n* (%)	225	198 (19.1)	121 (20.6)	77 (17.0)	0.145
Willing to remind blood pressure measurement information, *n* (%)	225	162 (15.6)	106 (18.1)	56 (12.4)	0.012
Smoking, *n* (%)	38	395 (39.5)	186 (32.9)	209 (40.1)	<0.001
Weekly high-intensity exercise, *n* (%)	226	641 (61.7)	328 (55.9)	313 (69.3)	<0.001
Wechat used by patients, *n* (%)	217	128 (12.3)	66 (11.3)	62 (13.7)	0.22
Wechat used by patients' family, *n* (%)	222	455 (43.8)	242 (41.2)	213 (47.1)	0.06
Medicine prescribed in above second-class hospital, *n* (%)	4	230 (22.1)	119 (20.3)	111 (24.6)	0.1

RESULTS

Totally 1,039 patients were included in the final analysis. The average age was 61 ± 13 years old, there were 549 (53.9%) male, the average body mass index was 24.3 ± 3.2 kg/m^2, and 153 (15%) were unmarried, the total uncontrolled hypertension rate (≥140/90 mm Hg) 452 (43.5%). Compared with patients with controlled hypertension, patients with uncontrolled hypertension and receiving antihypertensive treatment were younger (59 vs. 62 years), more likely to be unmarried (19.5% vs. 12.3%), full-time employed (51.1 vs. 37.5%), and have self-financed medical care (17.6 vs. 5.0%). However, they have

TABLE 2 | Logistics univariate analysis of substandard blood pressure control.

Variables	vs.	OR	CI	*P* value
Age > 75 years	≥75 vs. <75	0.63	0.44–0.89	<0.01
Marital status	Non-marital status status vs. marital status status	1.92	1.35–2.71	0.0002
Employment status	Full time working vs. not working	1.74	1.36–2.24	<0.001
Medical insurance	Self-financed vs. others	4.04	2.59–6.31	<0.001
Knowledge of hypertension diagnosis	poor vs. well	3.82	2.94–4.95	<0.001
Knowledge of anti-hypertension medicine	poor vs. well	2.62	2.00–3.42	<0.001
BP monitoring	Good vs. not	0.93	0.72–1.20	0.573
Willing to remind medication information	Yes vs. not	0.791	0.58–1.09	0.15
Willing to remind blood pressure measurement information	Yes vs. not	0.64	0.45–0.91	0.01
Weekly high-intensity exercise	Enough vs. no	1.78	1.37–2.30	<0.001
Smoking	Yes vs. no	1.89	1.46–2.44	<0.001
Wechat used by patients	Yes vs. not	1.26	0.87–1.82	0.23
Wechat used by patients' family	Yes vs. not	1.27	0.99–1.63	0.06
Medicine adherence	Good vs. not	0.49	0.37–0.64	<0.001
Medicine prescribed in above second-class hospital	second-class hospital and above vs. others	1.28	0.95–1.72	0.1
Knowledge of hypertension treatment	Well vs. poor	0.31	0.24–0.40	<0.001
Daily measurement of BP	Yes vs. not	0.59	0.42–0.83	0.003
BMI	/	0.97	0.93–1.01	0.09

TABLE 3 | Multivariate analysis of risk factors of drug non-compliance in patients with hypertension in community.

Risk factors	Test group vs. reference group	OR	95%CI	*P*	Weighted integral
Employment status	Full time working vs. others	1.98	1.46–2.69	<0.001	4
Medical insurance	Self-financed vs. medical insurance	3.47	2.08–5.80	<0.001	7
Marital status	Non-marital status vs. married	2.01	1.35–3.27	<0.001	4
Knowledge of hypertension diagnosis	Poor vs. well	3.28	2.42–4.45	<0.001	6
Medicine adherence	Bad vs. good	1.51	1.08–2.11	0.016	3
Knowledge of hypertension treatment	Wrong vs. right	2.94	2.16–3.99	<0.001	6
Willing to remind blood pressure measurement information	Not vs. yes	1.64	1.08–2.50	0.02	3

less knowledge of hypertension diagnosis (41.2 vs. 72.7%), hypertension treatment (31.4 vs. 60%), and hypertension medications (56.9 vs. 77.5%). In addition, they were less complied with antihypertensive medications (22.9 vs. 38%), and less willing to remind of blood pressure measurements (56.9 vs. 77.5%), more smoke (40.1 vs. 32.9%), and less exercise (69.3 vs. 55.9%) ($p < 0.05$ above), but no difference in gender, body mass index, blood pressure measurement compliance, drug purchase location, etc., (**Table 1**).

Univariate analysis results were shown in **Table 2**. Multivariable analysis showed that: full-time work [odds ratio (OR): 1.98, 95% CI: 1.46–2.69], self-financed medical care (OR: 3.47, 95% CI: 2.08–5.80), non-marital status (OR: 2.01, 95% CI: 1.35–3.27), poor knowledge of hypertension diagnosis (OR: 3.28, 95% CI: 2.42–4.45), poor drug compliance (OR: 1.51, 95% CI: 1.08–2.11), poor knowledge of hypertension treatment (OR: 2.94, 95% CI: 2.16–3.99), and reluctance to remind blood pressure measurement information (OR: 1.64, 95% CI: 1.08–2.50) were identified independent predictors of uncontrolled hypertension among patients in the community who received antihypertensive treatment (**Table 3**). The receiver operating characteristic (ROC) curve analysis showed the area under the ROC curve was 0.781 (**Figure 2**).

The risk model includes the following seven factors: full-time work (4 points), self-pay medical care (7 points), non-marital status (4 points), poor cognitive diagnosis of hypertension (6 points), poor drug compliance (3 points), poor cognition of hypertension treatment (6 points), and unwilling reminder of blood pressure measurement (3 points) (**Table 3**), Hosmer Lemeshow statistics of multivariate models do not suggest lack of appropriateness ($x^2 = 6.5649$, $P = 0.5842$). Based on the frequency of uncontrolled hypertension with different risk scores, 1,039 patients were classified into three groups in the modeling data set: low risk [<7 points, $n = 227$ (12%)], moderate risk [8–14 points, $n = 244$ (36%)], high risk [15–22 points, $n = 174$ (53%)], and extremely high risk [>22 points, $n = 262$ (74%)] (**Figure 3**). In the verification data set, the risk score showed good discriminating ability and predicting ability for the incidence of uncontrolled hypertension (C-static: 0.771) by the bootstrap method.

DISCUSSION

To the best of our knowledge, this study was the first study to estimate a risk-prediction model for uncontrolled hypertension in the Chinese community. Our data suggested that nearly half of the hypertension patients who have received antihypertensive treatment in the community suffered from uncontrolled hypertension. Bad hypertension health cognition and lifestyle behavior were independent risk factors for uncontrolled hypertension. We have established a prediction model based on 7 key predictors of uncontrolled hypertension among community patients receiving antihypertensive treatment, with good predictability and high discriminatory ability.

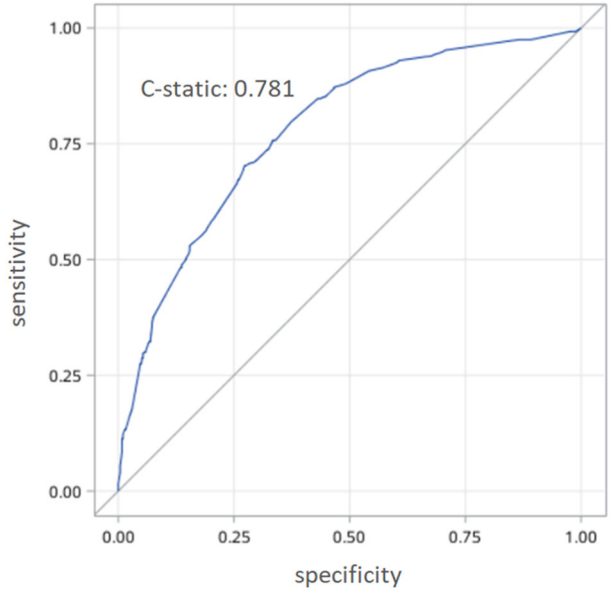

FIGURE 2 | The receiver operating characteristic (ROC) analyses of the prediction model.

This study found the uncontrolled hypertension rate was 43.5% (in 2008), in the developed region in South China, in line with recent trends of the United States, where near half of patients achieved hypertension control across 20 American primary care centers, and the hypertension control increased from 27.3% in 1988–1994 to 50.1% in 2007–2008 in recent NHANES survey (10–12). Wang et al. conducted MMM China project among 364,000 participants in 394 inside hospitals or community health centers and found that the awareness, treatment, and control rates of hypertension were 60.1, 42.5, and 25.4%, respectively, with the rate of uncontrolled hypertension (59.8%) moderately higher than our study (13). We only investigated the patients receiving antihypertensive treatment in the community, not patients in hospitals, which might present with higher control proportion.

The patients with uncontrolled hypertension in our study were younger, more unmarried, have more full-time work, more self-paying medical treatment, less knowledge of hypertension, less medication compliance, more smoking, and less exercise.

Our analysis also showed that the independent risk factors of uncontrolled hypertension are full-time work, self-financed medical care, non-marital status, poor cognition of hypertension diagnosis, poor cognition of hypertension treatment, poor drug compliance, and unwillingness for blood pressure measurement information reminder among patients in the community receiving antihypertensive treatment. MMM China project also showed that current smoking, no diabetes, no coronary heart disease, and older were independent risk factors of uncontrolled hypertension (13), while the above variables were not collected in our study. A recent review highlighted the important role of

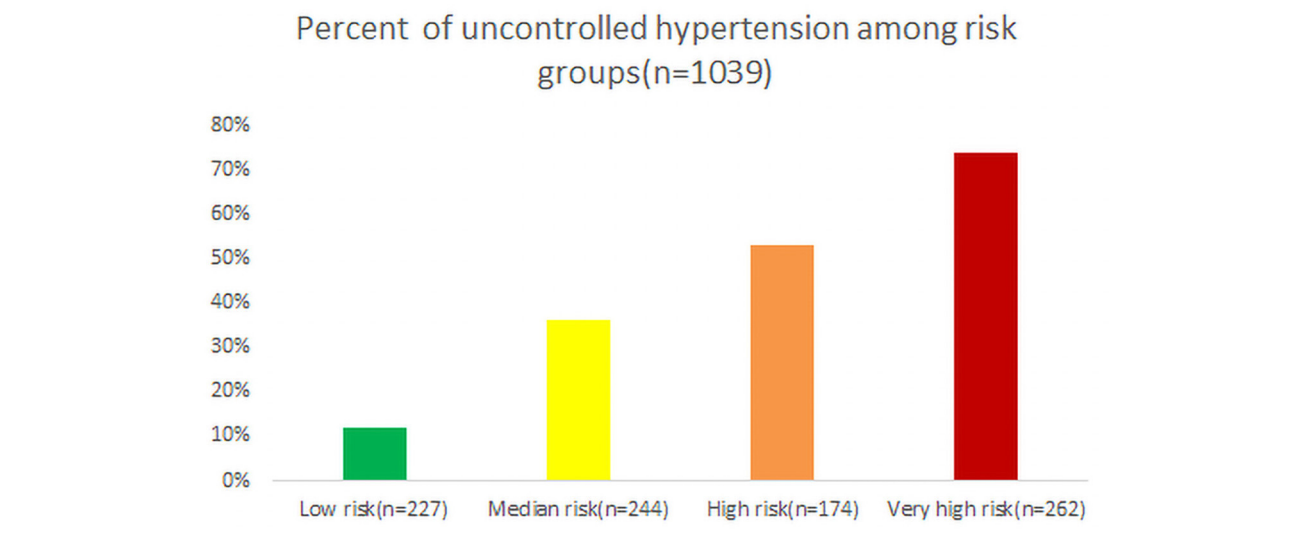

FIGURE 3 | Uncontrolled hypertension with different risk scores.

an education intervention on healthy behaviors and showed a moderate effect in the adherence to dietary recommendations and physical activity recommendations (7). Finnish Public Sector study with 41,225 participants suggested that those hypertensive patients were less likely to adhere to lifestyle modifications after the initiation of medications, as evidence of an increasingly becoming obese and physically inactive (14).

The WHO recommended more attention on nursing in the community, which could greatly contribute to many kinds of population groups in the community. Because of the role of a healthy lifestyle in hypertension control, we need to explore effective components of lifestyle modification educational intervention in terms of better delivery mode, use of the theoretical framework, and use of supportive methods. Theory-based educational interventions, such as knowledge of hypertension diagnosis, hypertension treatment, and consistent measures of adherence behavior, such as medicine compliance, blood pressure measurement, are needed to be adopted in future practice or studies. Monthly or weekly group education can be conducted in the community to promote hypertension control, especially for the patients on working or patients under non-marital status.

Besides the patients under treatment, we also should emphasize the inadequate use of antihypertensive treatment in the community in China. A combination of antihypertensive therapy would be a choice of approaches for improving control of hypertension in China. (13). Wang et al. established a web-based and WeChat-linked blood pressure measuring system in China mainly in public areas, such as office buildings, shopping malls, airports, railway stations, and so on, where younger people often walk around (15). Adherence to blood pressure measurement was a barrier to hypertension control. However, there were knowledge gaps, such as appropriate arm and body positioning, frequency of readings, the timing of measurements, duration of rest before measurement, proper cuff size and placement, the necessity of voiding before measurement, and the importance of refraining from other activities when obtaining reading (16). Canada study showed that only 8% of patients with hypertension were trained with the home blood pressure monitoring (HBPM) technique (17). Another American study with the HBPM program found that 13% of patients were sufficiently compliant with BP measurement guidelines to ensure reliable readings (18). Similarly, one Chinese cross-sectional survey collected data among 2,272 patients with hypertension aged $\geq$35 years from 20 communities across three cities and six townships in three provinces and found that only 45.3% owned a home blood pressure (BP) monitor. In addition, $\sim$4.4% of participants had achieved optimal HBPM method (duplicate measurements in the morning and evening for 1 week), and only 16.0% of participants actively reported their HBPM readings to doctors (19).

We have established a prediction model based on 7 key predictors of uncontrolled hypertension among community patients receiving antihypertensive treatment, with good predictability and high discriminatory ability. A recent review suggested that mHealth apps can be beneficial in terms of improving hypertension self-assessment, treatment, and control (20). One American study used electronic medical record (EMR) data from patients at two urban safety-net clinical systems and suggested that stable insurance of any type was associated with better hypertension control than no or unstable insurance. Therefore, we should pay more attention to hypertensive patients without health insurance and provide more economic antihypertension medicines. Our prediction model provides a good evaluation tool for community hypertension prevention and treatment to identify high-risk populations of uncontrolled hypertension, but still, needs evaluation and external validation/clinical promotion in future large-scale multicenter trials. We provided a new type of intervention target (hypertension health cognition and lifestyle behavior) for community hypertension prevention and control. Future studies, including large-scale randomized clinical trials with patients-centered design, are crucial to further evaluate the potential and effectiveness of interventions, such as mHealth apps-based patients' education, community levels, or integrate interventions in the hypertension control in the community.

LIMITATION

First, this study was a cross-sectional survey in the developed region (Guangzhou city and Dongguan city), which lacked the outcome among these participants, such as cardiovascular events and could not investigate associations of real office-BP control and their characteristics, while survey with questionnaire characteristics may be easier to recruit more patients in Chinese community economically. Second, there is a lack of information about important comorbidities that may affect blood pressure, such as drug use, kidney failure, or diabetes. This information may give us a better understanding of blood pressure. Third, the subjects were patients under anti-hypertension treatment, missing the information among patients without treatment in primary care practice or patients prescribed outside the primary care. But we recruited most the hypertensive participants in the clinic of primary care or community resident with hypertension by the following phones. Fourth, the risk factors in this study were defined in the questionnaire, such as evaluating the knowledge of the patient on hypertension, diagnostic criteria, hypertension drug, hypertension complication, etc. according to the subjective assessment of the patient, but the study found that the hypertension health knowledge level of hypertension patients is closely related to hypertension standards, defining new risk factors as future community hypertension control to provide intervention targets. Fifth, because we only recruited patients in 5 urban communities and communities are not randomly sampled from the general community, our results cannot be generalized to all communities (e.g., rural areas).

CONCLUSION

This study showed that nearly half of the patients receiving antihypertensive treatment suffered from uncontrolled

hypertension in the Chinese developed community. We also found the independent risk factors for uncontrolled hypertension, including full-time work, self-financed medical care, non-marital status, poor cognition of hypertension diagnosis, poor cognition of hypertension treatment, poor drug compliance, and unwillingness for blood pressure measurement information reminder, and established a prediction model based on 7 keys predictors of uncontrolled hypertension, with good predictability and high discriminatory ability. Our prediction model provides a good evaluation tool for community hypertension prevention and treatment to identify high-risk populations of uncontrolled hypertension, but still, needs evaluation and external validation/clinical promotion in future large-scale studies.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

All the traceable personal identifiers were removed from the analytic dataset to protect patients' privacy. The study protocol was approved by the Guangdong Provincial People's Hospital Ethics Committee, and this study was performed according to the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

ZG, SC, YL, and SL contributed to conception and design. ZG, SC, and YL contributed to manuscript writing. ZG, SC, YL, JY, JL, and ZH contributed to administrative support. All authors contributed to provision of study materials or patients, collection and assembly of data, data analysis and interpretation, and approval of final version of the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2021.808071/full#supplementary-material

REFERENCES

1. Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK, He J. Global burden of hypertension: analysis of worldwide data. *Lancet.* (2005) 365:217–23. doi: 10.1016/S0140-6736(05)17741-1
2. Forouzanfar MH, Alexander L, Anderson HR, Bachman VF, Biryukov S, Brauer M, et al. Global, regional, and national comparative risk assessment of 79 behavioural, environmental and occupational, and metabolic risks or clusters of risks in 188 countries, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet.* (2015) 386:2287–323. doi: 10.1016/S0140-6736(15)00128-2
3. Mills KT, Bundy JD, Kelly TN, Reed JE, Kearney PM, Reynolds K, et al. Global disparities of hypertension prevalence and control: a systematic analysis of population-based studies from 90 countries. *Circulation.* (2016) 134:441–50. doi: 10.1161/CIRCULATIONAHA.115.018912
4. Wang Z, Chen Z, Zhang L, Wang X, Hao G, Zhang Z, et al. Status of hypertension in china: results from the China hypertension survey, 2012-2015. *Circulation.* (2018) 137:2344–56. doi: 10.1161/CIRCULATIONAHA.117.032380
5. Lin FL, Zhan YQ, Jia GX, Ming YJ, University F, Shanghai, et al. Blood pressure control rate and its influencing factors in Chinese hypertensive outpatients. *Chinese J Hypertension.* (2013) 43:62–7.
6. Zeng XY, Zhang M, Li YC, Huang ZJ, Wang LM. [Study on effects of community-based management of hypertension patients aged ≥35 years and influencing factors in urban and rural areas of China, 2010]. *Zhonghua liu xing bing xue za zhi = Zhonghua liuxingbingxue zazhi.* (2016) 37:612.
7. Tam HL, Wong EML, Cheung K. Effectiveness of educational interventions on adherence to lifestyle modifications among hypertensive patients: an integrative review. *Int J Environ Res Public Health.* (2020) 17:7. doi: 10.3390/ijerph17072513
8. World Medical Association Declaration of Helsinki. Ethical principles for medical research involving human subjects. *Jama.* (2013) 310:2191–4. doi: 10.1001/jama.2013.281053
9. Chen S, Sun G, Cen X, Liu J, Ye J, Chen J, et al. Characteristics and requirements of hypertensive patients willing to use digital health tools in the Chinese community: a multicentre cross-sectional survey. *BMC public health.* (2020) 20:1333. doi: 10.1186/s12889-020-09462-2
10. Egan BM, Zhao Y, Axon RN. US trends in prevalence, awareness, treatment, and control of hypertension, 1988-2008. *Jama.* (2010) 303:2043–50. doi: 10.1001/jama.2010.650
11. Shelley D, Tseng TY, Andrews H, Ravenell J, Wu D, Ferrari P, et al. Predictors of blood pressure control among hypertensives in community health centers. *Am J Hypertension.* (2011) 24:1318–23. doi: 10.1038/ajh.2011.154
12. Ornstein SM, Nietert PJ, Dickerson LM. Hypertension management and control in primary care: a study of 20 practices in 14 states. *Pharmacotherapy.* (2004) 24:500–7. doi: 10.1592/phco.24.5.500.33359
13. Chen X, Xu SK, Guo QH, Hu Z, Wang HY, Yu J, et al. Barriers to blood pressure control in China in a large opportunistic screening. *J Clinic.* (2020) 22:835–41. doi: 10.1111/jch.13850
14. Korhonen MJ, Pentti J, Hartikainen J, Ilomäki J, Setoguchi S, Liew D, et al. Lifestyle changes in relation to initiation of antihypertensive and lipid-lowering medication: a cohort study. *J Am Heart Assoc.* (2020) 9:e014168. doi: 10.1161/JAHA.119.014168
15. Wang JG. Unique approaches to hypertension control in China. *Annals Transl Med.* (2018) 6:296. doi: 10.21037/atm.2018.07.27
16. Liyanage-Don N, Fung D, Phillips E, Kronish IM. Implementing home blood pressure monitoring into clinical practice. *Curr Hypertens Rep.* (2019) 21:14. doi: 10.1007/s11906-019-0916-0
17. Logan AG, Dunai A, McIsaac WJ, Irvine MJ, Tisler A. Attitudes of primary care physicians and their patients about home blood pressure monitoring in Ontario. *J Hypertens.* (2008) 26:446–52. doi: 10.1097/HJH.0b013e3282f2fdd4
18. Doane J, Buu J, Penrod MJ, Bischoff M, Conroy MB, Stults B. Measuring and managing blood pressure in a primary care setting: a pragmatic implementation study. *JABFM.* (2018) 31:375–88. doi: 10.3122/jabfm.2018.03.170450
19. Zuo HJ, Ma JX, Wang JW, Chen XR. Assessing the routine-practice gap for home blood pressure monitoring among Chinese adults with hypertension. *BMC Public Health.* (2020) 20:1770. doi: 10.1186/s12889-020-09901-0
20. Santo K, Redfern J. The potential of mhealth applications in improving resistant hypertension self-assessment, treatment and

control. *Curr Hypertens Rep.* (2019) 21:81. doi: 10.1007/s11906-019-0986-z

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frontiers in Physiology

REVIEW
published: 25 January 2022
doi: 10.3389/fphys.2021.791848

Pyroptosis and Its Regulation in Diabetic Cardiomyopathy

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Diabetic cardiomyopathy (DbCM) is a prevalent disease, characterized by contractile dysfunction and left ventricular hypertrophy. Patients with DbCM have high morbidity and mortality worldwide. Recent studies have identified that pyroptosis, a kind of cell death, could be induced by hyperglycemia involved in the formation of DbCM. This review summarizes the regulatory mechanisms of pyroptosis in DbCM, including NOD-like receptor3, AIM2 inflammasome, long non-coding RNAs, microRNAs, circular RNA, autophagy, and some drugs.

Keywords: diabetic cardiomyopathy, pyroptosis, NLRP3 inflammasome, caspase-1, regulation

OPEN ACCESS

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Specialty section:
This article was submitted to Redox Physiology, a section of the journal Frontiers in Physiology

Received: *11 October 2021*
Accepted: *10 December 2021*
Published: *25 January 2022*

Citation:
Lu Y, Lu Y, Meng J and Wang Z (2022) Pyroptosis and Its Regulation in Diabetic Cardiomyopathy. Front. Physiol. 12:791848. doi: 10.3389/fphys.2021.791848

INTRODUCTION

Diabetes mellitus (DM) potentially causes increased risks of heart failure in the absence of the traditional impetus to heart failure such as hypertension and coronary heart disease, including, diabetic cardiomyopathy (DbCM) (Murtaza et al., 2019). In 1972, Rubler and his colleagues first described DbCM from the postmortem pathological findings of four diabetic patients who died of heart failure without evidence of hypertension and coronary artery disease (Jia et al., 2018b). In general, DbCM has two stages, namely, early stage featured by left ventricular hypertrophy and diastolic dysfunction and later stage featured by cardiac fibrosis and impaired systolic function (Paolillo et al., 2019). Multiple hallmarks have been reviewed to contribute to DbCM, including hyperglycemia, insulin resistance, lipid peroxidation, increased oxidative stress, mitochondrial dysfunction, cardiomyocyte calcium handling, endothelial dysfunction (Jia et al., 2018a), and cell death (Cai and Kang, 2003).

Based on the morphological features, cell death can be clearly classified into four types, namely, necrosis, autophagy, entosis, and apoptosis (Chen et al., 2020). Necrosis, an energy-independent and uncontrolled type of cell death, can be induced by some external injury such as inflammation and hypoxia (D'Arcy, 2019). Autophagy is a catabolic and regulated process associated with the formation of the autophagosome to engulf the cytoplasmic content. Entosis demonstrates "cell-in-cell" cytological features through lysosomal degradation (Martins et al., 2017). Apoptosis is featured by characteristic morphological changes such as cell shrinkage, pyknosis, and karyorrhexis. Some studies have shown that DbCM involves pyroptosis, which is a proinflammatory apoptotic process that differs from classic apoptosis (Li et al., 2014; Yang et al., 2018b; D'Arcy, 2019).

PATHOGENESIS OF DIABETIC CARDIOMYOPATHY

Of note, inflammation is involved in the pathogenesis of DbCM. Diabetes is a proinflammatory process, and several cytokines including interleukin-1β (IL-1β), IL-18, and tumor necrosis factor-alpha (TNF-α) are highly expressed in DbCM (Bugger and Abel, 2014). Insulin resistance and hyperglycemia in the heart have been found to be associated with the pathogenesis of DbCM.

In physiological states, insulin regulates the myocardial metabolism, glucose transport, glycogen synthesis, and lipid metabolism. In a diabetic heart, the decreases in glucose transporter 4 and its abnormal translocation caused by insulin resistance limit the glucose uptake (Xia and Song, 2020). In contrast, cluster of differentiation 36 is preferentially located to the sarcolemma, which increases fatty acid oxidation in the diabetic heart. The changes in metabolisms prompt the deposition of lipids and advanced glycation end-products (AGEs) (Jia et al., 2016). Excessive lipids contribute to the mitochondrial dysfunction by affecting the respiratory function and mitochondrial biogenesis. Increased AGEs are associated with elevated generation of reactive oxygen species (ROS), which instigates inflammasome and fibrosis. ACEs result in crosslinks in collagen molecules, hence promoting collagen to be impaired and causing increased fibrosis, followed by elevated myocardial stiffness (Bugger and Abel, 2014). Furthermore, AGEs induced by hyperglycemia can lead to protein misfolding, which increases cytosolic calcium (Ca^{2+}) from the endoplasmic reticulum and then induces Ca^{2+} disturbance in the mitochondria (Fiorentino et al., 2013). In addition, AGEs participate in the activation of nuclear factor κB (NF-κB), which controls DNA transcription and the formation of proinflammatory cytokine. Insulin resistance and hyperglycemia result in stress to the endoplasmic reticulum, which implicates impaired Ca^{2+}handling. Impaired Ca^{2+} reuptake by the sarcoplasmic reticulum increases the duration of the action potential and decreases the diastolic relaxation of cardiomyopathy (Jia et al., 2018a). These pathophysiological abnormalities together cause cardiomyocyte death and finally cardiac stiffness and heart failure. Obviously, myocardial death is a crucial element in DbCM. A study showed that the expression of apoptosis and necrosis markers is high in the heart tissue of patients with type 2 diabetic (Frustaci et al., 2000). Recently, increasing studies have indicated the roles of non-coding RNAs (ncRNAs) in the pathogenesis of DbCM, including cell death, oxidative stress, myocardial fibrosis, and inflammation. For example, miR-1 could induce apoptosis in DbCM, while miR-22 could regulate hyperglycemia-induced oxidative stress (Zhang et al., 2019; **Figure 1**).

PYROPTOSIS

The term pyroptosis comes from the Greek word "pyro," meaning fever or fire, and ptosis, meaning falling. It depicts proinflammatory programmed cell death (Cookson and Brennan, 2001). According to the definition, pyroptosis is a proinflammatory type of programmed cell death, depending on the enzymatic activity with the involvement of inflammatory proteases, which are a part of the caspase family (Vande Walle and Lamkanfi, 2016). Pyroptosis leads to DNA damage, less DNA laddering, and chromatin condensation with an intact nucleus. Compared with apoptotic cells, pyroptotic cells are positive in TUNEL staining. Although DNA damage relies on caspase-activated DNase (CAD) and restricted CAD inhibitor during apoptosis, CAD is not needed during pyroptosis (Jorgensen and Miao, 2015; Fang et al., 2020). Furthermore, pyroptosis induces the formation of caspase-1-dependent membrane pores with 1.1–2.4 nm diameter, which causes swelling and osmotic lysis (Fink and Cookson, 2006). Pyroptosis responds to infectious diseases and protects host cells against microbial pathogens; however, caspase activation is harmful to the body. Traditionally named caspase-1-mediated cell death, pyrotosis involves human caspase-4 and caspase-5, mouse caspase-11 (Kayagaki et al., 2015), and caspase-3 (a hallmark of apoptosis) (Cao et al., 2017). These caspases cleave and activate some specific members of the gasdermin gene family, leading to the occurrence of pyroptosis. The molecular mechanisms of pyroptosis are shown in **Figure 2**.

Inflammasomes are multimolecular protein complexes that typically contain a sensor protein pattern-recognition receptor (PRR), a proinflammatory caspase, and an adaptor protein apoptosis-associated speck-like protein containing a caspase recruitment domain (CARD) (ASC) (Rathinam and Fitzgerald, 2016). The PRR family includes Toll-like receptors, absent in melanoma 2 (AIM2) and NOD-like receptors (NLRs) (Dolasia et al., 2018; Zhao et al., 2020), which recognize danger-associated molecular patterns (DAMPs) induced by endogenous pathogens or pathogen-associated molecular patterns (PAMPs) derived from invading pathogens (Man and Kanneganti, 2015; Fang et al., 2020). ASC connects the sensor protein and pro-caspase-1 after PRR recognizes DAMP/PAMP, further promoting pyroptosis. Previous studies have demonstrated that there are generally canonical and non-canonical inflammasome pathways triggering pyroptosis.

Canonical inflammasomes include NOD-like receptor 1 (NLPR1), NLRP3, NLRC4, and AIM2, triggering pyroptosis by activating caspase-1 (Aachoui et al., 2013). NLRP1, NLRP3, and AIM2 have a pyrin domain (PYD) (Wang B. et al., 2019), and NLRC4 contains an N-terminal (NT) CARD, which enables them to recruit pro-caspase-1. Various inflammasomes are activated by special DAMPs and PAMPs. NLPR1 activates muramyl dipeptide and anthrax lethal toxin in response to parasite *Toxoplasma gondii* (Franchi et al., 2009; Ewald et al., 2014). AIM2 is a member of the AIM2-like receptor family containing one or two HIN domains and NT PYD (Wang and Yin, 2017). The HIN domain contains two oligosaccharide folds that can recognize nucleic acids, while the PYD domain regulates the homotypic protein-protein interaction. In homeostasis, AIM2 senses self-DNA by conserved mechanisms, which have been evolved to degrade mislocalized DNA or separate self-DNA to the nucleus (Kawane et al., 2014). Once double-stranded DNA (dsDNA) is recognized by PYD, AIM2 recruits ASC, activating caspase-1 in turn (Wang and Yin, 2017; Wang B. et al., 2019). AIM2 detects cytoplasmic dsDNA, which could be derived from bacteria, viruses, or host (Wang and Yin, 2017). NLRC4 recognizes bacterial proteins including flagellin, a component protein of type III secretion system. NLRP3 senses wide-ranging stimuli, including endogenous and infectious DAMPs such as microbial components; pore-forming toxins; nucleic acids; endogenous molecules such as uric acid crystals and ATP; and common cellular distress molecules like mitochondrial dysfunction, ROS, Ca^{2+}, and rupture of lysosomes (Malik and Kanneganti, 2017;

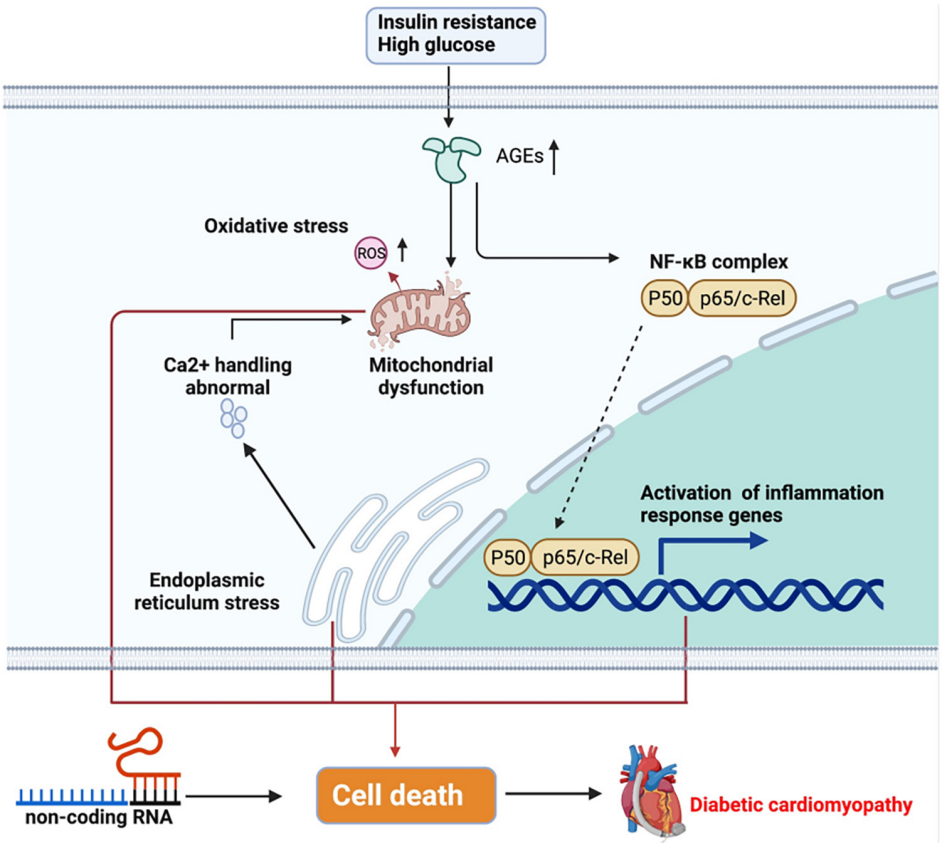

FIGURE 1 | Pathogenesis of diabetic cardiomyopathy (DbCM). Insulin resistance and high glucose trigger cell metabolism disorder, further causing inflammation and cell death, which finally results in heart failure. ER, endoplasmic reticulum; AGEs, advanced glycated end-products; ROS, reactive oxygen species; NF-κB, nuclear factor-κB.

Kelley et al., 2019). The AIM2 or NLR signaling domains (CARD or PYD) connect ASC *via* homotypic interactions, inducing the formation of the ASC focus, which further recruits procaspase-1 (Aachoui et al., 2013). Subsequently, procaspase-1 is self-cleaved into bioactive caspase-1 composed of two p10 and two p20 subunits, which promote the maturation of IL-1β and IL-18 and induces the cleavage of gasdermin D (GSDMD) to form the membrane pore (Fink et al., 2008; Boucher et al., 2018). The formation of membrane pores results in water influx, cell swelling, and lysis, in turn inducing pyroptotic cells to release inflammatory cytokines and DAMPs (Tsuchiya et al., 2019).

The non-canonical inflammasome pathway induces the activation of human caspase-4/5 and mouse caspase-11 (Broz and Dixit, 2016). As apical activators, caspase-4/5/11 directly detect lipopolysaccharide (LPS) in the host cytoplasm derived from Gram-negative bacteria by their CARD domains, causing the activation of GSDMD (Man et al., 2017). Furthermore, studies showed that caspase-4/5/11 cannot process IL-18 and IL-1β directly, but they induce GSDMD-mediated potassium efflux to promote the formation of the NLRP3 inflammasome and IL-1β activation (Ramirez et al., 2018; Frank and Vince, 2019). Wang et al. (2020) showed that caspase-4/11 autoprocess p10, which promotes the cleavage of GSDMD.

As the effector of pyroptosis, GSDMD was found to form pores in the membrane. Caspase-1/4/5/11 cleaves the linker sequence between the C-terminal (CT) and NT domains of GSDMD (Pandeya et al., 2019). GSDMD-CT is considered to bind to GSDMD-NT to restrict its activation (Liu et al., 2016). GSDMD-NT targets cardiolipin and phosphoinositide and oligomerizes to form pores with a diameter of 10–33 nm. Different from most pore-forming proteins, GSDMD lyses cell membranes from the cytosol (Ding et al., 2016; Man et al., 2017). GSDMD-NT also activates canonical NLRP3 inflammasome possibly by inducing K + efflux (Rühl and Broz, 2015).

Apoptosis is controlled by the caspase family and Bcl-2 protein family. Two pathways induce apoptosis, namely, extrinsic death pathway and intrinsic mitochondrial pathway. In the extrinsic pathway, caspase-8 is activated *via* Fas-associated death domain, which further activates caspase-3 after the combination of some substrates such as TNF-α and Fas-ligand-induced receptor trimerization (Sohns et al., 2010). In the intrinsic pathway, a number of proteins named the permeability transition pore complex form a pore in mitochondrial membranes, facilitating the release of mitochondrial proteins such as cytochrome C into the cytosol, which is involved in the formation of the apoptosome.

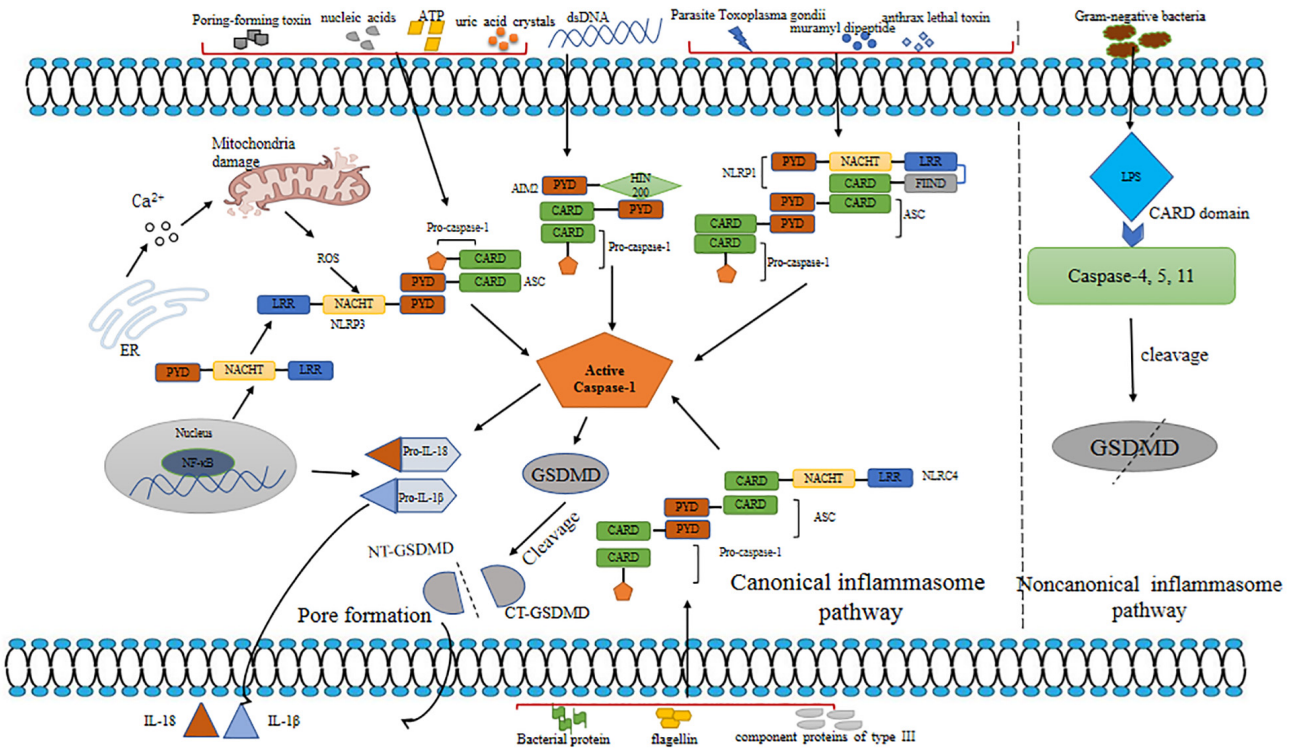

FIGURE 2 | Mechanisms of pyroptosis. Pyroptosis is triggered by canonical and non-canonical inflammasome pathways. Various inflammasomes involved in canonical pathway are activated by different DAMPs and PAMPs. Canonical inflammasomes bind to ASC to activate caspsase-1. Caspase-1 plays a role in maturation of IL-1β and IL-18 and cleavage of GSDMD. N-GSDMD releases and forms a membrane pore, leading to the secretion of IL-1β and IL-18. In non-canonical pathway, LPS binds to human caspase-4/5 and mice caspase-11 to promote cleavage of GSDMD. dsDNA, double-stranded DNA; ROS, reactive oxygen species; AIM2, absent in melanoma 2; ASC, apoptosis-associated speck-like protein containing a caspase recruitment domain; NLRP3, NOD-like receptor; PYD, pyrin domain; LRR, leucine-rich repeat; NACHT, nucleotide binding and oligomerization; HIN-200, the interferon (IFN)-inducible p200-protein; GSDMD, gasdermin D; NT-GSDMD, N-terminal gasdermin D; CT-GSDMD, C-terminal gasdermin D; LPS; lipopolysaccharide; DAMPs, danger-associated molecular patterns; PAMPs, pathogen-associated molecular patterns; IL-18, interleukin-18; IL-1β, interleukin-1β.

Hereafter, the apoptosome activates procaspase-9 and caspase-3 (Chen et al., 2020).

Apoptosis leads to cell shrinkage, blebbing, and regular DNA fragmentation maintaining the membrane integrity, which protects normal surrounding cells from inflammatory response. GSDMD-dependent pyroptosis is characterized by integrated nuclear changes, cell swelling, lysis, and the release of cytokines, promoting inflammatory response. Moreover, during apoptosis, signal molecules activate proapoptotic proteins including Bad and Bid, which further repress the antiapoptotic protein Bcl family and facilitate Bax/Bak oligomerization, affecting the mitochondria (Sohns et al., 2010). Emerging studies found that GSDMD and gasdermin E (GSDME) permeabilize *via* the mitochondrial membrane and form pores in the mitochondrial membrane, releasing cytochrome C in pyroptosis (Rogers and Alnemri, 2019). Zheng et al. (2020) demonstrated that highly expressed BH3-only protein Bcl-2/adenovirus EIB 19-KDa-interacting protein 3 activates caspase-3, further inducing GSDME to facilitate pyroptosis in cardiomyocytes treated with doxorubicin. These results show that caspase-3 can regulate not only pyroptosis but also apoptosis in cardiomyocytes, and the generation of GSDME may be a sign to clarify these two kinds of cell death. Nevertheless, whether caspase-3 induces GSDME in cardiomyocytes treated with high glucose remains unknown.

PYROPTOSIS IN DIABETIC CARDIOMYOPATHY

Previous studies found the overexpression of NLRP3, caspase-1, and IL-1 in the heart of rats with diabetes (Zhou et al., 2018; Xie et al., 2020). Increasing pyroptosis is commonly observed in patients with diabetes. In addition, except hyperglycemia, high lipid, insulin resistance, and hyperinsulinemia all contribute to pyroptosis in DbCM *via* increasing ROS (Lee and Kim, 2017; Kar et al., 2019; Urdaneta Perez et al., 2020), which is seen as a significant activator inducing the NLRP3 inflammasome. Pyroptosis has been identified to occur in various heart cell types in DbCM including cardiomyocytes, endothelial cells (ECs), and cardiac fibroblasts (Qiu et al., 2017; Zhaolin et al., 2019; Chen et al., 2020). These various pyroptotic cells present similar and different features in DbCM.

Cardiomyocytes maintain the heart systolic and diastole function and help the heart pump blood to the body. ELAV-like protein 1 (ELAVL1) is a kind of RNA stabilizing protein that binds to AU-abundant elements in the 3′-untranslated region of different cytokines (Zhou et al., 2019). In high-glucose condition, high levels of caspase-1 and ELAVL1 were detected in cardiomyocytes. Furthermore, cells with ELAVL1 knockdown express lesser NLRP3 and caspase-1 than those with ELAVL1. ELAVL1 has been proved to modulate pyroptosis in cardiomyocytes (Jeyabal et al., 2016). Cardiomyocyte pyroptosis triggers cardiac fibroblasts to repair injury by releasing cytokines such as IL-1β, ultimately causing heart hypertrophy and systolic and diastolic dysfunction. In high-glucose condition, metabolic disorders are easily found in cardiomyocytes. Under hyperglycemia condition, increasing glycolysis supplies a source of ATP, which may disturb mitochondria by upregulating glucose-6-phospate (G6PD). G6PD produces NADPH, whose dehydrogenase triggers superoxide anion and NADPH oxidase to further damage the mitochondria. Mitochondrial dysfunction plays a pivotal role in the activation of the NLRP3 inflammasome. However, as the NLRP3 inflammasome also promotes apoptosis in cardiomyocytes (Zhang et al., 2018) and considerable evidence proves myocardial apoptosis in DbCM, it makes sense to distinguish apoptosis and pyroptosis.

Endothelial cells separate blood and tissue, which is a part of the inner layer of the blood vessel. ECs play a vital role in maintaining the vascular tone and structure. Moreover, as a natural anticoagulant tissue, ECs release several kinds of anticoagulant molecules such as heparin. Meanwhile, ECs participate in the immune system, secreting cytokines and swallowing bacteria and necrotic tissues. Previously, Chen H. et al., 2016 found that cadmium triggers NLRP3-mediated pyroptosis in ECs. This study indicated that high glucose induces the formation of NLRP3 inflammasome in coronary arterial ECs of mice. In addition, endothelial dysfunction was observed, and the deficiency of NLRP3 inflammation alleviated the injury of ECs (Chen Y. et al., 2016). This study illustrated that hyperglycemia-induced NLRP3 inflammasome is involved in the EC dysfunction. NLRP3 inflammasome-induced IL-1 targets ECs to induce various inflammatory activities such as increased expression of adhesion molecules. Moreover, ECs produce IL-1 to increase inflammation (Grebe et al., 2018; Bai et al., 2020), which further aggravates DbCM. Furthermore, high glucose triggers ECs to reduce the secretion of nitric oxide (NO), which is recognized as the most important vasodilator of ECs. In certain conditions, nitric oxide synthase (NOS) reduces L-arginine to L-citrulline and NO, which prevent inflammation and maintain vascular homeostasis. However, acute inflammatory cells and ECs are able to express arginase, which locally decomposes L-arginine to decrease the substrate of eNOS. Moreover, eNOS merely plays a role in producing NO in the presence of tetrahydrobiopterin (BH4) and heme. Otherwise, it works as NADPH oxidase in oxidative reaction (Cyr et al., 2020). BH4 deficiency is closely associated with inflammation in the retina. Thus, high-glucose-induced inflammatory process inhibits NO production of ECs by oxidizing BH4. Along with NO deficiency, more platelets adhere and gather in the blood vessel to promote thrombus, further aggravating heart failure.

Cardiac fibroblasts remodel the heart by promoting the excretion of collagen and other extracellular matrix components when the heart suffers damage and inflammation. However, if the damage is too large, too much collagen causes cardiac fibrosis, stiffness, and dysfunction. In DM, heart failure occurs partly because of the excessive excretion of the extracellular matrix from cardiac fibroblasts. Micro-RNA 21 was demonstrated to target dual specific phosphatase 8 to increase the production of collagen in the cardiac fibroblasts in high glucose (Liu et al., 2014). Moreover, high expression of NLRP3 and caspase-1 was found in cardiac fibrosis tissue of rats with diabetes. Another study indicated that miR-21-3p was expressed in high glucose. The inhibitor of miR-21-3p reduces caspase-1 in hyperglycemia, which regulates cardiac fibroblast pyroptosis. These findings show that hyperglycemia-induced pyroptosis mediated by miR-21-3p is associated with the dysfunction of cardiac fibroblast.

REGULATION OF PYROPTOSIS IN DIABETIC CARDIOMYOPATHY

Pyroptosis is involved in the pathogenesis of DbCM. Previous and emerging studies indicated that the regulations of pyroptosis in DbCM are complicated with the involvement of NLRP3 and AIM2 inflammasome, long non-coding RNAs (lncRNAs), micro RNA, circular RNA (circRNA), autophagy, and some drugs (**Table 1**).

NLRP3 REGULATION

As mentioned previously, the NLRP3 inflammasome comprises protein complexes, NLRP3, ASC, and pro-caspase-1. NLRP3, a sensor of PAMPs and DAMPs, consists of three domains, namely, PYD, nucleoside triphosphatase domain (NACHT), and leucine-rich repeat. NACHT, known as adenosine triphosphatase domain, is composed of helical domain 1, helical domain 2, winged helix domain, and nucleotide-binding domain (NBD) (Sharif et al., 2019). ASC contains CARD and PYD. Pro-caspase-1 is composed of p10, p20, and CARD (Luo et al., 2017). The sensor recruits ASC after detection of stimuli. Subsequently, the multimeric complex interacts with procaspase-1 and forms the inflammasome, which promotes procaspase-1 autocatalytic cleavage into caspase-1 (Malik and Kanneganti, 2017). The activation of the NLRP3 inflammasome includes two processes, namely, induction of transcription of NLRP3, predecessors of caspase-1 and IL-1β, and subsequent assembly of NLRP3 with pro-caspase-1 and ASC (Zeng et al., 2020). In high glucose, NF-κB showed a high expression in cardiomyocytes. The NF-κB inhibitor decreased the expression of NF-κB in mRNA and the levels of NLRP3 and caspase-1 (Luo et al., 2014). Thus, the transcriptional activation of NLRP3 can be controlled by NF-κB (He et al., 2016). The assembly can be activated by a plethora of stimuli, including ATP, pore-forming toxins, and particulate matter, which may explain the activation of

TABLE 1 | Drugs/genes, their targets, and function in diabetic cardiomyopathy (DbCM).

Drug/Gene	MiRNA	Target	*Vivo* trial	Target function	Expression
Metformin	/	AMPK	Yes	Reduce blood glucose	/
Empagliflozin	/	SGC	Yes	Reduce blood glucose	/
EUK-134	/	TXNIP NLRP3	No	Inhibit NLRP3 inflammasome	/
Skimmin	/	/	Yes	Enhance autophagy and inhibit pyroptosis	/
MNS	/	ATPase	Yes	Inhibit interaction of NEK7 and NLRP3	/
NSA	/	GSDMD	NO	Inhibit GSDMD cleavage and IL-1βrelease	/
BMP-7	/	/	Yes	Inhibit pyroptosis and improve cardiac remodeling	/
MALAT1	miR-141-3p		No	Promote pyroptosis	↓
GAS5	miR-34b-3p	AHR	Yes	Inhibit NLRP3-mediated pyroptosis	↓
Kcnq1ot1	miR-214-3P	/	Yes	Promote pyroptosis	↑
/	miR-30d	foxo3a ARC	Yes	Promote pyroptosis	↑
/	miR-9	ELAVL1	No	Inhibit pyroptosis	↓
CACR	miR-214-3p	/	No	Promote pyroptosis	↑
/	miR-21-3p	AR	Yes	Promote pyroptosis	↑
circRNA-010567	miR-141	/	Yes	Promote fibrosis	↑

AMPK, AMP-activated protein kinase; MNS, 3,4-methylenedioxy-β-nitrostyrene; TXNIP, thioredoxin interacting protein; BMP-7, bone morphogenetic protein-7; MALAT1, metastasis associated lung adenocarcinoma transcript1; GAS5, growth arrest-specific transcript 5; AHR, aryl hydrocarbon receptor; Kcnq1ot1, KCNQ1 opposite strand/antisense transcript 1; foxo3a, Forkhead box o3; ARC, caspase recruitment domain; ELAVL1, ELAV-like protein 1; CACR, caspase-1-associated circRNA; AR, androgen receptor.

the inflammasome in multiple cardiovascular diseases. Unlikely to be sensed directly by PRRs, these stimuli trigger a series of cellular signaling events, mitochondrial dysfunction, Ca^{2+} mobilization, and ROS production (Kelley et al., 2019; Yang et al., 2019b).

Fibrosis is the chronic aberrant accumulation of extracellular matrix components from fibroblasts and myofibroblasts derived from various sources, including epithelial to mesenchymal transition (EMT, a process wherein the epithelial cells change into mesenchymal cells and lose adhesion and migration ability) (Stone et al., 2016; Alyaseer et al., 2020). After the binding of TGF-β to its receptor complex, Smad2 and Smad3 are activated through CT phosphorylation and then together form a polymer along with Smad4 to transport into the nucleus, which consequently activates the transcription of EMT. Studies have well supported that TGF-β is among the mediators of fibrosis of in DbCM. According to studies, the NLRP3 inflammasome promotes fibrosis by upregulating TGF-β *via* mature IL-1. In addition, the NLRP3 protein promotes the TGF-β/Smad pathway in the absence of activation of NLRP3 inflammasome (Alyaseer et al., 2020). Che et al. indicated that the NLRP3 inflammasome may also induce fibrosis through the TGF-β/Smad pathway, in view of the increasing levels of the TGF-β and Smad phosphorylation under IL-1 exposure (Che et al., 2020). Hence, the clear links between NLRP3 inflammasome and fibrosis remain to be further studied.

The activation of the inflammasome also promotes apoptosis, which further exacerbates DbCM. Zhang et al. (2018) found that high-glucose-induced apoptosis relies on the inflammasome and IL-1β in cardiomyocytes, and preventing inflammasome formation can ameliorate myocardial injury. The NLRP3 inflammasome also plays a crucial role in metabolic disturbances mentioned above. Activated NLRP3 may be associated with the shift toward the aliphatic acid metabolism, which is detrimental to the heart (Sharma et al., 2018). In a nutshell, the NLRP3 inflammasome partly contributes to structural and functional alternations in the diabetic heart.

ABSENT IN MELANOMA 2 INFLAMMASOME REGULATION

Besides the NLRP3 inflammasome, the AIM2 inflammasome has been demonstrated to be involved in the regulation of pyroptosis in DbCM. dsDNA released by pyroptotic cells subsequently activates AIM2, further aggravating the lesion tissue. Lugrin and Martinon (2018) found that activated AIM2 induces apoptotic caspase-8, resulting in caspase-1-mediated cell death in caspase-1-deficient mice *via* a process similar to apoptosis. A study showed that rats with DM exhibited high AIM2 protein and cardiac dysfunction. The inhibition of AIM2 reduces caspase-1, IL-1β, and collagen I and III of heart tissue. Of note, high levels of ROS can activate AIM2 (Wang X. et al., 2019). Previous studies have shown that prolonged ROS induced by hyperglycemia impairs mitochondrial function and decreases mitochondrial DNA copy number (mtDNA-CN) in retinal ECs (Al-Kafaji et al., 2016). The mtDNA-CN contains coding genes, which encode crucial proteins maintaining normal function of the mitochondria. The mtDNA-CN indicates the quantity of the mitochondria (Al-Kafaji et al., 2018). The mtDNA is released during mitochondrial stress caused by potentially dietary lipid overload or infection, leading to the formation of the AIM2 inflammasome (Sharma et al., 2019). The possibility is that ROS induced by hyperglycemia damage the mitochondria, subsequently inducing increased cytoplasmic mtDNA and finally activating AIM2. Oxidized low-density lipoprotein activates AIM2. However, compared with the NLRP3 inflammasome in DbCM, the pathways associated

with the induction of myocardial fibrosis by AIM2 remain unknown. The association between TGF-β and AIM2 needs to be further investigated.

NcRNA REGULATION

Recently, ncRNAs have attracted considerable attention due to their ability to regulate gene expression. NcRNAs are translated from genome and regulate several cellular events *via* regulating the genetic network to affect protein effectors, including differentiation, cell metabolism, transcription, and proliferation (Wang J. et al., 2019). Increasing studies have exhibited the role of ncRNAs in the pathogenesis of pyroptosis in the diabetic heart, including microRNA (miRNA), circular RNA (circRNA), and long ncRNA (lncRNA) (Zhang et al., 2019).

MicroRNAs are endogenous ncRNAs with nearly 22 nt RNAs that regulate gene expression by targeting mRNAs for degradation or translational repression (Bartel, 2004). The research of miRNAs began in 1993, and now miRNAs serves as important therapeutic targets for several diseases (Almeida et al., 2011). Of note, about 90% of the total miRNAs in the human heart are constituted of 18 miRNA families and linked with cardiac diseases (Kumarswamy and Thum, 2013). Numerous miRNAs are involved in the process of DbCM, such as regulating cardiac hypertrophy, myocardial fibrosis, inflammation, and pyroptosis (Zhang et al., 2019). Remarkably, recent studies have revealed the KCNQ1 opposite strand/antisense transcript 1 (Kcnq1ot1) regulates pyroptosis in DbCM. MiRNAs associated with pyroptosis include miR-30d, miR-21-3p, and miR-9. Pan et al. (2013) found that increased circulating miR-30 can be used to diagnose cardiac hypertrophy. In high glucose, miR-30d is upregulated in cardiomyocytes with remarkably increased expression of caspase-1 and downregulation of Forkhead box O3 (foxo3a). Mir-30d directly targets Foxo3a, a crucial regulator of the cell cycle, oxidative clearance, and apoptosis. Elevated miR-30d in diabetic hearts suppresses foxo3a and caspase recruitment domain (ARC) expression and enhances the expression of inflammatory molecules to promote pyroptosis. As an miR-30d downstream protein, ARC was proven to protect cardiomyocytes against oxidative stress and doxorubicin cardiotoxicity (Zhang and Herman, 2006; An et al., 2009). Therefore, cardiomyocyte pyroptosis could be induced by miR–30d/foxo3a/ARC/caspase-1 pathway (Li et al., 2014). Androgen receptor (AR), mir-21-3p, NLRP3, and caspase-1 were elevated in high-glucose-treated cardiac fibroblasts. Furthermore, the expression of NLRP3 and caspase-1 decreased after silencing miR-21-3p. A previous study found that miR-21-3p can enhance caspase-1 and NLRP3 expression to aggravate pyroptosis of diabetic cardiac fibroblasts by suppressing AR (Shi et al., 2021). AR, a kind of steroid hormone receptor, plays a vital role in maintaining the structure and function of the cardiac tissue. ARs in myocardial tissues recognize androgens to regulate the cardiac phenotype and hypertrophy (Huang et al., 2016). Moreover, miR-9 plays a potential role in pyroptosis through targeting ELAVL1. The downregulation of miR-9 in diabetic heart corresponds with an increase of ELAVL1, caspase-1, and IL-1β, while miR-9 mimic transfection inhibits their expression (Jeyabal et al., 2016). These findings may validate the modulation of miR-9 in ELAVL1-dependent pyroptosis.

Long non-coding RNAs are widely defined as RNAs that are longer than 200 nt (Shi et al., 2013). CircRNAs are characterized by covalently closed loop structures without 3′ tails and 5′ caps (Chen and Yang, 2015). The competitive endogenous RNA (ceRNA) theory explains how lncRNAs and circRNA function as sponging miRNAs (Tay et al., 2014). Studies have reported the ceRNAs in the process of pyroptosis. Wu et al. (2021) found that increased metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) enhances pyroptosis in high-glucose-induced cardiomyocytes *via* binding to miR-141-3p, which indicates that MALAT1 potentially has an impact in the pyroptosis of DbCM. Moreover, increased MALAT1 was found to enhance cardiac apoptosis in diabetic mice. The suppression of MALAT1 showed the opposite effects (Wang et al., 2021). Therefore, the knockdown of MELAT1 may effectively reduce myocardial injuries in DbCM. However, no distinctive evidence points out that MALAT1 mainly promotes pyroptosis or apoptosis in DbCM. With regard to the experiment using high-glucose-induced cardiomyocytes by Wu et al. (2021) further experiments on animal models are needed to learn more about the role of MALAT1 in DbCM. Kcnq1ot1, a kind of lncRNA, is present on human chromosome 11p15.5 (Kanduri, 2011). Kcnq1ot1 downregulates multiple genes located in the kcnq1ot1 domain *via* DNA-modifying proteins, and recruitment of chromatin. Kcnq1ot1 is closely associated with many heart disorders. Lai et al. (2020) indicated that Kcnq1ot1 regulates cardiomyocyte apoptosis in heart failure by targeting fusion in sarcoma. Li et al. (2017) elucidated that the down-regulation of Kcnq1ot1 inhibits myocardial ischemia reperfusion injury with acute myocardial infarction. In addition, emerging studies have proven that Kcnq1ot1 is closely related with pyroptosis in DbCM. Kcnq1ot1 is upregulated in the diabetic heart, which increases caspase-1 by targeting miR-214-3p. Silencing Kcnq1ot1 alleviates DNA fractionation and reduces calcium overload and hyperglycemia-treated cardiac fibroblasts (Yang et al., 2018a). Elevated caspase-1-associated circRNAs (CACR) in cardiomyocytes induced by high glucose also drive pyroptosis by targeting miR-214-3p. The knockdown of CACR can ameliorate caspase-1 activation. Meanwhile, CACR is also increased in the serum of patients with DM (Yang et al., 2019a), indicating that it can be used as a biomarker for DbCM. LncRNA growth arrest-specific transcript 5 (GAS5) is repressed in cardiomyocytes in DbCM. GAS5 is closely related to cardiovascular diseases. Wang et al. (2016) elucidated that GAS5 promotes hypertension-induced vascular remodeling. Moreover, Liu et al. (2019) found that the overexpression of GAS5 decreases cardiac fibrosis in mice *via* the PTEN/MMP-2 pathway. Xu et al. (2020) found that GAS5 is repressed in the cardiomyocytes of mice with DbCM, the upregulation of which enhances cardiac function and inhibits NLRP3 and caspase-1. Highly expressed GAS5 represses the expression of miR-34b-3p. Meanwhile elevated expression of miR-34b-3p induces the expression of aryl hydrocarbon receptor (AHR). Thus, this study suggested that GAS5 overexpression inhibits NLRP3-mediated pyroptosis *via* the miR-34b-3p/AHR

pathway (Xu et al., 2020). As a negative regulator of NLRP3, AHR binds to the xenobiotic response element regions to block NF-κB to inhibit the expression of NLRP3 (Huai et al., 2014). Therefore, GAS5 and AHR may be vital targets for the treatment of DbCM.

The process of cardiac fibrosis is a vital event in DbCM. Tang et al., indicated that circRNA-000203 is over-expressed in the diabetic myocardium, which increases the expression of α-SMA, Col1a2, and Col3a1 in cardiac fibroblasts. Zhou and Yu (2017) suggested that another circRNA could promote myocardial fibrosis. They found that circRNA_010567 modulates miR-141 and the expression of TGF-β1, mediating fibrosis-associated protein resection (Zhou and Yu, 2017). However, compared with lncRNAs and miRNAs, we know less about the molecular mechanisms of circRNA in DbCM.

Taken together, the upregulation and downregulation of these ncRNAs in cardiomyocytes play an important role in the progression of DbCM. Moreover, the network of lncRNA/circRNA–miRNA–mRNA may provide insights into the therapeutic targets for DbCM (Li et al., 2019).

AUTOPHAGY REGULATION

Recent studies indicated the potential interplay between pyroptosis and other forms of death. Zhang et al. (2020) found that LC3-II/I is reduced and p62 is increased in the myocardium of diabetic rats, suggesting that autophagy is inhibited. Along with suppressive autophagy, increased NLRP3 inflammasome is detected. Further study demonstrated that rapamycin, an autophagy activator, enhances the level of LC3-II/I, decreases p62, and inhibits the activation of NLRP3 inflammasome to alleviate myocardium injury. In addition, autophagy can clear injured tissues and stress products such as ROS, which can induce NLRP3 inflammasome activation. Therefore, autophagy can inhibit NLRP3 inflammasome induced by high glucose, which can be a new target for DbCM treatment (Zhang et al., 2020). However, how high glucose suppresses autophagy and whether high glucose promotes cells undergoing autophagy to embark in pyroptosis remain unknown. Song et al. (2021) found that sirtuin3 (SIRT3) is inhibited in the myocardium of diabetic mice, which further triggers severe cardiac injury. The level of necroptotic-related proteins receptor-interacting protein kinase (RIPK) 1/3 is enhanced in cardiomyocytes of SIRT3-knockout diabetic mice. Moreover, the expression of NLRP3 inflammasome in SIRT3-knockout diabetic mice is much higher than that in wild-type diabetic mice (Song et al., 2021). Furthermore, previous studies confirmed that PIPK3 participates in inflammasome activation and cytokine release. The PIPK1-PIPK3 cell death platform called necrosome forms after some related receptors are activated. Then, PIPK3 phosphorylates its downstream enzyme mixed lineage kinase domain-like protein, which combines to membrane and forms membrane pores. This results in the release of cytoplasmic contents and DAMPs, further triggering NLRP3 inflammasome (Frank and Vince, 2019). Evidence proves that ROS can activate necroptosis, which indicates the promotional function of pyroptosis to necroptosis in DbCM.

DRUG REGULATION

Pyroptosis is detrimental to the heart and speeds up the progression of DbCM. In this review, we summarized several treatment ideas to alleviate DbCM by hindering pyroptosis.

First, initial glycemia and metabolic disorders induce pyroptosis in cardiomyocytes, which may be prevented by early use of hypoglycemia drugs to protect cardiomyocytes. For example, previous studies have reported that metformin, an antidiabetic drug, could play a cardioprotective role. Studies have shown that metformin can upregulate the phosphorylation of AMP-activated protein kinase (AMPK), which develops glycolysis to maintain cellular energy balance (Choi and Park, 2013; Driver et al., 2018). In addition, Yang et al. (2019a) implicated that metformin represses the NLRP3 inflammasome *via* the AMPK/mTOR pathway in DbCM. Empagliflozin highly selectively inhibits sodium glucose cotransporter-2. Xue et al. (2019) suggested that empagliflozin inhibits cardiomyopathy through the SGC-CGMP-PKG pathway in mice with type 2 diabetes. Clinical trials have shown the efficiency of empagliflozin in patients with type 2 diabetes (Frampton, 2018; Xue et al., 2019).

Second, the inhibition of inflammasomes offers potential therapeutic targets for DbCM, including transcriptional suppression of inflammasome components, assembling prevention and blockade of active caspase-1 and GSDMD cleavage (Zeng et al., 2019). The NF-κB inhibitor BAY-11-7082 decreases the expression of inflammasome components in cardiomyocytes treated with high glucose. On the basis of previous reports, BAY-11-7082 exhibits strong anti-inflammatory effects by inhibiting κB kinase of NLRP3 priming process and reducing the translocation of NF-κB subunit (Lee et al., 2012). Regarded as the second messenger in the activation of NLRP3, ROS may affect NF-κB signaling and later assembly process. In cardiomyocytes treated with high glucose, increased ROS can promote thioredoxin interacting protein, which binds NLRP3 inflammasome directly and promotes its assembly (Luo et al., 2014). Studies have found that the ROS scavenger Eukarion (EUK-134) can block inflammasome dissociating TXNIP and NLRP3 inflammasome (Sho and Xu, 2019). Skimmin has recently been found to play a significant role in DbCM. It can reduce blood glucose and improve fibrosis in diabetic rats. Of note, skimmin enhances autophagy, which diminishes ROS generation and inflammatory reaction in a diabetic heart with decreased pyroptosis-related cytokines (Liang et al., 2021). Skimmin could serve as a promising protective medicine in DbCM. Previously, EUK134 has been synthesized and tested n heart protection as a mitochondria-targeted antioxidant (Ajith and Jayakumar, 2014). In this way, mitochondrial-targeted agents have great potential for cardioprotection. Novel NLRP3 inhibitors were recently found to inhibit inflammasome assembly. ATP is indispensable in the role of NIMA (never in mitosis gene)-related kinase 7 (NEK7) in activating inflammasome. The cryo-EM structure of NLRP3 in humans can bind to NEK7, a ser/thr kinase. NEK7 is phosphorylated to make NLRP3 active conformation when ATP binds to the NBD domain (Neha et al., 2019). Thus, according to the process, inhibitors of the inflammasome are produced by blocking the interaction of NEK7 and NLRP3 and active

conformation for copolymerization, which prevents pyroptosis (El-Sharkawy et al., 2020). Parthenolide and 3,4-methylenedioxy-β-nitrostyrene suppress the ATPase of NLRP3 and thus inhibit the interaction of NEK7 and NLRP3. Caspase-1 inhibitors have also been researched widely. VX765 was demonstrated to inhibit the expression of GSDMD and IL-1β in the brain of mice (Xu et al., 2019). However, further experiments are required to determine the effects of caspase-1 inhibitors such as VX765 in cardiomyocytes. Necrosulfonamide can bind to GSDMD directly and suppress membrane pore formation and IL-1β release in murine monocytes/macrophages, which provides ideas for the treatment of DbCM (Rathkey et al., 2018). Exogenous bone morphogenetic protein-7 was proved to inhibit NLRP3-related cell deaths and inflammatory response in a diabetic heart. Moreover, it improved heart remodeling, which makes it useful in several heart diseases related to adverse heart remodeling (Elmadbouh and Singla, 2021).

Third, the upregulation and downregulation of related ncRNAs also serve as a target. As AAV9-shNlrp3 alleviates atrial fibrillation, specific shRNAs may exert an influence on DbCM amid silencing the gene of caspase-1, NLRP3, and ASC (Zeng et al., 2019).

Fourth, the inhibition of AIM2 inflammasome provides a novel target. Connection with dsDNA is an essential factor for activation of AIM2 inflammasome. Thus, the molecules inhibiting the recognition of dsDNA and HIN domain to restrain AIM2 could be a therapeutic target of DbCM. Having a similar structure with mouse p202 human IF16-designated IFI16-β lacks a PYD but includes two HIN domains. P202 and IFI16 have higher affinity to connect dsDNA than AIM2 and prevents AIM2 oligomerization and ASC clustering by binding to HIN of AIM2 (Wang et al., 2018). CARD-only proteins and PYD-only proteins have been demonstrated to negatively regulate AIM2, which provides new ideas for the therapy of inflammatory disease (Indramohan et al., 2018). One tegument protein pUL83 released by human cytomegalovirus connects human AIM2 to attenuate AIM2 inflammasome activation (Huang et al., 2017). Likewise, VP22 released by herpes simplex virus-1 has been shown to inhibit human AIM2 (Maruzuru et al., 2018; **Table 1**).

CONCLUSION AND OUTLOOK

Diabetic cardiomyopathy is a prevalent disease worldwide causing heart failure in patients with diabetes. Pyroptosis features the formation of membrane pore and leakage of proinflammatory intracellular contents such as IL-1β and IL-18, which is mainly activated by caspase-1 signaling pathways. Previous and emerging studies have shown that pyroptosis could be triggered by the canonical pathway with the involvement of caspase-1 and non-canonical pathway induced by caspase-11/4/5. In canonical inflammasome pathway, NLRP1/3, NLRC4, and AIM2 are triggered by sensing variety of DAMPs and subsequently collecting ASC, which recruits pro-caspase-1. In the non-canonical pathway, related caspase family members directly recognize LPS without the involvement of inflammasome assembly. Several factors including abnormal nutrient metabolism such as glucose and lipid; production of toxic metabolic waste products; and imbalanced electrolytes such as $Ca^{2+}$and cell death such as apoptosis and necrosis contribute to DbCM. Evidence has supported that pyroptosis is involved in the pathogenesis of DbCM. Related studies found the interplay between pyroptosis and other kinds of cell death such as autophagy and necroptosis. Activated autophagy inhibits NLRP3 inflammasome activation to repress pyroptosis. Moreover, necroptosis and pyroptosis could promote each other. In our review, we summarized the regulations of pyroptosis in DbCM, including NLRP3 inflammasome, lncRNAs, miRNAs, circRNAs, and AIM2 inflammasome. As a molecular marker, NLRP3 inflammasome plays a significant role in DbCM. NF-κB modulates NLRP3, and mitochondrial dysfunction and overproduction of ROS are also involved in the activation of NLPR3 inflammation activation. Various miRNAs, circRNAs, and lcnRNAs such as MALAT1, Kcnq1ot1, and GAS5 have been proved to regulate pyroptosis in DbCM *via* different pathways. Given the regulatory mechanisms that participate in pyroptosis, hypoglycemic drugs and gene targeting may be new treatment in the future. However, the role of pyroptosis in DbCM is still not very clear. In accordance with numerous studies, there are closed crosslinks between pyroptosis and apoptosis. Apoptosis could serve as the second signal of NLRP3 activation under ER stress. Caspase-3 is also proved to participate in pyroptosis. Moreover, apoptosis does not release cytokines and apoptotic cells that send signals to attract macrophage. While pyroptosis enlarges inflammatory size, it is seen as a kind of dirty cell death way. Therefore, the characteristic molecule to distinguish pyroptosis and apoptosis may be gasdermin family members such as GSDMD and GSDME, which promote the formation of membrane pores to facilitate pyroptosis. And the point is, what can regulate caspase-3 to promote GSDME? However, no study has proved that caspase-3 induces GSDME in cardiomyocytes treated with high glucose. Does ROS play a role in increasing cytoplasmic mtDNA to activate AIM2 inflammasome by impairing mitochondria? Further studies are needed to determine the molecular mechanisms. Some viral proteins could bind to AIM2 to inhibit AIM2 inflammasome formation, which may alleviate pyroptosis in DbCM. Given this, related vaccinations would be used in the treatment of DbCM in the future.

AUTHOR CONTRIBUTIONS

YFL and YQL wrote this review. ZW and JM modified this review. All authors contributed to the article and approved it for publication.

FUNDING

This study was supported by the National Natural Science Foundation of China (Grant No. 81970389), the Natural Science Foundation of Hunan Province China (Grant No. 2021JJ30626), and Scientific Research Project of Hunan Provincial Department of Education (Grant No. 18B283).

REFERENCES

Aachoui, Y., Sagulenko, V., Miao, E. A., and Stacey, K. J. (2013). Inflammasome-mediated pyroptotic and apoptotic cell death, and defense against infection. *Curr. Opin. Microbiol.* 16, 319–326. doi: 10.1016/j.mib.2013.04.004

Ajith, T. A., and Jayakumar, T. G. (2014). Mitochondria-targeted agents: future perspectives of mitochondrial pharmaceutics in cardiovascular diseases. *World J. Cardiol.* 6, 1091–1099. doi: 10.4330/wjc.v6.i10.1091

Al-Kafaji, G., Aljadaan, A., Kamal, A., and Bakhiet, M. (2018). Peripheral blood mitochondrial DNA copy number as a novel potential biomarker for diabetic nephropathy in type 2 diabetes patients. *Exp. Ther. Med.* 16, 1483–1492. doi: 10.3892/etm.2018.6319

Al-Kafaji, G., Sabry, M. A., and Skrypnyk, C. (2016). Time-course effect of high-glucose-induced reactive oxygen species on mitochondrial biogenesis and function in human renal mesangial cells. *Cell Biol. Int.* 40, 36–48.

Almeida, M. I., Reis, R. M., and Calin, G. A. (2011). MicroRNA history: discovery, recent applications, and next frontiers. *Mutat. Res.* 717, 1–8. doi: 10.1016/j.mrfmmm.2011.03.009

Alyaseer, A. A. A., de Lima, M. H. S., and Braga, T. T. (2020). The role of NLRP3 inflammasome activation in the epithelial to mesenchymal transition process during the fibrosis. *Front. Immunol.* 11:883. doi: 10.3389/fimmu.2020.00883

An, J., Li, P., Li, J., Dietz, R., and Donath, S. (2009). ARC is a critical cardiomyocyte survival switch in doxorubicin cardiotoxicity. *J. Mol. Med.* 87, 401–410. doi: 10.1007/s00109-008-0434-z

Bai, B., Yang, Y., Wang, Q., Li, M., Tian, C., Liu, Y., et al. (2020). NLRP3 inflammasome in endothelial dysfunction. *Cell Death Dis.* 11:776. doi: 10.1038/s41419-020-02985-x

Bartel, D. P. (2004). MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 116, 281–297. doi: 10.1016/S0092-8674(04)00045-5

Boucher, D., Monteleone, M., Coll, R. C., Chen, K. W., Ross, C. M., Teo, J. L., et al. (2018). Caspase-1 self-cleavage is an intrinsic mechanism to terminate inflammasome activity. *J. Exp. Med.* 215, 827–840. doi: 10.1084/jem.20172222

Broz, P., and Dixit, V. M. (2016). Inflammasomes: mechanism of assembly, regulation and signalling. *Nat. Rev. Immunol.* 16, 407–420. doi: 10.1038/nri.2016.58

Bugger, H., and Abel, E. D. (2014). Molecular mechanisms of diabetic cardiomyopathy. *Diabetologia* 57, 660–671. doi: 10.1007/s00125-014-3171-6

Cai, L., and Kang, Y. J. (2003). Cell death and diabetic cardiomyopathy. *Cardiovasc. Toxicol.* 3, 219–228. doi: 10.1385/CT:3:3:219

Cao, X., Zhang, L., Chen, C., Wang, Q., Guo, L., Ma, Q., et al. (2017). The critical role of ABCG1 and PPARgamma/LXRalpha signaling in TLR4 mediates inflammatory responses and lipid accumulation in vascular smooth muscle cells. *Cell Tissue Res.* 368, 145–157. doi: 10.1007/s00441-016-2518-3

Che, H., Wang, Y., Li, H., Li, Y., Sahil, A., Lv, J., et al. (2020). Melatonin alleviates cardiac fibrosis via inhibiting lncRNA MALAT1/miR-141-mediated NLRP3 inflammasome and TGF-β1/Smads signaling in diabetic cardiomyopathy. *FASEB J.* 34, 5282–5298. doi: 10.1096/fj.201902692R

Chen, H., Lu, Y., Cao, Z., Ma, Q., Pi, H., Fang, Y., et al. (2016). Cadmium induces NLRP3 inflammasome-dependent pyroptosis in vascular endothelial cells. *Toxicol. Lett.* 246, 7–16. doi: 10.1016/j.toxlet.2016.01.014

Chen, L. L., and Yang, L. (2015). Regulation of circRNA biogenesis. *RNA Biol.* 12, 381–388. doi: 10.1080/15476286.2015.1020271

Chen, Y., Hua, Y., Li, X., Arslan, I. M., Zhang, W., and Meng, G. (2020). Distinct types of cell death and the implication in diabetic cardiomyopathy. *Front. Pharmacol.* 11:42. doi: 10.3389/fphar.2020.00042

Chen, Y., Wang, L., Pitzer, A. L., Li, X., Li, P. L., and Zhang, Y. (2016). Contribution of redox-dependent activation of endothelial Nlrp3 inflammasomes to hyperglycemia-induced endothelial dysfunction. *J. Mol. Med.* 94, 1335–1347. doi: 10.1007/s00109-016-1481-5

Choi, Y. K., and Park, K. G. (2013). Metabolic roles of AMPK and metformin in cancer cells. *Mol. Cells* 36, 279–287. doi: 10.1007/s10059-013-0169-8

Cookson, B. T., and Brennan, M. A. (2001). Pro-inflammatory programmed cell death. *Trends Microbiol.* 9, 113–114. doi: 10.1016/S0966-842X(00)01936-3

Cyr, A. R., Huckaby, L. V., Shiva, S. S., and Zuckerbraun, B. S. (2020). Nitric oxide and endothelial dysfunction. *Crit. Care Clin.* 36, 307–321. doi: 10.1016/j.ccc.2019.12.009

D'Arcy, M. S. (2019). Cell death: a review of the major forms of apoptosis, necrosis and autophagy. *Cell Biol. Int.* 43, 582–592. doi: 10.1002/cbin.11137

Ding, J., Wang, K., Liu, W., She, Y., Sun, Q., Shi, J., et al. (2016). Pore-forming activity and structural autoinhibition of the gasdermin family. *Nature* 535, 111–116. doi: 10.1038/nature18590

Dolasia, K., Bisht, M. K., Pradhan, G., Udgata, A., and Mukhopadhyay, S. (2018). TLRs/NLRs: Shaping the landscape of host immunity. *Int. Rev. Immunol.* 37, 3–19. doi: 10.1080/08830185.2017.1397656

Driver, C., Bamitale, K. D. S., Kazi, A., Olla, M., Nyane, N. A., and Owira, P. M. O. (2018). Cardioprotective effects of metformin. *J. Cardiovasc. Pharmacol.* 72, 121–127. doi: 10.1097/FJC.0000000000000599

Elmadbouh, I., and Singla, D. K. (2021). BMP-7 attenuates inflammation-induced pyroptosis and improves cardiac repair in diabetic cardiomyopathy. *Cells* 10:2640. doi: 10.3390/cells10102640

El-Sharkawy, L. Y., Brough, D., and Freeman, S. (2020). Inhibiting the NLRP3 Inflammasome. *Molecules* 25:5533. doi: 10.3390/molecules25235533

Ewald, S. E., Chavarria-Smith, J., and Boothroyd, J. C. (2014). NLRP1 is an inflammasome sensor for Toxoplasma gondii. *Infect. Immun.* 82, 460–468. doi: 10.1128/IAI.01170-13

Fang, Y., Tian, S., Pan, Y., Li, W., Wang, Q., Tang, Y., et al. (2020). Pyroptosis: a new frontier in cancer. *Biomed. Pharmacother.* 121:109595. doi: 10.1016/j.biopha.2019.109595

Fink, S. L., Bergsbaken, T., and Cookson, B. T. (2008). Anthrax lethal toxin and *Salmonella* elicit the common cell death pathway of caspase-1-dependent pyroptosis via distinct mechanisms. *Proc. Natl. Acad. Sci. U.S.A.* 105, 4312–4317. doi: 10.1073/pnas.0707370105

Fink, S. L., and Cookson, B. T. (2006). Caspase-1-dependent pore formation during pyroptosis leads to osmotic lysis of infected host macrophages. *Cell. Microbiol.* 8, 1812–1825. doi: 10.1111/j.1462-5822.2006.00751.x

Fiorentino, T. V., Prioletta, A., Zuo, P., and Folli, F. (2013). Hyperglycemia-induced oxidative stress and its role in diabetes mellitus related cardiovascular diseases. *Curr. Pharm. Des.* 19, 5695–5703. doi: /10.2174/1381612811319320005

Frampton, J. E. (2018). Empagliflozin: a review in Type 2 Diabetes. *Drugs* 78, 1037–1048. doi: 10.1007/s40265-018-0937-z

Franchi, L., Eigenbrod, T., Muñoz-Planillo, R., and Nuñez, G. (2009). The inflammasome: a caspase-1-activation platform that regulates immune responses and disease pathogenesis. *Nat. Immunol.* 10, 241–247.

Frank, D., and Vince, J. E. (2019). Pyroptosis versus necroptosis: similarities, differences, and crosstalk. *Cell Death Differ.* 26, 99–114. doi: 10.1038/s41418-018-0212-6

Frustaci, A., Kajstura, J., Chimenti, C., Jakoniuk, I., Leri, A., Maseri, A., et al. (2000). Myocardial cell death in human diabetes. *Circ. Res.* 87, 1123–1132. doi: 10.1161/01.RES.87.12.1123

Grebe, A., Hoss, F., and Latz, E. (2018). NLRP3 inflammasome and the IL-1 pathway in atherosclerosis. *Circ. Res.* 122, 1722–1740. doi: 10.1161/CIRCRESAHA.118.311362

He, Y., Hara, H., and Núñez, G. (2016). Mechanism and Regulation of NLRP3 inflammasome activation. *Trends Biochem. Sci.* 41, 1012–1021. doi: 10.1016/j.tibs.2016.09.002

Huai, W., Zhao, R., Song, H., Zhao, J., Zhang, L., Zhang, L., et al. (2014). Aryl hydrocarbon receptor negatively regulates NLRP3 inflammasome activity by inhibiting NLRP3 transcription. *Nat. Commun.* 5:4738. doi: 10.1038/ncomms5738

Huang, C. K., Lee, S. O., Chang, E., Pang, H., and Chang, C. (2016). Androgen receptor (AR) in cardiovascular diseases. *J. Endocrinol.* 229, R1–R16. doi: 10.1530/JOE-15-0518

Huang, Y., Ma, D., Huang, H., Lu, Y., Liao, Y., Liu, L., et al. (2017). Interaction between HCMV pUL83 and human AIM2 disrupts the activation of the AIM2 inflammasome. *Virol. J.* 14:34. doi: 10.1186/s12985-016-0673-5

Indramohan, M., Stehlik, C., and Dorfleutner, A. (2018). COPs and POPs patrol inflammasome activation. *J. Mol. Biol.* 430, 153–173. doi: 10.1016/j.jmb.2017.10.004

Jeyabal, P., Thandavarayan, R. A., Joladarashi, D., Suresh Babu, S., Krishnamurthy, S., Bhimaraj, A., et al. (2016). MicroRNA-9 inhibits hyperglycemia-induced pyroptosis in human ventricular cardiomyocytes by targeting ELAVL1. *Biochem. Biophys. Res. Commun.* 471, 423–429. doi: 10.1016/j.bbrc.2016.02.065

Jia, G., DeMarco, V. G., and Sowers, J. R. (2016). Insulin resistance and hyperinsulinaemia in diabetic cardiomyopathy. *Nat. Rev. Endocrinol.* 12, 144–153. doi: 10.1038/nrendo.2015.216

Jia, G., Whaley-Connell, A., and Sowers, J. R. (2018a). Diabetic cardiomyopathy: a hyperglycaemia- and insulin-resistance-induced heart disease. *Diabetologia* 61, 21–28. doi: 10.1007/s00125-017-4390-4

Jia, G., Hill, M. A., and Sowers, J. R. (2018b). Diabetic Cardiomyopathy: an update of mechanisms contributing to this clinical entity. *Circ. Res.* 122, 624–638. doi: 10.1161/CIRCRESAHA.117.311586

Jorgensen, I., and Miao, E. A. (2015). Pyroptotic cell death defends against intracellular pathogens. *Immunol. Rev.* 265, 130–142. doi: 10.1111/imr.12287

Kanduri, C. (2011). Kcnq1ot1: a chromatin regulatory RNA. *Semin. Cell Dev. Biol.* 22, 343–350. doi: 10.1016/j.semcdb.2011.02.020

Kar, S., Shahshahan, H. R., Hackfort, B. T., Yadav, S. K., Yadav, R., Kambis, T. N., et al. (2019). Exercise training promotes cardiac hydrogen sulfide biosynthesis and mitigates pyroptosis to prevent high-fat diet-induced diabetic cardiomyopathy. *Antioxidants* 8:638. doi: 10.3390/antiox8120638

Kawane, K., Motani, K., and Nagata, S. (2014). DNA degradation and its defects. *Cold Spring Harb. Perspect. Biol.* 6:a016394. doi: 10.1101/cshperspect.a016394

Kayagaki, N., Stowe, I. B., Lee, B. L., O'Rourke, K., Anderson, K., Warming, S., et al. (2015). Caspase-11 cleaves gasdermin D for non-canonical inflammasome signalling. *Nature* 526, 666–671. doi: 10.1038/nature15541

Kelley, N., Jeltema, D., Duan, Y., and He, Y. (2019). The NLRP3 inflammasome: an overview of mechanisms of activation and regulation. *Int. J. Mol. Sci.* 20:3328. doi: 10.3390/ijms20133328

Kumarswamy, R., and Thum, T. (2013). Non-coding RNAs in cardiac remodeling and heart failure. *Circ. Res.* 113, 676–689. doi: 10.1161/CIRCRESAHA.113.3002261

Lai, L., Xu, Y., Kang, L., Yang, J., and Zhu, G. (2020). LncRNA KCNQ1OT1 contributes to cardiomyocyte apoptosis by targeting FUS in heart failure. *Exp. Mol. Pathol.* 115:104480. doi: 10.1016/j.yexmp.2020.104480

Lee, J., Rhee, M. H., Kim, E., and Cho, J. Y. (2012). BAY 11-7082 is a broad-spectrum inhibitor with anti-inflammatory activity against multiple targets. *Mediators Inflamm.* 2012:416036. doi: 10.1155/2012/416036

Lee, W. S., and Kim, J. (2017). Diabetic cardiomyopathy: Where we are and where we are going. *Korean J. Intern. Med.* 32, 404–421. doi: 10.3904/kjim.2016.208

Li, M., Duan, L., Li, Y., and Liu, B. (2019). Long noncoding RNA/circular noncoding RNA-miRNA-mRNA axes in cardiovascular diseases. *Life Sci.* 233:116440. doi: 10.1016/j.lfs.2019.04.066

Li, X., Dai, Y., Yan, S., Shi, Y., Han, B., Li, J., et al. (2017). Down-regulation of lncRNA KCNQ1OT1 protects against myocardial ischemia/reperfusion injury following acute myocardial infarction. *Biochem. Biophys. Res. Commun.* 491, 1026–1033. doi: 10.1016/j.bbrc.2017.08.005

Li, X., Du, N., Zhang, Q., Li, J., Chen, X., Liu, X., et al. (2014). MicroRNA-30d regulates cardiomyocyte pyroptosis by directly targeting foxo3a in diabetic cardiomyopathy. *Cell Death Dis.* 5:e1479. doi: 10.1038/cddis.2014.430

Liang, R. K., Zhao, Y. Y., Shi, M. L., Zhang, G., Zhao, Y. J., Zhang, B. G., et al. (2021). Skimmin protects diabetic cardiomyopathy in streptozotocin-induced diabetic rats. *Kaohsiung J. Med. Sci.* 37, 136–144. doi: 10.1002/kjm2.12305

Liu, H. L., Chen, C. H., and Sun, Y. J. (2019). Overexpression of lncRNA GAS5 attenuates cardiac fibrosis through regulating PTEN/MMP-2 signal pathway in mice. *Eur. Rev. Med. Pharmacol. Sci.* 23, 4414–4418.

Liu, S., Li, W., Xu, M., Huang, H., Wang, J., and Chen, X. (2014). Micro-RNA 21Targets dual specific phosphatase 8 to promote collagen synthesis in high glucose-treated primary cardiac fibroblasts. *Can. J. Cardiol.* 30, 1689–1699. doi: 10.1016/j.cjca.2014.07.747

Liu, X., Zhang, Z., Ruan, J., Pan, Y., Magupalli, V. G., Wu, H., et al. (2016). Inflammasome-activated gasdermin D causes pyroptosis by forming membrane pores. *Nature* 535, 153–158. doi: 10.1038/nature18629

Lugrin, J., and Martinon, F. (2018). The AIM2 inflammasome: sensor of pathogens and cellular perturbations. *Immunol. Rev.* 281, 99–114. doi: 10.1111/imr.12618

Luo, B., Huang, F., Liu, Y., Liang, Y., Wei, Z., Ke, H., et al. (2017). NLRP3 inflammasome as a molecular marker in diabetic cardiomyopathy. *Front. Physiol.* 8:519. doi: 10.3389/fphys.2017.00519

Luo, B., Li, B., Wang, W., Liu, X., Xia, Y., Zhang, C., et al. (2014). NLRP3 gene silencing ameliorates diabetic cardiomyopathy in a type 2 diabetes rat model. *PLoS One* 9:e104771. doi: 10.1371/journal.pone.0104771

Malik, A., and Kanneganti, T. D. (2017). Inflammasome activation and assembly at a glance. *J. Cell Sci.* 130, 3955–3963. doi: 10.1242/jcs.207365

Man, S. M., and Kanneganti, T. D. (2015). Regulation of inflammasome activation. *Immunol. Rev.* 265, 6–21. doi: 10.1111/imr.12296

Man, S. M., Karki, R., and Kanneganti, T. D. (2017). Molecular mechanisms and functions of pyroptosis, inflammatory caspases and inflammasomes in infectious diseases. *Immunol. Rev.* 277, 61–75. doi: 10.1111/imr.12534

Martins, I., Raza, S. Q., Voisin, L., Dakhli, H., Law, F., De Jong, D., et al. (2017). Entosis: the emerging face of non-cell-autonomous type IV programmed death. *Biomed. J.* 40, 133–140. doi: 10.1016/j.bj.2017.05.001

Maruzuru, Y., Ichinohe, T., Sato, R., Miyake, K., Okano, T., Suzuki, T., et al. (2018). Herpes Simplex Virus 1 VP22 Inhibits AIM2-Dependent Inflammasome Activation to Enable Efficient Viral Replication. *Cell Host Microbe* 23, 254–265.e7. doi: 10.1016/j.chom.2017.12.014

Murtaza, G., Virk, H. U. H., Khalid, M., Lavie, C. J., Ventura, H., Mukherjee, D., et al. (2019). Diabetic cardiomyopathy - A comprehensive updated review. *Prog. Cardiovasc. Dis.* 62, 315–326. doi: 10.1016/j.pcad.2019.03.003

Neha, K., Haider, M. R., Pathak, A., and Yar, M. S. (2019). Medicinal prospects of antioxidants: a review. *Eur. J. Med. Chem.* 178, 687–704.

Pan, W., Zhong, Y., Cheng, C., Liu, B., Wang, L., Li, A., et al. (2013). MiR-30-regulated autophagy mediates angiotensin II-induced myocardial hypertrophy. *PLoS One* 8:e53950. doi: 10.1371/journal.pone.0053950

Pandeya, A., Li, L., Li, Z., and Wei, Y. (2019). Gasdermin D (GSDMD) as a new target for the treatment of infection. *Medchemcomm* 10, 660–667. doi: 10.1039/C9MD00059C

Paolillo, S., Marsico, F., Prastaro, M., Renga, F., Esposito, L., De Martino, F., et al. (2019). Diabetic cardiomyopathy: definition, diagnosis, and therapeutic implications. *Heart Fail. Clin.* 15, 341–347. doi: 10.1016/j.hfc.2019.02.003

Qiu, Z., Lei, S., Zhao, B., Wu, Y., Su, W., Liu, M., et al. (2017). NLRP3 inflammasome activation-mediated pyroptosis aggravates myocardial ischemia/reperfusion injury in diabetic rats. *Oxid. Med. Cell. Longev.* 2017:9743280. doi: 10.1155/2017/9743280

Ramirez, M. L. G., Poreba, M., Snipas, S. J., Groborz, K., Drag, M., and Salvesen, G. S. (2018). Extensive peptide and natural protein substrate screens reveal that mouse caspase-11 has much narrower substrate specificity than caspase-1. *J. Biol. Chem.* 293, 7058–7067. doi: 10.1074/jbc.RA117.001329

Rathinam, V. A., and Fitzgerald, K. A. (2016). Inflammasome complexes: emerging mechanisms and effector functions. *Cell* 165, 792–800. doi: 10.1016/j.cell.2016.03.046

Rathkey, J. K., Zhao, J., Liu, Z., Chen, Y., Yang, J., Kondolf, H. C., et al. (2018). Chemical disruption of the pyroptotic pore-forming protein gasdermin D inhibits inflammatory cell death and sepsis. *Sci. Immunol.* 3:eaat2738. doi: 10.1126/sciimmunol.aat2738

Rogers, C., and Alnemri, E. S. (2019). Gasdermins: novel mitochondrial pore-forming proteins. *Mol. Cell. Oncol.* 6:e1621501. doi: 10.1080/23723556.2019.1621501

Rühl, S., and Broz, P. (2015). Caspase-11 activates a canonical NLRP3 inflammasome by promoting K(+) efflux. *Eur. J. Immunol.* 45, 2927–2936. doi: 10.1002/eji.201545772

Sharif, H., Wang, L., Wang, W. L., Magupalli, V. G., Andreeva, L., Qiao, Q., et al. (2019). Structural mechanism for NEK7-licensed activation of NLRP3 inflammasome. *Nature* 570, 338–343. doi: 10.1038/s41586-019-1295-z

Sharma, A., Tate, M., Mathew, G., Vince, J. E., Ritchie, R. H., and de Haan, J. B. (2018). Oxidative stress and NLRP3-inflammasome activity as significant drivers of diabetic cardiovascular complications: therapeutic implications. *Front. Physiol.* 9:114. doi: 10.3389/fphys.2018.00114

Sharma, B. R., Karki, R., and Kanneganti, T. D. (2019). Role of AIM2 inflammasome in inflammatory diseases, cancer and infection. *Eur. J. Immunol.* 49, 1998–2011. doi: 10.1002/eji.201848070

Shi, P., Zhao, X. D., Shi, K. H., Ding, X. S., and Tao, H. (2021). MiR-21-3p triggers cardiac fibroblasts pyroptosis in diabetic cardiac fibrosis via inhibiting androgen receptor. *Exp. Cell Res.* 399:112464. doi: 10.1016/j.yexcr.2020.112464

Shi, X., Sun, M., Liu, H., Yao, Y., and Song, Y. (2013). Long non-coding RNAs: a new frontier in the study of human diseases. *Cancer Lett.* 339, 159–166. doi: 10.1016/j.canlet.2013.06.013

Sho, T., and Xu, J. (2019). Role and mechanism of ROS scavengers in alleviating NLRP3-mediated inflammation. *Biotechnol. Appl. Biochem.* 66, 4–13. doi: 10.1002/bab.1700

Sohns, W., van Veen, T. A., and van der Heyden, M. A. (2010). Regulatory roles of the ubiquitin-proteasome system in cardiomyocyte apoptosis. *Curr. Mol. Med.* 10, 1–13. doi: 10.2174/156652410791065426

Song, S., Ding, Y., Dai, G. L., Zhang, Y., Xu, M. T., Shen, J. R., et al. (2021). Sirtuin 3 deficiency exacerbates diabetic cardiomyopathy via necroptosis enhancement and NLRP3 activation. *Acta Pharmacol. Sin.* 42, 230–241. doi: 10.1038/s41401-020-0490-7

Stone, R. C., Pastar, I., Ojeh, N., Chen, V., Liu, S., Garzon, K. I., et al. (2016). Epithelial-mesenchymal transition in tissue repair and fibrosis. *Cell Tissue Res.* 365, 495–506. doi: 10.1007/s00441-016-2464-0

Tay, Y., Rinn, J., and Pandolfi, P. P. (2014). The multilayered complexity of ceRNA crosstalk and competition. *Nature* 505, 344–352. doi: 10.1038/nature12986

Tsuchiya, K., Nakajima, S., Hosojima, S., Thi Nguyen, D., Hattori, T., Manh Le, T., et al. (2019). Caspase-1 initiates apoptosis in the absence of gasdermin D. *Nat. Commun.* 10:2091. doi: 10.1038/s41467-019-09753-2

Urdaneta Perez, M. G., Garwe, T., Stewart, K., Sarwar, Z., and Morris, K. T. (2020). Obesity is an Independent Risk Factor for Mortality in Otherwise Healthy Patients After Hepatectomy. *J. Surg. Res.* 255, 50–57. doi: 10.1016/j.jss.2020.05.024

Vande Walle, L., and Lamkanfi, M. (2016). Pyroptosis. *Curr. Biol.* 26, R568–R572. doi: 10.1016/j.cub.2016.02.019

Wang, B., Tian, Y., and Yin, Q. (2019). AIM2 inflammasome assembly and signaling. *Adv. Exp. Med. Biol.* 1172, 143–155. doi: 10.1007/978-981-13-9367-9_7

Wang, B., and Yin, Q. (2017). AIM2 inflammasome activation and regulation: a structural perspective. *J. Struct. Biol.* 200, 279–282.

Wang, C., Liu, G., Yang, H., Guo, S., Wang, H., Dong, Z., et al. (2021). MALAT1-mediated recruitment of the histone methyltransferase EZH2 to the microRNA-22 promoter leads to cardiomyocyte apoptosis in diabetic cardiomyopathy. *Sci. Total Environ.* 766:142191. doi: 10.1016/j.scitotenv.2020.142191

Wang, J., Zhu, S., Meng, N., He, Y., Lu, R., and Yan, G. R. (2019). ncRNA-encoded peptides or proteins and cancer. *Mol. Ther.* 27, 1718–1725. doi: 10.1016/j.ymthe.2019.09.001

Wang, K., Sun, Q., Zhong, X., Zeng, M., Zeng, H., Shi, X., et al. (2020). Structural Mechanism for GSDMD Targeting by Autoprocessed Caspases in Pyroptosis. *Cell* 180, 941–955.e20. doi: 10.1016/j.cell.2020.02.002

Wang, P. H., Ye, Z. W., Deng, J. J., Siu, K. L., Gao, W. W., Chaudhary, V., et al. (2018). Inhibition of AIM2 inflammasome activation by a novel transcript isoform of IFI16. *EMBO Rep.* 19:e45737. doi: 10.15252/embr.201845737

Wang, X., Pan, J., Liu, H., Zhang, M., Liu, D., Lu, L., et al. (2019). AIM2 gene silencing attenuates diabetic cardiomyopathy in type 2 diabetic rat model. *Life Sci.* 221, 249–258. doi: 10.1016/j.lfs.2019.02.035

Wang, Y. N., Shan, K., Yao, M. D., Yao, J., Wang, J. J., Li, X., et al. (2016). Long Noncoding RNA-GAS5: a novel regulator of hypertension-induced vascular remodeling. *Hypertension* 68, 736–748. doi: 10.1161/HYPERTENSIONAHA.116.07259

Wu, A., Sun, W., and Mou, F. (2021). lncRNA-MALAT1 promotes high glucose-induced H9C2 cardiomyocyte pyroptosis by downregulating miR-141-3p expression. *Mol. Med. Rep.* 23:259. doi: 10.3892/mmr.2021.11898

Xia, L., and Song, M. (2020). Role of non-coding RNA in diabetic cardiomyopathy. *Adv. Exp. Med. Biol.* 1229, 181–195. doi: 10.1007/978-981-15-1671-9_10

Xie, Y., Huang, Y., Ling, X., Qin, H., Wang, M., and Luo, B. (2020). Chemerin/CMKLR1 axis promotes inflammation and pyroptosis by activating NLRP3 inflammasome in diabetic cardiomyopathy rat. *Front. Physiol.* 11:381. doi: 10.3389/fphys.2020.00381

Xu, X. E., Liu, L., Wang, Y. C., Wang, C. T., Zheng, Q., Liu, Q. X., et al. (2019). Caspase-1 inhibitor exerts brain-protective effects against sepsis-associated encephalopathy and cognitive impairments in a mouse model of sepsis. *Brain Behav. Immun.* 80, 859–870. doi: 10.1016/j.bbi.2019.05.038

Xu, Y., Fang, H., Xu, Q., Xu, C., Yang, L., and Huang, C. (2020). LncRNA GAS5 inhibits NLRP3 inflammasome activation-mediated pyroptosis in diabetic cardiomyopathy by targeting miR-34b-3p/AHR. *Cell Cycle* 19, 3054–3065. doi: 10.1080/15384101.2020.1831245

Xue, M., Li, T., Wang, Y., Chang, Y., Cheng, Y., Lu, Y., et al. (2019). Empagliflozin prevents cardiomyopathy via sGC-cGMP-PKG pathway in type 2 diabetes mice. *Clin. Sci.* 133, 1705–1720. doi: 10.1042/CS20190585

Yang, F., Qin, Y., Wang, Y., Meng, S., Xian, H., Che, H., et al. (2019b). Metformin Inhibits the NLRP3 Inflammasome via AMPK/mTOR-dependent effects in diabetic cardiomyopathy. *Int. J. Biol. Sci.* 15, 1010–1019. doi: 10.7150/ijbs.29680

Yang, F., Li, A., Qin, Y., Che, H., Wang, Y., Lv, J., et al. (2019a). A novel circular RNA mediates pyroptosis of diabetic cardiomyopathy by functioning as a competing endogenous RNA. *Mol. Ther. Nucleic Acids* 17, 636–643. doi: 10.1016/j.omtn.2019.06.026

Yang, F., Qin, Y., Lv, J., Wang, Y., Che, H., Chen, X., et al. (2018b). Silencing long non-coding RNA Kcnq1ot1 alleviates pyroptosis and fibrosis in diabetic cardiomyopathy. *Cell Death Dis.* 9:1000. doi: 10.1038/s41419-018-1029-4

Yang, F., Qin, Y., Wang, Y., Li, A., Lv, J., Sun, X., et al. (2018a). LncRNA KCNQ1OT1 mediates pyroptosis in diabetic cardiomyopathy. *Cell. Physiol. Biochem.* 50, 1230–1244. doi: 10.1159/000494576

Zeng, C., Duan, F., Hu, J., Luo, B., Huang, B., Lou, X., et al. (2020). NLRP3 inflammasome-mediated pyroptosis contributes to the pathogenesis of non-ischemic dilated cardiomyopathy. *Redox Biol.* 34:101523. doi: 10.1016/j.redox.2020.101523

Zeng, C., Wang, R., and Tan, H. (2019). Role of pyroptosis in cardiovascular diseases and its therapeutic implications. *Int. J. Biol. Sci.* 15, 1345–1357. doi: 10.7150/ijbs.33568

Zhang, D., He, Y., Ye, X., Cai, Y., Xu, J., Zhang, L., et al. (2020). Activation of autophagy inhibits nucleotide-binding oligomerization domain-like receptor protein 3 inflammasome activation and attenuates myocardial ischemia-reperfusion injury in diabetic rats. *J. Diabetes Investig.* 11, 1126–1136. doi: 10.1111/jdi.13235

Zhang, H., Chen, X., Zong, B., Yuan, H., Wang, Z., Wei, Y., et al. (2018). Gypenosides improve diabetic cardiomyopathy by inhibiting ROS-mediated NLRP3 inflammasome activation. *J. Cell. Mol. Med.* 22, 4437–4448. doi: 10.1111/jcmm.13743

Zhang, W., Xu, W., Feng, Y., and Zhou, X. (2019). Non-coding RNA involvement in the pathogenesis of diabetic cardiomyopathy. *J. Cell. Mol. Med.* 23, 5859–5867. doi: 10.1111/jcmm.14510

Zhang, Y. Q., and Herman, B. (2006). ARC protects rat cardiomyocytes against oxidative stress through inhibition of caspase-2 mediated mitochondrial pathway. *J. Cell. Biochem.* 99, 575–588. doi: 10.1002/jcb.20946

Zhao, Z. Z., Zheng, X. L., and Jiang, Z. S. (2020). Emerging roles of absent in melanoma 2 in cardiovascular diseases. *Clin. Chim. Acta* 511, 14–23. doi: 10.1016/j.cca.2020.08.031

Zhaolin, Z., Guohua, L., Shiyuan, W., and Zuo, W. (2019). Role of pyroptosis in cardiovascular disease. *Cell Prolif.* 52:e12563. doi: 10.1111/cpr.12563

Zheng, X., Zhong, T., Ma, Y., Wan, X., Qin, A., Yao, B., et al. (2020). Bnip3 mediates doxorubicin-induced cardiomyocyte pyroptosis via caspase-3/GSDME. *Life Sci.* 242:117186. doi: 10.1016/j.lfs.2019.117186

Zhou, B., and Yu, J. W. (2017). A novel identified circular RNA, circRNA_010567, promotes myocardial fibrosis via suppressing miR-141 by targeting TGF-β1. *Biochem. Biophys. Res. Commun.* 487, 769–775. doi: 10.1016/j.bbrc.2017.04.044

Zhou, W., Chen, C., Chen, Z., Liu, L., Jiang, J., Wu, Z., et al. (2018). NLRP3: a novel mediator in cardiovascular disease. *J. Immunol. Res.* 2018:5702103. doi: 10.1155/2018/5702103

Zhou, X., Wang, S., Zheng, M., Kuver, A., Wan, X., Dai, K., et al. (2019). Phosphorylation of ELAVL1 (Ser219/Ser316) mediated by PKC is required for erythropoiesis. *Biochim. Biophys. Acta Mol. Cell Res.* 1866, 214–224. doi: 10.1016/j.bbamcr.2018.10.021

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frontiers in Cardiovascular Medicine

ORIGINAL RESEARCH
published: 27 January 2022
doi: 10.3389/fcvm.2021.778038

A Non-Linear Association of Triglyceride Glycemic Index With Cardiovascular and All-Cause Mortality Among Patients With Hypertension

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OPEN ACCESS

Edited by:
Modar Kassan, University of Tennessee Health Science Center (UTHSC), United States

Reviewed by:
Yang Peng, The University of Queensland, Australia
Masahide Hamaguchi, Kyoto Prefectural University of Medicine, Japan

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Specialty section:
This article was submitted to Hypertension, a section of the journal Frontiers in Cardiovascular Medicine

Received: *16 September 2021*
Accepted: *27 December 2021*
Published: *27 January 2022*

Citation:
Zhou D, Liu X-c, Kenneth L, Huang Y-q and Feng Y-q (2022) A Non-Linear Association of Triglyceride Glycemic Index With Cardiovascular and All-Cause Mortality Among Patients With Hypertension. Front. Cardiovasc. Med. 8:778038. doi: 10.3389/fcvm.2021.778038

Background: To investigate the association between insulin resistance (IR), quantified by triglyceride glycemic index (TyG index), cardiovascular mortality (CVM), and all-cause mortality (ACM) in hypertension patients.

Methods: We included 8,554 patients with hypertension aged ≥18 years old from the 1999–2014 National Health and Nutrition Examination Surveys (NHANES). The status of CVM and ACM of participants were followed through December 31, 2015. Cox proportional hazards models and Kaplan-Meier survival curves were used to evaluate the relationship between TyG index, CVM, and ACM.

Results: During a median of 82 months follow-up, 1,882 mortality cases had occurred, 434 of which were due to cardiovascular disease. The patients with hypertension with TyG ≥ 10 were older, had a higher chance of being smokers, were obese, had higher blood pressure, and had risk or had cardiovascular disease. In Cox proportional hazards models, compared with the patients with TyG < 8, those with TyG ≥ 10 had 56% increased risk for ACM. On the other hand, no significant difference for CVM between the four groups were observed. In the restricted cubic spline regression models, the relationship between TyG index and ACM was non-linear. Subgroup analysis showed non-linear relationship between TyG index and ACM in elderly patients aged ≥60 years. The cut-off value of TyG for ACM was 9.45, and those with higher or lower than 9.45 had more risk of ACM. When TyG index was more than 9.52, the risk for CVM would increase among the whole group. Kaplan-Meier survival curves showed patients with TyG ≥ 10 had higher risk of ACM and CVM (Log rank $P < 0.05$).

Conclusions: We demonstrated that the association between ACM and TyG index in elderly patients with hypertension aged ≥60 years was non-linear. However, TyG index was only more than 9.52, hence, the risk for CVM would increase among the whole hypertension group.

Keywords: insulin resistance, triglyceride-glucose index, hypertension, all-cause mortality, cardiovascular mortality

BACKGROUND

Hypertension is a primary risk factor for cardiovascular disease and all-cause mortality (ACM) (1, 2). Careful risk factor assessment plays an important role in hypertension patients. The metabolic effects of insulin resistance (IR), including hyperglycemia and dyslipidemia, appear to interact synergistically with increased blood pressure to cause vascular and kidney injury (3). IR is proposed to be important contributors to hypertension-caused organ damage, arterial stiffness (4), and left ventricular diastolic dysfunction (5) due to hypertension. Hence, higher IR is a risk factor for hypertension patients.

At present, Homeostatic model assessment IR (HOMA-IR), a method for assessing β-cell function and IR, is frequently applied, but its high cost and inconvenience make it difficult to use in large cohort study. Therefore, there has been no large cohort study examinate the prognosis value of IR in hypertension group.

Triglyceride-Glucose index (TyG index), first published by South American authors, showed a good correlation with the insulin clamp technique and HOMA-IR index. The TyG index, described to be a simple, convenient, and low-cost method, is of research interest in many countries in Asia and can be used to screen for IR in the Asian hypertensive community (6). Despite this, there is no evidence between TyG index and prognosis value in North America hypertensive community. Therefore, we conducted a retrospective cohort study to certify the relationship of cardiovascular mortality (CVM) and ACM in hypertension patients.

Abbreviations: IR, insulin resistance; CVM, cardiovascular mortality; ACM, all-cause mortality; TyG index, triglyceride glycemic index; HOMA-IR, homeostatic model assessment IR; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; FBG, fasting blood glucose; eGFR, estimated glomerular filtration rate; BMI, body mass index; HbA1C, hemoglobin A1c.

METHODS

Study Population

The National Health and Nutrition Examination Surveys (NHANES), sponsored by the Centers for Disease Control and Prevention, is designed to assess the health status of US citizens. Data for analyses were taken from the NHANES 1999–2014 with a total of 82,091 participants. In our analysis, we included people aged ≥ 18 years old. Subjects without blood lipid data,

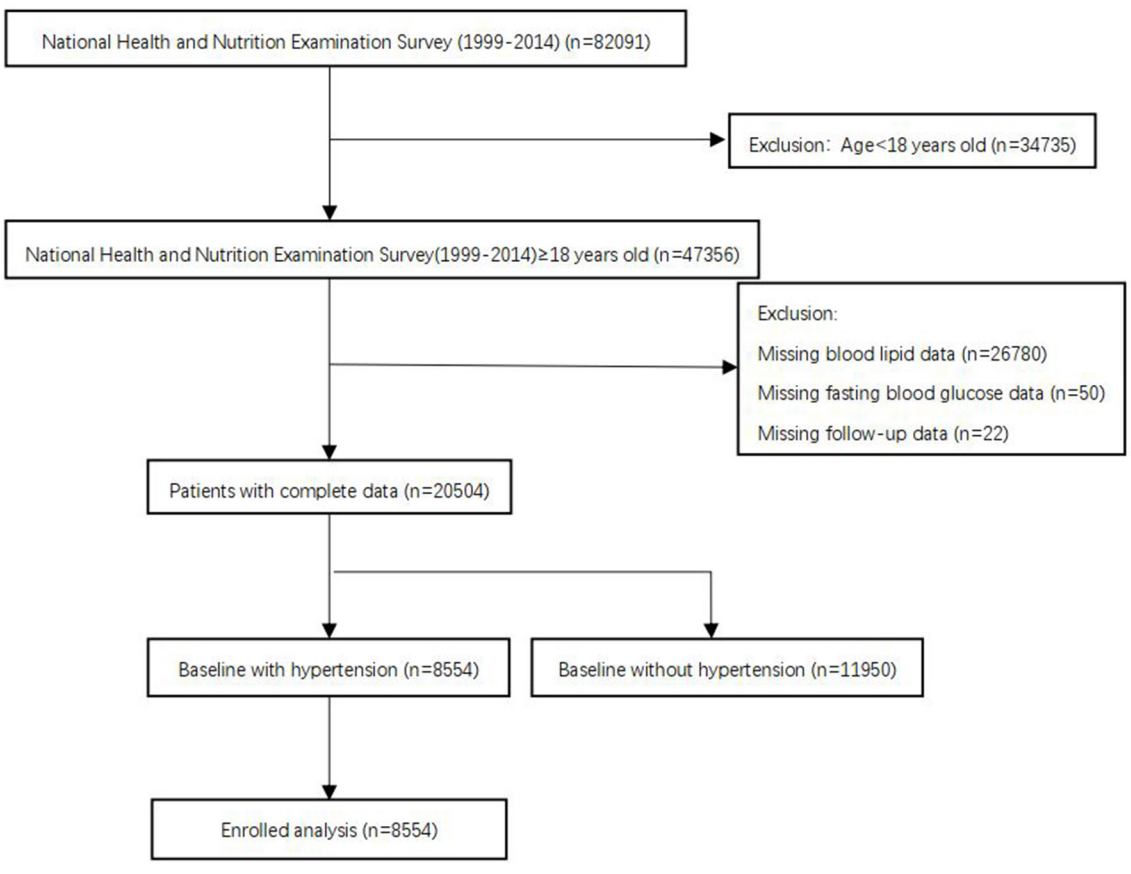

FIGURE 1 | Study cohort.

TABLE 1 | Demographic and clinical characteristics according to triglyceride-glucose index level.

	Total	TyG < 8	8 ≤ T yG < 9	9 ≤ TyG < 10	TyG ≥ 10	*p*
Number	8,554	690	4,655	2,743	466	
Age, years	60.12 ± 16.07	54.13 ± 18.33	60.70 ± 16.32	60.92 ± 15.01	58.50 ± 13.96	<0.001
Gender, n (%)						<0.001
Male	4,216 (49.29)	327 (47.39)	2,203 (47.33)	1,412 (51.48)	274 (58.80)	
Female	4,338 (50.71)	363 (52.61)	2,452 (52.67)	1,331 (48.52)	192 (41.20)	
Race, n (%)						<0.001
Non-white	4,245 (49.63)	432 (62.61)	2,335 (50.16)	1,238 (45.13)	240 (51.50)	
White	4,309 (50.37)	258 (37.39)	2,320 (49.84)	1,505 (54.87)	226 (48.50)	
Marital status, n (%)						<0.001
Married	4,632 (54.91)	329 (48.53)	2,493 (54.31)	1,552 (57.29)	258 (56.33)	
Other	3,803 (45.09)	349 (51.47)	2,097 (45.69)	1,157 (42.71)	200 (43.67)	
Education level, n (%)						<0.001
Less than high school	2,727 (32.30)	173 (26.13)	1,412 (30.70)	945 (34.77)	197 (42.55)	
High school or above	5,716 (67.70)	489 (73.87)	3,188 (69.30)	1,773 (65.23)	266 (57.45)	
Smoking, n (%)						<0.001
No	4,178 (49.40)	365 (54.80)	2,366 (51.38)	1,251 (45.93)	196 (42.33)	
Yes	4,280 (50.60)	301 (45.20)	2,239 (48.62)	1,473 (54.07)	267 (57.67)	
Body mass index, kg/m^2	30.31 ± 7.09	28.11 ± 7.30	29.76 ± 7.26	31.49 ± 6.51	32.15 ± 6.80	<0.001
Systolic blood pressure, mmHg	135.62 ± 21.35	133.21 ± 20.58	135.11 ± 21.62	136.53 ± 20.96	138.89 ± 21.55	<0.001
Diastolic blood pressure, mmHg	71.00 ± 16.07	71.07 ± 16.05	70.44 ± 16.21	71.79 ± 15.69	71.93 ± 16.69	0.004
eGFR, mg/min/1.73m^2	79.31 ± 26.93	86.21 ± 27.81	79.29 ± 26.98	77.24 ± 25.85	81.55 ± 29.48	<0.001
Total cholesterol, mg/dL	197.68 ± 43.43	179.68 ± 39.45	193.14 ± 40.25	204.94 ± 43.47	226.94 ± 56.40	<0.001
HDL cholesterol, mg/dL	52.87 ± 16.31	66.46 ± 19.88	56.03 ± 15.75	46.36 ± 12.26	39.46 ± 11.65	<0.001
LDL cholesterol, mg/dL	115.31 ± 36.57	102.64 ± 32.30	116.05 ± 35.35	117.83 ± 38.74	109.09 ± 38.95	<0.001
Triglycerides, mg/dL	150.58 ± 117.66	52.23 ± 11.80	104.85 ± 28.96	203.81 ± 65.30	439.76 ± 291.68	<0.001
Fasting blood glucose, mg/dL	115.87 ± 42.86	92.76 ± 12.14	104.34 ± 18.47	125.30 ± 40.63	209.87 ± 94.41	<0.001
TyG index	8.86 ± 0.68	7.76 ± 0.21	8.56 ± 0.27	9.37 ± 0.26	10.51 ± 0.47	<0.001
Comorbidities, n (%)						
diabetes						<0.001
No	5,958 (69.67)	613 (88.84)	3,695 (79.39)	1,560 (56.89)	90 (19.31)	
Yes	2,594 (30.33)	77 (11.16)	959 (20.61)	1,182 (43.11)	376 (80.69)	
Cardiovascular disease						<0.001
No	6,821 (80.64)	570 (85.84)	3,776 (81.98)	2,124 (77.92)	351 (75.81)	
Yes	1,638 (19.36)	94 (14.16)	830 (18.02)	602 (22.08)	112 (24.19)	
Treatment, n (%)						
Antihypertensive drugs						<0.001
No	3,254 (38.04)	333 (48.26)	1,854 (39.83)	919 (33.50)	148 (31.76)	
Yes	5,300 (61.96)	357 (51.74)	2,801 (60.17)	1,824 (66.50)	318 (68.24)	
Hypoglycemic agents						<0.001
No	7,115 (83.18)	647 (93.77)	4,149 (89.13)	2,089 (76.16)	230 (49.36)	
Yes	1,439 (16.82)	43 (6.23)	506 (10.87)	654 (23.84)	236 (50.64)	
Lipid-lowering drugs						<0.001
No	6,309 (73.75)	569 (82.46)	3,498 (75.15)	1,936 (70.58)	306 (65.67)	
Yes	2,245 (26.25)	121 (17.54)	1,157 (24.85)	807 (29.42)	160 (34.33)	
Antiplatelet drugs						0.024
No	8,185 (95.69)	666 (96.52)	4,476 (96.15)	2,600 (94.79)	443 (95.06)	
Yes	369 (4.31)	24 (3.48)	179 (3.85)	143 (5.21)	23 (4.94)	
Outcomes, n (%)						
Cardiovascular disease mortality						<0.001
No	8,120 (94.93)	659 (95.51)	4,445 (95.49)	2,595 (94.60)	421 (90.34)	
Yes	434 (5.07)	31 (4.49)	210 (4.51)	148 (5.40)	45 (9.66)	
All-cause mortality						<0.001
No	6,672 (78.00)	582 (84.35)	3,650 (78.41)	2,125 (77.47)	315 (67.60)	
Yes	1,882 (22.00)	108 (15.65)	1,005 (21.59)	618 (22.53)	151 (32.40)	

Q, quintiles; n, number; HDL, high density lipoprotein; LDL, low density lipoprotein; TyG, Triglyceride-glucose.
Values are mean ± standardized differences or n (%).

TABLE 2 | Multivariate Cox regression analysis of triglyceride-glucose index with cause-specific mortality.

	Event rate/1,000 person-years	Model I HR (95%CI), *p* value	Model II HR (95%CI), *p* value	Model III HR (95%CI), *p* value
All-cause mortality				
TyG index group				
TyG < 8	24.07	1.0	1.0	1.0
8 ≤ TyG < 9	29.16	1.19 (0.98, 1.45), 0.0841	0.84 (0.69, 1.02), 0.0794	0.90 (0.72, 1.13), 0.3626
9 ≤ TyG < 10	29.02	1.18 (0.96, 1.45), 0.1153	0.84 (0.68, 1.03), 0.0923	0.85 (0.66, 1.10), 0.2127
TyG ≥ 10	43.45	1.78 (1.39, 2.28), <0.0001	1.56 (1.22, 1.99), 0.0005	1.56 (1.14, 2.15), 0.0061
P for trend		<0.001	0.002	0.099
Cardiovascular mortality				
TyG index group				
TyG < 8	6.91	1.0	1.0	1.0
8 ≤ TyG < 9	6.09	0.87 (0.60, 1.27), 0.4776	0.62 (0.42, 0.90), 0.0125	0.55 (0.36, 0.82), 0.0039
9 ≤ TyG < 10	6.95	0.99 (0.67, 1.46), 0.9543	0.72 (0.49, 1.07), 0.1011	0.53 (0.33, 0.86), 0.0092
TyG ≥ 10	12.95	1.84 (1.17, 2.91), 0.0088	1.69 (1.07, 2.67), 0.0252	1.23 (0.67, 2.25), 0.5044
P for trend		0.002	0.002	0.438

TyG, triglyceride-glucose; HR, hazard ratio; CI, confidence interval; Q, quintiles.
Model I adjust for none.
Model II adjust for age, gender, and race.
Model III adjust for age, gender, race, smoking, marital status, education level, body mass index, systolic blood pressure, estimated glomerular filtration rate, total cholesterol, high density lipoprotein cholesterol, comorbidities (cardiovascular disease and diabetes), and medicine use (antihypertensive drugs, hypoglycemic agents, lipid-lowering drugs, and antiplatelet drugs).

fasting blood glucose data, follow-up data, or baseline without hypertension were excluded. After applying the criteria, we enrolled 8,554 participants for final analysis (**Figure 1**). The survival status of participants was followed up to December 31, 2015. The NHANES study protocol was approved by the Institutional Review Board of the Centers for Disease Control and Prevention. Informed consent was signed by all participants.

Data Collection

Questionnaires were collected at baseline to acquire demographic information (age, gender, race, marital status, and education level), smoking status, personal medical history (cardiovascular diseases and diabetes), and medication history (antihypertensive drugs, hypoglycemic agents, lipid-lowering drug, and antiplatelet drugs).

Physical assessments were performed to examine height, weight, systolic blood pressure (SBP), and diastolic blood pressure (DBP). Blood samples were collected on an empty stomach in the morning and after 8 h. Total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), fasting blood glucose (FBG), and estimated glomerular filtration rate (eGFR) were also collected. Body mass index (BMI) was calculated using weight (kg) divided by the square of height (m^2). Hypertension was defined as having an SBP ≥ 140 or/and DBP ≥ 90 mmHg, is confirmed to be taking antihypertensive medications, or has a self-reported history of hypertension (7). Diabetes was defined as having an FBG ≥ 126 mg/dl, having a self-reported hemoglobin A1c(HbA1C) ≥ 6.5%, or using hypoglycemic drug (8). TyG index was calculated by ln [fasting triglycerides (mg/dl) *fasting glucose (mg/dl)/2] (9). eGFR was computed using Modification of Diet in Renal Disease (MDRD) formula (10).

Clinical Outcome

All-cause mortality refer to death from any cause, cardiovascular disease, or cerebrovascular disease until December 31, 2015 were the primary outcomes. Mortality data were extracted from the 1999–2014 NHANES public-use linked mortality files. We examined the time from enrollment (date of interview) to mortality for censoring. The International Classification of Diseases, Tenth Revision codes (I00-I09, I11, I13, I20-I51) were used to define cardiovascular deaths. Any participant who was not matched with any death records was considered to be alive throughout the follow-up period.

Statistical Analysis

Continuous variables were expressed as mean ± standard deviation for normally distributed variables or median (interquartile range) if the data were not normally distributed. Categorical variables were presented as number (n) and percentage (%). The one-way ANOVA, Kruskal–Wallis *H*-test or chi-square tests were used to assess differences according to baseline TyG index (TyG < 8, 8 ≤ TyG < 9, 9 ≤ TyG < 10, TyG ≥ 10) in groups. Multivariable Cox regression analysis were used to estimate adjusted hazard ratio (HR) and 95% CI for mortality according to baseline TyG index in groups. Model I adjust for none. Model II adjust for age, gender, and race. Age, gender, race, smoking, marital status, education level, BMI, SBP, eGFR, TC, HDL-C, comorbidities (cardiovascular disease and diabetes), and medicine use (antihypertensive drugs, hypoglycemic agents, lipid-lowering drugs, and antiplatelet drugs) were included in Model III. We initially performed survival analysis using standardized Kaplan–Meier curves and Log rank test. The association between TyG index, ACM, and CVM was then examined by multivariate adjusted Cox restricted

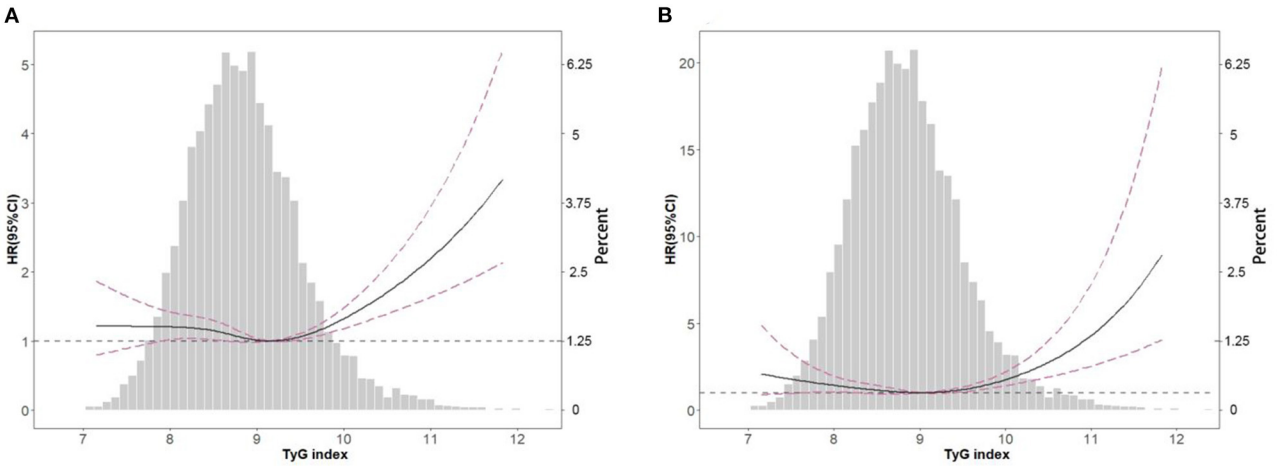

FIGURE 2 | Spline analyses of all-cause **(A)** and cardiovascular **(B)** mortality by TyG index in the overall cohort and the probability distribution histogram is represented in the background (spline analyses were adjusted for age, gender, race, smoking, marital status, education level, body mass index, systolic blood pressure, estimated glomerular filtration rate, total cholesterol, high density lipoprotein cholesterol, cardiovascular disease, diabetes, antihypertensive drugs, hypoglycemic agents, lipid-lowering drugs, and antiplatelet drugs).

TABLE 3 | The results of two-piecewise linear regression model between triglyceride-glucose index and cause-specific mortality.

	All-cause mortality	Cardiovascular disease mortality
	HR (95% CI), *p* value	HR (95% CI), *p* value
Cutoff value	9.45	9.52
< Cut-off value	0.87 (0.76, 0.99), 0.0329	0.81 (0.62, 1.06), 0.1277
≥ Cut-off value	1.73 (1.44, 2.09), <0.0001	2.85 (2.05, 3.98), <0.0001
p for log likelihood ratio test	<0.001	<0.001

TyG, triglyceride-glucose; HR, hazard ratio; CI, confidence interval.
The two-piecewise linear regression model were adjusted for age, gender, race, smoking, marital status, education level, body mass index, systolic blood pressure, estimated glomerular filtration rate, total cholesterol, high density lipoprotein cholesterol, comorbidities (cardiovascular disease and diabetes), and medicine use (antihypertensive drugs, hypoglycemic agents, lipid-lowering drugs, and antiplatelet drugs).

cubic spline regression models and used a generalized additive model to explore the non-linear relationship between TyG index and mortality. If non-linear relationships were identified, we used two-piecewise linear regression models to elucidate how the associations differed by the threshold point. The threshold value was estimated by trying all possible value and choosing the threshold point with highest likelihood. Finally, we conducted subgroup analyses, including age (<65 or ≥65 years), gender (male or female), race (white or non-white), and BMI (<25 or ≥25 kg/m^2). $P < 0.05$ was considered statistically significant. R version 3.3.2 (R Foundation for Statistical Computing, Vienna, Austria) was used for all statistical analyses.

RESULTS

Baseline Characteristics

The baseline characteristics of the analytical cohort according to TyG index in groups were showed in **Table 1**. In total, 8,554 patients (49.29% men) were included in this analysis with mean age of 60.12 ± 16.07 years. Of these, 4,309 (50.37%) participants were white, 4,632 (54.91%) married, 5,716 (67.70%) received high school or above education, and 4,178 (49.40%) never smoked. The patients with hypertension in group of TyG ≥ 10 were older, were more likely to be smokers, were obese, had higher blood pressure, and had higher risk or had cardiovascular disease. In addition, the proportion of participants with diabetes and cardiovascular disease was 30.33 and 19.36%, respectively. During a median follow up of 82 months, 1,882 cases of death have occurred, 434 of which were due to cardiovascular disease. All baseline variables differed significantly among the TyG index in groups (all $p < 0.05$).

Hazard Ratios (HRs) of TyG Index for ACM and CVM Risk

Table 2 reveals the estimated HRs and CIs of TyG index in relation to ACM and CVM. In the Model III, compared with the lowest group of TyG index (TyG < 8), the HRs for ACM from other groups (8 ≤ TyG < 9, 9 ≤ TyG < 10, TyG ≥ 10) in the fully adjusted model were 0.90 (0.72 to −1.13, $p = 0.36$), 0.85 (0.66–1.10, $p = 0.21$), and 1.56 (1.14–2.15, $p < 0.05$), respectively (P for trend = 0.09).

Population with TyG ≥ 10 showed a 56% increased risk of ACM compared with those TyG < 8. After an adjustment for confounders, the HRs and CIs for CVM from the three groups (8 ≤ TyG < 9, 9 ≤ TyG < 10, TyG ≥ 10) were 0.55 (0.36, 0.82), 0.53 (0.33, 0.86), and 1.23 (0.67, 2.25), respectively (P for trend = 0.43). After excluding type 2 diabetes mellitus or cardiovascular disease in the baseline, in **Supplementary Table 1**, we found that when comparing with the lowest group of TyG index (TyG < 8), the HRs for ACM from TyG ≥ 10 in the fully adjusted model were 2.05 (1.04, 4.04) ($p = 0.03$). However, it was not associated with any change in the risk of CVM.

In the restricted cubic spline regression models with full adjustment for confounders, the relationships between TyG

TABLE 4 | Subgroups analysis.

	N	All-cause mortality		P for log likelihood ratio test	Cardiovascular disease mortality		P for log likelihood ratio test
		HR (95% CI), *p* value			HR (95% CI), *p* value		
Cutoff value, mmol/L		**<9.45**	**≥9.45**		**<9.52**	**≥9.52**	
Age							
≥65	3,442	0.81 (0.70, 0.95), 0.0080	1.48 (1.14, 1.93), 0.0032	<0.001	0.79 (0.58, 1.08), 0.1364	2.36 (1.43, 3.90), 0.0008	0.001
<65	4,402	1.08 (0.83, 1.41), 0.5575	1.59 (1.20, 2.10), 0.0011	0.071	0.86 (0.47, 1.55), 0.6134	2.34 (1.42, 3.87), 0.0009	0.022
Gender							
Male	3,889	0.86 (0.72, 1.03), 0.0975	1.78 (1.40, 2.27), <0.0001	<0.001	0.92 (0.65, 1.30), 0.6382	3.18 (2.12, 4.78), <0.0001	<0.001
Female	3,955	0.86 (0.70, 1.05), 0.1439	1.68 (1.25, 2.24), 0.0005	0.001	0.72 (0.46, 1.13), 0.1541	2.23 (1.16, 4.27), 0.0158	0.015
Race							
Non-white	3,847	0.83 (0.67, 1.02), 0.0757	1.76 (1.35, 2.28), <0.0001	<0.001	0.71 (0.47, 1.08), 0.1114	3.58 (2.30, 5.57), <0.0001	<0.001
White	3,997	0.91 (0.76, 1.08), 0.2892	1.63 (1.25, 2.13), 0.0003	0.001	0.88 (0.61, 1.26) 0.4722	2.09 (1.21, 3.62), 0.0087	0.020
Body mass index, kg/m^2							
<25	1,683	0.91 (0.71, 1.18), 0.4867	3.08 (1.98, 4.79), <0.0001	<0.001	0.82 (0.49, 1.37), 0.4400	4.72 (2.29, 9.71), <0.0001	<0.001
≥25	6,161	0.85 (0.73, 1.00), 0.0477	1.56 (1.26, 1.92), <0.0001	<0.001	0.79 (0.57, 1.09) 0.1511	2.58 (1.74, 3.80), <0.0001	<0.001

TyG, triglyceride-glucose; CI, confidence interval.

When analyzing a subgroup variable, age, gender, race, smoking, body mass index, systolic blood pressure, estimated glomerular filtration rate, total cholesterol, high density lipoprotein cholesterol, comorbidities (cardiovascular disease, diabetes, and hypertension), and medicine use (antihypertensive drugs, hypoglycemic agents, lipid-lowering drugs, and antiplatelet drugs) were all adjusted except the variable itself.

index, ACM (**Figure 2A**), and CVM (**Figure 2B**) were both non-linear in participants with hypertension. The results of two piecewise linear regression model are demonstrated in **Table 3**. The relationship between TyG index and ACM was non-linear. Subgroup analysis (**Table 4**) showed non-linear relationship between TyG index and ACM in elderly patients with aged ≥60 years. The cut-off value of TyG for ACM was 9.45. Values higher or lower than 9.45 had more risk of ACM. When TyG index was more than 9.52, the risk for CVM would increase among the whole group.

As showed in Kaplan-Meier survival curves (**Figure 3**), hypertension patients with TyG ≥ 10 had significantly higher ACM (**Figure 3A**) and CVM (**Figure 3B**) in the following life (log rank $p < 0.05$).

Subgroup Analyses

Table 4 has explored the relationship between TyG index, ACM, and CVM as stratified by gender, age, BMI, and race. Only for age ≥ 60 years old patients, the association was significant different between TyG index <9.45 or ≥9.45. When index ≥9.45, risk increased by 48% at every 1 SD increase of TyG index. When index <9.45, risk increased by 19% at every 1 SD decrease of TyG index. Other subgroups only showed a higher TyG index, more than the cut-off value, would present higher risk for ACM and CVM. When TyG index was less than the cut-off value, it showed no difference.

DISCUSSION

In this retrospective study, we, for the first time, revealed association between IR (TyG index), ACM, and CVM among patients with hypertension in a large cohort study. We demonstrated that the association was non-linear between ACM and TyG index in elderly patient with aged ≥60 years old. But for CVM, only when TyG index was more than 9.52 will the risk for CVM increase among the whole hypertension group.

We also demonstrated the association was non-linear between ACM and TyG index in elderly with aged ≥60 years old. When TyG index ≥9.45, the risk of ACM increased by 48% at every 1 SD increase of TyG index. In contrast, for TyG index < 9.45, the risk of ACM increased by 19% as the TyG index decreased by 1 SD. TyG index, a composite indicator based on TG level and FBG value, was shown to be used as a surrogate marker of IR (9, 11, 12). Previous studies probed the relationship between IR and ACM in different population. An observational prospective cohort study that enrolled 15,773 patients with type 2 diabetes showed that highest IR predicts ACM in type 2 diabetes (13). High IR, as measured by HOMA-IR, identified postmenopausal women at higher risk for cancer-specific and ACM (14). In addition, A meta-analysis included seven articles involving 26,976 non-diabetic adults showed that the highest HOMA-IR increased the risk of ACM by 34% when compared with the lowest category (15). For elderly people, there were different conclusions. Among community-dwelling older individuals over 65 years, HOMA-IR and low-grade systemic inflammation was associated with a 9-year ACM and CVM risk (16). Aging and high C-reactive protein levels were, usually,

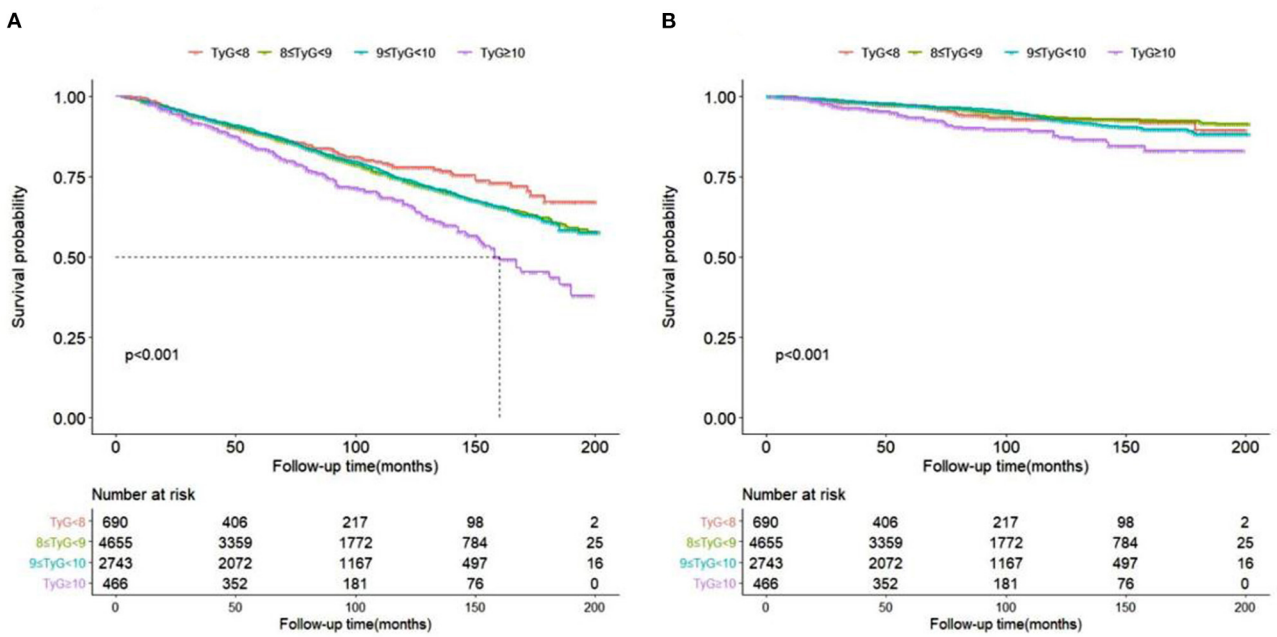

FIGURE 3 | Kaplan-Meier survival curve for all-cause **(A)** and cardiovascular **(B)** mortality by TyG index.

risk factors for cardiovascular disease mortality. We did not evaluate systemic inflammation in our study. A community-based prospective study selected participants with age ≥65 years in Korea (17). Elderly subjects in the fifth and first quintile of HOMA-IR values had increased rates of ACM and CVM as there is a threshold level of HOMA-IR in relation to mortality. The HOMA-IR was lower in Korea study (1st quintile ≤ 0.67 and 5th quintile > 1.50). Our work showed similar with the study in Korea; however, we used the TyG index to evaluate IR, not HOMA-IR, in elderly hypertension patients.

A previous study showed that there was a striking U-shaped relationship between FBG levels and in-hospital and 3-year mortality in older patients with acute myocardial infarction (AMI) (18). The study observed that mild to moderately low FBG levels (≤5 mmol/L, 90 mg/dl) were associated with a relative increase in mortality risk. Hypoglycemia and rapid changes in blood glucose level were shown to increase levels of counterregulatory hormones, such as epinephrine and norepinephrine, which may induce vasoconstriction and platelet aggregation, resulting in ischemia of cardiovascular or cerebrovascular (19). In the elderly population, the decrease in skeletal muscle mass is generally accompanied by weight loss. A study that recruited elderly individuals (≥65 years) in Taiwan showed that elderly subjects with sarcopenic obesity had the highest ACM (19). In contrast, except for the TG level, none of the other lipid profile indices were related to ACM in patients aged over 75 years (20). The mortality risk decreased by 17% for each 1 mmol/L increase serum level of TG in ZODIAC study (20). The lower TG had higher risk of ACM for elderly population. One study, which enrolled individuals aged 65 and older residing in northern Manhattan in 1992–1994 and 1999–2002, showed low cholesterol level was a robust predictor of mortality in the non-demented elderly and may be a surrogate of frailty or subclinical disease (21). Our study was collected from 1999 to 2014 in the same country as the study (21), so the life diet structure and level were similar. The mean TG of the patients with mortality was 155 mg/dl (21). Therefore, the finding of our study, specifically of the 1 SD decrease of TyG index when TyG index <9.45 in elderly with aged ≥60 years and 19% increased risk of ACM was persuasive. Other factors, such as body composition and nutritional state, might be predictors of mortality in the elderly population (17). Older age, anemia, lower baseline hemoglobin level (22), and lower BMI had a greater effect on mortality.

Only when TyG index ≥9.52, TyG-index showed significant with CVM. A previous study had reported that IR was associated with cardiovascular disease (23). The relationship between IR and endothelial dysfunction may the underlying biological mechanism of cardiovascular. This mostly includes inflammation (24) and functional impairment in the endothelium of blood vessels (25) which leads to atherosclerosis and cardiovascular disease. This simple, convenient, and low-cost TyG index was of research interest in many Countries and could be used to screen for IR in the hypertensive community. Furthermore, Park et al. (26) reported that TyG-index is an independent predictor of coronary artery calcium progression. We first investigate relationship between TyG index and CVM and ACM in hypertension. The Kaplan-Meier survival curve by TyG index groups also confirmed that the higher TyG index was associated with increased higher incident of ACM and CVM.

Our study still had several limitations that need to be mentioned. First, we did not have multiple-time monitoring of the TyG index along the follow-up, which may provide more information. Secondly, we did not compare the TyG index with HOMA-IR and the hyperinsulinemic-euglycemic clamp test. Thirdly, nutritional habits or energy intake were not recorded. Although we did not adjust for these potential confounding variables, we used other variables, such as BMI or cholesterol levels, which are indirectly related to nutritional habits or energy intake. Finally, this was a population-based study conducted among hypertension participants in the United States. Therefore, our findings may not be generalizable to other populations.

CONCLUSION

We first time revealed association between IR (TyG index), ACM, and CVM among patients with hypertension. We demonstrated that the association was non-linear between ACM and TyG index in elderly with aged ≥60 years. But for CVM, higher TyG index had high risk among patients with hypertension only when TyG index ≥9.52.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/**Supplementary Material**.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Review Board of the Centers for Disease Control and Prevention. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

DZ and Y-qF contributed to the conception or design of the study and drafted the manuscript. X-cL contributed to the acquisition of data, interpretation of data, and analysis of data. Y-qH and LK contributed to the interpretation of data and critical revision of the article for important intellectual content. All authors gave final approval of the article.

FUNDING

This research was supported by Science and Technology Plan Program of Guangzhou (201803040012), the Key Area R&D Program of Guangdong Province (No. 2019B020227005), Guangdong Provincial People's Hospital Clinical Research Fund (Y012018085), the Fundamental and Applied Basic Research Foundation Project of Guangdong Province (2020A1515010738), High-level Hospital Construction Project of Guangdong Provincial People's Hospital (DFJH2020022), and Guangdong Provincial Clinical Research Center for Cardiovascular Disease (2020B111 1170011).

ACKNOWLEDGMENTS

We gratefully acknowledge the contributions of all staffs who work on the National Health and Nutrition Examination Surveys.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2021.778038/full#supplementary-material

REFERENCES

1. Kjeldsen SE, Narkiewicz K, Burnier M, Oparil S. The global burden of disease study 2015 and blood pressure. *Blood Press.* (2017) 26:1. doi: 10.1080/08037051.2016.1267557
2. Bromfield S, Muntner P. High blood pressure: the leading global burden of disease risk factor and the need for worldwide prevention programs. *Curr Hypertens Rep.* (2013) 15:134–6. doi: 10.1007/s11906-013-0340-9
3. da Silva AA, do Carmo JM, Li X, Wang Z, Mouton AJ, Hall JE. Role of hyperinsulinemia and insulin resistance in hypertension: metabolic syndrome revisited. *Can J Cardiol.* (2020) 36:671–82. doi: 10.1016/j.cjca.2020.02.066
4. Bello-Chavolla OY, Antonio-Villa NE, Vargas-Vázquez A, Martagón AJ, Mehta R, Arellano-Campos O, et al. Prediction of incident hypertension and arterial stiffness using the non-insulin-based metabolic score for insulin resistance (METS-IR) index. *J Clin Hypertens.* (2019) 21:1063–70. doi: 10.1111/jch.13614
5. Bamaiyi AJ, Woodiwiss AJ. Insulin resistance influences the impact of hypertension on left ventricular diastolic dysfunction in a community sample. *Clin Cardiol.* (2019) 42:305–11. doi: 10.1002/clc.23145
6. Minh HV, Tien HA, Sinh CT, Thang DC, Chen CH. Assessment of preferred methods to measure insulin resistance in Asian patients with hypertension. *J Clin Hypertension.* (2021) 23:529–37. doi: 10.1111/jch.14155
7. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL Jr., et al. The seventh report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure: the JNC 7 report. *JAMA.* (2003) 289:2560–72. doi: 10.1001/jama.289.19.2560
8. American Diabetes Association. Standards of medical care for patients with diabetes mellitus. *Diab Care.* (2003) 26 Suppl 1:S33–50. doi: 10.2337/diacare.26.2007.S33
9. Simental-Mendía LE, Rodríguez-Morán M, Guerrero-Romero F. The product of fasting glucose and triglycerides as surrogate for identifying insulin resistance in apparently healthy subjects. *Metab Syndr Relat Disord.* (2008) 6:299–304. doi: 10.1089/met.2008.0034
10. Levey AS, Coresh J, Bolton K, Culleton B, Harvey KS, Ikizler TA, et al. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Am J Kidney Dis.* (2002) 39(2 Suppl 1):S1-266.
11. Khan SH, Sobia F, Niazi NK, Manzoor SM, Fazal N, Ahmad F. Metabolic clustering of risk factors: evaluation of triglyceride-glucose index (TyG index) for evaluation of insulin resistance.

Diabetol Metab Syndr. (2018) 10:74. doi: 10.1186/s13098-018-0376-8
12. Guerrero-Romero F, Simental-Mendía LE, González-Ortiz M, Martínez-Abundis E, Ramos-Zavala MG, Hernández-González SO, et al. The product of triglycerides and glucose, a simple measure of insulin sensitivity. Comparison with the euglycemic-hyperinsulinemic clamp. *J Clin Endocrinol Metab.* (2010) 95:3347–51. doi: 10.1210/jc.2010-0288
13. Penno G, Solini A, Orsi E, Bonora E, Fondelli C, Trevisan R. et al. Insulin resistance, diabetic kidney disease, and all-cause mortality in individuals with type 2 diabetes: a prospective cohort study. *BMC Med.* (2021) 19:66. doi: 10.1186/s12916-021-01936-3
14. Pan K, Nelson RA, Wactawski-Wende J, Lee DJ, Manson JE, Aragaki AK, et al. Insulin resistance and cancer-specific and all-cause mortality in postmenopausal women: the women's health initiative. *J Natl Cancer Inst.* (2020) 112:170–8. doi: 10.1093/jnci/djz069
15. Zhang X, Li J, Zheng S, Luo Q, Zhou C, Wang C. Fasting insulin, insulin resistance, and risk of cardiovascular or all-cause mortality in non-diabetic adults: a meta-analysis. *Biosci. Rep.* (2017) 37:BSR20170947. doi: 10.1042/BSR20170947
16. Zuliani G, Morieri ML, Volpato S, Maggio M, Cherubini A, Francesconi D, et al. Insulin resistance and systemic inflammation, but not metabolic syndrome phenotype, predict 9 years mortality in older adults. *Atherosclerosis.* (2014) 235:538–45. doi: 10.1016/j.atherosclerosis.2014.05.959
17. Kim HJ Ryu J, Ahn SY, Kim S, Lim S, Na KY, et al. Association of insulin resistance with lower glomerular filtration rate and all-cause mortality in the Korean elderly population: a community-based prospective cohort study. *Tohoku J Exp Med.* (2013) 231:271–9. doi: 10.1620/tjem.231.271
18. Yang SW, Zhou YJ, Nie XM, Liu YY, Du J, Hu DY, et al. Effect of abnormal fasting plasma glucose level on all-cause mortality in older patients with acute myocardial infarction: results from the Beijing Elderly Acute Myocardial Infarction Study (BEAMIS). *Mayo Clin Proc.* (2011) 86:94–104. doi: 10.4065/mcp.2010.0473
19. Galassetti P, Davis SN. Effects of insulin per se on neuroendocrine and metabolic counter-regulatory responses to hypoglycaemia. *Clin Sci.* (2000) 99:351–62. doi: 10.1042/cs0990351
20. van Hateren KJ, Landman GW, Kleefstra N, Logtenberg SJ, Groenier KH, Kamper AM, et al. The lipid profile and mortality risk in elderly type 2 diabetic patients: a ten-year follow-up study (ZODIAC-13). *PLoS ONE.* (2009) 4:e8464. doi: 10.1371/journal.pone.0008464
21. Schupf N, Costa R, Luchsinger J, Tang MX, Lee JH, Mayeux R. Relationship between plasma lipids and all-cause mortality in nondemented elderly. *J Am Geriatr Soc.* (2005) 53:219–26. doi: 10.1111/j.1532-5415.2005.53106.x
22. Zakai NA, French B, Arnold AM, Newman AB, Fried LF, Robbins J, et al. Hemoglobin decline, function, and mortality in the elderly: the cardiovascular health study. *Am J Hematol.* (2013) 88:5–9. doi: 10.1002/ajh.23336
23. Salazar MR, Carbajal HA, Espeche WG, Aizpurúa M, Dulbecco CA, Reaven GM. Comparison of two surrogate estimates of insulin resistance to predict cardiovascular disease in apparently healthy individuals. *Nutr Metab Cardiovasc Dis.* [2017] 27:366–73. doi: 10.1016/j.numecd.2016.12.002
24. Lee DY, Rhee EJ, Chang Y, Sohn CI, Shin HC Ryu S, et al. Impact of systemic inflammation on the relationship between insulin resistance and all-cause and cancer-related mortality. *Postgrad Med.* (2018) 81:52–62. doi: 10.1016/j.metabol.2017.11.014
25. Ormazabal V, Nair S, Elfeky O, Aguayo C, Salomon C. Association between insulin resistance and the development of cardiovascular disease. *Cardiovasc Diabetol.* (2018) 17:122. doi: 10.1186/s12933-018-0762-4
26. Park K, Ahn CW, Lee SB, Kang S. Elevated TyG index predicts progression of coronary artery calcification. *Diab Care.* (2019) 42:1569–73. doi: 10.2337/dc18-1920

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frontiers in Cardiovascular Medicine

REVIEW
published: 28 February 2022
doi: 10.3389/fcvm.2022.836111

Current Studies of Mitochondrial Quality Control in the Preeclampsia

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Mitochondria are cellular energy powerhouses that play important roles in regulating cellular processes. Mitochondrial quality control (mQC), including mitochondrial biogenesis, mitophagy, mitochondrial fusion and fission, maintains physiological demand and adapts to changed conditions. mQC has been widely investigated in neurodegeneration, cardiovascular disease and cancer because of the high demand for ATP in these diseases. Although placental implantation and fetal growth similarly require a large amount of energy, the investigation of mQC in placental-originated preeclampsia (PE) is limited. We elucidate mitochondrial morphology and function in different pregnancy stages, outline the role of mQC in cellular homeostasis and PE and summarize the current findings of mQC-related PE studies. This review also provides suggestions on the future investigation of mQC in PE, which will lead to the development of new prevention and therapy strategies for PE.

Keywords: mitochondrial quality control, mitophagy, biogenesis, fusion, fission, preeclampsia

OPEN ACCESS

Edited by:
María Galán,
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Reviewed by:
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Specialty section:
This article was submitted to Hypertension, a section of the journal Frontiers in Cardiovascular Medicine

Received: *15 December 2021*
Accepted: *02 February 2022*
Published: *28 February 2022*

Citation:
Peng X, Hou R, Yang Y, Luo Z and Cao Y (2022) Current Studies of Mitochondrial Quality Control in the Preeclampsia. Front. Cardiovasc. Med. 9:836111. doi: 10.3389/fcvm.2022.836111

PREECLAMPSIA

Preeclampsia (PE) is a leading cause of neonatal and maternal morbidity and mortality, affecting 2–8% of pregnant women worldwide (1, 2). Preeclampsia is diagnosed by new-onset hypertension (systolic>140 mmHg and diastolic>90 mmHg) after 20 weeks of gestation accompanied by one or more other features: proteinuria, other maternal organ dysfunction (including liver, kidney and neurological), hematological involvement, and/or uteroplacental dysfunction, such as fetal growth restriction and/or abnormal Doppler ultrasound findings of utero-placental blood flow (3). Pre-term delivery is often the only definite treatment for PE, which is associated with adverse short- and long-term health outcomes in offspring, including a high prevalence of subsequent endocrine and metabolic diseases in children (4). Other effective treatment options are limited.

PE is a placental interface-originated disease affecting multiple organ systems (5). Abundant evidence suggests that defective implantation of placentation is the core risk factor for PE, characterized by abnormal trophoblast invasion and remodeling of the spiral arteries (6). Under normal conditions, the blastocyst is encapsulated by a shell of cytotrophoblast (CT) cells, which adhere to the uterus, penetrate into the decidua and continue to proliferate and differentiate in the first trimester. CT is an undifferentiated and proliferative trophoblast that either fuses into multinucleated syncytiotrophoblast (ST) on the surface of the shell or differentiates into extravillous cytotrophoblast (EVT) through a partial epithelial-mesenchymal transition at the interface between the outer surface of the shell and the tips of anchoring villi. ST cells facilitate the uptake of nutrients and oxygen from maternal blood and produce large quantities of placental hormones

(including progesterone and hCG) to maintain pregnancy. There are two types of EVT. Interstitial EVT migrates into the lumen of the maternal decidua via the invasion of the endometrium, while endovascular EVT invades the myometrial spiral arteries involving the remodeling spiral arteries (**Figure 1**) (7). The myometrial spiral arteries are remodeled from high-resistance, coiled vessels to dilated low-resistance vessels because of the intervillous space at the terminal portion entered by endovascular EVT. Remodeling of the myometrial spiral arteries adapts to the increased cardiac output during pregnancy and slows the blood flow into the intervillous space of placenta, meeting the oxygen and nutrition requirements of the developing fetus (8).

Shallow placental implantation and defective spiral artery remodeling lead to placental ischemia, releasing angiogenic markers such as soluble fms-like tyrosine kinase 1 (sFlt-1) and soluble endoglin (sEng) (9). Impaired placental implantation could be triggered by the abnormalities of CT fusion into ST or abnormalities of ST differentiation into EVT (10). Flt-1 is a receptor of vascular endothelial growth factor (VEGF) and placental growth factor (PIGF), which are mediators of the transformation from epithelial to endothelial phenotype to regulate the endothelial cell function. sFlt-1, a splice variant of Flt1 lacking the trans-membrane and cytoplasmic domains, performs as an antagonist of VEGF and PIGF resulting in endothelial dysfunction (11). Similarly, sEng is the shed Eng from the endothelial cell surface into maternal circulation, which binds to transforming growth factor beta 1 in circulation. Thus, free transforming growth factor beta 1 is decreased, and the migration and proliferation of endothelial cells are inhibited (9). Elevated sFlt-1 and sEng have been found in PE in dozens of human studies (9) and thus have been recognized as predictive or diagnostic biomarkers of PE (12, 13). These antiangiogenic factors lead to the following vasoconstrictive state, oxidative stress and microemboli that contribute to the clinical features of PE (9). Moreover, the administration of sEng or sFlt-1 has been shown to induce severe PE signs or adverse birth outcomes in pregnant rats (11, 14).

MITOCHONDRIAL MORPHOLOGY AND FUNCTION DURING PREGNANCY

Mitochondria are the main resource of energy production for placental implantation and development. Adenosine triphosphate (ATP) synthesis requires five subunit protein complexes (i.e., complexes I-V) of the electron transport chain through oxidative phosphorylation in the inner membrane of the mitochondrion (IMM). Nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide 2 (FADH2) produced from the tricarboxylic acid cycle expedite electrons to the electron transport chain at complex I (NADH dehydrogenase) and complex III (ubiquinone cytochrome c reductase), respectively. The electrons flow to Complex IV, reducing O_2 to H_2O. Meanwhile, protons (H^+) are transferred from the mitochondrial matrix to the intermembrane space at complexes I, III, and IV, leading to the proton gradient and transmembrane electrical potential. The energy stored at proton gradient is used to synthesize ATP (15).

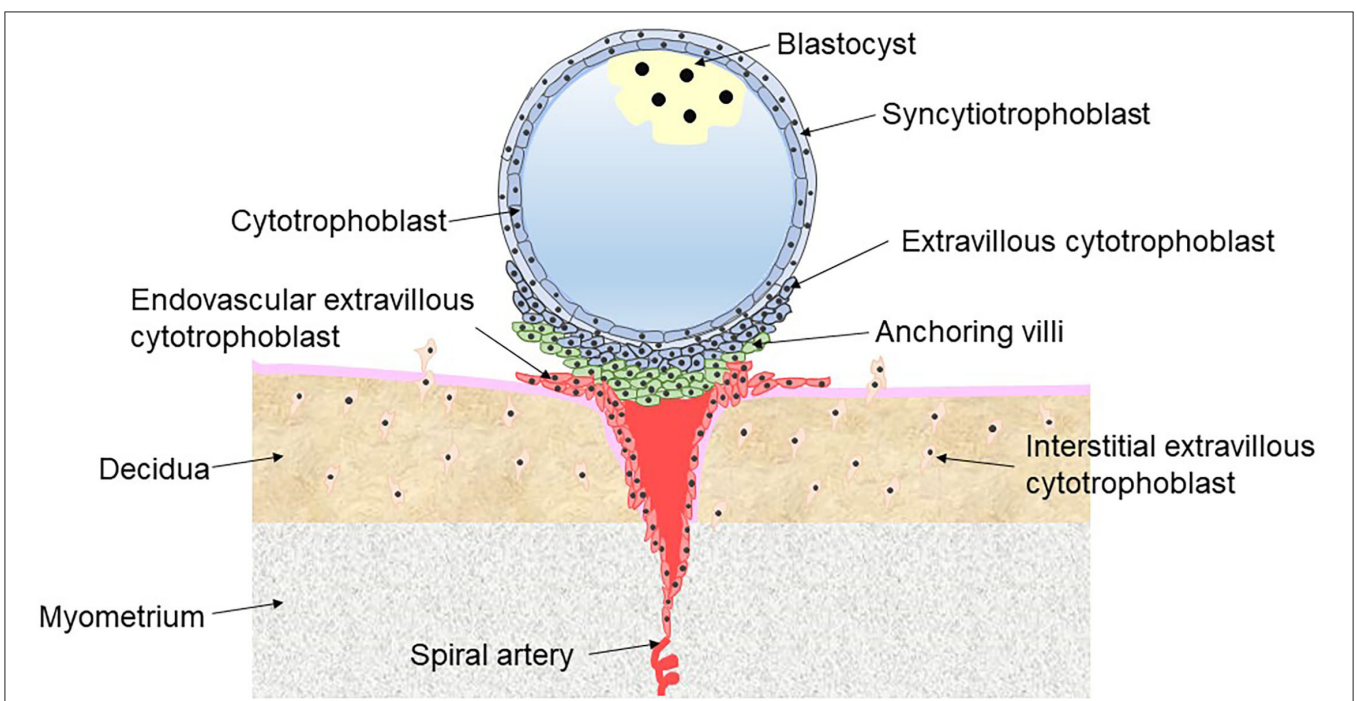

FIGURE 1 | Different types of trophoblast in placental implantation.

The mitochondrion is a double-membrane organelle with an ion-permeable IMM and an outer mitochondrial membrane (OMM) (16). Mitochondrial morphology and function vary in different trimesters of pregnancy. In the first trimester, CT differentiation into ST leads to a shift from the classical morphology of mitochondria (0.2–0.8 μm) with lamellar cristae into small (<0.1 μm), irregular shapes with no defined cristae and low-density matrix, and this adaptation meets the increased requirement of energy production in mitochondria for embryo development (17). The smaller mitochondria in ST might facilitate the transport of cholesterol and steroidogenesis, which requires cytochrome P450scc and 3 s-hydroxysteroiddehydrogenase-Δ^{4-5} isomerase type I located in the IMM, to transform cholesterol into pregnenolone and then convert into progesterone (18). Sufficient steroid hormone progesterone synthesized in human placental mitochondria is essential for the maintenance of pregnancy (19). With the development of the placenta, the mitochondrial content is greater in the third trimester than in the first trimester (20). However, the respiratory rate in the third trimester is similar to that in the first trimester. Thus, the efficiency of mitochondrial respiration using oxygen is lower in the later trimester after normalization to the mitochondrial content (20).

Mitochondria consume 90% cellular O_2 to synthesize ATP and are thus sensitive to oxygen tension. In the early first trimester (6–10 weeks), the spiral arteries are plugged by endovascular EVT so that oxygen tension is lower around the placenta, ∼20 mmHg in the placenta and ∼60 mmHg in the decidua (21). Relative hypoxia limits ATP synthesis by mitochondria, but the endometrial glands consume D-glucose to supply a large amount of ATP (22). Low O_2 pressure promotes trophoblast proliferation and angiogenesis in the placenta (23, 24). With embryo growth, the spiral arteries become unblocked at the end of the first trimester, and the oxygen tension rises to ∼60 mm Hg at the placenta and ∼70 mm Hg at the decidua through the villous tress to meet the increased metabolic requirement (21). High O_2 pressure promotes CT fusion into the ST and further invasion in the placenta (23, 25).

Hypoxic condition increases the secretion of sFlt-1 that related to the pathogenesis of PE (26). Hypoxia has been shown to reduce mitochondrial content, mitochondrial oxidative capacity and the expression of key molecules involved in the electron transport chain (27, 28). However, the dynamic alteration of mitochondrial morphology and function in PE has never been observed, which should be investigated in the future to comprehensively understand the role of mitochondria in the pathology of PE.

REACTIVE FREE RADICALS AND PE

Reactive free radicals (ROS) are byproducts of oxidative phosphorylation, including superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH) and peroxynitrite ($ONOO^-$). Most ROS are produced when electrons leak from complexes I/III: the leaked electrons reduce O_2 to generate O_2^-, of those generated at complex I are delivered into the matrix and of those generated at complex III are released into both the matrix and the intermembrane space, and then the dismutation of O_2^- to H_2O_2 is induced by superoxide dismutase 2 (SOD2) in the matrix and SOD1 in the intermembrane space. Glutathione peroxidases and peroxiredoxins are antioxidant enzymes that decompose H_2O_2 to O_2^-. The balance between ROS and antioxidant defense maintains cellular physical function. During pregnancy, a low level of ROS upregulates transcription factor E26 transformation-specific oncogene homolog 1 and VEGF to promote angiogenesis (29) and increases mitogen-activated protein kinase (MAPK) signaling to facilitate trophoblast differentiation and placental development (30).

Excessive ROS overwhelm antioxidant defense, leading to detrimental effects on cell physiologies such as lipids, proteins and DNAs. Excessive ROS production and an impaired enzymatic antioxidant system are detected in PE (31). Both the direct measurement of O_2^-/H_2O_2 and the indirect measurement of oxidative phosphorylation capacity (complex I-IV, cytochrome c oxidase) are reduced in PE (32, 33). Moreover, alterations in various proteins involved in oxidative phosphorylation have been found (34, 35). On the other hand, the expression and activity of antioxidant enzymes, including SODs, GPXs, thioredoxin reductases and catalase, are suppressed in PE placentas and trophoblasts (36). The decreased expression and activity of antioxidant enzymes result in the low efficiency of ATP synthesis, leading to electronic leakage and subsequent high production of ROS (37). ROS accumulation then triggers increased lipid peroxidation, including malondialdehyde (MDA), thiobarbituric acid reactive substances (i.e., a production of MDA) and 4-hydroxynonenal-modified proteins (38, 39).

Several well-known antioxidant nutrient supplementations have been found to prevent PE in small randomized trials, but a meta-analysis of randomized controlled trials revealed that vitamin C and E, selenium, L-arginine, allicin, lycopene or coenzyme Q10 did not effectively prevent PE (40). This could be because that antioxidants increase the concentration of circulating antioxidants but cannot repair the imbalance between ROS and antioxidants (41). Moreover, a recent study (42) found that potent antioxidant MitoQ administration during late gestation alleviated PE, but treatment during early gestation exacerbated reduced uterine perfusion pressure (RUPP)-induced PE in mice. Mild ROS has been shown to improve the proliferation, invasion and migration of CT-characterized HTR8-S/Veno cells for early placental implantation, and this could be blunted by antioxidants (42). Because mitochondrion is the main source of ROS, the lack of an effect of antioxidant therapy on PE brings out the consideration whether that mitochondrial-targeted interventions would be effective in preventing PE (40).

MITOCHONDRIAL QUALITY CONTROL AND PE

Mitochondria cannot be synthesized *de novo* but contain their own self-replicating genome. Coordination between mitochondrial autophagy (mitophagy) and biogenesis to deal with irreparably damaged mitochondria is essential for maintaining the mitochondrial volumes and determining the

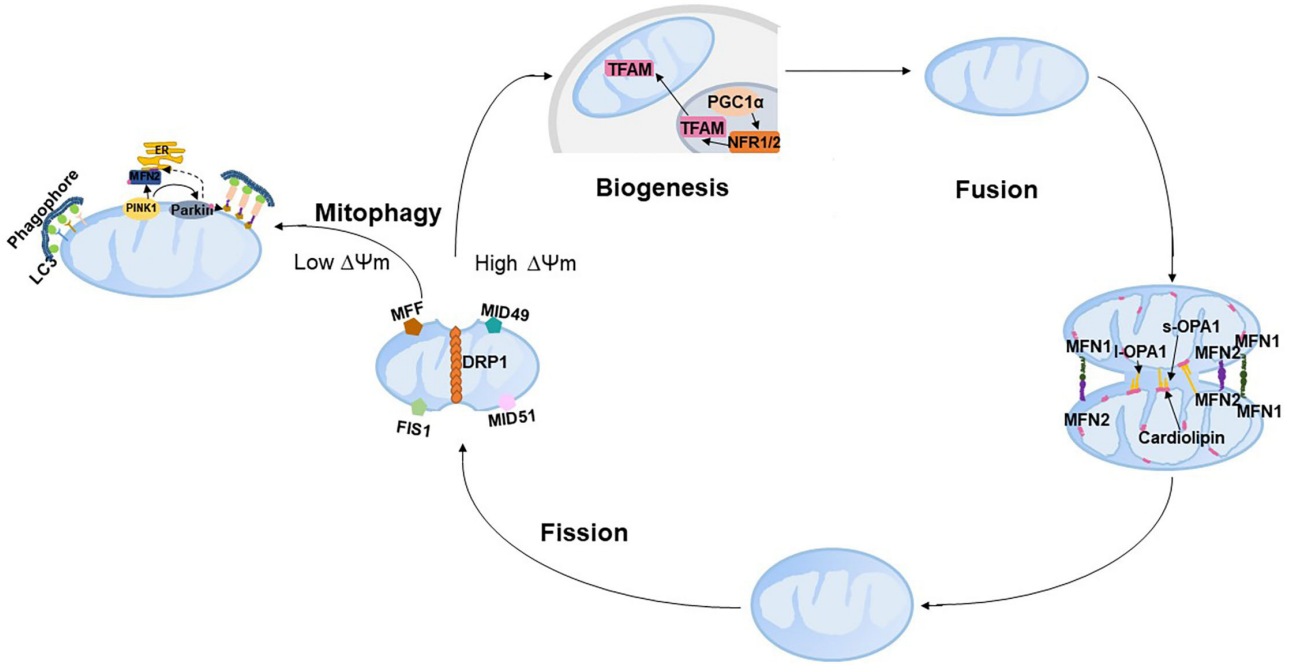

FIGURE 2 | Schematic representation of mitochondrial quality control. PINK, PTEN-induced putative kinase 1; LC3, microtubule-associated protein 1 light chain 3; ER, endoplasmic reticulum; DRP1, dynamin-related protein 1; FIS1, fission 1; MFF, mitochondrial fission factor; MID49, mitochondrial dynamics protein of 49 kDa; and MID51, mitochondrial dynamics protein of 51 kDa.

rate of mitochondrial turnover. Damaged mitochondrial proteins or parts of mitochondrial organelles are removed by mitophagy, and damaged components are renewed by adding proteins and lipids through biogenesis. Both mitochondrial biogenesis and mitophagy require mitochondrial dynamics fusion and fission. Mitochondrial dynamics, including mitochondrial fission and fusion nested in the tube-like mitochondrial network, continuously occur in response to metabolic or environmental stresses such as caloric restriction and low temperature. The integration of fusion, fission, mitophagy and mitochondrial biogenesis is referred to as mitochondrial quality control (mQC, **Figure 2**). The following text will introduce the process of mQC processes and the related predominant proteins. To our knowledge, 10 articles reported the expression of mQC-related genes in PE (**Table 1**), and this will also be overviewed.

Mitochondria Biogenesis

Mitochondrial biogenesis produces new mitochondria based on pre-existing mitochondria to respond to internal and external stresses such as oxidative stress, inflammation and mitochondrial drug toxicity. Mitochondrial biogenesis involves synthesis of IMM and OMM and mitochondrial encoded proteins; replication of mitochondrial DNA (mtDNA); and synthesis and import of nuclear encoded mitochondrial proteins. The vast majority of mitochondrial proteins are encoded by the nuclear genome, and thus mitochondrial biogenesis requires exquisite coordination of both mitochondrial and nuclear genomes, such as target, importation and correction of mRNA from nuclear to mitochondria (43). Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) is the master regulator of mitochondrial biogenesis, which is activated by either phosphorylation or deacetylation in the cytoplasm and then translocates to the nucleus (44, 45). Activated c-1α in the nucleus stimulates the expression of two key transcription factors, nuclear respiratory factor 1 (NRF1) and NRF2, and further interacts with NFR1/2 to increase their transcriptional activity, leading to the increased activity of mitochondrial transcription factor A (TFAM) to replicate mtDNA and encode mitochondrial proteins (46). Moreover, NRF1/2 are essential for nuclear-mitochondrial crosstalk to adapt to mitochondrial biomass and oxidative metabolism, especially in the developmental stage. NRF1-null mice exhibit lethality at early embryos due to the dramatic lack of mtDNA content and mitochondrial membrane potential in blastocysts (47). Embryos homozygous for the null NRF2 allele die prior to implantation, which highlights the critical mitochondrial roles of NRF2 during cleavage events of the embryo (48). PGC-1α also interacts with other nuclear transcription factors, such as estrogen-related receptors, thyroid hormone, peroxisome proliferator-activated receptors, and glucocorticoids, to regulate mitochondrial energy metabolism, respiration, and biogenesis (43).

Mitochondrial biogenesis can be regulated by several cell signaling pathways (49). AMP-activated kinase (AMPK) activated by exercise and starvation directly phosphorylates PGC-1a or indirectly deacetylates PGC-1a to stimulate biogenesis (50). The human Sirtuin isoforms SIRT1-2 have been shown to

TABLE 1 | Summary of current studies in humans related to mitochondrial quality control molecules.

Articles	Groups (*n* and gestation weeks)	Parts	Sites	Alteration	Non-alteration
Wangkheimayum et al. (41)	13 30.5 ± 2.9 wks eoPE, 11 37.8 ± 1.0 wks loPE, 14 39.2 ± 0.9 wks Ctrl	Placenta	1.5–2 cm next to the umbilical cord insertion, 1 cm in depth	mRNA and protein of OPA1 in eoPE	mRNA of MFN1, MFN2, NRF among three groups; mRNA and protein of OPA1 in eoPE; mRNA of TFAM in loPE; Protein of DRP1 among three groups.
				mRNA of TFAM in lope protein of TRAM in eoPE	
Yang et al. (42)	16 36.4 ± 2.26 wks PE, 16 36.7 ± 1.96 wks Ctrl	Villous tissues	NA	mRNA of MFN2	
Ventura-Clapier et al. (43)	10 33.7 ± 1.2 wks eoPE, 10 30.2 ± 1.1 wks loPE, 10 32.7±1.4 wks Ctrl	Myometrial biopsy (0.5 × 0.5 × 0.5 cm)	the upper edge of lower segment uterine incision	Protein of TFAM, PGC-1α in lope; mRNA of OPA in eoPE; Protein of L-OPA1:S-OPA1 in eoPE.	mRNA of NRF1 and NRF2 among three groups; Protein of MFN1, MFN2, DRP1, PINK1 and BNIP3 among three groups
				Protein of s-OPA in both PE	
Brenmoehl and Hoeflich (44)	11 30.0 ± 3.9 wks sPE, 11 31.0 ± 4.3 wks Ctrl,	Placenta	the maternal side of the placental villous tissue	Protein of MFN1, MFN2, OPA1, BNIP3, and PGC-1α	Protein of DRP1 and FIS1
Ryan and Hoogenraad (45)	33 29.3 ± 3.0 wks PE, 30 29.7 ± 2.3 wks Ctrl	Placenta	NA	Protein of DRP; protein of p-DRP1 in MIs	NA
				Protein of OPA1	
Virbasius and Scarpulla (46)	14 37.88 ± 2.10 wks term PE, 20 38.75 ± 0.84 wks term Ctrl, 8 29.73 ± 3.21 wks pre-term PE, 10 29.29 ± 3.83 wks pre-term Ctrl	Placenta	NA	Protein of l-OPA1:s-OPA1 in term PE vs. term Ctrl; Protein of MFN1 in term PE vs. term Ctrl.	Protein of DRP1 in placenta and placental MIs between two term groups and between two pre-term groups; Protein of MFN2 between two term groups and between two pre-term groups.
				Protein of FIS1 in term PE vs. term Ctrl;	
Matsubara et al. (32)	20 32.45 ± 1.81 wks eoPE, 20 38.29±1.60 wks Ctrl,	Placenta	NA	Protein of BNIP and MFN2	NA
Huo and Scarpulla (47)	19 <34 wks pre-term PE, 20 <34 wks pre-term Ctrl	Placenta	NA	mRNA and protein of PGC1α	NA
Ristevski et al. (48)	12 33 ± 3 wks PE, 11 39 ± 1 wks Ctrl	Placenta (<1 cm^2)	the paracentral region of the placenta at the maternal side	Protein of NRF1, BNIP3, BCL2, BNIP3L; mRNA and protein of DNM1.	mRNA of PGC1α, NRF2α, FUNDC1, FIS1, MFN1, MFN2 and OPA1; Protein of TFAM, FUNDC1, PINK1, PARK2.
				Protein of PGC1α; mRNA of NRF1, TFAM, BNIP3, BCL2, BNIP3L, PINK1, and PARK2	
Li et al. (49)	10 PE, 10 Ctrl	Placenta	NA	Ubiquitination level of FUNDC1	

wks, weeks; eoPE, early-onset PE; loPE, late-onset PE; Ctrl, normotensive controls; MIs, mitochondrial isolates; the listed molecules with highlight in gray refers increase in those molecules, and the listed molecule highlighted in gray refers decrease in those molecules; the mRNA or protein were in default extracted from placentas unless specified to MIs; and, the alteration was in default compared with controls unless specified otherwise.

deacetylate PGC-1a and then increase the activity of PGC-1a (51, 52); SIRT3 has been shown to increase the expression of PGC-1a through AMPK (**Figure 3**) (44). Recently, a decreasing trend of SIRT1 and activated AMPK/PGC-1a protein have been found in PE placentas (53), and inhibited SIRT1 and PGC-1a have been found in a group of patients with both intrauterine growth restriction and PE (54). Moreover, downregulated proteins of both SIRT3 and PGC1a were found in severe PE (55). Numerous regulators, including calcium/calmodulin-dependent protein kinase IV, Akt, AMP-dependent protein kinase (PKA), NO, and PPARa, also activate mitochondrial biogenesis (56).

Recent studies have shown decreased protein levels of PGC-1α in PE patients compared with controls (53, 55, 57), and the transcriptional level of PGC-1α was reduced in pre-term PE and pre-term controls (53) but was not altered between pre-term PE relative to term counterparts (57). All the current studies

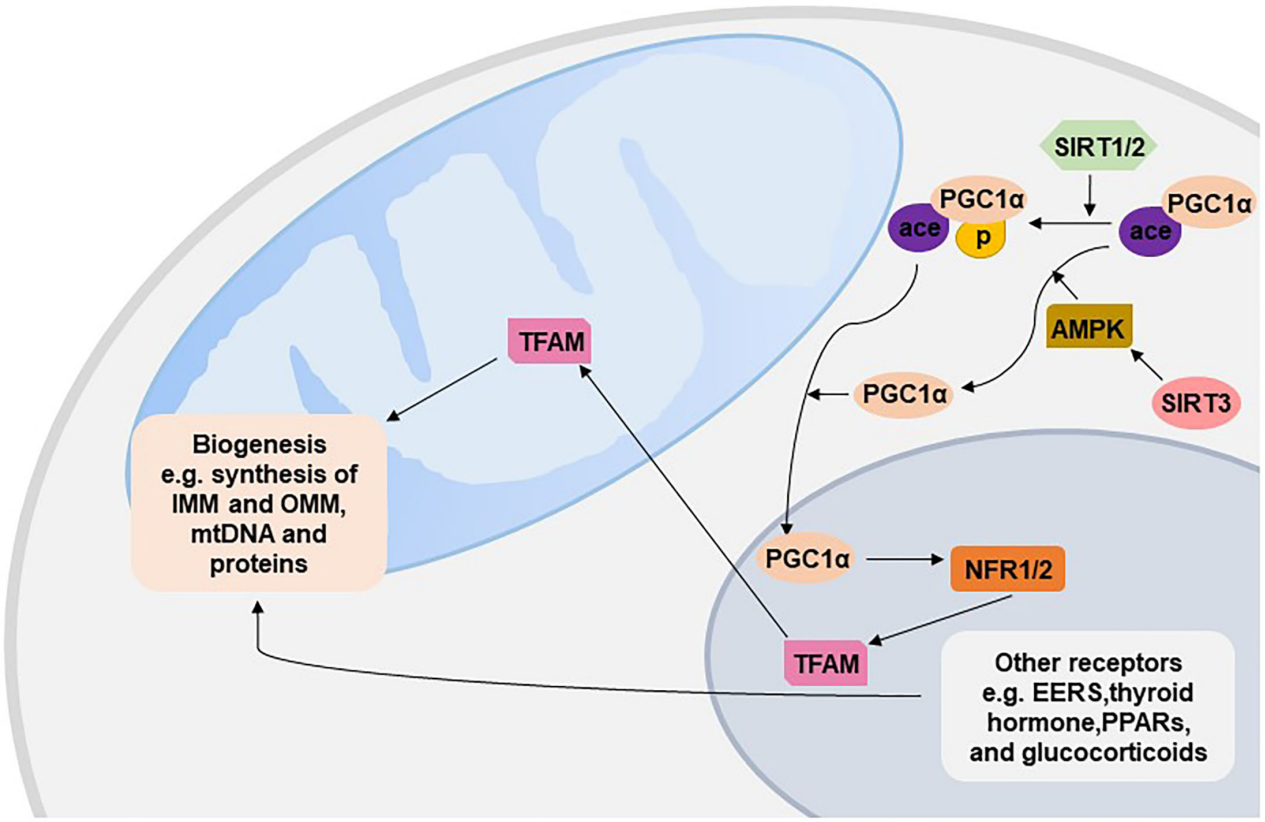

FIGURE 3 | Mitochondrial biogenesis. EERs, estrogen-related receptors; PPARs, peroxisome proliferator-activated receptors; TFAM, mitochondrial transcription factor A; PGC1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; NFR, nuclear respiratory factor; SIRT, Sirtuin; AMPK, AMP-activated kinase; IMM, inner membrane of the mitochondrion; OMM, outer membrane of the mitochondrion.

only assessed the mRNA or protein level of PGC-1α; however, activated PGC-1α (i.e., phosphorylated or deacetylated PGC-1α) has never been reported, and the expression of PGC-1α was not specified in the cytoplasm or mitochondrion. The protein level of NRF1 in pre-term PE patients has been shown to be higher than that in term controls, while the mRNA level of NRF1 was lower (57). Another study reported no difference in either the protein or mRNA expression of NRF1 between pre-term PE and pre-term counterparts (58). The number of cases of each group in the above three studies was relatively similar (i.e., 10–20), and the inconsistent results might result from the unmatched gestational age at delivery and unspecified subcellular organelles (e.g., nucleus or cytoplasm) from which PGC-1α was extracted. NFR2 has only been found to be decreased in hypoxia-induced BeWo cells (27). TFAM was 1.8-fold lower in late-onset PE placentas but not altered in early-onset PE placentas compared with control placentas (59).

Mitophagy

Mitophagy is the selective autophagic degradation of damaged mitochondria by autophagosomes and lysosomes. The best studied mitophagic process is the PTEN-induced putative kinase 1 (PINK1)-Parkin mitophagy pathway. PINK1 is a serine/thereonine kinase mainly localized to the OMM (60). Under normal conditions, PINK1 located at the OMM is imported into the IMM through the translocase of the inner and outer membrane complex and then cleaved and degraded by proteases, including mitochondrial-processing protease and inner membrane presenilin-associated rhomboid-like protease (PARL) (61–63). In contrast, damaged mitochondria with an indication of depolarized membrane potential are unable to import and degrade PINK1, resulting in the accumulation of PINK1 on the OMM (61). The accumulated PINK1 on the OMM is activated by autophosphorylation, which phosphorylates the E3 ubiquitin ligase Parkin by phosphorylating Thr175 and Thr217 on Parkin's linker region and translocates Parkin from the cytosol to mitochondria (61). Activated Parkin binds to phosphorylated ubiquitin tethering to the OMM, leading to the conjugation of ubiquitin to various substrates and the formation of polyubiquitin (poly-Ub) chains (64). The poly-Ub chain provokes the recruitment of Ub-binding autophagy receptors, including p62/sequestosome 1, nuclear dot protein 52 and optineurin, to connect with microtubule-associated protein 1 light chain 3 (LC3) and further facilitates the selective engulfment of ubiquitinated mitochondria by the autophagosome (**Figure 4**). A recent study has revealed that ubiquitination of the mitofusion

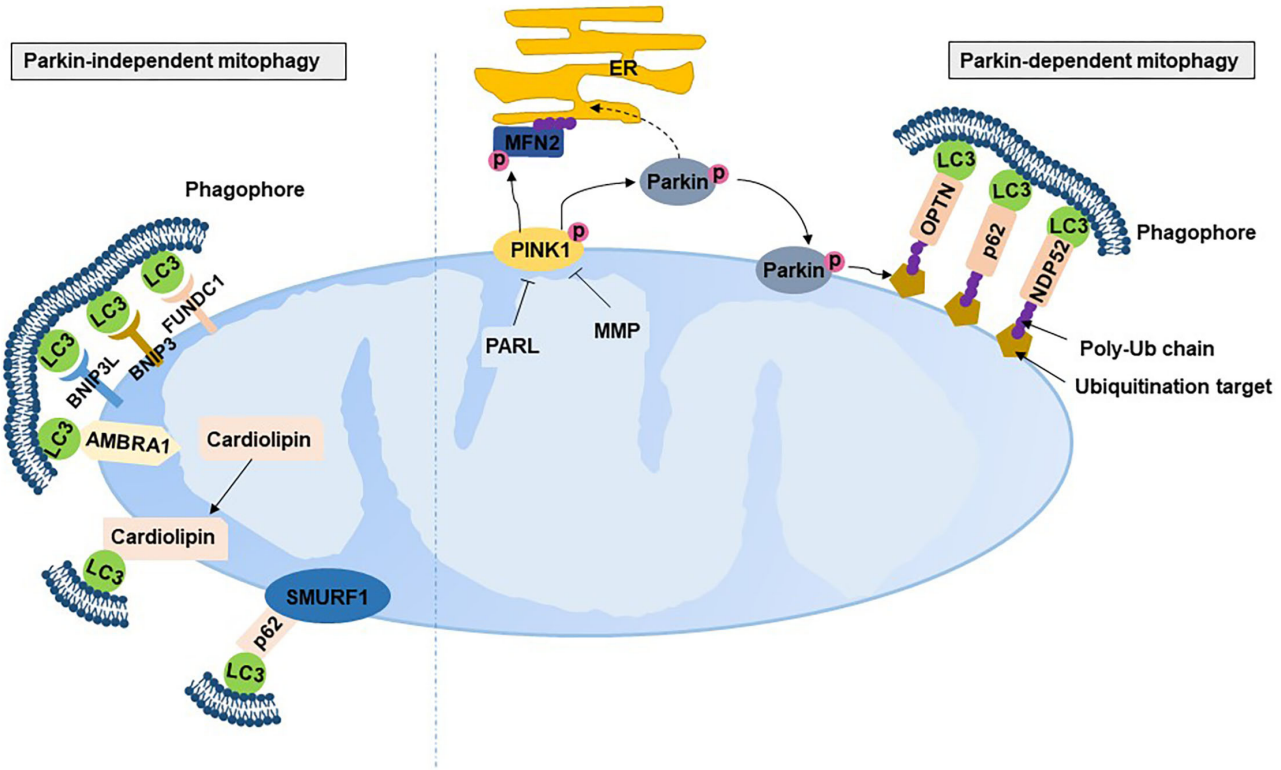

FIGURE 4 | Mitophagy. PARL, presenilin-associated rhomboid-like protease; MMP, mitochondrial-processing protease; PINK, PTEN-induced putative kinase 1; NDP52, nuclear dot protein 52; OPTN, optineurin; LC3, microtubule-associated protein 1 light chain 3; BNIP3, Bcl2/adenovirus E1B 19 kDa protein-interacting protein 3; BNIP3L, Bcl2/adenovirus E1B 19 kDa protein-interacting protein 3-like; FUNDC1, fun14 domain containing 1; AMBRA1, autophagy and beclin 1 regulator 1; SMURF1, SMAD specific E3 ubiquitin protein ligase 1.

2 (MFN2) is one of the very first step of mitophagy that occurs prior to autophagosomal engulfment of the organelle (65). PINK1 can explicitly facilitate Parkin-dependent mitophagy and be activated in other manners. This is evidenced by Parkin mutants having more severe phenotypes than PINK-null flies (66), while Parkin overexpression rescued mitochondrial morphology (67) and arrested mitochondrial motility (68). Parkin also simulates mitochondrial biogenesis, presumably to replace damaged mitochondria with healthy and functional organelles by degrading transcriptional repression (i.e., parkin-interacting substrate) on the depolarized mitochondrion (69).

Mounting evidence suggests that there are Parkin-independent mitophagic mechanisms. Several Parkin-independent proteins localize to mitochondria to recruit autophagosomes by interacting with LC3, including Bcl2/adenovirus E1B 19 kDa protein-interacting protein 3 (BNIP3), BNIP3-like (BNIP3L) and Fun14 domain containing 1 (FUNDC1) (70). BNIP3 and BNIP3L bonding to Bcl-2 separates the complex of Bcl-2 with Beclin-2, resulting in the initiation of autophagosomes (71). BNIP3 and BNIP3L have been shown to be complementary to mitophagy (71) and protect against excessive ROS (72). FUNDC1 localized on the OMM can be dephosphorylated under hypoxia to interact with LC3 on autophagosome membranes (73, 74). In addition, SMAD-specific E3 ubiquitin protein ligase 1 and autophagy and beclin 1 regulator 1 also induce LC3 dependence in a Parkin-independent mitophagic manner (75, 76). Cardiolipin, a membrane lipid in IMM, is also an LC3 receptor. Cardiolipin translates from the IMM to the OMM with external adverse stimulation and then interacts with the N-terminal helices of LC3 (77), indicating that cardiolipin also participates in mitophagy. Studies have found that mitochondria-derived vesicles stimulated by ROS instead of mitochondrial depolarization induced a faster rate of mitochondrial turnover with the requirement of Pink1/Parkin by delivering the mitochondrial content to the lysosome, where degradation of the mitochondrial content occurs (78, 79).

Compared with Parkin-dependent mitophagy, Parkin-independent mitophagy tends to play a more crucial role in PE. One study found that $PINK1_{63kDa/53kDa}$ ratio was increased in line with the increase in Parkin in the placentas of PE (80). PINK1 is 63 kDa under normal conditions, and cleaved PINK1 is 53 kDa. Mitophagy, as indicated by the PINK1 and Parkin proteins, was exhibited in the placentas of PE mice (81). In contrast, BNIP3-mediated mitophagy has been found to be involved in PE, evidenced by the higher protein expression of BNIPB and BNIP3L and higher mRNA expression of FUNDC1

in pre-term PE placentas compared to term controls (57). BNIP3 was inhibited in term severe PE placentas compared with term controls (55), while BNIP3 was upregulated in the pre-term early-onset PE placentas compared with the term controls (34). The ubiquitination level of FUNDC1 was low in hypoxic HTR8-S/Veno cells and the placenta of pregnant women with PE (82).

Mitochondrial Fusion

Mitochondrial fusion helps to mitigate metabolic or environmental stresses by distributing the mitochondrial contents between partially damaged mitochondria and healthy mitochondria (83). The fused mitochondria can be prevented from mitophagy (84). Mitochondrial fusion includes the fusion of both OMM and IMM and a mixture of mitochondrial contents. Mitochondrial fusion in mammalian cells is regulated by the fusion proteins MFN1/2 on the OMM and optic atrophy 1 (OPA1) on the IMM, which all belong to the dynamin-related family of large nucleotide guanosine triphosphates (GTPases). The GTPase domain of MFN1/2 hydrolyses GTP, which promotes homo- and hetero-oligomerization of MFN to dock on two OMMs and initiates OMM fusion (85–87) (**Figure 5**). Although the GTPase activity of MFN1 was ∼eightfold higher than that of MFN2, the affinity for GTP of MFN2 was more than 100-fold higher than that of MFN1 (85). This could be because that the internal interaction between the first and second heptad repeat domains of MFN2 resulted in the closed conformation of MFN2 and sequent fusion-deficiency. The closed conformation is activated by the phosphorylation at MFN2 Ser 378 (88). Replacing Ser 378 with Asp that cannot be phosphorylated has normal MFN2-mediated fusion features (88). Moreover, genetic mutations of MFN2 in murine embryonic fibroblasts interrupt mitochondrial fusion and produce large mitochondrial fragments (89). Interestingly, mutation in human MFN2 but not MFN1 results in Charcot–Marie–Tooth disease type 2A, a neurodegenerative disorder disease (90, 91). However, in MFN1-deficient Hela cells, mitochondrion failed to bind to each other and resulted in fragmentation of mitochondrion (92). Loss of either MFN1 or MFN2 causes lethality in mice, and the extracted cells from these mice display obviously fragmented mitochondria (87). These evidences suggest that MFN1/2 are dispensable for mitochondrial fusion.

OPA1 typically has two isoforms: long/membrane-bound (l-OPA1) and short/soluble OPA1 (s-OPA1). l-OPA1 is located in the IMM, and s-OPA1 is integral in the intermembrane space. l-OPA1 can be further cleaved to s-OPA1, while overexpression of s-OPA1 leads to mitochondrial fragmentation, which might be the result of mitochondrial fission (93). The interaction between l-OPA1 on one IMM and cardiolipin on another IMM has been shown to be essential to mitochondrial fusion *in vitro*, with evidence that the absence of cardiolipin caused the loss of membrane fusion activity (94). Whether s-OPA1 is required for IMM fusion is still controversial. Ishihara et al. (95) and Tondera et al. (96) found that l-OPA1 is sufficient to facilitate IMM fusion, while other studies showed that both s-OPA1 and l-OPA1 are required for efficient and fast fusion (97, 98). Moreover, the l-OPA1:s-OPA1 ratio is thought to mediate the balance between fission and fusion (97). OPA1 has also been discovered to be involved in mitochondrial crista remodeling with inner membrane organization (99).

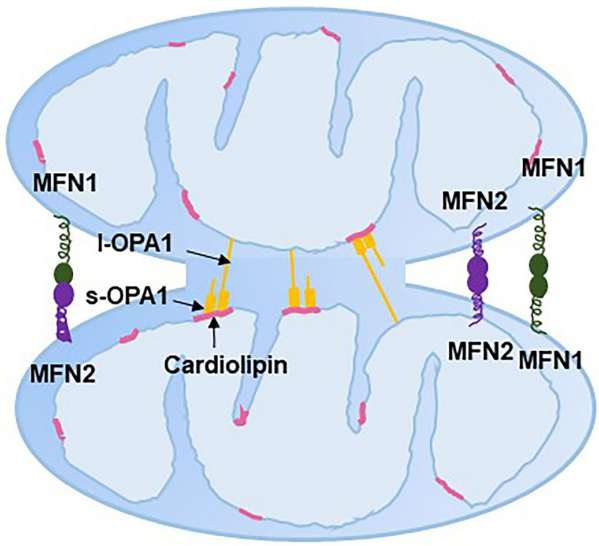

FIGURE 5 | Mitochondrial fusion. ER, endoplasmic reticulum; MFN, mitofusin; l-OPA1, long-optic atrophy 1; s-OPA1, short-optic atrophy 1.

Decreased mRNA levels of MFN2 have been found in term PE placentas (100). However, a proteomics analysis found the upregulated expression of MFN2 in pre-term early-onset PE placentas compared with pre-term controls (34). The gene expression of OPA increased 2.5-fold in pre-term early-onset PE placentas compared with term controls, but there was no difference between term early-onset PE and term late-onset PE placentas (59). In contrast, the protein expression of the l-OPA1/s-OPA1 ratio and OPA1 significantly increased in placenta from term PE patients compared with term controls, but the difference was not observed between pre-term PE placenta and pre-term normal placenta (101). Another study found decreased protein expression of OPA1 in pre-term PE placentas compared with pre-term controls (80). Neither MFN1/2 nor OPA1 was altered in term PE placentas compared with controls (27), but OPA1 was upregulated in the myometrium of pre-term early-onset PE compared to pre-term controls (58). OPA1 and MFN1/2 were downregulated in severe PE placentas (55). Although the findings of mitochondrial fusion-related genes in PE patients are inconsistent, the results in PE-like trophoblast cells are coincident. Decreased mRNA and protein levels of MFN2 have been confirmed in hypoxia-induced TEV-1 cells (100), and decreased transcript levels of MFN1 and MFN2 have been found in hypoxia-induced BeWo cells (27).

Mitochondrial Fission

Fission segregates and uncouples damaged mitochondrial sub-organelles by dividing mitochondria from mitochondrial networks to maintain adequate numbers of mitochondria.

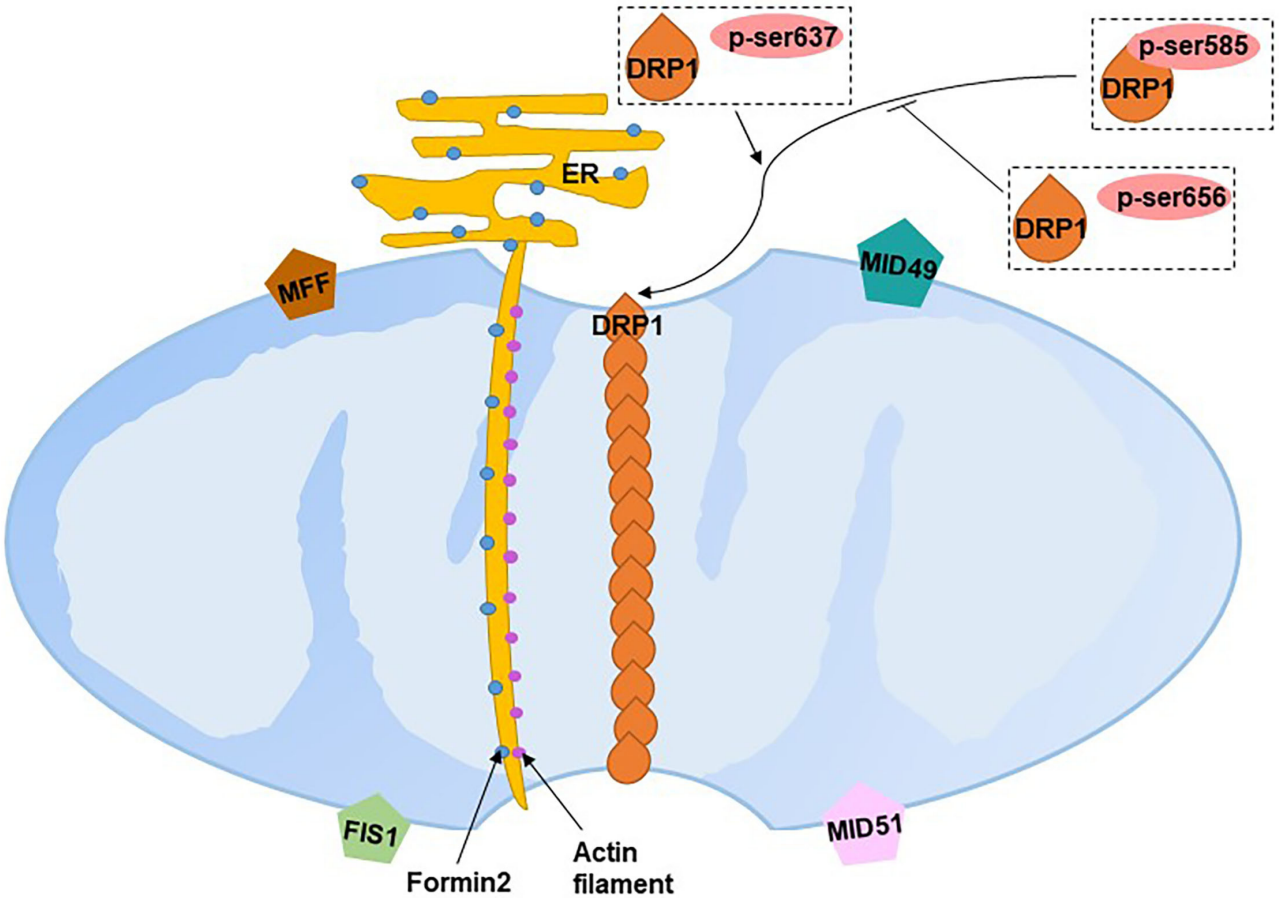

FIGURE 6 | Mitochondrial fission. ER, endoplasmic reticulum; DRP1, dynamin-related protein 1; FIS1, fission 1; MFF, mitochondrial fission factor; MID49, mitochondrial dynamics protein of 49 kDa; MID51, mitochondrial dynamics protein of 51 kDa. Blue dots on the ER refer to formin 2, purple dots between the mitochondria and endoplasmic reticulum refer to actin filaments.

Mitochondrial fission is primarily carried out by dynamin-related protein 1 (DRP1), a large GTPase. The translocation of DRP1 from the cytosol to mitochondria interacts with four receptor proteins in the OMM: fission 1 (FIS1), mitochondrial fission factor (MFF), mitochondrial dynamics protein of 49 kDa (MID49) and MID51, which initiate fission by constricting mitochondria (102). The translocation of DRP1 requires the phosphorylation-dephosphorylation at Ser of DRP1: Ser585 is phosphorylated by Cdk1/Cyclin B leading to the increased DRP1 GTPase activity at the onset of mitosis (103); the Ser656 phosphatased by PKA inhibits mitochondrial scission (104); and, dephosphorylation at Ser637 by the mitochondrial phosphatase phosphoglycerate mutase family member 5 recruits DRP1 to mitochondria to drive scission (105–107). However, DRP1 recruitment at the reticulum–mitochondria contact site has been found to occur prior to recruitment at the mitochondria constrict site, where the mitochondrion starts to segregate (108). The endoplasmic reticulum tubules first wrap around the constricted parts of mitochondria in the form of rings (108). Actin filaments then accumulate between mitochondrial and inverted formin 2-enriched endoplasmic reticulum membranes at the constriction sites, which initially recruits DRP1 to drive mitochondrial fission (109) (**Figure 6**). DRP1, FIS1 and MFF have been found to localize to peroxisomal membranes involved in peroxisomal fission (110).

A recent study found two distinct types of fission on African green monkey Cos-7 cells and mouse cardiomyocytes (111). Fission at the mitochondrial periphery (<25% from a tip) divides damaged mitochondria into smaller daughter mitochondria for sequent mitophagy, whereas division at the mid-zone of mitochondria (within the central 50%) leads to mitochondrial biogenesis (111). Compared with mid-zone fission, mitochondrial fission occurring at the periphery tends to have the following characteristics: reduced mitochondrial membrane potential, matrix pH, elevated ROS and increased Ca^{+}. Although both types are regulated by DRP1, mitochondrial-endoplasmic reticulum contact, actin preconstruction and MFF play crucial roles in mid-zone fission, whereas mitochondrial-lysosomal contact and FIS1 play essential roles in peripheral fission. Divided mitochondrial

fragmentation has two fates. Daughter mitochondria with higher membrane potential (presumably good quality mitochondria) proceed to fusion, while depolarized daughter mitochondria (presumably bad quality mitochondria) are degraded by mitophagy (112).

PE placentas show increased numbers of mitochondria in CTs, but with reduced size (80, 113), which is suggestive of increased mitochondrial fission. Although the mRNA transcript level of FIS-1 was not changed (55, 57), the fission-related dynamin-1-like protein (DNM1L) protein and mRNA transcript levels were increased in placentas complicated with PE (57). The protein expression of FIS1 was decreased in term PE relative to term controls (101). Increases in DRP1 expression, activation and phosphorylation have been found in pre-term PE placentas compared with pre-term controls (80). However, there was no difference in DRP1 expression levels in either pre-term PE or term PE compared with corresponding controls (55, 59). Neither FIS-1 nor DNM1L was unaltered in hypoxia-induced placental explants, but both were increased in hypoxia-induced BeWo cells (27).

The Interaction Between Mitochondrial Dynamics and Biogenesis/Mitophagy

Many studies have found that mitochondrial biogenesis, mitophagy, fusion or fission could be simultaneously affected by external stimuli, but the interaction between mitochondrial dynamics and biogenesis/mitophagy has rarely been confirmed by intervention one molecule to observe the effect on another mQC molecule. Mitochondrial biogenesis has been found to be mediated by fusion- and fission-related proteins (114), but this lacks further confirmation. Mitochondrial dynamics are closely related to mitophagy. MFN2 phosphorylated by PINK1 regulates the recruitment of Parkin to depolarize mitochondria and facilitates Parkin-mediated ubiquitination (115). Chen and Dorn (115). found that the absence of MFN2 in mouse cardiac myocytes prevented the translocation of Parkin to depolarized mitochondria and then inhibited mitophagy OPA1 has also been shown to interact with lysine 70 of FUNDC1, which facilitates OPA1-mediated fusion, moreover, mutants of lysine 70 inhibit the interaction and thus promote FUNDC1-regulated mitophagy (116). Overexpressing OPA1 reduces the majority of mitophagy (117). Fission is required for mitophagy, as evidenced by mitophagy being prevented with a dominant-negative mutant of DRP1 (117, 118). Inhibition of mitochondrial fission by lowering the expression of FIS1 also reduces mitochondrial mitophagy (117, 118). Fusion and fission have been shown to be paired consecutive events, and fission quickly follows fusion (117). Furthermore, DRP1 interacts with MFN to increase elongated mitochondria by promoting fusion and inhibiting fission (119). However, the interaction between mitochondrial dynamics and biogenesis/mitophagy in PE has not been investigated, and this requires further studies to be helpful to explore the mQC-targeted treatment.

SUMMARY OF CURRENT STUDIES IN HUMANS AND FUTURE DIRECTIONS

Ten human studies have reported the alteration of mQC-related molecules in PE since 2015, while there are several limitations: (1) The numbers of clinical cases were small, ranging from 10 to 33. (2) 60% of these studies did not elucidate where the examined tissues were collected (e.g., at the maternal side or the fetal side). (3) In 30% of these studies, the gestational week at delivery between control and PE groups was not comparable. The expression of mQC-related molecules varies on the different gestational weeks, and thus the gestational week should be equivalent for comparison. (4) Only two studies isolated mitochondrial mRNA and protein from total mRNA and protein. The subcellular organelle where the mRNA or protein extracted is critical for molecular examination because several mQC-related molecules widely distribute in the cytosol, nucleus and mitochondrion, but the research scope of the above studies limit to the mitochondria. (5) The assessment of mQC-related molecules in humans should be verified in trophoblast cells or RUPP rats to reduce the bias of species differences and large fluctuation of human individuals. Therefore, future studies should be performed with larger sample size, comparable gestational weeks, clear distinction of the site where tissues were collected and the subcellular organelle where the molecules were assessed, and verification of results in other species. The future comprehensive human mQC-related PE studies will help to provide the clinical basis for mQC-targeted treatment of PE.

CONCLUSION

mQC has been emerged as the treatment target of several diseases, including neurodegeneration, cardiovascular disease and cancer. Current studies have revealed that mQC-related molecules are associated with PE, although there are several drawbacks of these studies. These findings suggest that mQC plays an important role in the progression of PE, but further investigations for deeper elucidation are required. The future investigation of mQC in PE may provide a new insight on prevention and therapy strategies for PE.

AUTHOR CONTRIBUTIONS

XP contributed to relevant studies collection and summarisation and drafted manuscript. RH and YY: contributed to relevant studies collection and summarisation. ZL and YC: contributed to project conception and critical revision of manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This work has been supported by National Natural Science Foundation of China (NSFC-82000399).

REFERENCES

1. Steegers EA, von Dadelszen P, Duvekot JJ, Pijnenborg R. Pre-eclampsia. *Lancet*. (2010) 376:631–44. doi: 10.1016/S0140-6736(10)60279-6
2. ACOG Practice Bulletin No. 202: gestational hypertension and preeclampsia. *Obstet Gynecol*. (2019) 133:e1–25. doi: 10.1097/AOG.0000000000003018
3. Brown MA, Magee LA, Kenny LC, Karumanchi SA, McCarthy FP, Saito S, et al. The hypertensive disorders of pregnancy: ISSHP classification, diagnosis & management recommendations for international practice. *Pregn Hypertens*. (2018) 13:291–310. doi: 10.1016/j.preghy.2018.05.004
4. Walani SR. Global burden of pre-term birth. *Int J Gynaecol Obstet*. (2020) 150:31–3. doi: 10.1002/ijgo.13195
5. Ives CW, Sinkey R, Rajapreyar I, Tita ATN, Oparil S. Preeclampsia-pathophysiology and clinical presentations: JACC state-of-the-art review. *J Am Coll Cardiol*. (2020) 76:1690–702. doi: 10.1016/j.jacc.2020.08.014
6. Sircar M, Thadhani R, Karumanchi SA. Pathogenesis of preeclampsia. *Curr Opin Nephrol Hypertens*. (2015) 24:131–8. doi: 10.1097/MNH.0000000000000105
7. Boss AL, Chamley LW, James JL. Placental formation in early pregnancy: how is the centre of the placenta made? *Hum Reprod Update*. (2018) 24:750–60. doi: 10.1093/humupd/dmy030
8. Abbas Y, Turco MY, Burton GJ, Moffett A. Investigation of human trophoblast invasion *in vitro*. *Hum Reprod Update*. (2020) 26:501–13. doi: 10.1093/humupd/dmaa017
9. Levine RJ, Lam C, Qian C, Yu KF, Maynard SE, Sachs BP, et al. Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. *N Engl J Med*. (2006) 355:992–1005. doi: 10.1056/NEJMoa055352
10. Farah O, Nguyen C, Tekkatte C, Parast MM. Trophoblast lineage-specific differentiation and associated alterations in preeclampsia and fetal growth restriction. *Placenta*. (2020) 102:4–9. doi: 10.1016/j.placenta.2020.02.007
11. Maynard SE, Min J-Y, Merchan J, Lim K-H, Li J, Mondal S, et al. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. *J Clin Invest*. (2003) 111:649–58. doi: 10.1172/JCI17189
12. Leanos-Miranda A, Navarro-Romero CS, Sillas-Pardo LJ, Ramirez-Valenzuela KL, Isordia-Salas I, Jimenez-Trejo LM. Soluble endoglin as a marker for preeclampsia, its severity, and the occurrence of adverse outcomes. *Hypertension*. (2019) 74:991–7. doi: 10.1161/HYPERTENSIONAHA.119.13348
13. Zeisler H, Llurba E, Chantraine F, Vatish M, Staff AC, Sennstrom M, et al. Predictive value of the sFlt-1:PlGF ratio in women with suspected preeclampsia. *N Engl J Med*. (2016) 374:13–22. doi: 10.1056/NEJMoa1414838
14. Chang X, Yao J, He Q, Liu M, Duan T, Wang K. Exosomes from women with preeclampsia induced vascular dysfunction by delivering sFlt (Soluble Fms-Like Tyrosine Kinase)-1 and sEng (Soluble Endoglin) to endothelial cells. *Hypertension*. (2018) 72:1381–90. doi: 10.1161/HYPERTENSIONAHA.118.11706
15. Hu XQ, Zhang L. Hypoxia and mitochondrial dysfunction in pregnancy complications. *Antioxidants*. (2021) 10:405. doi: 10.3390/antiox10030405
16. Balaban RS. Regulation of oxidative phosphorylation in the mammalian cell. *Am J Physiol*. (1990) 258(3 Pt 1):C377–89. doi: 10.1152/ajpcell.1990.258.3.C377
17. Holland O, Dekker Nitert M, Gallo LA, Vejzovic M, Fisher JJ, Perkins AV. Review: placental mitochondrial function and structure in gestational disorders. *Placenta*. (2017) 54:2–9. doi: 10.1016/j.placenta.2016.12.012
18. Tuckey RC, Kostadinovic Z, Cameron KJ. Cytochrome P-450scc activity and substrate supply in human placental trophoblasts. *Mol Cell Endocrinol*. (1994) 105:103–9. doi: 10.1016/0303-7207(94)90041-8
19. Tuckey RC. Progesterone synthesis by the human placenta. *Placenta*. (2005) 26:273–81. doi: 10.1016/j.placenta.2004.06.012
20. Holland OJ, Hickey AJR, Alvsaker A, Moran S, Hedges C, Chamley LW, et al. Changes in mitochondrial respiration in the human placenta over gestation. *Placenta*. (2017) 57:102–12. doi: 10.1016/j.placenta.2017.06.011
21. Jauniaux E, Watson A, Burton G. Evaluation of respiratory gases and acid-base gradients in human fetal fluids and uteroplacental tissue between 7 and 16 weeks' gestation. *Am J Obstet Gynecol*. (2001) 184:998–1003. doi: 10.1067/mob.2001.111935
22. Frolova AI, O'Neill K, Moley KH. Dehydroepiandrosterone inhibits glucose flux through the pentose phosphate pathway in human and mouse endometrial stromal cells, preventing decidualization and implantation. *Mol Endocrinol*. (2011) 25:1444–55. doi: 10.1210/me.2011-0026
23. Genbacev O, Zhou J, Ludlow JW, Fisher SJ. Regulation of human placental development by oxygen tension. *Science*. (1997) 277:1669–72. doi: 10.1126/science.277.5332.1669
24. Burton GJ, Charnock-Jones DS, Jauniaux E. Regulation of vascular growth and function in the human placenta. *Reproduction*. (2009) 138:895–902. doi: 10.1530/REP-09-0092
25. Aye I, Aiken CE, Charnock-Jones DS, Smith GCS. Placental energy metabolism in health and disease-significance of development and implications for preeclampsia. *Am J Obstet Gynecol*. (2020) 226:S928–44. doi: 10.1016/j.ajog.2020.11.005
26. Li H, Gu B, Zhang Y, Lewis DF, Wang Y. Hypoxia-induced increase in soluble Flt-1 production correlates with enhanced oxidative stress in trophoblast cells from the human placenta. *Placenta*. (2005) 26:210–7. doi: 10.1016/j.placenta.2004.05.004
27. Vangrieken P, Al-Nasiry S, Bast A, Leermakers PA, Tulen CBM, Janssen GMJ, et al. Hypoxia-induced mitochondrial abnormalities in cells of the placenta. *PLoS ONE*. (2021) 16:e0245155. doi: 10.1371/journal.pone.0245155
28. Sferruzzi-Perri AN, Higgins JS, Vaughan OR, Murray AJ, Fowden AL. Placental mitochondria adapt developmentally and in response to hypoxia to support fetal growth. *Proc Natl Acad Sci USA*. (2019) 116:1621–6. doi: 10.1073/pnas.1816056116
29. Yasuda M, Ohzeki Y, Shimizu S, Naito S, Ohtsuru A, Yamamoto T, et al. Stimulation of *in vitro* angiogenesis by hydrogen peroxide and the relation with ETS-1 in endothelial cells. *Life Sci*. (1999) 64:249–58. doi: 10.1016/S0024-3205(98)00560-8
30. Mudgett JS, Ding J, Guh-Siesel L, Chartrain Na, Yang L, Gopal S, et al. Essential role for p38alpha mitogen-activated protein kinase in placental angiogenesis. *Proc Natl Acad Sci USA*. (2000) 97:10454–9. doi: 10.1073/pnas.180316397
31. Bharadwaj SK, Vishnu Bhat B, Vickneswaran V, Adhisivam B, Bobby Z, Habeebullah S. Oxidative stress, antioxidant status and neurodevelopmental outcome in neonates born to pre-eclamptic mothers. *Indian J Pediatr*. (2018) 85:351–7. doi: 10.1007/s12098-017-2560-5
32. Matsubara S, Minakami H, Sato I, Saito T. Decrease in cytochrome c oxidase activity detected cytochemically in the placental trophoblast of patients with pre-eclampsia. *Placenta*. (1997) 18:255–9. doi: 10.1016/S0143-4004(97)80059-8
33. Furui T, Kurauchi O, Tanaka M, Mizutani S, Ozawa T, Tomoda Y. Decrease in cytochrome c oxidase and cytochrome oxidase subunit I messenger RNA levels in preeclamptic pregnancies. *Obstet Gynecol*. (1994) 84:283–8.
34. Xu Z, Jin X, Cai W, Zhou M, Shao P, Yang Z, et al. Proteomics analysis reveals abnormal electron transport and excessive oxidative stress cause mitochondrial dysfunction in placental tissues of early-onset preeclampsia. *Proteomics Clin Appl*. (2018) 12:e1700165. doi: 10.1002/prca.201700165
35. Shi Z, Long W, Zhao C, Guo X, Shen R, Ding H. Comparative proteomics analysis suggests that placental mitochondria are involved in the development of pre-eclampsia. *PLoS ONE*. (2013) 8:e64351. doi: 10.1371/journal.pone.0064351
36. Kumar CA, Das UN. Lipid peroxides, anti-oxidants and nitric oxide in patients with pre-eclampsia and essential hypertension. *Med Sci Monit*. (2000) 6:901–7.
37. Vaka VR, McMaster KM, Cunningham MW Jr, Ibrahim T, Hazlewood R, Usry N, et al. Role of mitochondrial dysfunction and reactive oxygen species in mediating hypertension in the reduced uterine perfusion pressure rat model of preeclampsia. *Hypertension*. (2018) 72:703–11. doi: 10.1161/HYPERTENSIONAHA.118.11290
38. Shibata ENH, Ejima K, Araki M, Fukuda J, Yoshimura K, Toki N, et al. Enhancement of mitochondrial oxidative stress and up-regulation of antioxidant protein peroxiredoxin III/SP-22 in the mitochondria of human pre-eclamptic placentae. *Placenta*. (2003) 24:698–705. doi: 10.1016/S0143-4004(03)00083-3
39. Wang Y, Walsh SW. Placental mitochondria as a source of oxidative stress in pre-eclampsia. *Placenta*. (1998) 19:581–6. doi: 10.1016/S0143-4004(98)90018-2

40. Tenorio MB, Ferreira RC, Moura FA, Bueno NB, Goulart MOF, Oliveira ACM. Oral antioxidant therapy for prevention and treatment of preeclampsia: meta-analysis of randomized controlled trials. *Nutr Metab Cardiovasc Dis*. (2018) 28:865–76. doi: 10.1016/j.numecd.2018.06.002
41. Wangkheimayum S, Kumar S, Suri V. Effect of vitamin E on sP-selectin levels in pre-eclampsia. *Indian J Clin Biochem*. (2011) 26:169–71. doi: 10.1007/s12291-010-0102-2
42. Yang Y, Xu P, Zhu F, Liao J, Wu Y, Hu M, et al. The potent antioxidant mitoq protects against preeclampsia during late gestation but increases the risk of preeclampsia when administered in early pregnancy. *Antioxid Redox Signal*. (2021) 34:118–36. doi: 10.1089/ars.2019.7891
43. Ventura-Clapier R, Garnier A, Veksler V. Transcriptional control of mitochondrial biogenesis: the central role of PGC-1alpha. *Cardiovasc Res*. (2008) 79:208–17. doi: 10.1093/cvr/cvn098
44. Brenmoehl J, Hoeflich A. Dual control of mitochondrial biogenesis by sirtuin 1 and sirtuin 3. *Mitochondrion*. (2013) 13:755–61. doi: 10.1016/j.mito.2013.04.002
45. Ryan MT, Hoogenraad NJ. Mitochondrial-nuclear communications. *Annu Rev Biochem*. (2007) 76:701–22. doi: 10.1146/annurev.biochem.76.052305.091720
46. Virbasius JV, Scarpulla RC. Activation of the human mitochondrial transcription factor A gene by nuclear respiratory factors: a potential regulatory link between nuclear and mitochondrial gene expression in organelle biogenesis. *Proc Natl Acad Sci USA*. (1994) 91:1309–13. doi: 10.1073/pnas.91.4.1309
47. Huo L, Scarpulla RC. Mitochondrial DNA instability and peri-implantation lethality associated with targeted disruption of nuclear respiratory factor 1 in mice. *Mol Cell Biol*. (2001) 21:644–54. doi: 10.1128/MCB.21.2.644-654.2001
48. Ristevski S, O'Leary DA, Thornell AP, Owen MJ, Kola I, Hertzog PJ. The ETS transcription factor GABPalpha is essential for early embryogenesis. *Mol Cell Biol*. (2004) 24:5844–9. doi: 10.1128/MCB.24.13.5844-5849.2004
49. Li PA, Hou X, Hao S. Mitochondrial biogenesis in neurodegeneration. *J Neurosci Res*. (2017) 95:2025–9. doi: 10.1002/jnr.24042
50. Cantó C, Auwerx J. PGC-1alpha, SIRT1 and AMPK, an energy sensing network that controls energy expenditure. *Curr Opin Lipidol*. (2009) 20:98–105. doi: 10.1097/MOL.0b013e328328d0a4
51. Rodgers JT, Lerin C, Haas W, Gygi SP, Spiegelman BM, Puigserver P. Nutrient control of glucose homeostasis through a complex of PGC-1alpha and SIRT1. *Nature*. (2005) 434:113–8. doi: 10.1038/nature03354
52. Krishnan J, Danzer C, Simka T, Ukropec J, Walter KM, Kumpf S, et al. Dietary obesity-associated Hif1α activation in adipocytes restricts fatty acid oxidation and energy expenditure via suppression of the Sirt2-NAD+ system. *Genes Dev*. (2012) 26:259–70. doi: 10.1101/gad.180406.111
53. Hastie R, Brownfoot FC, Pritchard N, Hannan NJ, Cannon P, Nguyen V, et al. EGFR (Epidermal Growth Factor Receptor) signaling and the mitochondria regulate sFlt-1 (Soluble FMS-Like Tyrosine Kinase-1) secretion. *Hypertension*. (2019) 73:659–70. doi: 10.1161/HYPERTENSIONAHA.118.12300
54. Poidatz D, Dos Santos E, Duval F, Moindjie H, Serazin V, Vialard F, et al. Involvement of estrogen-related receptor-γ and mitochondrial content in intrauterine growth restriction and preeclampsia. *Fertil Steril*. (2015) 104:483–90. doi: 10.1016/j.fertnstert.2015.05.005
55. Zhou X, Han TL, Chen H, Baker PN, Qi H, Zhang H. Impaired mitochondrial fusion, autophagy, biogenesis and dysregulated lipid metabolism is associated with preeclampsia. *Exp Cell Res*. (2017) 359:195–204. doi: 10.1016/j.yexcr.2017.07.029
56. Jornayvaz FR, Shulman GI. Regulation of mitochondrial biogenesis. *Essays Biochem*. (2010) 47:69–84. doi: 10.1042/bse0470069
57. Vangrieken P, Al-Nasiry S, Bast A, Leermakers PA, Tulen CBM, Schiffers PMH, et al. Placental mitochondrial abnormalities in preeclampsia. *Reprod Sci*. (2021) 28:2186–99. doi: 10.1007/s43032-021-00464-y
58. Vishnyakova PA, Volodina MA, Tarasova NV, Marey MV, Kan NE, Khodzhaeva ZS, et al. Alterations in antioxidant system, mitochondrial biogenesis and autophagy in preeclamptic myometrium. *BBA Clin*. (2017) 8:35–42. doi: 10.1016/j.bbacli.2017.06.002
59. Vishnyakova PA, Volodina MA, Tarasova NV, Marey MV, Tsvirkun DV, Vavina OV, et al. Mitochondrial role in adaptive response to stress conditions in preeclampsia. *Sci Rep*. (2016) 6:32410. doi: 10.1038/srep32410
60. Youle RJ, Narendra DP. Mechanisms of mitophagy. *Nat Rev Mol Cell Biol*. (2011) 12:9–14. doi: 10.1038/nrm3028
61. Narendra DP, Jin SM, Tanaka A, Suen DF, Gautier CA, Shen J, et al. PINK1 is selectively stabilized on impaired mitochondria to activate Parkin. *PLoS Biol*. (2010) 1:e1000298. doi: 10.1371/journal.pbio.1000298
62. Deas E, Plun-Favreau H, Gandhi S, Desmond H, Kjaer S, Loh SH, et al. PINK1 cleavage at position A103 by the mitochondrial protease PARL. *Hum Mol Genet*. (2011) 5:867–79. doi: 10.1093/hmg/ddq526
63. Greene AW, Grenier K, Aguileta MA, Muise S, Farazifard R, Haque ME, et al. Mitochondrial processing peptidase regulates PINK1 processing, import and Parkin recruitment. *EMBO Rep*. (2012) 4:378–85. doi: 10.1038/embor.2012.14
64. Okatsu K, Koyano F, Kimura M, Kosako H, Saeki Y, Tanaka K, et al. Phosphorylated ubiquitin chain is the genuine Parkin receptor. *J Cell Biol*. (2015) 209:111–28. doi: 10.1083/jcb.201410050
65. McLelland GL, Goiran T, Yi W, Dorval G, Chen CX, Lauinger ND, et al. Mfn2 ubiquitination by PINK1/parkin gates the p97-dependent release of ER from mitochondria to drive mitophagy. *Elife*. (2018) 7:e32866. doi: 10.7554/eLife.32866
66. Poole AC, Thomas RE, Andrews LA, McBride HM, Whitworth AJ, Pallanck LJ. The PINK1/Parkin pathway regulates mitochondrial morphology. *Proc Natl Acad Sci USA*. (2008) 105:1638–43. doi: 10.1073/pnas.0709336105
67. Greene JC, Whitworth AJ, Kuo I, Andrews LA, Feany MB, Pallanck LJ. Mitochondrial pathology and apoptotic muscle degeneration in Drosophila parkin mutants. *Proc Natl Acad Sci USA*. (2003) 100:4078–83. doi: 10.1073/pnas.0737556100
68. Wang X, Winter D, Ashrafi G, Schlehe J, Wong YL, Selkoe D, et al. PINK1 and Parkin target Miro for phosphorylation and degradation to arrest mitochondrial motility. *Cell*. (2011) 147:893–906. doi: 10.1016/j.cell.2011.10.018
69. Shin JH, Ko HS, Kang H, Lee Y, Lee YI, Pletinkova O, et al. PARIS (ZNF746) repression of PGC-1α contributes to neurodegeneration in Parkinson's disease. *Cell*. (2011) 144:689–702. doi: 10.1016/j.cell.2011.02.010
70. Zhang J, Ney PA. Role of BNIP3 and NIX in cell death, autophagy, and mitophagy. *Cell Death Differ*. (2009) 16:939–46. doi: 10.1038/cdd.2009.16
71. Bellot G, Garcia-Medina R, Gounon P, Chiche J, Roux D, Pouysségur J, et al. Hypoxia-induced autophagy is mediated through hypoxia-inducible factor induction of BNIP3 and BNIP3L via their BH3 domains. *Mol Cell Biol*. (2009) 29:2570–81. doi: 10.1128/MCB.00166-09
72. Zhang H, Bosch-Marce M, Shimoda LA, Tan YS, Baek JH, Wesley JB, et al. Mitochondrial autophagy is an HIF-1-dependent adaptive metabolic response to hypoxia. *J Biol Chem*. (2008) 283:10892–903. doi: 10.1074/jbc.M800102200
73. Liu L, Feng D, Chen G, Chen M, Zheng Q, Song P, et al. Mitochondrial outer-membrane protein FUNDC1 mediates hypoxia-induced mitophagy in mammalian cells. *Nat Cell Biol*. (2012) 14:177–85. doi: 10.1038/ncb2422
74. Chen G, Han Z, Feng D, Chen Y, Chen L, Wu H, et al. A regulatory signaling loop comprising the PGAM5 phosphatase and CK2 controls receptor-mediated mitophagy. *Mol Cell*. (2014) 54:362–77. doi: 10.1016/j.molcel.2014.02.034
75. Orvedahl A, Sumpter R Jr, Xiao G, Ng A, Zou Z, Tang Y, et al. Image-based genome-wide siRNA screen identifies selective autophagy factors. *Nature*. (2011) 480:113–7. doi: 10.1038/nature10546
76. Strappazzon F, Nazio F, Corrado M, Cianfanelli V, Romagnoli A, Fimia GM, et al. AMBRA1 is able to induce mitophagy via LC3 binding, regardless of PARKIN and p62/SQSTM1. *Cell Death Differ*. (2015) 22:419–32. doi: 10.1038/cdd.2014.139
77. Chu CT, Ji J, Dagda RK, Jiang JF, Tyurina YY, Kapralov AA, et al. Cardiolipin externalization to the outer mitochondrial membrane acts as an elimination signal for mitophagy in neuronal cells. *Nat Cell Biol*. (2013) 15:1197–205. doi: 10.1038/ncb2837

78. McLelland G-L, Soubannier V, Chen CX, McBride HM, Fon EA. Parkin and PINK1 function in a vesicular trafficking pathway regulating mitochondrial quality control. *EMBO J*. (2014) 4:282–95. doi: 10.1002/embj.201385902
79. Martinus RD, Garth GP, Webster TL, Cartwright P, Naylor DJ, Hoj PB, et al. Selective induction of mitochondrial chaperones in response to loss of the mitochondrial genome. *Eur J Biochem*. (1996) 240:98–103. doi: 10.1111/j.1432-1033.1996.0098h.x
80. Ausman J, Abbade J, Ermini L, Farrell A, Tagliaferro A, Post M, et al. Ceramide-induced BOK promotes mitochondrial fission in preeclampsia. *Cell Death Dis*. (2018) 9:298. doi: 10.1038/s41419-018-0360-0
81. Chen G, Lin Y, Chen L, Zeng F, Zhang L, Huang Y, et al. Role of DRAM1 in mitophagy contributes to preeclampsia regulation in mice. *Mol Med Rep*. (2020) 22:1847–58. doi: 10.3892/mmr.2020.11269
82. Chen G, Chen L, Huang Y, Zhu X, Yu Y. Increased FUN14 domain containing 1 (FUNDC1) ubiquitination level inhibits mitophagy and alleviates the injury in hypoxia-induced trophoblast cells. *Bioengineered*. (2021) 3:3620–33. doi: 10.1080/21655979.2021.1997132
83. Youle RJ, Van Der Bliek AM. Mitochondrial fission, fusion, and stress. *Science*. (2012) 337:1062–5. doi: 10.1126/science.1219855
84. Rambold AS, Kostelecky B, Elia N, Lippincott-Schwartz J. Tubular network formation protects mitochondria from autophagosomal degradation during nutrient starvation. *Proc Natl Acad Sci USA*. (2011) 108:10190–5. doi: 10.1073/pnas.1107402108
85. Ishihara N, Eura Y, Mihara K. Mitofusin 1 and 2 play distinct roles in mitochondrial fusion reactions via GTPase activity. *J Cell Sci*. (2004) 117(Pt 26):6535–46. doi: 10.1242/jcs.01565
86. Ishihara N, Otera H, Oka T, Mihara K. Regulation and physiologic functions of GTPases in mitochondrial fusion and fission in mammals. *Antioxid Redox Signal*. (2013) 19:389–99. doi: 10.1089/ars.2012.4830
87. Chen H, Detmer SA, Ewald AJ, Griffin EE, Fraser SE, Chan DC. Mitofusins Mfn1 and Mfn2 coordinately regulate mitochondrial fusion and are essential for embryonic development. *J Cell Biol*. (2003) 160:189–200. doi: 10.1083/jcb.200211046
88. Rocha AG, Franco A, Krezel AM, Rumsey JM, Alberti JM, Knight WC, et al. MFN2 agonists reverse mitochondrial defects in preclinical models of Charcot-Marie-Tooth disease type 2A. *Science*. (2018) 360:336–41. doi: 10.1126/science.aao1785
89. Franco A, Kitsis RN, Fleischer JA, Gavathiotis E, Kornfeld OS, Gong G, et al. Correcting mitochondrial fusion by manipulating mitofusin conformations. *Nature*. (2016) 540:74–9. doi: 10.1038/nature20156
90. Züchner S, Mersiyanova IV, Muglia M, Bissar-Tadmouri N, Rochelle J, Dadali EL, et al. Mutations in the mitochondrial GTPase mitofusin 2 cause Charcot-Marie-Tooth neuropathy type 2A. *Nat Genet*. (2004) 36:449–51. doi: 10.1038/ng1341
91. Rouzier C, Bannwarth S, Chaussenot A, Chevrollier A, Verschueren A, Bonello-Palot N, et al. The MFN2 gene is responsible for mitochondrial DNA instability and optic atrophy 'plus' phenotype. *Brain*. (2012) 135(Pt 1):23–34. doi: 10.1093/brain/awr323
92. Eura Y, Ishihara N, Yokota S, Mihara K. Two mitofusin proteins, mammalian homologues of FZO, with distinct functions are both required for mitochondrial fusion. *J Biochem*. (2003) 134:333–44. doi: 10.1093/jb/mvg150
93. Anand R, Wai T, Baker MJ, Kladt N, Schauss AC, Rugarli E, et al. The i-AAA protease YME1L and OMA1 cleave OPA1 to balance mitochondrial fusion and fission. *J Cell Biol*. (2014) 204:919–29. doi: 10.1083/jcb.201308006
94. Ban T, Ishihara T, Kohno H, Saita S, Ichimura A, Maenaka K, et al. Molecular basis of selective mitochondrial fusion by heterotypic action between OPA1 and cardiolipin. *Nat Cell Biol*. (2017) 19:856–63. doi: 10.1038/ncb3560
95. Ishihara N, Fujita Y, Oka T, Mihara K. Regulation of mitochondrial morphology through proteolytic cleavage of OPA1. *EMBO J*. (2006) 25:2966–77. doi: 10.1038/sj.emboj.7601184
96. Tondera D, Grandemange S, Jourdain A, Karbowski M, Mattenberger Y, Herzig S, et al. SLP-2 is required for stress-induced mitochondrial hyperfusion. *EMBO J*. (2009) 28:1589–600. doi: 10.1038/emboj.2009.89
97. Ge Y, Shi X, Boopathy S, McDonald J, Smith AW, Chao LH. Two forms of Opa1 cooperate to complete fusion of the mitochondrial inner-membrane. *Elife*. (2020) 9:e50973. doi: 10.7554/eLife.50973
98. Song Z, Chen H, Fiket M, Alexander C, Chan DC. OPA1 processing controls mitochondrial fusion and is regulated by mRNA splicing, membrane potential, and Yme1L. *J Cell Biol*. (2007) 178:749–55. doi: 10.1083/jcb.200704110
99. Meeusen S, DeVay R, Block J, Cassidy-Stone A, Wayson S, McCaffery JM, et al. Mitochondrial inner-membrane fusion and crista maintenance requires the dynamin-related GTPase Mgm1. *Cell*. (2006) 127:383–95. doi: 10.1016/j.cell.2006.09.021
100. Yu J, Guo X, Chen R, Feng L. Downregulation of mitofusin 2 in placenta is related to preeclampsia. *Biomed Res Int*. (2016) 2016:6323086. doi: 10.1155/2016/6323086
101. Holland OJ, Cuffe JSM, Dekker Nitert M, Callaway L, Kwan Cheung KA, Radenkovic F, et al. Placental mitochondrial adaptations in preeclampsia associated with progression to term delivery. *Cell Death Dis*. (2018) 9:1150. doi: 10.1038/s41419-018-1190-9
102. Ni HM, Williams JA, Ding WX. Mitochondrial dynamics and mitochondrial quality control. *Redox Biol*. (2015) 4:6–13. doi: 10.1016/j.redox.2014.11.006
103. Taguchi N, Ishihara N, Jofuku A, Oka T, Mihara K. Mitotic phosphorylation of dynamin-related GTPase Drp1 participates in mitochondrial fission. *J Biol Chem*. (2007) 282:11521–9. doi: 10.1074/jbc.M607279200
104. Cribbs JT, Strack S. Reversible phosphorylation of Drp1 by cyclic AMP-dependent protein kinase and calcineurin regulates mitochondrial fission and cell death. *EMBO Rep*. (2007) 8:939–44. doi: 10.1038/sj.embor.7401062
105. Kalia R, Wang RY, Yusuf A, Thomas PV, Agard DA, Shaw JM, et al. Structural basis of mitochondrial receptor binding and constriction by DRP1. *Nature*. (2018) 558:401–5. doi: 10.1038/s41586-018-0211-2
106. Wang Z, Jiang H, Chen S, Du F, Wang X. The mitochondrial phosphatase PGAM5 functions at the convergence point of multiple necrotic death pathways. *Cell*. (2012) 148:228–43. doi: 10.1016/j.cell.2011.11.030
107. Moujalled DM, Cook WD, Murphy JM, Vaux DL. Necroptosis induced by RIPK3 requires MLKL but not Drp1. *Nature*. (2015) 517:311–20. doi: 10.1038/cddis.2014.18
108. Friedman JR, Lackner LL, West M, DiBenedetto JR, Nunnari J, Voeltz GK. ER tubules mark sites of mitochondrial division. *Science*. (2011) 334:358–62. doi: 10.1126/science.1207385
109. Farida Korobova VR, Henry Higgs N. An Actin-dependent step in mitochondrial fission mediated by the ER-associated formin INF2. *Science*. (2013) 339:464–7. doi: 10.1126/science.1228360
110. Camoes F, Bonekamp NA, Delille HK, Schrader M. Organelle dynamics and dysfunction: a closer link between peroxisomes and mitochondria. *J Inherit Metab Dis*. (2009) 32:163–80. doi: 10.1007/s10545-008-1018-3
111. Kleele T, Rey T, Winter J, Zaganelli S, Mahecic D, Perreten Lambert H, et al. Distinct fission signatures predict mitochondrial degradation or biogenesis. *Nature*. (2021) 593:435–9. doi: 10.1038/s41586-021-03510-6
112. Zorov DB, Vorobjev IA, Popkov VA, Babenko VA, Zorova LD, Pevzner IB, et al. Lessons from the discovery of mitochondrial fragmentation (fission): a review and update. *Cells*. (2019) 8:175. doi: 10.3390/cells8020175
113. Zsengellér ZK, Rajakumar A, Hunter JT, Salahuddin S, Rana S, Stillman IE, et al. Trophoblast mitochondrial function is impaired in preeclampsia and correlates negatively with the expression of soluble fms-like tyrosine kinase 1. *Pregn Hypertens*. (2016) 6:313–9. doi: 10.1016/j.preghy.2016.06.004
114. Yin K, Zhu R, Wang S, Zhao RC. Low-level laser effect on proliferation, migration, and antiapoptosis of mesenchymal stem cells. *Stem Cells Dev*. (2017) 26:762–75. doi: 10.1089/scd.2016.0332
115. Chen Y, Dorn GW II. PINK1-phosphorylated mitofusin 2 is a Parkin receptor for culling damaged mitochondria. *Science*. (2013) 340:471–5. doi: 10.1126/science.1231031
116. Chen M, Chen Z, Wang Y, Tan Z, Zhu C, Li Y, et al. Mitophagy receptor FUNDC1 regulates mitochondrial dynamics and mitophagy. *Autophagy*. (2016) 12:689–702. doi: 10.1080/15548627.2016.1151580
117. Twig G, Elorza A, Molina AJ, Mohamed H, Wikstrom JD, Walzer G, et al. Fission and selective fusion govern mitochondrial

segregation and elimination by autophagy. *EMBO J.* (2008) 27:433–46. doi: 10.1038/sj.emboj.7601963
118. Shirihai OS, Song M, Dorn GW II. How mitochondrial dynamism orchestrates mitophagy. *Circ Res.* (2015) 116:1835–49. doi: 10.1161/CIRCRESAHA.116.306374
119. Huang P, Galloway CA, Yoon Y. Control of mitochondrial morphology through differential interactions of mitochondrial fusion and fission proteins. *PLoS ONE.* (2011) 6:e20655. doi: 10.1371/journal.pone.0020655

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frontiers in Physiology

REVIEW
published: 28 February 2022
doi: 10.3389/fphys.2022.792292

The Molecular Mechanism of Aerobic Exercise Improving Vascular Remodeling in Hypertension

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The treatment and prevention of hypertension has been a worldwide medical challenge. The key pathological hallmark of hypertension is altered arterial vascular structure and function, i.e., increased peripheral vascular resistance due to vascular remodeling. The aim of this review is to elucidate the molecular mechanisms of vascular remodeling in hypertension and the protective mechanisms of aerobic exercise against vascular remodeling during the pathological process of hypertension. The main focus is on the mechanisms of oxidative stress and inflammation in the pathological condition of hypertension and vascular phenotypic transformation induced by the trilaminar structure of vascular endothelial cells, smooth muscle cells and extracellular matrix, and the peripheral adipose layer of the vasculature. To further explore the possible mechanisms by which aerobic exercise ameliorates vascular remodeling in the pathological process of hypertension through anti-proliferative, anti-inflammatory, antioxidant and thus inhibiting vascular phenotypic transformation. It provides a new perspective to reveal the intervention targets of vascular remodeling for the prevention and treatment of hypertension and its complications.

Keywords: hypertension, aerobic exercise, vascular remodeling, vascular smooth muscle cells, endothelial cells

OPEN ACCESS

Edited by:
Soo-Kyoung Choi,
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Specialty section:
This article was submitted to Vascular Physiology, a section of the journal Frontiers in Physiology

Received: *10 October 2021*
Accepted: *13 January 2022*
Published: *28 February 2022*

Citation:
Song Y, Jia H, Hua Y, Wu C, Li S, Li K, Liang Z and Wang Y (2022) The Molecular Mechanism of Aerobic Exercise Improving Vascular Remodeling in Hypertension. Front. Physiol. 13:792292. doi: 10.3389/fphys.2022.792292

INTRODUCTION

According to the World Health Organization (WHO), cardiovascular diseases (CVDs) are the number one cause of death worldwide. The number of deaths due to CVDs is expected to rise to approximately 23.2 million in 2030, with cardiovascular deaths accounting for 31% of all global deaths. Hypertension increases patient's risk of cardiovascular, brain, kidney, and other diseases. WHO recommends 25% relative reduction in prevalence of hypertension in public health targets by 2020 to reduce global disease burden (Diem et al., 2016). Hypertension endangers the health of the vascular system, as evidenced by vascular pathological remodeling. A characteristic pathological alteration of hypertension is augmented vasoconstrictor and attenuated vasodilator responses to various physiological stimuli, resulting in elevated vascular tone in arteries and arterioles that are exposed to persistent high blood pressure. Initially, the vascular remodeling caused by increased blood pressure allows the vasculature to adapt to short-term hemodynamic changes. However, sustained increases in blood pressure leads to chronic vascular maladaptation and dysfunction. This is manifested by structural and functional changes in the vascular endothelium, smooth muscle cells

(VSMCs), extracellular matrix (ECM), and perivascular adipose tissue (PVAT) (**Figure 1**; Ghaffari et al., 2015; Wang and Khalil, 2018).

Hypertension damages blood vessels, which in turn leads to pathological changes in blood vessels—vascular remodeling. In 1994, Gibbons and Dzau introduced the concept of vascular remodeling, which is characterized by vascular dysfunction, vessel wall thickening, and increased wall-to-lumen ratio (Gibbons and Dzau, 1994). Angiotensin II (Ang II), endothelin (ET), nitric oxide (NO), local growth factors (fibroblast growth factor, platelet-derived growth factor, and transforming growth factor beta), and metalloproteinases have been shown to be closely involved in the regulation of hypertension (Brown et al., 2018). Excessive activation of the renin-angiotensin system (RAS) causes diseases such as hypertension. AngII and aldosterone levels lead to vascular fibrosis, inflammation and proliferation. The interaction of oxidative stress and inflammation also leads to vascular remodeling (Schiffrin and Touyz, 2004). United States and European hypertension guidelines encourage regular aerobic exercise in hypertensive patients because of its effectiveness in improving hypertension (Mancia et al., 2007). Aerobic exercise significantly reduces systolic 24-h blood pressure, systolic systemic vascular resistance, and small artery elasticity index (Pagonas et al., 2017). This review summarizes the molecular mechanisms of changes in vascular endothelial cells, smooth muscle cells, extracellular matrix, and vascular peripheral fat during pathological alterations. And further explored the molecular mechanism of aerobic exercise to improve vascular remodeling for the prevention and treatment of hypertension, providing a theoretical basis for the prevention and treatment of hypertension (**Figure 2**).

ENDOTHELIAL CELLS AND THE AREOBIC EXERCISE ON VASCULAR REMODELING

Endothelial injury is a critical early step in the development and progression of hypertension. Endothelial damage/repair imbalance causes endothelial dysfunction which in turn induces hypertension. In addition, endothelial cells (ECs) signaling disorders lead to endothelial dysfunction, which is characterized by arterial vascular remodeling (Konukoglu and Uzun, 2017).

Endothelial Dysfunction

Endothelial cells are seen as the first line of defense between risk factors and vascular disease. Endothelial cells are thought to play an important role in the regulation of local vascular tone. In 1980, Furchgott and Zawadzki (1980) discovered endothelium-derived relaxing factor (EDRF). EDRF is chemically identified as endogenous nitric oxide (NO) (Ignarro et al., 1987). Since then, endothelial dysfunction has become synonymous with reduced NO bioactivity. Furthermore, hemodynamics is ubiquitous and essential physiological stimulus for vascular cells and is thought to exert an important influence on the pathological course of hypertension by regulating endothelial cell function. Shear stress plays a role in the control of endothelial cell proliferation and apoptosis; for example, stable flow reduces EC proliferation, whereas disturbed flow increases EC turnover and stimulates apoptosis (Davies et al., 1986; Akimoto et al., 2000). An increase in shear stress usually causes vasodilation, mostly mediated by an increase in endothelial nitric oxide synthase (eNOS) activity and NO production (Rubanyi et al., 1986; Redmond et al., 1998). Indeed, shear stress is thought to be the primary physiological stimulus for this potent vasodilator molecule. Other endothelium-derived vasoactive substances altered by shear stress include PGI2 (Redmond et al., 1998; Hendrickson et al., 1999) and endothelin-1 (ET-1) (Kuchan and Frangos, 1993; Malek et al., 1993).

Hypertension is associated with endothelial dysfunction (Konukoglu and Uzun, 2017). The main factors of endothelial dysfunction are reduced bioavailability of NO, increased sensitivity of ECs to vasoconstrictors, increased production of vasoconstrictor substances and elevated shear stress (Zhou et al., 2014; Cyr et al., 2020).Bone marrow secretes and releases endothelial progenitor cells (EPCs), which migrate to the peripheral circulation and differentiate into mature vascular endothelial cells (VECs) to maintain vascular integrity. EPC levels are a risk factor for cardiovascular disease and are associated with endothelial endothelium-dependent vasodilation (Vasa et al., 2001). VECs secretes active substances such as NO and ET to maintain vascular homeostasis. In moderate and severe hypertension, VECs damage and imbalance of reactive substances result in decreased NO secretion, increased ET vasoconstrictor, decreased diastolic system function, and vasoconstriction (Iwakiri and Groszmann, 2007).

The nitric oxide synthase (NOS) enzyme catalyzes the eventual production of NO from L-arginine. Mammals have three NOS isoforms: neuronal (nNOS), endothelial (eNOS), and inducible (iNOS). Infection and chronic inflammation induce increased NO production by iNOS. Under hypertensive pathology, increased NO concentration generates reactive nitrogen oxides (RNOS) with oxygen radicals, which indirectly cause apoptosis and tissue damage. In contrast, eNOS, a calcium-dependent protein, has a diastolic effect. Shear stress, acetylcholine, bradykinin, and histamine stimulate eNOS activity and NO production through calcium-dependent and non-dependent way (Zhao et al., 2015). In addition, NO channels are present in the myoendothelial junction (MEJ), a cellular extension that promotes crosstalk connections between endothelial cells and vascular smooth muscle in small arteries and arterioles. eNOS expression in the MEJ limits long-distance diffusion of NO and reduces the scavenging of NO by reactive oxygen species (ROS) (Shu et al., 2019). In addition to targeting eNOS to the MEJ, hemoglobin-α (Hb-α) is enriched in the MEJ by unbiased proteomic screening. Functionally, Hb-α acts as a "NO uptake pool" by buffering NO diffusion from endothelium to smooth muscle cells through the formation of a dioxygenation reaction between nitrate and methemoglobin-α, which further regulates NOS-mediated signaling to control vascular remodeling (Straub et al., 2012). Disruption of eNOS and Hb-α binding with Hb-α mimetic peptide enhances NO signaling and lowers blood pressure *in vivo*. Thereby identifying

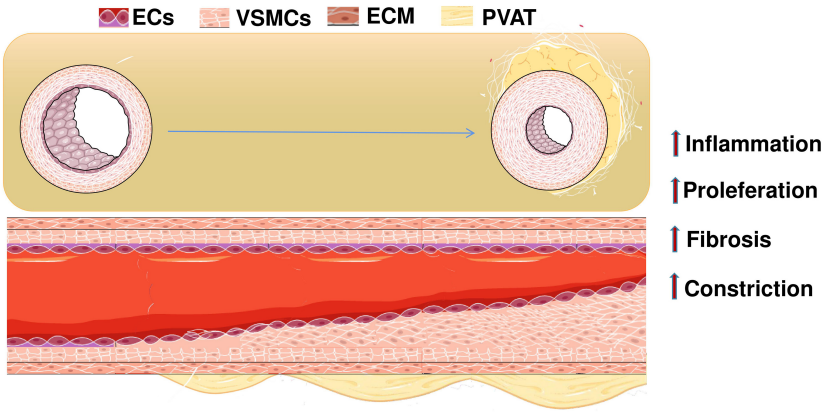

FIGURE 1 | When hypertension occurs, the continuous increase of blood pressure leads to chronic poor vascular adaptation and dysfunction. The specific manifestations are changes in the structure and function of vascular endothelial cells, smooth muscle cells, extracellular matrix, and perivascular adipose tissue. ECs, endothelial cells; VSMCs, vascular smooth muscle cells; ECM, extracellular matrix; PVAT, perivascular adipose tissue.

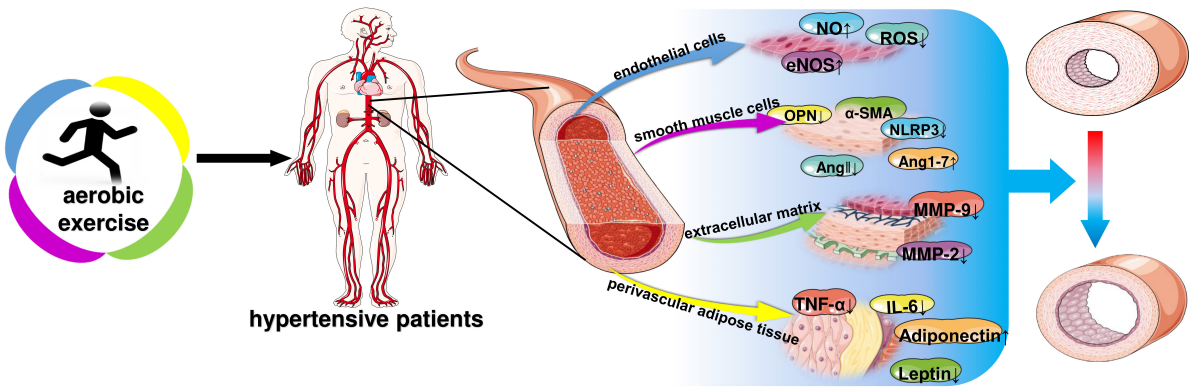

FIGURE 2 | Aerobic exercise improves molecular changes in vascular remodeling of hypertension. NO, nitric oxide; ROS, reactive oxygen species; eNOS, endothelial nitric oxide synthase; OPN, osteopontin; α-SMA, α-smooth muscle actin; NLRP3, NOD-like receptor thermal protein domain associated protein 3; ANG, angiotensin; MMP-9, matrix metallopeptidase 9; MMP-2, matrix metallopeptidase 2; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α; Adiponectin; Leptin.

new targets for the treatment of hypertensive vascular remodeling (Straub et al., 2014).

Reactive Oxygen Species

Impaired endothelium-dependent vasodilatory function in hypertension is associated with oxidative stress and ROS together with other pathways reduce NO bioavailability (Virdis et al., 2013).

Reactive oxygen species alter gene expression by regulating the activation of transcription factors, with subsequent effects on downstream target proteins, and also regulate the production and degradation of extracellular matrix, inactivate NO function, and stimulate the expression of multiple kinases and pro-inflammatory genes (Monteiro et al., 2019).

Elevated levels of oxidative stress in hypertensive patients lead to an imbalance in the production/accumulation of ROS (Montezano et al., 2015). Nicotinamide adenine dinucleotide phosphate oxidase (Nox) is a major source of ROS in the vascular wall and has been identified as playing a key role in the pathogenesis of hypertension (Magnani and Mattevi, 2019). NOx induces increased ROS production in response to inflammation. In ECs, superoxide reacts with NO to generate peroxynitrite to inhibit oxidative capacity leading to oxidative stress. This further leads to vascular inflammation, fibrosis and remodeling in hypertension (Lopes et al., 2015). In addition, the mechanical forces on the vessel wall are altered in patients with hypertension. Increased stretch leads to endothelial cell proliferation and the release of Interleukin-6 (IL-6), Interleukin-8 (IL-8), ROS, ET, and other pro-inflammatory mediators also contribute to impaired endothelial cell function in hypertensive vessels (Jufri et al., 2015).

Aerobic Exercise Improves Vascular Remodeling Through Endothelial Cell Regulation

The effect of aerobic exercise on the maintenance of endothelial barrier function is due to the increased heart rate, blood flow

and shear stress associated with aerobic exercise, which in turn releases vascular protective molecules, such as NO (Laughlin et al., 2008). This immediately leads to a downregulation of endothelial angiotensin II type 1 receptor expression, which leads to a decrease in nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity and superoxide anion production, thereby reducing ROS production and maintaining endothelial NO bioavailability (Ramkhelawon et al., 2009). This ultimately allows vasodilation and slows down the vascular remodeling of the hypertensive pathological process.

Aerobic exercise for 16 weeks reduced blood pressure and promoted eNOS expression in 29-week-old rats. And exercise also reduced protein levels of insulin-like growth factor-1 (IGF-1), PI3K, and phosphorylated protein kinase B (p-Akt) (Jufri et al., 2015). Long-term aerobic exercise promotes eNOS expression and reduces hypertension via IGF-1/PI3K/p-Akt pathway (Zhang et al., 2018).

Melatonin (MT) acts as an antioxidant and anti-hypertensive. By activating melatonin receptor 2 (MT2). It can increase ca^{2+} levels in endothelial cells, which in turn plays a key role in activating eNOS to increase NO production and NO bioavailability. Studies have shown that exercise can increase MT levels (Escames et al., 2012). In addition, skeletal muscle hypertrophy induced by exercise training increases the production of follicle-stimulating hormone 1 (Follistatin1, Fstl1) (Escames et al., 2012), which improves the repair of vascular endothelial cell damage and reduces the expression of inflammatory cytokines (Miyabe et al., 2014). Aerobic exercise also induces an increase in eNOS expression and thus improves vascular function by increasing shear force (Suvorava and Cortese-Krott, 2018).

AEROBIC EXERCISE IMPROVES THE EFFECT OF VSMCs ON VASCULAR REMODELING

Vascular remodeling in hypertension is manifested in the midmembrane by a shift from contractile phenotype to synthetic phenotype in VSMCs, which is a hallmark of vascular dysfunction in hypertension (Touyz et al., 2018). Multiple factors such as growth factors, ROS, and mechanical injury have been shown to be involved in VSMCs growth and phenotype conversion (Nishio and Watanabe, 1997; Luo et al., 2012; Hald and Alford, 2014).

Effects of VSMC-Specific Factors and Signaling Pathway Modulation on Vascular Phenotype Transformation

Vascular endothelium, smooth muscle cells phenotypic transition is regulated by specific factors and signaling pathways such as phosphatidylinositol kinase signaling pathway (PI3K/Akt/eNOS) and mitogen-activated protein kinase cascade reaction (MAPK). VSMC phenotypic features perform functions by virtue of different proteins, such as α-SMA, calreticulin, smooth muscle myosin heavy chain, and SM22α (Zhang et al., 2019). Osteopontin (OPN) and epithelial regulatory proteins are associated with cell growth, synthesis, proliferation, and migration (Seo et al., 2015). Vasoactive stimulation, growth factors and epidermal growth factors are involved in VSMC phenotypic conversion through activation of membrane receptors and intracellular and extracellular signaling pathways (Kennedy et al., 2016). Platelet-derived growth factor-BB (PDGF-BB) binds to PDGF receptors and subsequently activates intracellular signaling cascades such as the protein kinase B (Akt), extracellular signal-regulated kinase (ERK), and p38MAPK pathways (Chen et al., 2015). Akt is a major downstream target of phosphatidylinositol 3-kinase (PI3K). MAPK contains three major members: ERK, p38 MAPK, and c-Jun N-terminal kinase (JNK), of which ERK and p38MAPK are involved in VSMCs phenotype conversion (Ma and Wells, 2014).

Inflammation Is Involved in VSMCs Phenotype Conversion

Increased concentrations of pro-inflammatory cytokines were observed in smooth muscle cells of hypertensive patients (Chi et al., 2019). Nucleotide-binding oligomerization domain-like receptor protein 3 (NLRP3) inflammatory vesicles activate caspase-1 and thus induce inflammation, thus becoming another new focus for triggering hypertension (Sun et al., 2017).

Nucleotide-binding oligomerization domain-like receptor protein 3forms a complex with atypical squamous cells (ASC) prompting the conversion of procaspase-1 to active caspase-1. Activated caspase-1 prompts the conversion of pro interleukin-1beta (IL-1β) to mature IL-1β ultimately inducing inflammation. Elevated levels of the pro-inflammatory cytokine IL-1β in the vasculature under hypertensive pathology suggest that inflammation is highly associated with hypertensive vascular remodeling (Slaats et al., 2016). Multiple signaling and metabolic dysregulation cause NLRP3 inflammasome activation, such as ca^{2+}, ROS, NO, Ang II, and endoplasmic reticulum stress and mitochondrial dysfunction (He et al., 2016). NLRP3 inflammasome activation leads to nuclear factor-kappaB (NF-κB) signaling activation involved in the development and progression of hypertension. NLRP3 gene deletion attenuates Ang II-induced inflammation, VSMC phenotypic transformation and proliferation, and Ang II-induced hypertension and vascular remodeling (Ren et al., 2017).

Renin-Angiotensin System-Induced Vascular Remodeling in Hypertension

The renin-angiotensin system (RAS) regulates vascular tone and plays a key role in vascular remodeling (Schiffrin, 2012). The RAS consists of series of enzymatic reactions culminating in the generation of AngII in plasma as well as in cardiovascular system. The Ang II/AT1 signaling has been shown to be aberrantly activated in vascular hypertrophy and remodeling by promoting VSMC growth, transdifferentiation and proliferation, eliciting a variety of biological actions of the RAS in the vascular homeostasis (Thomas et al., 2005; Mehta and Griendling, 2007; Zhong et al., 2010; Jin et al., 2012). As a specific Ang II-degredating enzyme, ACE2 suppresses VSMC proliferation and vascular hypertrophy. Loss of ACE2 led to vascular proliferation

and elevated migration of SMC while ACE2 overexpression inhibited vascular proliferation and hypertrophy by preventing aortic wall thickening (Strawn et al., 1999; Landon and Inagami, 2005; Ferreira et al., 2009; Zhang et al., 2009; Zhong et al., 2011; Jin et al., 2012; Patel et al., 2012).Excessive activation of RAS under hypertensive pathology causes upregulation of the classical pathway action of the Ang-converting enzyme ACE/Ang II/Ang type I receptor (AT1R) and impairs the protective effect of the ACE2/Ang 1–7/Mas receptor (MasR) pathway.

Patients with hypertension present with locally or systemically elevated Ang II levels, i.e., excessive activation of the classical pathway. Renin released from the kidney converts angiotensinogen (AGT) produced by the liver to Ang I, which is converted to Ang II by the action of Ang converting enzyme (ACE) (Li et al., 2017). Other enzymes may also be involved in Ang II production, such as histones, chymotrypsin, etc. (Passos-Silva et al., 2013). ACE also inactivates bradykinin, which has a vasodilatory effect. The physiological effects of Ang II are mediated by the G protein-coupled receptor family, whose types are type 1 (AT1R) and type 2 (AT2R) (Zhang et al., 2017). Activation of the ACE/Ang II/AT1R pathway stimulates vasoconstriction, sympathetic activation and ROS production, and triggers harmful effects such as endothelial dysfunction, inducing vascular inflammation, thrombosis, proliferation, and fibrosis (Kawai et al., 2017). In contrast, AT2R exerts histoprotective effects, including vasodilatation, anti-inflammatory, and anti-proliferative (Santos et al., 2018).

ACE2 hydrolyzes AngI to produce Ang1-9, which is cleaved by ACE to produce Ang 1–7 (Santos et al., 2018). Ang 1–7 mainly acts through ACE2. Ang 1–7 binds to the specific receptor MasR, a G protein-coupled receptor that triggers anti-inflammatory, anti-fibrotic and anti-proliferative and produces protective effects (Rodrigues Prestes et al., 2017).

ROS Participates in the Phenotypic Transition of Hypertensive VSMCs

Disruption of ROS signaling leads to the development of several diseases, such as hypertension. In hypertension, Ang II, NE, and ET-1 activate receptors located on the cell membrane, namely AT1, α-AR, and ET receptors. These receptors are coupled to G proteins and activate NADPH oxidase. Activated NADPH oxidases produce ROS, which in turn activate cellular phosphorylation pathways: MAPK, PI3K/Akt. Activated phosphorylation pathways activate transcription factors, such as activator protein-1 (AP-1), p53, NF-κB, and nuclear E2-related factor 2 (Nrf2), which promote post-entry gene transcription into the nucleus of the cell. These target genes encode proteins that subsequently mediate changes in cellular phenotypes, such as hypertrophy, inflammation, necrosis, and apoptosis (Das et al., 2018).

Although cells of different systems perform different functions, redox signaling is very similar. NADPH oxidase is a major source of ROS in endothelial cells, vascular smooth muscle cells, cardiomyocytes, renal cells, and cardiovascular neurons (Nowak et al., 2018). Ang II is an important activator of NADPH oxidase and a stimulator of ROS (Kang et al., 2019). ROS are produced through mechanical stress stimulation of vascular smooth muscle cells, and ROS act through MAPK production to cause cell proliferation, hypertrophy and apoptosis (Gusan and Anand-Srivastava, 2013).

Aerobic Exercise Improves Smooth Muscle Vascular Remodeling

The powerful stimuli generated by aerobic exercise are associated with vascular remodeling (Green, 2009; Green et al., 2017). Small arteries are the main resistance vessels that regulate flow to different tissues of the body and control blood pressure. Phenotypic conversion of VSMC in these vessels plays an important role in structural remodeling and can lead to various cardiovascular diseases, including hypertension (Owens et al., 2004).

Exercise induced the VSMCs of SHR to maintain a more contractile phenotype, with differentiation protein α-SM-actin and OPN, which is involved during VSMC migration and proliferation and as dedifferentiation marker being inhibited (Chaulet et al., 2001; Speer et al., 2002; Ye et al., 2009; Jiang et al., 2014).

After 8 weeks of aerobic exercise, the phenotype of spontaneously hypertensive rats was reversed, showing an increase in contractile protein expression and a decrease in synthetic protein expression. 12-week aerobic exercise increased the expression of eNOS protein in 3-month-old hypertensive rats, and decreased the expression of ERK and p38, thereby improving VSMC function. Aerobic exercise has a beneficial effect on vascular phenotyping by regulating the balance of Akt and MAPK signal pathways in VSMC. Aerobic exercise enhances the effect of PI3K/Akt/eNOS signaling pathway in normal rats, and maintains a good contractile phenotype of normal rat VSMC (Zhang et al., 2019). Aerobic exercise moves the role of RAS to the protective pathway in several disease models such as hypertension (ACE2/Ang 1–7/MasR) (Frantz et al., 2017). Eight weeks of aerobic exercise inhibits the activity of NF-κB p65, reduces the increase of norepinephrine, epinephrine and the expression of IL-1β and TNF-α in plasma (Qi et al., 2019).

Therefore, aerobic exercise is an effective intervention for hypertensive vascular remodeling. Aerobic exercise is involved in improving the vascular remodeling caused by vascular media injury in many aspects, such as reducing inflammation and activating the protective pathway of RAS from the specific signaling pathway.

HYPERTENSIVE EXTRAVASCULAR MEMBRANE AND THE AMELIORATIVE EFFECT OF AEROBIC EXERCISE

Adventitial fibroblast (AF) is the main cellular component of the adventitia of blood vessels. Under the pathology of hypertension, the ability of proliferation and migration is enhanced, and a variety of cytokines are secreted, which participates in inflammation and vascular remodeling (Qi et al., 2019). When adventitia fibroblasts are pathologically damaged,

ECM is secreted to participate in vascular remodeling. Excessive accumulation of collagen will increase the stiffness of blood vessels and accelerate the development of hypertension. In addition, ECM induces cell signals to regulate cell adhesion, proliferation, migration, and differentiation, and participates in the remodeling of hypertensive blood vessels, among which matrix metalloproteinases (MMPs) are the key factors leading to vascular maladaptation (Castro and Tanus-Santos, 2013; Hua and Nair, 2015). Gelatinase MMP-2 and MMP-9 are vascular disease-related proteins, which are involved in oxidative stress and cause cardiovascular dysfunction, and are involved in vascular remodeling in chronic maladaptive hypertension (Belo et al., 2015; Han et al., 2019).

Biologically active peptides, hemodynamics and reactive oxygen species regulate the expression and activity of MMP-2. Increased MMP-2 can cause poor vascular adaptability due to hypertension (Hardy et al., 2018). MMP-2 stimulates VSMC to interact with the newly formed ECM. ECM triggers intracellular signal transduction through integrin to induce phenotypic transition and continuous migration. VSMC changes from a contractile phenotype to a synthetic phenotype, leading to vascular remodeling under the pathology of hypertension. The tissue matrix metalloproteinase inhibitor TIMP is a secreted protein that can inhibit the activity of MMPs. AF-derived TIMP1 acts on the smooth muscle cells and inflammatory cells in the vascular part through paracrine, inhibiting the enzymatic activity of MMP-9, leading to increased synthesis and secretion of collagen in blood vessels. The expression of Ang II increases during hypertension. Ang II induces the expression and secretion of type I collagen in cultured adventitia fibroblasts (Somanna et al., 2016; Fu et al., 2018). Ang II regulates the expression of MMP-2 and TIMP1 in adventitia fibroblasts, and the changes in the expression of MMP-2 and TIMP1 are involved in the secretion of collagen by adventitia fibroblasts to participate in the process of vascular remodeling.

ROS Is Involved in the Regulation of Matrix Metalloproteinases

Researches have shown that ROS can regulate the activity of MMPs. Pro-MMP-2 and pro-MMP-9 secreted by VSMC are activated by ROS (Prado et al., 2018). The expression of MMPs genes is also regulated by ROS. When VSMCs are mechanically stretched, NAD(P)H oxidase-derived ROS increases the expression of MMP-2 mRNA (Yue et al., 2018). The strategy of adjusting the bioavailability of ROS can reverse vascular remodeling, effectively prevent vascular damage and reduce hypertension and its related end-organ damage (Prado et al., 2018).

Ameliorative Effect of Aerobic Exercise

Twelve weeks of exercise training increased collagen deposition in hypertensive rats, and reduced the size of pores in the intima, which explained the beneficial effects of exercise on vascular remodeling and vasodilation, especially the pressure exerted by elastin protein at low positions. The latest research on the aorta of hypertensive rats also shows that exercise training can normalize changes in the deposition of elastic components (Moraes-Teixeira Jde et al., 2010). The imbalance between synthesis and degradation of ECM protein can affect vascular remodeling. Sports training affects the expression of MMP to varying degrees. Under pathological conditions, ROS production will increase ECM proteins, such as collagen and fibronectin (Lee and Griendling, 2008). In addition, the reduction of oxidative stress in hypertension is related to the normalization of vascular remodeling and collagen deposition observed in arteries (Zhang et al., 2016).

PERIVASCULAR ADIPOSE TISSUE AND THE AMELIORATIVE EFFECT OF AEROBIC EXERCISE

PVAT Adipose Tissue Is Involved in Vascular Remodeling of Hypertension

Perivascular adipose tissue secretes a large number of metabolically vasoactive adipokines (e.g., lipocalin, leptin, resistin, endolipin, etc.) that exert endocrine and paracrine effects (Saxton et al., 2019). Vascular injury, infection leads to abnormal PVAT and inflammatory cell infiltration and imbalance in the release of harmful and beneficial adipokines. This is usually manifested by increased levels of leptin and decreased levels of adiponectin (Zhang et al., 2016). This in turn accelerates inflammation, oxidative stress causing endothelial dysfunction and VSMC proliferation.

The adipokines produced by PVAT are more likely to cause inflammation, proliferation, and then cause vascular remodeling (Schlich et al., 2013; Nosalski and Guzik, 2017). PVAT dysfunction activates the NLRP3/IL-1 signaling pathway after early vascular injury, leading to increased proliferation and differentiation of AF, thereby aggravating vascular adventitia remodeling. PVAT causes endothelial dysfunction by increasing the oxidative stress derived from NADPH oxidase and increasing the production of pro-inflammatory adipokines (such as leptin)

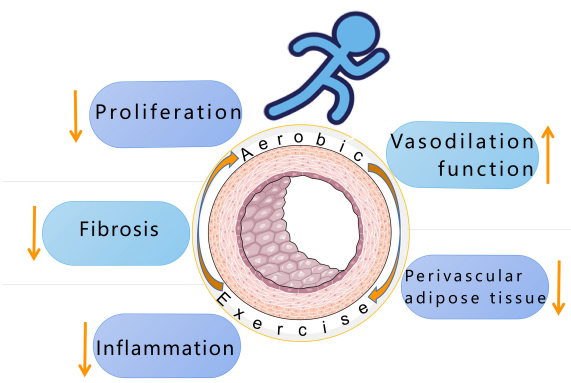

FIGURE 3 | Aerobic exercise improves hypertension by reducing inflammation, reducing fibrosis and proliferation, mediating vasodilation, and reducing perivascular adipose tissue.

(Gil-Ortega et al., 2014). The increase of tumor necrosis factor-α (TNF-α) gene expression in PVAT under hypertension is related to the increase of ET-1 and endothelin receptors. Increased TNF-α gene expression is related to NOS uncoupling and reduced NO release (Virdis et al., 2015). Under the pathology of hypertension, PVAT secretes a large amount of adipokines to accelerate inflammation and oxidative stress, aggravate vascular endothelial dysfunction and VSMC proliferation to accelerate vascular remodeling. Adipose tissue contains AGT and ACE, and the gene expression of AT1 receptor in PVAT is higher (Mikolajczyk et al., 2019). Systemic infusion of Ang II can cause local PVAT inflammation and participate in vascular remodeling of hypertension. Adiponectin induces AMP-activated protein kinase (AMPK) phosphorylation, inhibits the migration of mouse outer membrane fibroblasts and inhibits the expression of nitric oxide synthase (Ghantous et al., 2020).

Aerobic Exercise Regulating Vascular Remodeling by Ameliorating PVAT

Aerobic exercise can significantly reduce the serum leptin level in PVAT in patients with hypertension and improve leptin resistance, and the adiponectin content increases. Aerobic exercise can improve the low-grade inflammation in obese people and reduce the level of plasma inflammatory cytokines (Sousa et al., 2019).

The activation of endothelial cell mechanical sensors during aerobic exercise stimulates the production of eNOS and NO, reduces vascular oxidative stress, increases antioxidant response and improves NO bioavailability (Sponton et al., 2017; Ruegsegger and Booth, 2018). In addition, aerobic exercise changes the metabolic phenotype of adipose tissue and inhibits the expression of inflammatory markers (Boa et al., 2017). Aerobic exercise is beneficial to restore eNOS activation or reduce iNOS protein expression, both of which are related to the normalization of contractile vascular reactivity in obese rats (Araujo et al., 2018).

Exercise training reduces PVAT inflammation (Lee et al., 2016). Aerobic exercise training stimulates angiogenesis in adipose tissue, improves blood flow and reduces hypoxia and macrophage infiltration (You et al., 2013). It can also prevent or weaken the infiltration of immune cells into PVAT, thereby improving blood vessel function (Boa et al., 2017). At the same time, mechanical stimulation of exercise plays a basic role in preventing endothelial dysfunction by reducing ROS and increasing the bioavailability of NO. Exercise training increases the expression of eNOS protein in the aorta and prevents the up-regulation of iNOS in PVAT. Aerobic exercise also increases the expression of Mn-SOD protein in PVAT and reduces tissue ROS production (Huang et al., 2018).

CONCLUSION

To sum up, the pathological changes of the three-layer membrane structure of blood vessels and the increase of perivascular adipose tissue are the factors that lead to the development of hypertensive vascular remodeling. At present, clinically, antihypertensive drugs that may have a beneficial effect on vascular remodeling are being explored, such as neutral lysozyme inhibitors related to angiotensin receptor blockers, aldosterone synthase inhibitors, and renal denervation and baroreceptors Stimulate and other new drugs. In terms of exercise, it has been proven that aerobic exercise can improve vascular remodeling by improving the tunica intima, media, and adventitia thickening and fibrosis under the pathology of hypertension (**Figure 3**). Based on a large number of previous studies, the future research direction of aerobic exercise and hypertension can be as follows: (1) To further accurately grasp the exercise intensity and exercise time of people of different ages, races and degrees of vascular remodeling. (2) Regular aerobic exercise can reduce ROS in cells and increase the bioavailability of NO, but the mechanism of endothelial function improvement during exercise has not been fully elucidated. Or the protective effect of aerobic exercise in regulating DNA methylation on the cardiovascular system can be used as a further research direction.

AUTHOR CONTRIBUTIONS

YS wrote the manuscript. HJ and YH designed the figures along with YS. CW, ZL, SL, and KL reviewed the manuscript writing. YW supervised the manuscript writing and figure making processes. All authors contributed to the article and approved the submitted version.

FUNDING

This publication was supported by grants from the National Natural Science Foundation of China (Grant/Award Number: 81570449); Fundamental Research Funds for the Central Universities (Grant/Award Number: GK2020031); and College Students' Innovative and Training Project of Shaanxi Normal University (Grant/Award Number: S202110718230).

REFERENCES

Akimoto, S., Mitsumata, M., Sasaguri, T., and Yoshida, Y. (2000). Laminar shear stress inhibits vascular endothelial cell proliferation by inducing cyclin-dependent kinase inhibitor p21(Sdi1/Cip1/Waf1). *Circ. Res.* 86, 185–190. doi: 10.1161/01.res.86.2.185

Araujo, H. N., Jamaira, A., Victório, Carmem, P. V. S., Amanda, C. S. S., Jean, F. V., et al. (2018). Anti-contractile effects of perivascular adipose tissue in thoracic aorta from rats fed a high-fat diet: role of aerobic exercise training. *Clin. Exp. Pharmacol. Physiol.* 45, 293–302. doi: 10.1111/1440-1681.12882

Belo, V. A., Lacchini, R., Miranda, J. A., Lanna, C. M., Souza-Costa, D. C., and Tanus-Santos, J. E. (2015). Increased activity of MMP-2 in hypertensive obese children is associated with hypoadiponectinemia. *Obesity* 23, 177–182. doi: 10.1002/oby.20939

Boa, B. C. S., Yudkin, J. S., van Hinsbergh, V. W. M., Bouskela, E., and Eringa, E. C. (2017). Exercise effects on perivascular adipose tissue: endocrine and

paracrine determinants of vascular function. *Br. J. Pharmacol.* 174, 3466–3481. doi: 10.1111/bph.13732

Brown, I. A. M., Diederich, L., Good, M. E., DeLalio, L. J., Murphy, S. A., Cortese-Krott, M. M., et al. (2018). Vascular Smooth Muscle Remodeling in Conductive and Resistance Arteries in Hypertension. *Arterioscler. Thromb. Vasc. Biol.* 38, 1969–1985. doi: 10.1161/ATVBAHA.118.311229

Castro, M. M., and Tanus-Santos, J. E. (2013). Inhibition of matrix metalloproteinases (MMPs) as a potential strategy to ameliorate hypertension-induced cardiovascular alterations. *Curr. Drug Targets.* 14, 335–343. doi: 10.2174/1389450111314030005

Chaulet, H., Desgranges, C., Renault, M. A., Dupuch, F., Ezan, G., Peiretti, F., et al. (2001). Extracellular nucleotides induce arterial smooth muscle cell migration via osteopontin. *Circ. Res.* 89, 772–778. doi: 10.1161/hh2101.098617

Chen, S., Liu, B., Kong, D., Li, S., Li, C., Wang, H., et al. (2015). Atorvastatin calcium inhibits phenotypic modulation of PDGF-BB-induced VSMCs via down-regulation the Akt signaling pathway. *PLoS One* 10:e0122577. doi: 10.1371/journal.pone.0122577

Chi, C., Li, D. J., Jiang, Y. J., Tong, J., Fu, H., Wu, Y. H., et al. (2019). Vascular smooth muscle cell senescence and age-related diseases: state of the art. *Biochim. Biophys. Acta Mol. Basis Dis.* 1865, 1810–1821. doi: 10.1016/j.bbadis.2018.08.015

Cyr, A. R., Huckaby, L. V., Shiva, S. S., and Zuckerbraun, B. S. (2020). Nitric Oxide and Endothelial Dysfunction. *Crit. Care Clin.* 36, 307–321. doi: 10.1016/j.ccc.2019.12.009

Das, S., Zhang, E., Senapati, P., Amaram, V., Reddy, M. A., Stapleton, K., et al. (2018). A Novel Angiotensin II-Induced Long Noncoding RNA Giver Regulates Oxidative Stress, Inflammation, and Proliferation in Vascular Smooth Muscle Cells. *Circ. Res.* 123, 1298–1312. doi: 10.1161/CIRCRESAHA.118.313207

Davies, P. F., Remuzzi, A., Gordon, E. J., Dewey, C. F. Jr., and Gimbrone, M. A. Jr. (1986). Turbulent fluid shear stress induces vascular endothelial cell turnover *in vitro*. *Proc. Natl. Acad. Sci. U. S. A.* 83, 2114–2117. doi: 10.1073/pnas.83.7.2114

Diem, G., Brownson, R. C., Grabauskas, V., Shatchkute, A., and Stachenko, S. (2016). Prevention and control of noncommunicable diseases through evidence-based public health: implementing the NCD 2020 action plan. *Glob. Health Promot.* 23, 5–13. doi: 10.1177/1757975914567513

Escames, G., Ozturk, G., Baño-Otálora, B., Pozo, M. J., Madrid, J. A., Reiter, R. J., et al. (2012). Exercise and melatonin in humans: reciprocal benefits. *J. Pineal. Res.* 52, 1–11. doi: 10.1111/j.1600-079X.2011.00924.x

Ferreira, A. J., Shenoy, V., Yamazato, Y., Sriramula, S., Francis, J., Yuan, L., et al. (2009). Evidence for angiotensin-converting enzyme 2 as a therapeutic target for the prevention of pulmonary hypertension. *Am. J. Respir. Crit. Care Med.* 179, 1048–1054. doi: 10.1164/rccm.200811-1678OC

Frantz, E. D. C., Medeiros, R. F., Giori, I. G., Lima, J. B. S., Bento-Bernardes, T., Gaique, T. G., et al. (2017). Exercise training modulates the hepatic renin-angiotensin system in fructose-fed rats. *Exp. Physiol.* 102, 1208–1220. doi: 10.1113/EP085924

Fu, B., Su, Y., Ma, X., Mu, C., and Yu, F. (2018). Scoparone attenuates angiotensin II-induced extracellular matrix remodeling in cardiac fibroblasts. *J. Pharmacol. Sci.* 137, 110–115. doi: 10.1016/j.jphs.2018.05.006

Furchgott, R. F., and Zawadzki, J. V. (1980). The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 288, 373–376. doi: 10.1038/288373a0

Ghaffari, S., Leask, R. L., and Jones, E. A. (2015). Simultaneous imaging of blood flow dynamics and vascular remodelling during development. *Development* 142, 4158–4167. doi: 10.1242/dev.127019

Ghantous, C. M., Farhat, R., Djouhri, L., Alashmar, S., Anlar, G., Korashy, H. M., et al. (2020). Molecular Mechanisms of Adiponectin-Induced Attenuation of Mechanical Stretch-Mediated Vascular Remodeling. *Oxid. Med. Cell. Longev.* 2020:6425782. doi: 10.1155/2020/6425782

Gibbons, G. H., and Dzau, V. J. (1994). The emerging concept of vascular remodeling. *N. Engl. J. Med.* 330, 1431–1438. doi: 10.1056/NEJM199405193302008

Gil-Ortega, M., Condezo-Hoyos, L., García-Prieto, C. F., Arribas, S. M., González, M. C., Aranguez, I., et al. (2014). Imbalance between pro and anti-oxidant mechanisms in perivascular adipose tissue aggravates long-term high-fat diet-derived endothelial dysfunction. *PLoS One* 9:e95312. doi: 10.1371/journal.pone.0095312

Green, D. J. (2009). Exercise training as vascular medicine: direct impacts on the vasculature in humans. *Exerc. Sport Sci. Rev.* 37, 196–202. doi: 10.1097/JES.0b013e3181b7b6e3

Green, D. J., Hopman, M. T., Padilla, J., Laughlin, M. H., and Thijssen, D. H. (2017). Vascular Adaptation to Exercise in Humans: role of Hemodynamic Stimuli. *Physiol. Rev.* 97, 495–528. doi: 10.1152/physrev.00014.2016

Gusan, S., and Anand-Srivastava, M. B. (2013). cAMP attenuates the enhanced expression of Gi proteins and hyperproliferation of vascular smooth muscle cells from SHR: role of ROS and ROS-mediated signaling. *Am. J. Physiol. Cell Physiol.* 304, C1198–C1209. doi: 10.1152/ajpcell.00269.2012

Hald, E. S., and Alford, P. W. (2014). Smooth muscle phenotype switching in blast traumatic brain injury-induced cerebral vasospasm. *Transl. Stroke Res.* 5, 385–393. doi: 10.1007/s12975-013-0300-3

Han, B. H., Yoon, J. J., Kim, H. Y., Ahn, Y. M., Jin, S. N., Wen, J. F., et al. (2019). Inhibitory effects of herbal decoction Ojeoksan on proliferation and migration in vascular smooth muscle cells. *J. Physiol. Pharmacol.* 70, 287–94. doi: 10.26402/jpp.2019.2.12

Hardy, E., Hardy-Sosa, A., and Fernandez-Patron, C. (2018). MMP-2: is too low as bad as too high in the cardiovascular system? *Am. J. Physiol. Heart Circ. Physiol.* 315, H1332–H1340. doi: 10.1152/ajpheart.00198.2018

He, Y., Hara, H., and Núñez, G. (2016). Mechanism and Regulation of NLRP3 Inflammasome Activation. *Trends Biochem. Sci.* 41, 1012–1021. doi: 10.1016/j.tibs.2016.09.002

Hendrickson, R. J., Cappadona, C., Yankah, E. N., Sitzmann, J. V., Cahill, P. A., and Redmond, E. M. (1999). Sustained pulsatile flow regulates endothelial nitric oxide synthase and cyclooxygenase expression in co-cultured vascular endothelial and smooth muscle cells. *J. Mol. Cell. Cardiol.* 31, 619–629. doi: 10.1006/jmcc.1998.0898

Hua, Y., and Nair, S. (2015). Proteases in cardiometabolic diseases: pathophysiology, molecular mechanisms and clinical applications. *Biochim. Biophys. Acta.* 1852, 195–208. doi: 10.1016/j.bbadis.2014.04.032

Huang, T., Lu, C., Schumann, M., Le, S., Yang, Y., Zhuang, H., et al. (2018). Timing of Exercise Affects Glycemic Control in Type 2 Diabetes Patients Treated with Metformin. *J. Diabetes Res.* 2018:2483273. doi: 10.1155/2018/2483273

Ignarro, L. J., Byrns, R. E., Buga, G. M., and Wood, K. S. (1987). Endothelium-derived relaxing factor from pulmonary artery and vein possesses pharmacologic and chemical properties identical to those of nitric oxide radical. *Circ. Res.* 61, 866–879. doi: 10.1161/01.res.61.6.866

Iwakiri, Y., and Groszmann, R. J. (2007). Vascular endothelial dysfunction in cirrhosis. *J. Hepatol.* 46, 927–934. doi: 10.1016/j.jhep.2007.02.006

Jiang, H., Lun, Y., Wu, X., Xia, Q., Zhang, X., Xin, S., et al. (2014). Association between the hypomethylation of osteopontin and integrin beta3 promoters and vascular smooth muscle cell phenotype switching in great saphenous varicose veins. *Int. J. Mol. Sci.* 15, 18747–18761. doi: 10.3390/ijms151018747

Jin, H. Y., Song, B., Oudit, G. Y., Davidge, S. T., Yu, H. M., Jiang, Y. Y., et al. (2012). ACE2 deficiency enhances angiotensin II-mediated aortic profilin-1 expression, inflammation and peroxynitrite production. *PLoS One* 7:e38502. doi: 10.1371/journal.pone.0038502

Jufri, N. F., Mohamedali, A., Avolio, A., and Baker, M. S. (2015). Mechanical stretch: physiological and pathological implications for human vascular endothelial cells. *Vasc. Cell.* 7:8. doi: 10.1186/s13221-015-0033-z

Kang, Y., Ding, L., Dai, H., Wang, F., Zhou, H., Gao, Q., et al. (2019). Intermedin in Paraventricular Nucleus Attenuates Ang II-Induced Sympathoexcitation through the Inhibition of NADPH Oxidase-Dependent ROS Generation in Obese Rats with Hypertension. *Int. J. Mol. Sci.* 20:4217. doi: 10.3390/ijms20174217

Kawai, T., Forrester, S. J., O'Brien, S., Baggett, A., Rizzo, V., and Eguchi, S. (2017). AT1 receptor signaling pathways in the cardiovascular system. *Pharmacol. Res.* 125, 4–13. doi: 10.1016/j.phrs.2017.05.008

Kennedy, A. J., Yang, P., Read, C., Kuc, R. E., Yang, L., Taylor, E. J., et al. (2016). Chemerin Elicits Potent Constrictor Actions via Chemokine-Like Receptor 1 (CMKLR1), not G-Protein-Coupled Receptor 1 (GPR1), in Human and Rat Vasculature. *J. Am. Heart Assoc.* 238, R79–R94. doi: 10.1161/JAHA.116.004421

Konukoglu, D., and Uzun, H. (2017). Endothelial Dysfunction and Hypertension. *Adv. Exp. Med. Biol.* 856, 511–540. doi: 10.1007/5584_2016_90

Kuchan, M. J., and Frangos, J. A. (1993). Shear stress regulates endothelin-1 release via protein kinase C and cGMP in cultured endothelial cells. *Am. J. Physiol.* 264, H150–H156. doi: 10.1152/ajpheart.1993.264.1.H150

Landon, E. J., and Inagami, T. (2005). Beyond the G protein: the saga of the type 2 angiotensin II receptor. *Arterioscler. Thromb. Vasc. Biol.* 25, 15–16. doi: 10.1161/01.ATV.0000153047.93274.5c

Laughlin, M. H., Newcomer, S. C., and Bender, S. B. (2008). Importance of hemodynamic forces as signals for exercise-induced changes in endothelial cell phenotype. *J. Appl. Physiol.* 104, 588–600. doi: 10.1152/japplphysiol.01096.2007

Lee, M. Y., and Griendling, K. K. (2008). Redox signaling, vascular function, and hypertension. *Antioxid Redox Signal.* 10, 1045–1059. doi: 10.1089/ars.2007.1986

Lee, S., Norheim, F., Langleite, T. M., Noreng, H. J., Storås, T. H., Afman, L. A., et al. (2016). Effect of energy restriction and physical exercise intervention on phenotypic flexibility as examined by transcriptomics analyses of mRNA from adipose tissue and whole body magnetic resonance imaging. *Physiol. Rep.* 4:e13019. doi: 10.14814/phy2.13019

Li, X. C., Zhang, J., and Zhuo, J. L. (2017). The vasoprotective axes of the renin-angiotensin system: physiological relevance and therapeutic implications in cardiovascular, hypertensive and kidney diseases. *Pharmacol. Res.* 125, 21–38. doi: 10.1016/j.phrs.2017.06.005

Lopes, R. A., Neves, K. B., Tostes, R. C., Montezano, A. C., and Touyz, R. M. (2015). Downregulation of Nuclear Factor Erythroid 2-Related Factor and Associated Antioxidant Genes Contributes to Redox-Sensitive Vascular Dysfunction in Hypertension. *Hypertension.* 66, 1240–1250. doi: 10.1161/HYPERTENSIONAHA.115.06163

Luo, X., Xiao, Y., Song, F., Yang, Y., Xia, M., and Ling, W. (2012). Increased plasma S-adenosyl-homocysteine levels induce the proliferation and migration of VSMCs through an oxidative stress-ERK1/2 pathway in apoE(-/-) mice. *Cardiovasc. Res.* 95, 241–250. doi: 10.1093/cvr/cvs130

Ma, B., and Wells, A. (2014). The mitogen-activated protein (MAP) kinases p38 and extracellular signal-regulated kinase (ERK) are involved in hepatocyte-mediated phenotypic switching in prostate cancer cells. *J. Biol. Chem.* 289, 11153–11161. doi: 10.1074/jbc.M113.540237

Magnani, F., and Mattevi, A. (2019). Structure and mechanisms of ROS generation by NADPH oxidases. *Curr. Opin. Struct. Biol.* 59, 91–97. doi: 10.1016/j.sbi.2019.03.001

Malek, A. M., Greene, A. L., and Izumo, S. (1993). Regulation of endothelin 1 gene by fluid shear stress is transcriptionally mediated and independent of protein kinase C and cAMP. *Proc. Natl. Acad. Sci. U. S. A.* 90, 5999–6003. doi: 10.1073/pnas.90.13.5999

Mancia, G., De Backer, G., Dominiczak, A., Cifkova, R., Fagard, R., Germano, G., et al. (2007). 2007 Guidelines for the Management of Arterial Hypertension: the Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *J. Hypertens.* 25, 1105–1187. doi: 10.1097/HJH.0b013e3281fc975a

Mehta, P. K., and Griendling, K. K. (2007). Angiotensin II cell signaling: physiological and pathological effects in the cardiovascular system. *Am. J. Physiol. Cell. Physiol.* 292, C82–C97. doi: 10.1152/ajpcell.00287.2006

Mikolajczyk, T. P., Nosalski, R., Skiba, D. S., Koziol, J., Mazur, M., Justo-Junior, A. S., et al. (2019). 1,2,3,4,6-Penta-O-galloyl-beta-d-glucose modulates perivascular inflammation and prevents vascular dysfunction in angiotensin II-induced hypertension. *Br. J. Pharmacol.* 176, 1951–1965. doi: 10.1111/bph.14583

Miyabe, M., Ohashi, K., Shibata, R., Uemura, Y., Ogura, Y., Yuasa, D., et al. (2014). Muscle-derived follistatin-like 1 functions to reduce neointimal formation after vascular injury. *Cardiovasc. Res.* 103, 111–120. doi: 10.1093/cvr/cvu105

Monteiro, H. P., Rodrigues, E. G., Amorim Reis, A. K. C., Longo, L. S. Jr., Ogata, F. T., Moretti, A. I. S., et al. (2019). Nitric oxide and interactions with reactive oxygen species in the development of melanoma, breast, and colon cancer: A redox signaling perspective. *Nitric. Oxide.* 89, 1–13. doi: 10.1016/j.niox.2019.04.009

Montezano, A. C., Dulak-Lis, M., Tsiropoulou, S., Harvey, A., Briones, A. M., and Touyz, R. M. (2015). Oxidative stress and human hypertension: vascular mechanisms, biomarkers, and novel therapies. *Can. J. Cardiol.* 31, 631–641. doi: 10.1016/j.cjca.2015.02.008

Moraes-Teixeira Jde, A., Félix, A., Fernandes-Santos, C., Moura, A. S., Mandarim-de-Lacerda, C. A., and de Carvalho, J. J. (2010). Exercise training enhances elastin, fibrillin and nitric oxide in the aorta wall of spontaneously hypertensive rats. *Exp. Mol. Pathol.* 89, 351–357. doi: 10.1016/j.yexmp.2010.08.004

Nishio, E., and Watanabe, Y. (1997). The involvement of reactive oxygen species and arachidonic acid in alpha 1-adrenoceptor-induced smooth muscle cell proliferation and migration. *Br. J. Pharmacol.* 121, 665–670. doi: 10.1038/sj.bjp.0701171

Nosalski, R., and Guzik, T. J. (2017). Perivascular adipose tissue inflammation in vascular disease. *Br. J. Pharmacol.* 174, 3496–3513. doi: 10.1111/bph.13705

Nowak, K. L., Wang, W., Farmer-Bailey, H., Gitomer, B., Malaczewski, M., Klawitter, J., et al. (2018). Vascular Dysfunction, Oxidative Stress, and Inflammation in Autosomal Dominant Polycystic Kidney Disease. *Clin. J. Am. Soc. Nephrol.* 13, 1493–1501. doi: 10.2215/CJN.05850518

Owens, G. K., Kumar, M. S., and Wamhoff, B. R. (2004). Molecular regulation of vascular smooth muscle cell differentiation in development and disease. *Physiol. Rev.* 84, 767–801. doi: 10.1152/physrev.00041.2003

Pagonas, N., Vlatsas, S., Bauer, F., Seibert, F. S., Zidek, W., Babel, N., et al. (2017). Aerobic versus isometric handgrip exercise in hypertension: a randomized controlled trial. *J. Hypertens.* 35, 2199–2206. doi: 10.1097/HJH.0000000000001445

Passos-Silva, D. G., Verano-Braga, T., and Santos, R. A. (2013). Angiotensin-(1-7): beyond the cardio-renal actions. *Clin. Sci.* 124, 443–456. doi: 10.1042/CS20120461

Patel, V. B., Bodiga, S., Basu, R., Das, S. K., Wang, W., Wang, Z., et al. (2012). Loss of angiotensin-converting enzyme-2 exacerbates diabetic cardiovascular complications and leads to systolic and vascular dysfunction: a critical role of the angiotensin II/AT1 receptor axis. *Circ. Res.* 110, 1322–1335. doi: 10.1161/CIRCRESAHA.112.268029

Prado, A. F., Pernomian, L., Azevedo, A., Costa, R. A. P., Rizzi, E., Ramos, J., et al. (2018). Matrix metalloproteinase-2-induced epidermal growth factor receptor transactivation impairs redox balance in vascular smooth muscle cells and facilitates vascular contraction. *Redox. Biol.* 18, 181–190. doi: 10.1016/j.redox.2018.07.005

Qi, J., Yu, X. J., Fu, L. Y., Liu, K. L., Gao, T. T., Tu, J. W., et al. (2019). Exercise Training Attenuates Hypertension Through TLR4/MyD88/NF-kappaB Signaling in the Hypothalamic Paraventricular Nucleus. *Front. Neurosci.* 13:1138. doi: 10.3389/fnins.2019.01138

Ramkhelawon, B., Vilar, J., Rivas, D., Mees, B., de Crom, R., Tedgui, A., et al. (2009). Shear stress regulates angiotensin type 1 receptor expression in endothelial cells. *Circ. Res.* 105, 869–875. doi: 10.1161/CIRCRESAHA.109.204040

Redmond, E. M., Cahill, P. A., and Sitzmann, J. V. (1998). Flow-mediated regulation of G-protein expression in cocultured vascular smooth muscle and endothelial cells. *Arterioscler. Thromb. Vasc. Biol.* 18, 75–83. doi: 10.1161/01.atv.18.1.75

Ren, X. S., Tong, Y., Ling, L., Chen, D., Sun, H. J., Zhou, H., et al. (2017). NLRP3 Gene Deletion Attenuates Angiotensin II-Induced Phenotypic Transformation of Vascular Smooth Muscle Cells and Vascular Remodeling. *Cell. Physiol. Biochem.* 44, 2269–2280. doi: 10.1159/000486061

Rodrigues Prestes, T. R., Rocha, N. P., Miranda, A. S., Teixeira, A. L., and Simoes-E-Silva, A. C. (2017). The Anti-Inflammatory Potential of ACE2/Angiotensin-(1-7)/Mas Receptor Axis: evidence from Basic and Clinical Research. *Curr. Drug Targets* 18, 1301–1313. doi: 10.2174/1389450117666160727142401

Rubanyi, G. M., Romero, J. C., and Vanhoutte, P. M. (1986). Flow-induced release of endothelium-derived relaxing factor. *Am. J. Physiol.* 250, H1145–H1149. doi: 10.1152/ajpheart.1986.250.6.H1145

Ruegsegger, G. N., and Booth, F. W. (2018). Health Benefits of Exercise. *Cold Spring Harb Perspect. Med.* 8:a029694. doi: 10.1101/cshperspect.a029694

Santos, R. A. S., Sampaio, W. O., Alzamora, A. C., Motta-Santos, D., Alenina, N., Bader, M., et al. (2018). The ACE2/Angiotensin-(1-7)/MAS Axis of the Renin-Angiotensin System: focus on Angiotensin-(1-7). *Physiol. Rev.* 98, 505–553. doi: 10.1152/physrev.00023.2016

Saxton, S. N., Withers, S. B., Nyvad, J., Mazur, A., Matchkov, V., Heagerty, A. M., et al. (2019). Perivascular Adipose Tissue Contributes to the Modulation of Vascular Tone *in vivo*. *J. Vasc. Res.* 56:6. doi: 10.1159/000502689

Schiffrin, E. L. (2012). Vascular remodeling in hypertension: mechanisms and treatment. *Hypertension* 59, 367–374. doi: 10.1161/HYPERTENSIONAHA.111.187021

Schiffrin, E. L., and Touyz, R. M. (2004). From bedside to bench to bedside: role of renin-angiotensin-aldosterone system in remodeling of resistance arteries in hypertension. *Am. J. Physiol. Heart Circ. Physiol.* 287, H435–H446. doi: 10.1152/ajpheart.00262.2004

Schlich, R., Willems, M., Greulich, S., Ruppe, F., Knoefel, W. T., Ouwens, D. M., et al. (2013). VEGF in the crosstalk between human adipocytes and smooth muscle cells: depot-specific release from visceral and perivascular adipose tissue. *Med. Inflamm.* 13:982458. doi: 10.1155/2013/982458

Seo, K. W., Lee, S. J., Ye, B. H., Kim, Y. W., Bae, S. S., and Kim, C. D. (2015). Mechanical stretch enhances the expression and activity of osteopontin and MMP-2 via the Akt1/AP-1 pathways in VSMC. *J. Mol. Cell. Cardiol.* 85, 13–24. doi: 10.1016/j.yjmcc.2015.05.006

Shu, X., Ruddiman, C. A., Keller, T. C. S. IV, Keller, A. S., Yang, Y., Good, M. E., et al. (2019). Heterocellular Contact Can Dictate Arterial Function. *Circ. Res.* 124, 1473–1481. doi: 10.1161/CIRCRESAHA.118.313926

Slaats, J., Ten Oever, J., van de Veerdonk, F. L., and Netea, M. G. (2016). IL-1beta/IL-6/CRP and IL-18/ferritin: distinct Inflammatory Programs in Infections. *PLoS Pathog.* 12:e1005973. doi: 10.1371/journal.ppat.1005973

Somanna, N. K., Valente, A. J., Krenz, M., Fay, W. P., Delafontaine, P., and Chandrasekar, B. (2016). The Nox1/4 Dual Inhibitor GKT137831 or Nox4 Knockdown Inhibits Angiotensin-II-Induced Adult Mouse Cardiac Fibroblast Proliferation and Migration. AT1 Physically Associates With Nox4. *J. Cell. Physiol.* 231, 1130–1141. doi: 10.1002/jcp.25210

Sousa, A. S., Sponton, A. C. S., Trifone, C. B., and Delbin, M. A. (2019). Aerobic Exercise Training Prevents Perivascular Adipose Tissue-Induced Endothelial Dysfunction in Thoracic Aorta of Obese Mice. *Front. Physiol.* 10:1009. doi: 10.3389/fphys.2019.01009

Speer, M. Y., McKee, M. D., Guldberg, R. E., Liaw, L., Yang, H. Y., Tung, E., et al. (2002). Inactivation of the osteopontin gene enhances vascular calcification of matrix Gla protein-deficient mice: evidence for osteopontin as an inducible inhibitor of vascular calcification *in vivo*. *J. Exp. Med.* 196, 1047–1055. doi: 10.1084/jem.20020911

Sponton, A. C., Silva, F. H., Araujo, H. N., Valgas da Silva, C. P., de Moraes, C., Antunes, E., et al. (2017). Circulating Concentrations of Adipocytokines and Their Receptors in the Isolated Corpus Cavernosum and Femoral Artery from Trained Rats on a High-Fat Diet. *J. Vasc. Res.* 54, 33–50. doi: 10.1159/000457800

Straub, A. C., Butcher, J. T., Billaud, M., Mutchler, S. M., Artamonov, M. V., Nguyen, A. T., et al. (2014). Hemoglobin alpha/eNOS coupling at myoendothelial junctions is required for nitric oxide scavenging during vasoconstriction. *Arterioscler. Thromb. Vasc. Biol.* 34, 2594–2600. doi: 10.1161/ATVBAHA.114.303974

Straub, A. C., Lohman, A. W., Billaud, M., Johnstone, S. R., Dwyer, S. T., Lee, M. Y., et al. (2012). Endothelial cell expression of haemoglobin alpha regulates nitric oxide signalling. *Nature* 491, 473–477. doi: 10.1038/nature11626

Strawn, W. B., Ferrario, C. M., and Tallant, E. A. (1999). Angiotensin-(1-7) reduces smooth muscle growth after vascular injury. *Hypertension* 33, 207–211. doi: 10.1161/01.hyp.33.1.207

Sun, H. J., Ren, X. S., Xiong, X. Q., Chen, Y. Z., Zhao, M. X., Wang, J. J., et al. (2017). NLRP3 inflammasome activation contributes to VSMC phenotypic transformation and proliferation in hypertension. *Cell. Death Dis* 8:e3074. doi: 10.1038/cddis.2017.470

Suvorava, T., and Cortese-Krott, M. M. (2018). Exercise-Induced Cardioprotection via eNOS: a Putative Role of Red Blood Cell Signaling. *Curr. Med. Chem.* 25, 4457–4474. doi: 10.2174/0929867325666180307112557

Thomas, M. C., Burns, W. C., and Cooper, M. E. (2005). Tubular changes in early diabetic nephropathy. *Adv. Chronic. Kidney Dis.* 12, 177–186. doi: 10.1053/j.ackd.2005.01.008

Touyz, R. M., Alves-Lopes, R., Rios, F. J., Camargo, L. L., Anagnostopoulou, A., Arner, A., et al. (2018). Vascular smooth muscle contraction in hypertension. *Cardiovasc. Res.* 114, 529–539. doi: 10.1093/cvr/cvy023

Vasa, M., Fichtlscherer, S., Aicher, A., Adler, K., Urbich, C., Martin, H., et al. (2001). Number and migratory activity of circulating endothelial progenitor cells inversely correlate with risk factors for coronary artery disease. *Circ. Res* 89, E1–E7. doi: 10.1161/hh1301.093953

Virdis, A., Bacca, A., Colucci, R., Duranti, E., Fornai, M., Materazzi, G., et al. (2013). Endothelial dysfunction in small arteries of essential hypertensive patients: role of cyclooxygenase-2 in oxidative stress generation. *Hypertension* 62, 337–344. doi: 10.1161/HYPERTENSIONAHA.111.00995

Virdis, V., Emiliano Di, Chiara, R., Umberto, D. A., Eleonora, S., Marco, A., et al. (2015). Tumour necrosis factor-alpha participates on the endothelin-1/nitric oxide imbalance in small arteries from obese patients: role of perivascular adipose tissue. *Eur. Heart J.* 36, 784–794. doi: 10.1093/eurheartj/ehu072

Wang, X., and Khalil, R. A. (2018). Matrix Metalloproteinases, Vascular Remodeling, and Vascular Disease. *Adv. Pharmacol.* 81, 241–330. doi: 10.1016/bs.apha.2017.08.002

Ye, S., Sun, Y., Bie, A., Zhou, Y., Liu, J., and Liu, Q. (2009). Influence of osteopontin short hairpin RNA on the proliferation and activity of rat vascular smooth muscle cells. *J. Huazhong. Univ. Sci. Technol. Med. Sci.* 29, 144–149. doi: 10.1007/s11596-009-0202-5

You, T., Arsenis, N. C., Disanzo, B. L., and Lamonte, M. J. (2013). Effects of exercise training on chronic inflammation in obesity: current evidence and potential mechanisms. *Sports Med.* 43, 243–256. doi: 10.1007/s40279-013-0023-3

Yue, J., Chang, S., Xiao, Z., Qi, Y., and He, J. (2018). The protective effect of puerarin on angiotensin II-induced aortic aneurysm formation by the inhibition of NADPH oxidase activation and oxidative stress-triggered AP-1 signaling pathways. *Oncol. Lett.* 16, 3327–3332. doi: 10.3892/ol.2018.9021

Zhang, H., Han, G. W., Batyuk, A., Ishchenko, A., White, K. L., Patel, N., et al. (2017). Structural basis for selectivity and diversity in angiotensin II receptors. *Nature* 544, 327–332. doi: 10.1038/nature22035

Zhang, L., Zhang, Y., Wu, Y., Yu, J., Zhang, Y., Zeng, F., et al. (2019). Role of the Balance of Akt and MAPK Pathways in the Exercise-Regulated Phenotype Switching in Spontaneously Hypertensive Rats. *Int. J. Mol. Sci.* 20:5690. doi: 10.3390/ijms20225690

Zhang, L., Zheng, H., Luo, J., Li, L., Pan, X., Jiang, T., et al. (2018). Inhibition of endothelial nitric oxide synthase reverses the effect of exercise on improving cognitive function in hypertensive rats. *Hypertens Res.* 41, 414–425. doi: 10.1038/s41440-018-0033-5

Zhang, R., Wu, Y., Zhao, M., Liu, C., Zhou, L., Shen, S., et al. (2009). Role of HIF-1alpha in the regulation ACE and ACE2 expression in hypoxic human pulmonary artery smooth muscle cells. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 297, L631–L640. doi: 10.1152/ajplung.90415.2008

Zhang, Y., Yu, X. J., Chen, W. S., Gao, H. L., Liu, K. L., Shi, X. L., et al. (2016). Exercise training attenuates renovascular hypertension partly via RAS- ROS-glutamate pathway in the hypothalamic paraventricular nucleus. *Sci. Rep.* 6, 37467. doi: 10.1038/srep37467

Zhao, Y., Vanhoutte, P. M., and Leung, S. W. (2015). Vascular nitric oxide: beyond eNOS. *J. Pharmacol. Sci.* 129, 83–94. doi: 10.1016/j.jphs.2015.09.002

Zhong, J., Basu, R., Guo, D., Chow, F. L., Byrns, S., Schuster, M., et al. (2010). Angiotensin-converting enzyme 2 suppresses pathological hypertrophy, myocardial fibrosis, and cardiac dysfunction. *Circulation* 128, 717–28,18following728. doi: 10.1161/CIRCULATIONAHA.110.955369

Zhong, J., Guo, D., Chen, C. B., Wang, W., Schuster, M., Loibner, H., et al. (2011). Prevention of angiotensin II-mediated renal oxidative stress, inflammation, and fibrosis by angiotensin-converting enzyme 2. *Hypertension* 57, 314–322. doi: 10.1161/HYPERTENSIONAHA.110.164244

Zhou, J., Li, Y. S., and Chien, S. (2014). Shear stress-initiated signaling and its regulation of endothelial function. *Arterioscler. Thromb. Vasc. Biol.* 34, 2191–2198. doi: 10.1161/ATVBAHA.114.303422

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Cardiovascular Medicine

ORIGINAL RESEARCH
published: 02 March 2022
doi: 10.3389/fcvm.2022.791700

Proteomic Analysis Suggests Altered Mitochondrial Metabolic Profile Associated With Diabetic Cardiomyopathy

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OPEN ACCESS

Edited by:
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Yonsei University College of Medicine, South Korea

Reviewed by:
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Specialty section:
This article was submitted to Hypertension, a section of the journal Frontiers in Cardiovascular Medicine

Received: *08 October 2021*
Accepted: *24 January 2022*
Published: *02 March 2022*

Citation:
Gomes KP, Jadli AS, de Almeida LGN, Ballasy NN, Edalat P, Shandilya R, Young D, Belke D, Shearer J, Dufour A and Patel VB (2022) Proteomic Analysis Suggests Altered Mitochondrial Metabolic Profile Associated With Diabetic Cardiomyopathy. Front. Cardiovasc. Med. 9:791700. doi: 10.3389/fcvm.2022.791700

Diabetic cardiomyopathy (DbCM) occurs independently of cardiovascular diseases or hypertension, leading to heart failure and increased risk for death in diabetic patients. To investigate the molecular mechanisms involved in DbCM, we performed a quantitative proteomic profiling analysis in the left ventricle (LV) of type 2 diabetic mice. Six-month-old C57BL/6J-lepr/lepr (*db/db*) mice exhibited DbCM associated with diastolic dysfunction and cardiac hypertrophy. Using quantitative shotgun proteomic analysis, we identified 53 differentially expressed proteins in the LVs of *db/db* mice, majorly associated with the regulation of energy metabolism. The subunits of ATP synthase that form the F1 domain, and Cytochrome c1, a catalytic core subunit of the complex III primarily responsible for electron transfer to Cytochrome c, were upregulated in diabetic LVs. Upregulation of these key proteins may represent an adaptive mechanism by diabetic heart, resulting in increased electron transfer and thereby enhancement of mitochondrial ATP production. Conversely, diabetic LVs also showed a decrease in peptide levels of NADH dehydrogenase 1β subcomplex subunit 11, a subunit of complex I that catalyzes the transfer of electrons to ubiquinone. Moreover, the atypical kinase COQ8A, an essential lipid-soluble electron transporter involved in the biosynthesis of ubiquinone, was also downregulated in diabetic LVs. Our study indicates that despite attempts by hearts from diabetic mice to augment mitochondrial ATP energetics, decreased levels of key components of the electron transport chain may contribute to impaired mitochondrial ATP production. Preserved basal mitochondrial respiration along with the markedly reduced maximal respiratory capacity in the LVs of *db/db* mice corroborate the association between altered mitochondrial metabolic profile and cardiac dysfunction in DbCM.

Keywords: diabetes, diabetic cardiomyopathy, diastolic dysfunction, electron transport chain, shotgun proteomics

INTRODUCTION

Diabetes mellitus (DM) is one of the major risk factors for cardiovascular disease (CVD), and CVD is the leading cause of morbidity and mortality worldwide. By 2045, DM is expected to affect 700 million people worldwide, with a prevalence of around 10.9% (1). In 1972, Rubler and colleagues first reported a *post-mortem* study of four diabetic patients who died of heart failure (HF) without evidence of hypertension, coronary artery disease, or congenital or valvular heart disease (2). This unique form of CVD was termed "diabetic cardiomyopathy" (DbCM). Since then, the pathophysiology of DbCM has been under investigation. However, its underlying molecular mechanisms have not yet been fully elucidated. Elusive molecular pathophysiology has resulted in the lack of standard treatment for DbCM.

The occurrence of DbCM is thought to be multifactorial, and various mechanisms have been proposed to be involved in diabetes-induced cardiac dysfunction, including resistance to metabolic actions of insulin, compensatory hyperinsulinemia, and progression of hyperglycemia in cardiac tissue (3). Together, these alterations result in changes in substrate metabolism and cardiac lipotoxicity (4), deposition of advanced glycated end-products (AGE) (5), endothelial and microvascular impairment (6), inappropriate neurohormonal responses (7), oxidative stress (8), subcellular component abnormalities, and maladaptive immune response (9). These changes result in myocardial injury, fibrosis, and hypertrophy leading to diastolic, and eventually systolic, heart failure (10).

The prevalence of DbCM has been estimated between 30% and 60% in preclinical and clinical stages among the diabetic population (11). Although significant progress has been made in recent years in the diagnosis and management of DbCM, until now, there is no specific therapy for myocardial damage induced by DbCM. A better understanding of the underlying pathological mechanisms of DbCM is highly warranted to further improve the clinical management, and therapy, of DbCM. To decipher the underlying mechanisms involved in DbCM at the molecular level, advanced proteomic profiling of left ventricular (LV) tissue specimens from type 2 diabetic mice was carried out.

Abbreviations: DM, diabetes mellitus; CVD, cardiovascular disease; HF, heart failure; DbCM, diabetic cardiomyopathy; AGE, advanced glycated end-products; LV, left ventricular; WT, wild-type; *db/db*, membrane-bound leptin receptor deficient mice; FS, fractional shortening; EF, ejection fraction; E, early peak filling; A, late peak filling; IVRT, isovolumic relaxation time; LC-MS/MS, liquid chromatography and tandem mass spectrometry; SAMS, Southern Alberta Mass Spectrometry; Easy-Nlc, nanoflow liquid chromatography; AGC, auto gain control; STRING, search tool for the retrieval of interacting genes; IPA, ingenuity pathway analysis; LVPWd, LV posterior wall thickness at diastole; LVPWs, LV posterior wall thickness at systole; MPI, myocardial performance index; OXPHOS, oxidative phosphorylation system; ETC, electron transport chain; RICTOR, rapamycin-insensitive companion of mTOR; mTORC2, mTOR complex 2; PXN, paxillin; CLPP, caseinolytic peptidase p; ADIPOQ, adiponectin; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; ILK, Integrin-Linked Kinase.

MATERIALS AND METHODS

Experimental Animals

All experiments were performed in accordance with the University of Calgary institutional guidelines, which conform to guidelines published by the Canadian Council on Animal Care and the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (revised 2011). Animals were kept at the animal facilities of the Health Sciences Animal Resources Centre of the University of Calgary. Six-month old male C57BL/6J-lepr/lepr (*db/db*) and age-matched C57BL/6J (wild-type [WT]) mice were used as experimental units, and a total of 37 mice were used in the current study. Mice were housed in standard animal cages and maintained in a constant environment with controlled room temperature, humidity, and light-dark cycle. They had access to laboratory chow pellets and drinking water *ad libitum* throughout the study, except before the oral glucose tolerance test, when the animals were fasted for 6 h before the procedure. All studies were approved by the Animal Care Committee of the University of Calgary.

Oral Glucose Tolerance Test

An oral glucose tolerance test was performed in 6 h fasted conscious mice, as previously described (12). Briefly, mice were administered with glucose (1 g/kg) by oral gavage, and the blood glucose levels were monitored repeatedly at 0, 15, 30, 60, 90, and 120 min post-glucose administration. Blood glucose levels were plotted against the time curve to determine glucose tolerance.

Echocardiography and Tissue Doppler Imaging

Cardiac function was evaluated using the Vevo 3100 high-resolution imaging system equipped with a 30-MHz transducer (MX250, VisualSonics) (12–14). Mice were anesthetized with 1.5% isoflurane in 100% oxygen and kept on a heating pad, with body temperature maintained at 36.5–37.5°C. Pre-warmed ultrasound gel was placed on the shaved chest of the anesthetized mouse. The temperature and heart rate were constantly monitored during the scanning. M-mode echocardiography images were obtained to measure LV anterior and posterior wall thickness, and LV end-diastolic and end-systolic dimensions, which were used to calculate fractional shortening (FS) and ejection fraction (EF), measures of the LV systolic function. Diastolic transmitral LV inflow images were obtained from apical four-chamber views using color flow mapping-guided pulsed-wave Doppler and were used to measure early (E) and late (atrial, A) peak filling blood flow velocities (and calculate E/A ratio), isovolumic relaxation time (IVRT), and deceleration time, all commonly used indices of LV diastolic function). Transmitral flow and tissue Doppler imaging were used to assess the E/E' ratio. All echocardiographic images were analyzed using Vevo LAB ultrasound analysis software (v5.5.1).

Shotgun Proteomic Analysis

Mice were euthanized under ketamine and xylazine anesthesia. The hearts were immediately dissected, and the LVs were stored in a −80°C for proteomics analysis. Subsequently, protein

samples were lysed with 1% sodium dodecyl sulfalte (SDS), 0.1 M dithiothreitol (DTT) in 200 nM HEPES (pH 8), protease inhibitor tablets (Sigma Aldrich, ON, Canada) with a final concentration of 3 M guanidine HCl (pH 8), 100 mM HEPES, and 10 mM DTT. Samples were alkylated by incubation with a final concentration of 15 mM iodoacetamide (IAA) in the dark for 25 min at room temperature, and the pH was adjusted to 6. Samples were then trypsinized overnight at 37°C using Trypsin gold (Promega, WI, USA). The next day, samples were incubated for 18 h at 37°C with isotopically heavy [40 mM $13CD_2O$ + 20 mM $NaBH_3CN$ (sodium cyanoborohydride)] or light labels [40mM light formaldehyde (CH_2O) + 20 mM $NaBH_3CN$], to label peptide α- and ε-amines. Samples were passed through a C18 chromatography before being subjected to liquid chromatography and tandem mass spectrometry (LC-MS/MS).

High-Performance Liquid Chromatography and Mass Spectrometry

Liquid chromatography and mass spectrometry experiments were performed at the Southern Alberta Mass Spectrometry (SAMS) core facility at the University of Calgary, Canada. An Orbitrap Fusion Lumos Tribrid mass spectrometer (Thermo Scientific) operated with Xcalibur (version 4.0.21.10) and coupled to a Thermo Scientific Easy-nLC (nanoflow Liquid Chromatography) 1200 system was used for the analysis. Tryptic peptides (2 μg) were loaded into a C18 trap (75 um x 2 cm; Acclaim PepMap 100, P/N 164946; ThermoScientific) at a flow rate of 2 μl/min of solvent A (0.1% formic acid and 3% acetonitrile in LC-MS grade water). Peptides were eluted using a 120 min gradient from 5 to 40% (5 to 28% in 105 min followed by an increase to 40% B in 15 min) of solvent B (0.1% formic acid in 80% LC-MS grade acetonitrile) at a flow rate of 0.3 μL/min and separated on a C18 analytical column (75 um × 50 cm; PepMap RSLC C18; P/N ES803; Thermo Scientific).

Peptides were subsequently electrosprayed using a voltage of 2.3 kV into the ion transfer tube (300°C) of the Orbitrap Lumos operating in positive mode. Orbitrap first performed a full MS scan at a resolution of 120,000 FWHM to detect the precursor ion with an m/z between 375 and 1,575 and a +2 to +7 charge. The Orbitrap Auto Gain Control (AGC) and the maximum injection time were set at 4×10^5 and 50 ms, respectively. Orbitrap was operated using full speed mode with a 3 sec cycle time for precursor selection. The most intense precursor ions showing a peptidic isotopic profile and having an intensity threshold of at least 5,000 were isolated using the quadrupole and fragmented with HCD (30% collision energy) in the ion routing multipole. Fragment ions (MS2) were analyzed in the ion trap at a fast scan rate. The AGC and the maximum injection time were set at 1 x 10^4 and 35 ms, respectively, for the ion trap. Dynamic deletion was enabled for 45 sec to avoid acquiring the same precursor ion with a similar m/z (plus or minus 10 ppm).

Proteomic Data Analysis

Spectral matching of the resulting raw data was done in MaxQuant (15) software package (v.1.6.10.23) implemented with the Andromeda algorithm using a UniProt murine proteome database, at a peptide-spectrum match false discovery rate of <0.01. Search parameters included a mass tolerance of 20 p.p.m. for the parent ion, 0.5 Da for the fragment ion, carbamidomethylation of cysteine residues (+57.021464 Da), variable N-terminal modification by acetylation (+42.010565 Da), and variable methionine oxidation (+15.994915 Da). N-terminal and lysine heavy (+34.063116 Da) and light (+28.031300 Da) dimethylation were defined as labels for relative quantification. The cleavage site specificity was set to Trypsin/P for the proteomics data, with up to two missed cleavages allowed. Significant outlier cut-off values were determined after Log2 transformation by boxplot-and-whiskers analysis using the BoxPlotR tool (16). The dataset was deposited into the PRIDE database and is freely available using the accession code PXD029566.

Protein–Protein Interactions and Pathway Analysis Using Bioinformatics

The selected proteins were uploaded to Metascape (17) to validate various biological functions of the selected proteins. The Search Tool for the Retrieval of Interacting Genes (STRING) database was used to identify interconnectivity among proteins. The protein interaction relationship is encoded into networks in the STRING v11 database (https://string-db.org). *Mus musculus* was used as our model organism at a false discovery rate of 1%.

To further appreciate their biological significance, the differentially expressed proteins were subjected to protein network analysis using the Ingenuity Pathway Analysis (IPA) software (Qiagen Inc.) based on curated databases from the literature. These include binding, activation, inhibition, expression, and other protein interactions to generate pathways according to the function of the molecules involved. IPA is a powerful tool widely used in the omics field to suggest/predict the effects of specific conditions or drugs on biological outcomes. Datasets containing protein identifiers (UniProt) and corresponding expression values (Log2 [Fold change]) of *db/db* vs. age-matched control were uploaded, and predicted networks were analyzed.

Mitochondrial Bioenergetics

Bioenergetics profile of isolated mitochondria was assessed using Seahorse Analyzer XFe24 (Agilent technologies) (18, 19). Briefly, mitochondria were isolated from the LVs of *db/db* and age-matched WT mice using Dounce homogenization and differential centrifugation as directed by mitochondrial isolation kit for tissue (#ab110168, Abcam). Isolated mitochondria were resuspended in mitochondrial assay solution [MAS; 220 mM mannitol, 10 mM KH2PO4, 5 mM MgCl2, 2 mM HEPES, 1 mM EGTA, and 0.2% (w/v) fatty acid-free BSA, pH 7.2] containing 10 mM of Glutamate (#G8415, Sigma-Aldrich) and 5 mM Malate (#M6413, Sigma-Aldrich) for complex I-driven respiration. The total protein concentration of isolated mitochondria was determined by a BCA assay (#5000116, Bio-Rad). A stock solution of 40 mM ADP (#A2754, Sigma-Aldrich) was prepared in MAS. Stocks of inhibitors and uncouplers were prepared by dissolving 10 mM FCCP (#C2920, Sigma), 5 mg/mL of oligomycin (#O4876, Sigma), and 40 mM of antimycin A

(#A8674, Sigma) in DMSO. The mitochondrial coupling assay for isolated mitochondria using substrates specific for the respiratory chain complex I (RCCI) was performed as described previously (18–20). 50 µl suspension of 10 µg isolated mitochondria was loaded in each well of XFe24 plates, except the wells intended for the background correction. Following final concentrations of substrate, inhibitors and uncouplers were used in wells for RCCI-driven respiration: 4 nM ADP (Port A), 2.5 µg/ml oligomycin (Port B), 4 µM FCCP (Port C), and 4 µM Antimycin A (Port D). All data were analyzed using the XFe Wave software (version 2.6; Agilent Technologies) and displayed as point-to-point oxygen consumption rates (pmol/min/well). Data are presented as the average of 3 replicate wells ±SEM.

Western Blot

Western blot analysis was performed in the mitochondrial and cytoplasmic fractions obtained by the mitochondrial isolation kit for tissue (#ab110168, Abcam) as well as in the whole tissue lysates from LVs of *db/db* and WT mice to validate the purity of the mitochondrial isolation (**Figure 7F**). Briefly, equal amounts of proteins were separated using SDS-PAGE and electrophoretically transferred to PVDF membranes. Non-specific binding was blocked by incubation in 5% non-fat milk and 0.1% Tween 20 in Tris-buffered saline. The membranes were probed individually with specific primary antibodies against glyceraldehyde 3-phosphate dehydrogenase (GAPDH; 1:1000, #sc-32233, Santa Cruz Biotechnology), voltage-dependent anion channel 1 (VDAC1; 1:1000, #sc-390996, Santa Cruz Biotechnology) and NADH dehydrogenase (ubiquinone) 1 β subcomplex subunit 11 (NDUFB11; 1:1000, #sc-374370, Santa Cruz Biotechnology). After probing with the HRP-linked secondary antibody (anti-mouse IgG, 1:3000, #7076, Cell Signaling Technology), membranes were incubated with SuperSignal West Femto Maximum Sensitivity Substrate (#34096; Thermo Scientific), and chemiluminescence was recorded using iBright™ FL1500 Imaging System (Invitrogen). Immunoreactive bands were quantified by the iBright Analysis Software using the total protein detection (No-Stain Protein Labeling Reagent, #A44717, Invitrogen) as a normalization control.

Statistical Analysis

All data are presented as mean±SEM. The sample sizes were determined based on 95% confidence level. Hypothesis testing methods included unpaired and two-tailed Student's *t*-test (two independent groups) and repeated measures ANOVA followed by Sidak's multiple comparisons. Statistical comparisons were performed by GraphPad Prism software. Statistical significance is recognized at $p < 0.05$.

RESULTS

db/db Mice Exhibit Diastolic Dysfunction and Cardiac Remodeling

Leptin-receptor mutant *db/db* mice exhibit many of the clinical characteristics of type 2 diabetes and metabolic syndrome, including hyperglycemia, hyperinsulinemia, obesity, hypertension, hyperlipidemia, and glucose intolerance (21, 22).

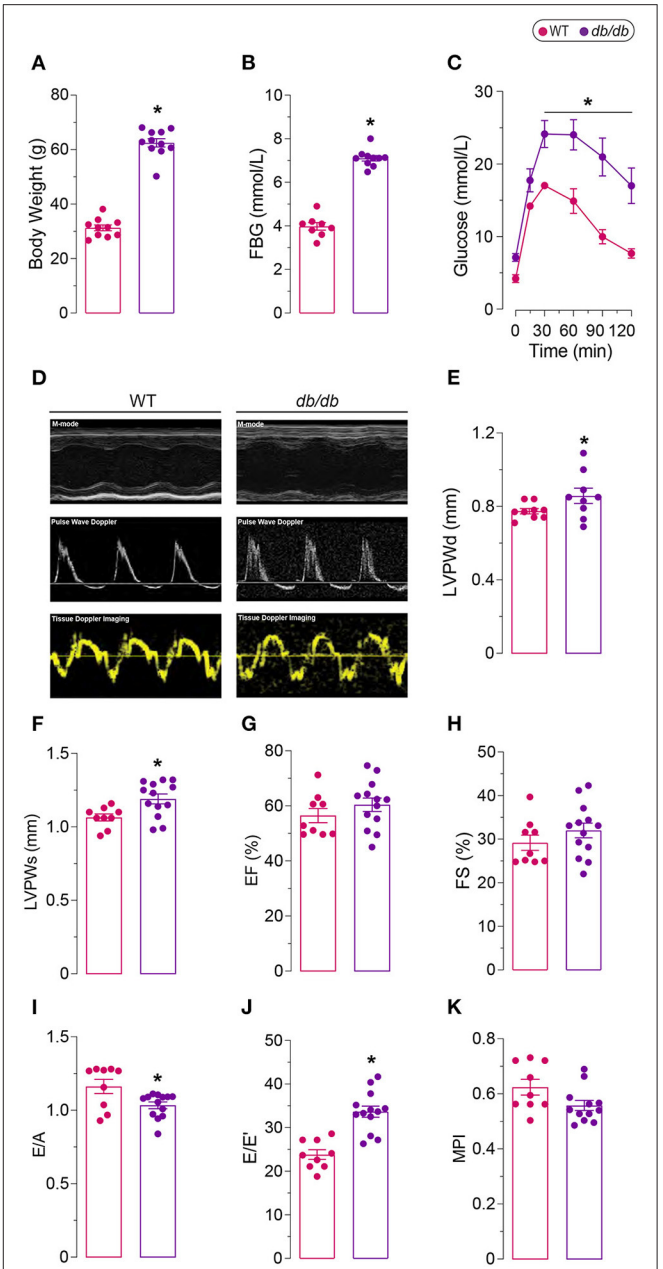

FIGURE 1 | Diabetic phenotype of 6 months old *db/db* mice. **(A)** Increased body weight, **(B)** fasting blood glucose (FBG), and **(C)** glucose intolerance in *db/db* mice compared to age-matched controls (WT). **(D)** Representative M-mode, Pulse Wave Doppler, and Tissue Doppler Imaging echocardiography. Increased left ventricle posterior wall thickness in **(E)** diastole (LVPWd) and in **(F)** systole (LVPWs) indicate left ventricular hypertrophy. **(G)** Ejection fraction (EF) and **(H)** fractional shortening (FS) remained unaffected, indicating preserved systolic function in *db/db* mice. **(I)** Decreased ratio of early to late filling (E/A) and **(J)** increased ratio of late filling in Pulse Wave Doppler to the late filling in Tissue Doppler (E/E') show abnormal diastolic function. **(K)** Myocardial performance index (MPI) was not different between the groups. *Represents *p*-value < 0.05 between WT (n = 8–10) and *db/db* (n = 9–13) mice. The scale bar shows 1.5 mm [y-axis of **(D)**], 100 ms [x-axis of **(D)**].

We observed significantly increased obesity in *db/db* mice at 6 months of age (**Figure 1A**). Increased obesity in *db/db* mice was also associated with increased fasting blood glucose levels

TABLE 1 | Body weight and echocardiographic parameters of 6-wk-old control (WT) and diabetic *(db/db)* mice.

	WT ($n = 13$)	*db/db* ($n = 9$)	*p*
HR, beats/min	428.6 ± 6.50	407.1 ± 5.05*	0.0157
LVPWd, mm	0.774 ± 0.0145	0.857 ± 0.0416*	0.0474
LVPWs, mm	1.064 ± 0.0236	1.191 ± 0.0333*	0.0106
LVIDd, mm	3.733 ± 0.1167	3.754 ± 0.0500	0.8643
LVIDs, mm	2.678 ± 0.1299	2.585 ± 0.0939	0.5574
IVSd, mm	0.877 ± 0.0364	0.915 ± 0.0421	0.5323
IVSs, mm	1.078 ± 0.0400	1.146 ± 0.0501	0.3342
EF, %	56.48 ± 2.568	60.42 ± 2.440	0.2917
FS, %	29.19 ± 1.737	32.01 ± 1.690	0.2722
Vcf, circs/s	0.611 ± 0.0388	0.608 ± 0.0287	0.9537
ET, ms	49.44 ± 0.9308	51.11 ± 0.9311	0.0745
IVCT, ms	14.29 ± 0.8149	13.02 ± 0.346	0.1229
IVRT, ms	15.16 ± 0.8235	15.10 ± 0.4649	0.9503
E/A	1.162 ± 0.0486	1.034 ± 0.0230*	0.0160
MPI	0.6244 ± 0.0284	0.5579 ± 0.0182	0.0538

*Values are mean ± SEM. HR, heart rate; LVPWd, left ventricular posterior wall thickness in diastole; LVPWs, left ventricular posterior wall thickness in systole; LVIDd, diastolic left ventricular (LV) internal dimension; LVIDs, systolic LV internal dimension; IVSd, Interventricular septal end diastole; IVSs, interventricular septal end systole; EF, ejection fraction; FS, fractional shortening; Vcf, velocity of circumferential shortening; ET, ejection time; IVCT, isovolumic contraction time; IVRT, Isovolumic relaxation time; E/A, early rapid filling/atrial contraction; MPI, myocardial performance index. *P < 0.05 compared with age-matched controls.*

and glucose intolerance, which validated the induction of severe type 2 diabetes in 6 months old *db/db* mice (**Figures 1B,C**). Chronic type 2 diabetes in 6 months old *db/db* mice resulted in the onset of DbCM (**Table 1**; **Figures 1D–K**). Quantitative assessments of transthoracic echocardiography and tissue Doppler imaging are presented in **Table 1**. Compared with age-matched WT mice, *db/db* mice exhibited significantly increased LV Posterior Wall thickness at diastole (LVPWd) (**Figures 1D,E**) and systole (LVPWs) (**Figures 1D,F**), indicative of cardiac hypertrophy in *db/db* mice. However, no significant differences were observed in systolic function among diabetic and non-diabetic mice (**Figures 1D,G,H**; **Table 1**). *db/db* mice showed reduced E/A ratio (**Figure 1I**), and markedly increased E/E' ratio, a sensitive indicator of diastolic dysfunction (**Figure 1J**). However, myocardial Performance Index (MPI), an index that incorporates both systolic and diastolic time intervals in expressing global systolic and diastolic ventricular function was not different between the groups (**Figure 1K**). Echocardiographic phenotyping validated the onset of DbCM characterized by diastolic dysfunction and cardiac hypertrophy, without any overt systolic dysfunction in 6 months old male *db/db* mice.

Differential Protein Expression in the LV of *db/db* Mice

Quantitative shotgun proteomics analysis performed after light (+28 Da) and heavy (+34 Da) formaldehyde labeling (demethylation) resulted in the identification of 715 proteins, which were subsequently used for comparative analysis (**Figure 2A**). As shown on the volcano map (**Figure 2B**), on the basis of an absolute fold change in expression levels and a corrected *p*-value ($p < 0.05$), we found 53 proteins that were differentially expressed in *db/db* LVs compared to the WT LVs. Among the 53 differentially expressed proteins in LV of *db/db* mice, 30 proteins were downregulated in response to chronic diabetes, while 23 were upregulated. All the differentially expressed proteins are listed in **Table 2**. In addition to the numerous peptides differently expressed in *db/db* mice revealed by the proteomic profile, the Metascape (17) analysis identified a top enrichment in the generation of precursor metabolites and energy production (GO:0006091) (**Figure 2C**).

To characterize the changes in the global protein network in DbCM, we investigated the functional interactions of altered proteins for each condition using STRING (23) protein-protein interaction networks and functional enrichment analysis. In the LV of WT mice, we identified two dominant clusters: six enriched proteins were involved in calcium ion binding, and two were involved in the striated muscle contraction (**Figure 3A**). However, in the LV of *db/db* mice we found enrichment for nine proteins involved in metabolism, seven involved in the citric acid cycle and respiratory electron transport, four involved in ATP synthesis-coupled proton transport, three of the tubulin family, and two involved in striated muscle contraction (**Figure 3B**).

Ingenuity Pathway Analysis Suggests Mitochondrial Dysfunction in *db/db* Mice Hearts

Specific canonical pathways and their networks functions were further explored using the IPA bioinformatics program to compare common-specific proteins and their pathological or functional implications. Canonical pathway analysis through IPA identified the top 10 pathways in *db/db* mice as described in **Figure 4A**. Predictive bioinformatics analysis revealed that the differentially expressed proteins participated in various biological processes, such as carbohydrate metabolism (**Figure 4B**), mitochondrial dysfunction, cardiac hypertrophy, cardiac necrosis/cell death, and cardiac fibrosis (**Figure 4C**), the biological responses that are previously known to occur in diabetic hearts (24–26).

Bioinformatics analysis predicted the mitochondrial dysfunction and oxidative phosphorylation (OXPHOS) to be the highest scoring protein networks impacted in diabetic hearts (**Figure 5A**). Furthermore, the proteomic analysis also identified the increased peptide levels of alpha, beta, and delta subunits of ATP synthase. Moreover, we also found a marked induction of Cytochrome c1 in *db/db* LVs compared to WT LVs. Conversely, compared to healthy controls, *db/db* mice showed a decrease in peptide levels of NDUFB11 and atypical kinase COQ8A/Adck3 (**Figure 5B**). These data showed both up and downregulation of key regulators of electron transfer in the electron transport chain (ETC) and mitochondrial ATP production (**Figure 5C**).

Subsequently, upstream regulator analysis was performed using the IPA program to determine the number of known targets of each transcription regulator present in the *db/db* dataset

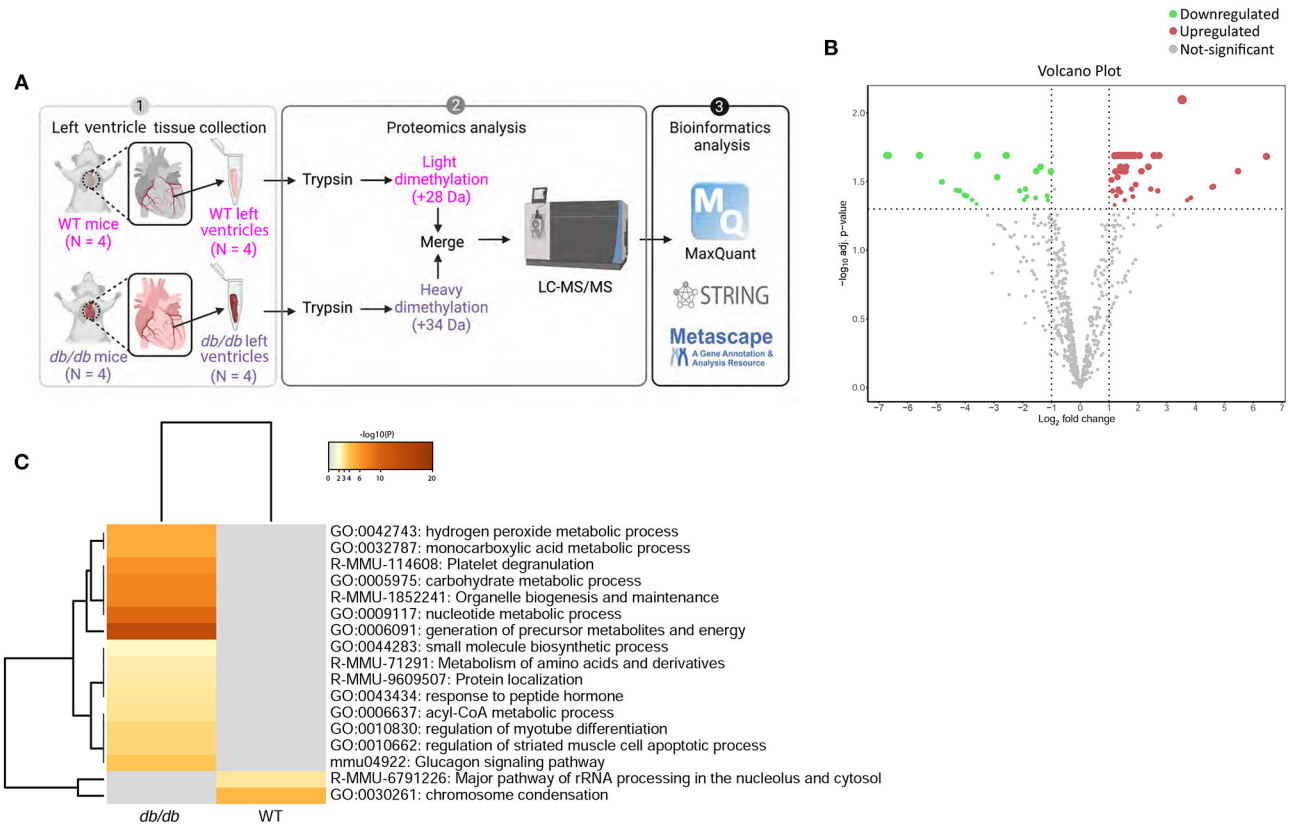

FIGURE 2 | Process enrichment analysis of differentially expressed proteins in *db/db* mice. **(A)** Experimental design. Left ventricles from WT-control (n = 4) and *db/db* (n = 4) mice were labeled with light or heavy formaldehyde, quantified by shotgun proteomics and analyzed using MaxQuant. **(B)** Volcano plot of all proteins identified. The points indicate different proteins that display both large magnitude fold-changes (x-axis) and high statistical significance (y-axis). The dashed horizontal line shows the *p*-values cut-off, and the two vertical dashed lines indicate downregulated (green) and upregulated (red) proteins in the left ventricle of *db/db* mice. Gray points show the non-significantly differently expressed proteins. **(C)** Heatmap of top 17 enriched terms across upregulated and downregulated proteins in *db/db* mice using Metascape. Darker color indicates a lower *p*-value.

obtained from the proteomic analysis. Upstream regulator analysis also allowed to compare each differentially expressed protein to the reported relationship in the literature. The top predicted inhibited upstream regulator in the DbCM was Rapamycin-Insensitive Companion of mTOR (RICTOR), a key regulatory subunit that binds to mTOR to form the mTOR Complex 2 (mTORC2) (27). RICTOR leads to inhibition of alpha, beta, and delta subunits of ATP synthase and Cytochrome c1 (28, 29), and its predicted inhibition in our study was associated with the upregulation of the ETC components in the LV of *db/db* mice. RICTOR also leads to activation of Paxillin (PXN) (30), a focal adhesion protein whose inactivation results in a progressive decrease of cardiac contractility and heart failure (31). PXN was decreased in diabetic hearts, validating the predicted inhibition of RICTOR (**Figure 6A**; **Table 2**). Another predicted inhibited upstream regulator found in the LV of *db/db* mice was Caseinolytic Peptidase P (CLPP), a mitochondrial matrix ATP-dependent peptidase. CLPP leads to inhibition of alpha and beta subunits of ATP synthase and Cytochrome c1 (32), in addition to Adiponectin (ADIPOQ) and Glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Proteomic analysis identified the upregulation of ATPF1A, ATPF1B, ADIPOQ, and GAPDH in the db/db LVs in our study, validating the predicted inhibition of CLPP (**Figure 6B**; **Table 2**).

Diabetic Cardiomyopathy-Associated Mitochondrial Dysfunction Exhibits Impaired Complex I-Driven Respiration

As our proteomic analysis suggested key regulations of proteins involved in mitochondrial metabolism, we sought to investigate the changes in mitochondrial bioenergetics and performed a mitochondrial coupling assay. Mitochondria were isolated from db/db and WT LVs, and their purity was validated using western blot analysis. The successful mitochondria isolation was corroborated by the presence and absence of mitochondria-specific (VDAC1 and NDUFB11) and non-mitochondrial (GAPDH) proteins in the mitochondrial fraction, respectively (**Figure 7A**). The proteomic analysis and bioinformatics prediction (**Table 2**) revealed reduced levels of NDUFB11 in *db/db* mice, a subunit of complex I that facilitates electron transfer to ubiquinone (20). The reduced protein levels

TABLE 2 | Selected peptides that were differentially regulated in the left ventricle of *db/db* mice.

Gene	Protein name	Log2 (db/db:WT)	Adj. *p* Value	
S100a1	Protein S100-A1	6.4633	0.0207	UPREGULATED
Rpl17	60S ribosomal protein L17	5.4742	0.0266	
Ehd1;Ehd3;Ehd4	EH domain-containing protein	4.6209	0.0343	
Tmx1	Thioredoxin-related transmembrane protein 1	4.5741	0.0347	
Zscan4b;Zscan4c;Zscan4f;Zscan4d	Zinc finger and SCAN domain-containing	3.8345	0.0415	
Hp	Haptoglobin	3.5337	0.0080	
B4galt1	Beta-1,4-galactosyltransferase 1	3.2367	0.0553	
Fam122a	P2R1A-PPP2R2A-interacting phosphatase regulator 1	2.7861	0.0661	
Gm20390;Nme2	Nucleoside diphosphate kinase B	2.7300	0.0204	
Nfs1;Gm28036	Cysteine desulfurase, mitochondrial	2.6907	0.0682	
Acta1;Actc1;Acta2	Actin, alpha skeletal muscle	2.6882	0.0370	
Ighg2b;Igh-3	Ig gamma-2B chain C region	2.6631	0.0553	
Atp5d	ATP synthase subunit delta	2.5640	0.0204	
Znfx1	NFX1-type zinc finger-containing protein 1	2.4443	0.0359	
Gm3839;Gapdh;Gapdhs	Glyceraldehyde-3-phosphate dehydrogenase	2.3627	0.0247	
2210016F16Rik	Queuosine salvage protein	2.1222	0.0266	
Adipoq	Adiponectin	2.0487	0.0204	
Igkv1-110;Igkv1-35;Igkv1-99;Igkv1-115	Immunoglobulin kappa variable	1.9245	0.1173	
Gm20425;Tf;Trf	Telomeric repeat-binding factor 1	1.9215	0.0333	
Psma3	Proteasome subunit alpha type-3	1.9042	0.1174	
Atp5i	ATP synthase subunit e, mitochondrial	1.8973	0.1174	
Gys1	Glycogen [starch] synthase, muscle	1.8882	0.0204	
Atp5b	ATP synthase subunit beta, mitochondrial	1.7560	0.0204	
Cyc1	Cytochrome c1, heme protein, mitochondrial	1.5430	0.0432	
Atp5a1	ATP synthase subunit alpha, mitochondrial	1.4200	0.0265	
Ndufb11	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 11, mitochondrial	−1.1490	0.0402	DOWNREGULATED
Adck3	Atypical kinase COQ8A, mitochondrial	−1.3760	0.0246	
Gorasp2	Golgi reassembly-stacking protein 2	−2.1490	0.0977	
Pxn	Paxillin	−2.1530	0.1426	
Myh7;Myh6;Myh4;Myh3;Myh1;Myh2;Myh8;M yh7b	Myosin	−2.1923	0.0948	
Rps4x;Gm15013	40S ribosomal protein S4, X isoform	−2.2322	0.0626	
Nedd8	NEDD8	−2.2950	0.0775	
Apoa1	Apolipoprotein A-I	−2.4082	0.0805	
Prosc	Pyridoxal phosphate-binding protein	−2.4441	0.0795	
Idh3a	Isocitrate dehydrogenase [NAD] subunit alpha, mitochondrial	−2.4736	0.2108	
C5	Complement C5	−2.5709	0.0204	
Cand1	Cullin-associated NEDD8-dissociated protein 1	−2.6097	0.0705	
Aldoa;Aldoc	Fructose-bisphosphate aldolase	−2.6662	0.0689	
Cmya5	Cardiomyopathy-associated protein 5	−2.7514	0.0661	
Mrps24	28S ribosomal protein S24, mitochondrial	−2.7644	0.0661	
Pabpc4;Gm10110	Polyadenylate-binding protein	−2.8658	0.0653	
Tpx2	Targeting protein for Xklp2	−2.8850	0.0294	
Idh1	Isocitrate dehydrogenase [NADP] cytoplasmic	−3.0045	0.0905	
Cfl1	Cofilin-1	−3.0594	0.1464	
Nrbp1	Nuclear receptor-binding protein	−3.2125	0.0556	
Fnbp1	Formin-binding protein 1	−3.5705	0.0204	
Map2	Microtubule-associated protein 2	−3.5947	0.0460	

(Continued)

TABLE 2 | Continued

Gene	Protein name	Log2 (db/db:WT)	Adj. *p* Value
Rps6ka5	Ribosomal protein S6 kinase alpha-5	−3.7585	0.0430
Atp2a1;Atp2a3	Sarcoplasmic/endoplasmic reticulum calcium ATPase 1	−3.9354	0.0402
Zfp280d;Znf280d	Zinc finger protein 280D	−3.9930	0.0402
Smc2	Structural maintenance of chromosomes protein 2	−4.0378	0.0395
Myo3a	Myosin-IIIa	−4.1878	0.0370
Pclo	Protein piccolo	−4.3033	0.0366
Noxred1	NADP-dependent oxidoreductase domain-containing protein 1	−4.8053	0.0318
Rdm1	RAD52 motif-containing protein 1	−5.5836	0.0204
Cblb	E3 ubiquitin-protein ligase CBL-B	−6.6663	0.0204
Cep162	Centrosomal protein of 162 kDa	−6.7192	0.0204

Color gradient represents the magnitude of the changes. Shades of red refer to upregulation and shades of green refer to downregulation.

of NDUFB11 were also confirmed by the western blot analysis performed on the mitochondrial fraction obtained from LVs of *db/db* mice (**Figures 7A,B**).

The mitochondrial coupling assay examines the degree of coupling between OXPHOS and ETC; an impaired coupling between OXPHOS and ETC would indicate mitochondrial dysfunction. In the Seahorse extracellular flux analysis, the respiratory chain complex I (RCCI)-driven respiration, which represents the respiration of mitochondria in the presence of substrates but without ADP, did not show any difference in basal respiration between WT and *db/db* groups (**Figure 7C**). State III, which represents the formation of ATP from ADP and inorganic phosphate, and State IVo, which represents the proton leak due to the inhibition of the ATP synthase by oligomycin, was moderately decreased in mitochondria-derived from *db/db* LVs compared to WT LVs (**Figures 7D,E**). The state IIIu, an indicator of the maximal respiratory capacity, was significantly decreased in the mitochondria isolated from *db/db* LVs compared to the WT LVs (**Figure 7F**). The respiratory control ratio (RCR), an index of mitochondrial coupling, which is obtained by dividing the corrected values of State IIIu/State Ivo, was markedly reduced in *db/db* group compared to WT (**Figure 7G**), indicating increased mitochondrial uncoupling in DbCM. Although the basal respiration remained unchanged, markedly decreased respiratory chain complex I (RCCI)-driven mitochondrial coupling and severely reduced maximal respiratory capacity suggest impaired mitochondrial respiration in *db/db* mice corroborating proteomic discoveries.

DISCUSSION

DbCM is a complex disorder caused by multifactorial pathology (33). The natural history of DbCM ranges from a short-term physiological adaptation to degenerative changes unable to be repaired by the myocardium, ultimately culminating in an irreversible pathological remodeling (34, 35). Induction of hyperglycemia and hyperlipidemia with progressive accumulation of the respective substrates in cardiomyocytes causes functional and structural changes (36, 37). These gradual changes often begin with diastolic dysfunction, followed by decreased left ventricular systolic function, resulting in HF (3, 38). In the present study, we validated the diabetic phenotype in 6 months old *db/db* mice, which was associated with structural and functional abnormalities of DbCM, including diastolic dysfunction and cardiac hypertrophy. These essential features are related to an established stage of DbCM, validating our experimental model.

HF-related to DbCM is evidently associated with abnormal myocardial energy metabolism with progressive myocardial hypertrophy and fibrosis (4, 39, 40). Despite the increasing number of studies in recent years that attempt to explain the potential pathophysiological mechanisms involved in the genesis of DbCM (21), there is still no clear and comprehensive integration of the pathways involved due to its multifactorial nature. In our research, we used quantitative shotgun proteomics to track the changes in the protein content, and subsequently, the proteomic profile in LVs of type 2 diabetic *db/db* mice. Proteomics analysis provides an unbiased experimental tool for the identification of aberrant protein expressions associated with disease, revealing potential signaling cascades that can be targeted therapeutically. Here we identified the shift in reactome priorities from calcium handling and muscle contraction in healthy mice to energy metabolism and mitochondrial dysfunction in DbCM. Mitochondria play a pivotal role in integrating cellular energy metabolism and cell survival (41). In type 2 DM, the impairment of mitochondrial function leads to a significant ROS production, further contributing to DM-induced myocardial dysfunction (42, 43). Proteomic analysis showed that the peptide levels of alpha, beta, and delta subunits of ATP synthase were upregulated in the LVs of *db/db* mice. These subunits form the F1 domain, a catalytic assembly of the enzyme critically involved in ATP synthesis (44). We also found a marked induction of Cytochrome c1 in LVs of *db/db* mice, which is a catalytic core subunit of the complex III, that catalyzes the transfer of electrons from coenzyme Q to Cytochrome c (45). By means of electron transfer, Cytochrome c1 plays an important role in the elevation of mitochondrial membrane potential, by using its heme group as a redox intermediate to transport electrons between complex III and complex IV (46). Importantly, studies have shown that increased levels of mitochondrial Cytochrome c are early events that precede the onset of apoptosis (47). We hypothesize

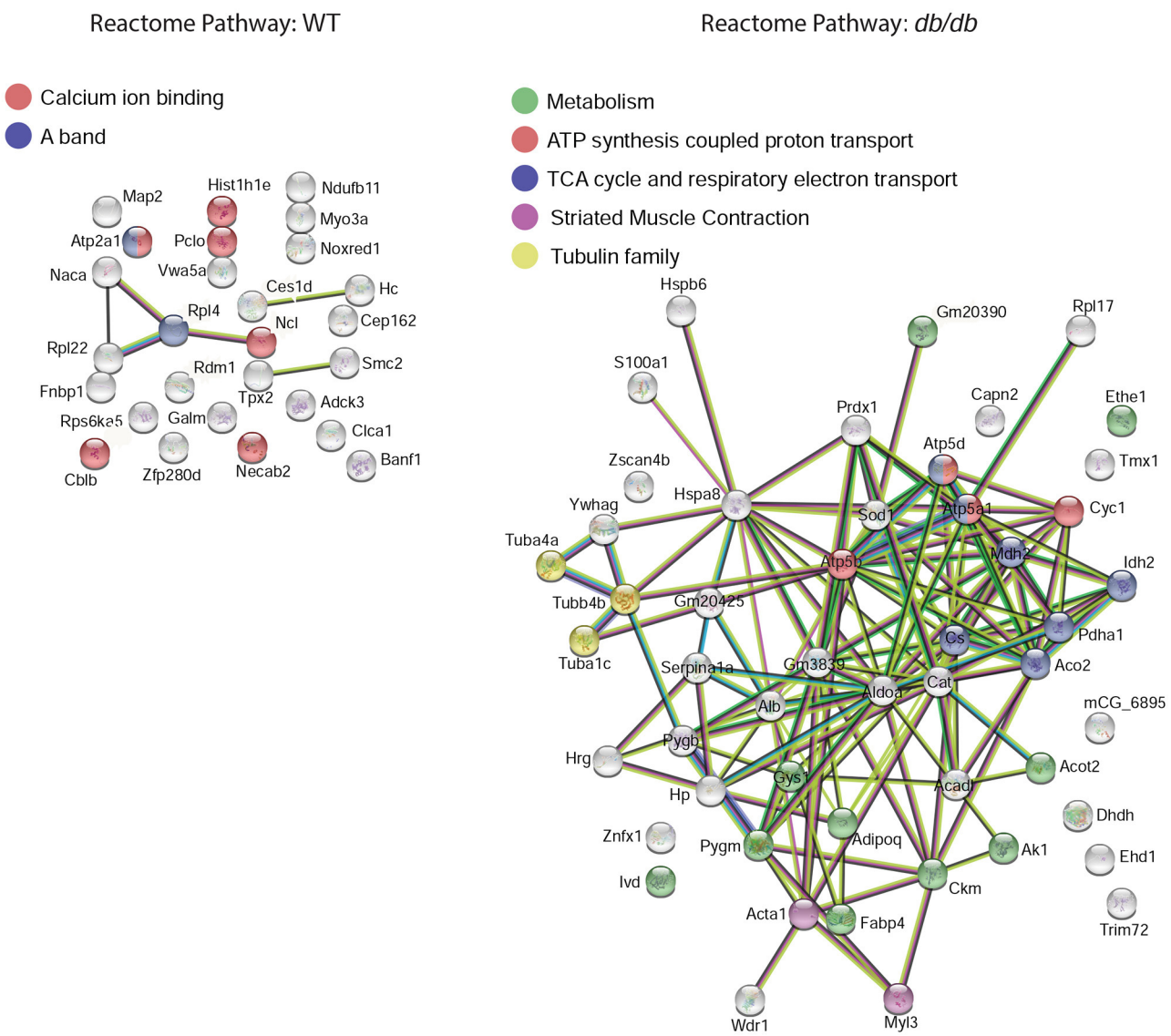

FIGURE 3 | Functional protein-protein network analysis by STRING database. Left ventricular elevated proteins in **(A)** WT or **(B)** *db/db* mice identified from the proteomic analysis were mapped by searching the STRING software with a confidence level of 1% false discovery rate. The global protein network shows the shift in reactome priorities from calcium handling and muscle contraction in healthy mice, to energy metabolism and mitochondrial dysfunction in DbCM. Colored lines between the proteins indicate different types of interaction evidence: known interactions (teal), experimentally determined (pink), predicted interactions gene neighborhood (green), gene fusions (red), gene co-occurrence (blue), text-mining (yellow), co-expression (black), protein homology (purple).

that increased Cytochrome c1 levels may represent an adaptive mechanism by which diabetic heart attempts to increase electron transfer, and thereby enhance mitochondrial ATP production.

Contrarily, in *db/db* mice, we found decreased peptide levels of NDUFB11, a subunit of complex I that catalyzes the transfer of electrons to ubiquinone, and is considered an important factor in the regulation of mitochondrial respiration (20). Although basal respiration remained unchanged in LVs of *db/db* mice, metabolic assessment exhibited severely reduced rate of state IIIu respiration (maximal respiratory capacity) and decreased respiratory control, during the oxidation of the complex I-linked substrates in isolated mitochondria from *db/db* LVs. Combination of proteomic and metabolic assessments suggest that the restricted proton pumping by complex I may induce a slower and prolonged proton entry into complex V after the addition of ADP, leading to alterations in mitochondrial oxidative capacity and coupling of oxygen consumption, eventually affecting ATP production (48). We also observed a downregulation of atypical kinase COQ8A in the proteomic profile of type 2 diabetic LVs. COQ8A is

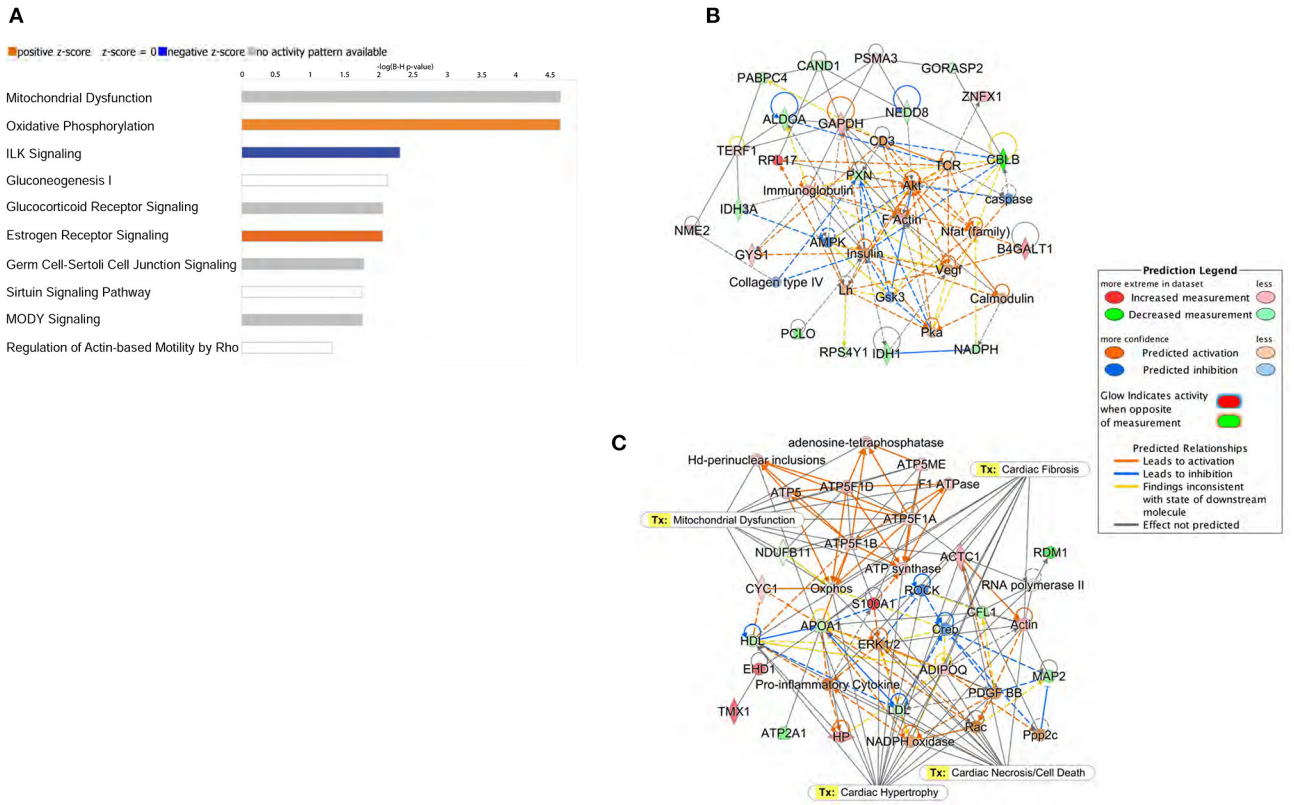

FIGURE 4 | Ingenuity Pathway Analysis (IPA) of proteomic data. **(A)** Top 10 canonical pathways identified by IPA of proteins differentially expressed in left ventricle of *db/db* mice. The most statistically significant canonical pathways identified in *db/db* mice are listed according to their *p*-value (–log). Blue bars: negative z-score; orange bars: positive z-score. Clear bars are indicative of a z-score of 0, and thus have no difference in activity. Gray shaded bars indicate that there is no activity pattern available identified in IPA, despite highly significant association of the proteins within the pathway. Predictive bioinformatics analysis revealed that the significantly changed proteins participated in various biological processes. **(B)** Carbohydrate metabolism, **(C)** energy production, and cellular assembly were the highest scoring molecular networks identified by IPA in *db/db* mice.

an essential lipid-soluble electron transporter involved in the biosynthesis of ubiquinone and in the energetic movement of electrons through the ETC (49). Interestingly, DM-associated mitochondrial dysfunction has also been linked with metabolic deficits. The cardiac demand for energy comes predominantly from mitochondrial OXPHOS, which accounts for 95% of total ATP produced. However, in the chronic diabetic state established in 6 months old *db/db* mice, the ability of the heart to switch between available oxidizable substrates is impaired, and in this condition, the heart depends almost exclusively on fatty acid metabolism, which increases mitochondrial damage (50, 51). In our study we found that despite attempts by hearts from the diabetic mice to upregulate some ETC elements, which results in preserved basal mitochondrial metabolism, decreased levels of key components contribute to impaired maximal respiratory capacity, critically affecting ability of the diabetic heart to respond to increased metabolic needs. It is likely that ATP synthase subunits and Cytochrome c1 may also be downregulated with prolonged persistence of the diabetic phenotype. At first, the protective mechanism of mitochondrial function seems to be present, favoring the myocardial redox environment essential for the resting contractile. However, our study suggests that diabetic hearts have altered expression of essential mitochondrial peptides, which may contribute to the impaired mitochondrial bioenergetics contributing to the establishment of DbCM in this model.

In type 2 DM, the accumulation of ectopic lipids in the heart has also been associated with reduced cardiac efficiency. As lipid accumulation and plasma free fatty acid levels increase in type 2 DM, adverse effects of lipid accumulation on cardiac structure and function have been discussed as a potential mechanism for DbCM. *Ex vivo* perfusion of murine hearts from obese mice with free fatty acid demonstrated increased oxygen consumption and reduced ATP-to-Oxygen ratio when compared to glucose perfusion; these changes in the ATP-to-Oxygen ratio were too large to be explained by changes in the substrate metabolism, and were found to be associated with increased mitochondrial uncoupling (52, 53). Moreover, increased fatty acid metabolism in these hearts was associated with increased expression of mitochondrial uncoupling proteins (54). Similarly, reduced mitochondrial oxidative capacity inspite of increased mitochondrial biogenesis

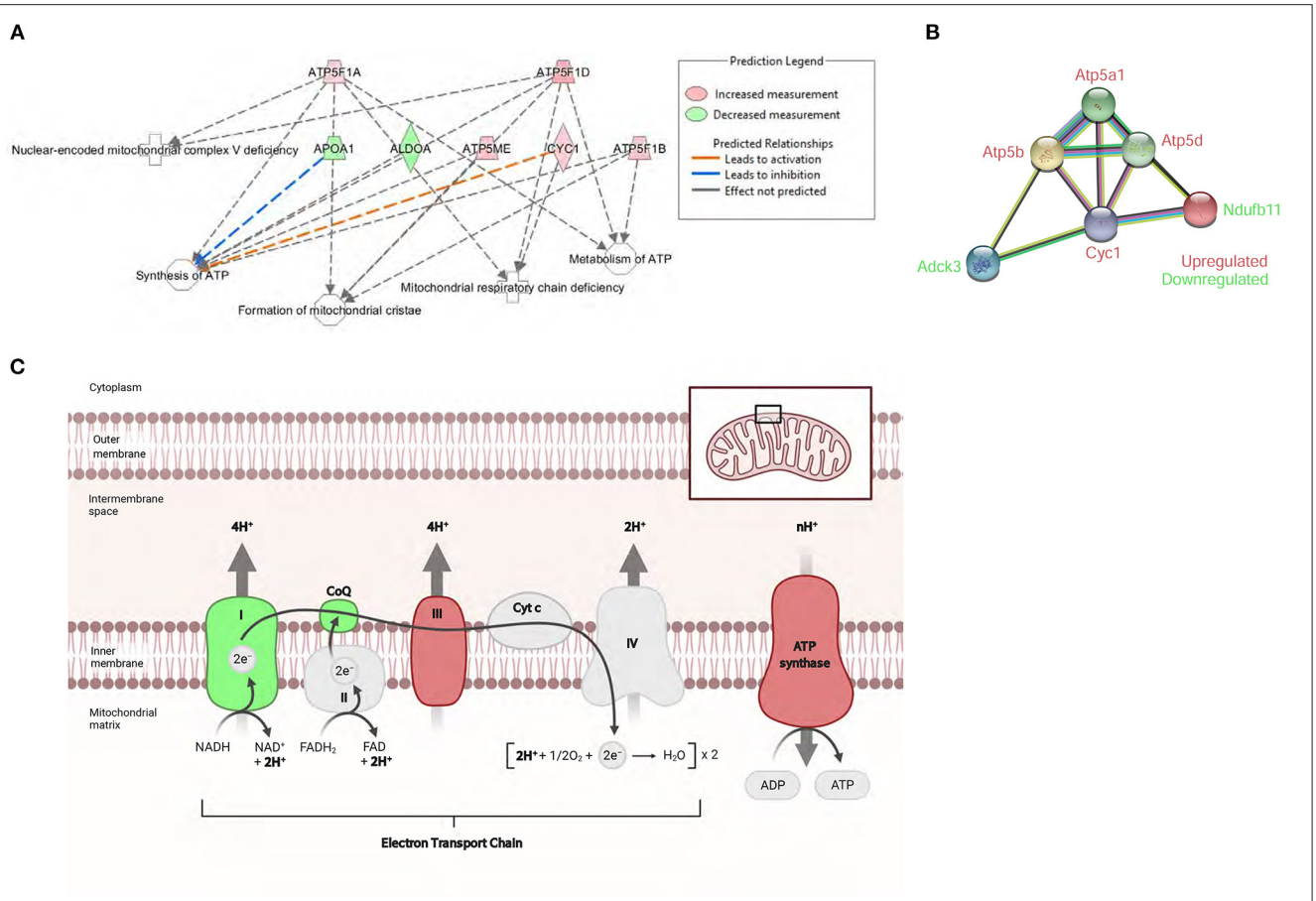

FIGURE 5 | Proteomic profile of *db/db* mice suggests severe mitochondrial metabolic dysregulation. **(A)** The top affected cellular function identified by IPA in *db/db* mice was the ATP synthesis network. **(B)** STRING interaction network of differentially expressed mitochondrial proteins in the left ventricle of *db/db* mice. **(C)** Schematic overview of the mitochondrial respiratory electron transport chain and the differentially expressed mitochondrial proteins associated with diabetic cardiomyopathy. Adapted from "Electron Transport Chain," by BioRender.com (2021). Retrieved from https://app.Biorender.com/Biorender-templates. Symbol color represents expression value, red indicating an upregulation, and green indicating downregulation in our dataset.

were observed in *db/db* hearts (55). Cytosolic as well as mitochondrial lipidic environment has been proposed to play a key role in regulation of mitochondrial metabolism, and remains to be investigated. Integrated proteomic and lipidomic analyses of diabetic heart may provide novel insight into the molecular state and underlying molecular mechanisms of DbCM.

IPA software was used to facilitate the organization and interpretation of the proteomic data in our study, which enabled prediction of upstream regulators to diseases and functions. RICTOR, an obligate regulatory subunit of mTORC2 (27), was predicted to be inhibited in DbCM. Activation of mTORC2 modulates mitochondrial function *via* Akt (28), and regulates cell survival *via* its anti-apoptotic effects in cardiac hypertrophy and myocardial ischemia (56). Inhibition of RICTOR expression has been demonstrated to block mTORC2 assembly and activity (57). RICTOR deletion from cardiomyocytes inactivates mTORC2, but does not modify basal cardiac function and geometry. However, RICTOR-deficient hearts display reduced cardiac performance when challenged by haemodynamic stress, which leads to cardiac dysfunction and dilatation (58). While studies indicate that activating autophagy in cardiomyocytes by inhibiting mTORC1 may prevent the aggravation of DbCM (59, 60), the role of RICTOR/mTORC2 in DbCM remains largely unknown. Additionally, IPA-based bioinformatics analysis also predicted CLPP to be inhibited in the LV of *db/db* mice. CLPP is a mitochondrial peptidase essential for maintaining protein quality control and mitochondrial function. Protease-mediated quality control is the first line of defense against mitochondrial damage and involves the degradation of non-assembled proteins that result from mitonuclear imbalance, and proteins that are damaged or misfolded as result of ROS (61).

Both RICTOR and CLPP are involved in the maintenance of cardiac homeostasis, and alterations in the levels of these regulators may contribute to pathological remodeling and cardiac dysfunction. Cells with genetic deletion of RICTOR exhibit defects in cell polarity and cytoskeletal architecture (62), whereas cardiac-specific knockdown of

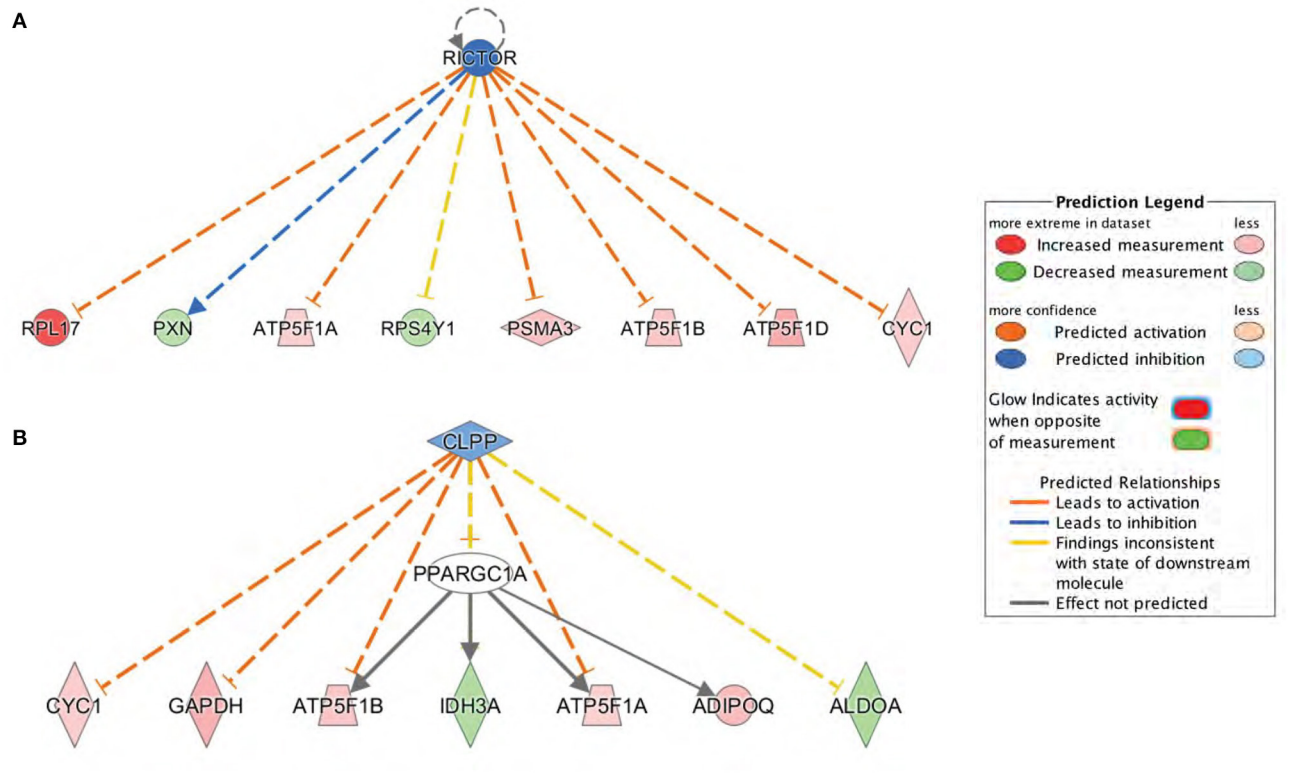

FIGURE 6 | Upstream regulators predicted by Ingenuity Pathway Analysis (IPA) at the proteome level. **(A)** Rapamycin-Insensitive Companion of mTOR (RICTOR) and **(B)** Caseinolytic Peptidase P (CLPP) were the top inhibited upstream regulators predicted in *db/db* mice. Downstream proteins are displayed as networks. Symbol color represents expression value, red indicating an upregulation and blue/green indicating downregulation in our dataset. RICTOR and CLPP modulates the expression or function of downstream proteins listed in figure.

RICTOR exacerbated cardiac remodeling and dysfunction after myocardial infarction (63). Accordingly, mTORC2 activation has been shown to mediate the cardioprotective effects of hydrogen sulfide in response to ischemia/reperfusion in rats (64). Muscle-specific CLPP deficiency can partially restore mitochondrial protein synthesis, improving mitochondrial respiratory activity and attenuating pathological cardiac remodeling (65). Whereas, CLPP knockout mice exhibit reduced prenatal/postnatal survival, growth retardation, movement impairment, mild respiratory defects, and female and male infertility (65, 66). Indeed, further investigations are highly warranted to better understand the role of RICTOR and CLPP in initiation, progression and establishment of DbCM. In addition to mitochondrial dysfunction, the Integrin-Linked Kinase (ILK) signaling has also been identified as a top canonical pathway through IPA analysis in *db/db* mice LVs. ILK is a broadly expressed serine/threonine-protein kinase that binds to the cytoplasmic tail of β integrins, linking the interactions of cellular matrix to signals that regulate cytoskeletal remodeling and cellular processes such as growth, proliferation, survival, and differentiation (67, 68). As a major regulator of cytoskeletal remodeling, ILK pathway remains of a major interest in various cardiovascular disease.

Growing experimental research has been conducted to better understand the sexual dimorphism in the molecular mechanisms and outcomes of DbCM. However, the sex-specific differences at the level of the myocardium remain largely unknown. A potential limitation of our study is the absence of female subjects, as this could support a better comprehension of the sexual dimorphism in DbCM. Interestingly, Estrogen Receptor Signaling has been identified as a top canonical pathway with predicted activation through IPA analysis in male *db/db* mice LVs. Further research is critical to ascertain the role of estrogen in cardiovascular complications of DM. Furthermore, the validation of our findings in other independent models of DbCM, and explanted human hearts to understand the translational potential of our investigations are paramount. Moreover, although the present study identified mitochondrial dysfunction in diabetic hearts, further studies at various stages of DbCM onset and progression may shed light on "cause-and-effect" relationship between mitochondrial (dys)function and diastolic (dys)function. In summary, the present study found that diabetic LVs displayed altered expression of peptides involved in key mitochondrial metabolic processes together with the coordinated downregulation of cytoskeletal proteins. Further investigations into cause-and-effect relationship may provide novel insight into molecular

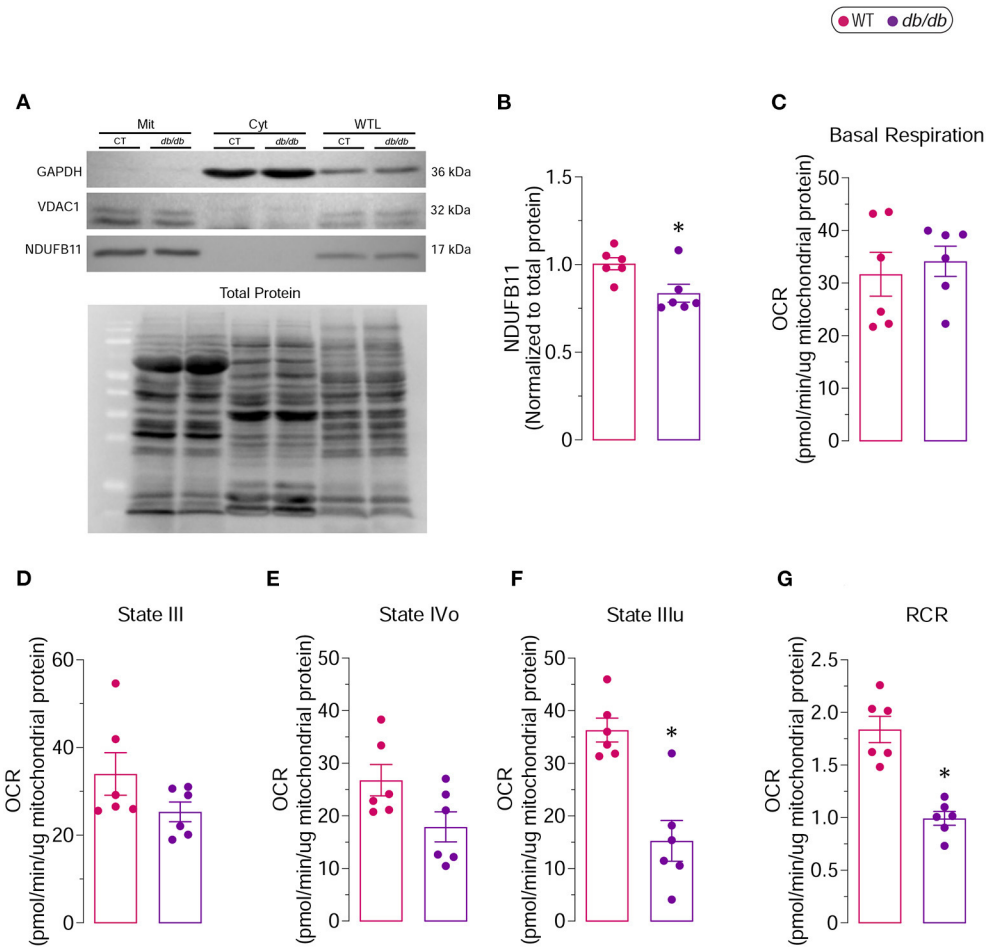

FIGURE 7 | Complex I-driven respiratory capacity in isolated mitochondria from 6 months old *db/db* mice **(A)** Representative immunoblot showing the purity of the mitochondrial (Mit) fraction used for determination of mitochondrial respiratory function. VDAC1 and NDUFB11 were used as mitochondrial markers, while GAPDH was used as cytoplasmic (Cyt) marker. Whole tissue lysate (WTL) protein levels were also evaluated. **(B)** Proteins levels of NDUFB11 normalized to total protein. Oxygen consumption rates (OCR) of isolated mitochondria were measured by Seahorse XF analyzer. **(C)** Basal Respiration, **(D)** State III, **(E)** State IVo, **(F)** State IIIu and **(G)** Respiratory Control Ratio (RCR) are shown. *Represents p-value < 0.05 between WT ($n = 6$) and *db/db* ($n = 6$) mice.

mechanism of DbCM, with a potential to developing novel therapeutic targets.

DATA AVAILABILITY STATEMENT

The data presented in the study are deposited in the PRIDE repository, accession number PXD029566.

ETHICS STATEMENT

The animal study was reviewed and approved by the Animal Care Committee, University of Calgary.

AUTHOR CONTRIBUTIONS

KG, AJ, and VP designed the research. KG, AJ, LA, NB, PE, RS, DY, DB, JS, and AD acquired and analyzed data. KG and VP wrote the manuscript. VP is the guarantor of this work and, as such, has full access to all the data and takes responsibility for the integrity of data and the accuracy of data analysis, and supervised and managed the funding. All authors critically revised and approved the final version of the manuscript.

FUNDING

This work received support from Canadian Institutes of Health Research (CIHR; operating grant to VP) Project Grant (PJT-165857), Libin Cardiovascular Institute, Cumming School of Medicine (Start-up operating fund to VP; postdoctoral scholarships to KG and AJ; graduate scholarship in Women Cardiovascular Health to PE), Alberta Diabetes Institute (operating funds to VP), and Natural Sciences and Engineering Research Council (NSERC; operating grant to AD) Discovery Grant (DGECR-2019-00112).

REFERENCES

1. Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, et al. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: results from the international diabetes federation diabetes atlas, 9(th) edition. *Diabetes Res Clin Pract.* (2019) 157:107843. doi: 10.1016/j.diabres.2019.107843
2. Rubler S, Dlugash J, Yuceoglu YZ, Kumral T, Branwood AW, Grishman A. New type of cardiomyopathy associated with diabetic glomerulosclerosis. *Am J Cardiol.* (1972) 30:595–602. doi: 10.1016/0002-9149(72)90595-4
3. Jia G, Whaley-Connell A, Sowers JR. Diabetic cardiomyopathy: a hyperglycaemia- and insulin-resistance-induced heart disease. *Diabetologia.* (2018) 61:21–8. doi: 10.1007/s00125-017-4390-4
4. Sung MM, Hamza SM, Dyck JR. Myocardial metabolism in diabetic cardiomyopathy: potential therapeutic targets. *Antioxid Redox Signal.* (2015) 22:1606–30. doi: 10.1089/ars.2015.6305
5. Nozynski J, Zakliczynski M, Konecka-Mrowka D, Nikiel B, Mlynarczyk-Liszka J, Zembala-Nozynska E, et al. Advanced glycation end products in the development of ischemic and dilated cardiomyopathy in patients with diabetes mellitus type 2. *Transplant Proc.* (2009) 41:99–104. doi: 10.1016/j.transproceed.2008.09.065
6. Knapp M, Tu X, Wu R. Vascular endothelial dysfunction, a major mediator in diabetic cardiomyopathy. *Acta Pharmacol Sin.* (2019) 40:1–8. doi: 10.1038/s41401-018-0042-6
7. Amour J, Loyer X, Le Guen M, Mabrouk N, David JS, Camors E, et al. Altered contractile response due to increased beta3-adrenoceptor stimulation in diabetic cardiomyopathy: the role of nitric oxide synthase 1-derived nitric oxide. *Anesthesiology.* (2007) 107:452–60. doi: 10.1097/01.anes.0000278909.40408.24
8. Dhalla NS, Shah AK, Tappia PS. Role of oxidative stress in metabolic and subcellular abnormalities in diabetic cardiomyopathy. *Int J Mol Sci.* (2020) 21(7). doi: 10.3390/ijms21072413
9. Mishra PK, Ying W, Nandi SS, Bandyopadhyay GK, Patel KK, Mahata SK. Diabetic cardiomyopathy: an immunometabolic perspective. *Front Endocrinol.* (2017) 8:72. doi: 10.3389/fendo.2017.00072
10. Lee MMY, McMurray JJV, Lorenzo-Almoros A, Kristensen SL, Sattar N, Jhund PS, et al. Diabetic cardiomyopathy. *Heart.* (2019) 105:337–45. doi: 10.1136/heartjnl-2016-310342
11. Authors/Task Force M, Ryden L, Grant PJ, Anker SD, Berne C, Cosentino F, et al. ESC Guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD: the task force on diabetes, pre-diabetes, and cardiovascular diseases of the european society of cardiology. (ESC) and developed in collaboration with the European Association for the Study of Diabetes. (EASD). *Eur Heart J.* (2013) 34:3035–87. doi: 10.1093/eurheartj/eht108
12. Patel VB, Mori J, McLean BA, Basu R, Das SK, Ramprasath T, et al. ACE2 deficiency worsens epicardial adipose tissue inflammation and cardiac dysfunction in response to diet-induced obesity. *Diabetes.* (2016) 65:85–95. doi: 10.2337/db15-0399
13. Mori J, Patel VB, Abo Alrob O, Basu R, Altamimi T, Desaulniers J, et al. Angiotensin 1-7 ameliorates diabetic cardiomyopathy and diastolic dysfunction in db/db mice by reducing lipotoxicity and inflammation. *Circ Heart Fail.* (2014) 7:327–39. doi: 10.1161/CIRCHEARTFAILURE.113.000672
14. Patel VB, Zhabyeyev P, Chen X, Wang F, Paul M, Fan D, et al. PI3Kalpha-regulated gelsolin activity is a critical determinant of cardiac cytoskeletal remodeling and heart disease. *Nat Commun.* (2018) 9:5390. doi: 10.1038/s41467-018-07812-8
15. Cox J, Neuhauser N, Michalski A, Scheltema RA, Olsen JV, Mann M. Andromeda: a peptide search engine integrated into the MaxQuant environment. *J Proteome Res.* (2011) 10:1794–805. doi: 10.1021/pr101065j
16. Spitzer M, Wildenhain J, Rappsilber J, Tyers M. BoxPlotR: a web tool for generation of box plots. *Nat Methods.* (2014) 11:121–2. doi: 10.1038/nmeth.2811
17. Zhou Y, Zhou B, Pache L, Chang M, Khodabakhshi AH, Tanaseichuk O, et al. Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. *Nat Commun.* (2019) 10:1523. doi: 10.1038/s41467-019-09234-6
18. Iuso A, Biagosch C, Terrile C, Prokisch H. "Assessing mitochondrial bioenergetics in isolated mitochondria from various mouse tissues using seahorse XF96 analyzer," In: *Methods in Molecular Biology* [Internet]. (2017). New York, NY: Humana Press.
19. Rogers GW, Brand MD, Petrosyan S, Ashok D, Elorza AA, Ferrick DA, et al. High throughput microplate respiratory measurements using minimal quantities of isolated mitochondria. *PLoS ONE.* (2011) 6:e21746. doi: 10.1371/journal.pone.0021746
20. Wirth C, Brandt U, Hunte C, Zickermann V. Structure and function of mitochondrial complex I. *Biochim Biophys Acta.* (2016) 1857:902–14. doi: 10.1016/j.bbabio.2016.02.013
21. Jia G, Hill MA, Sowers JR. Diabetic cardiomyopathy: an update of mechanisms contributing to this clinical entity. *Circ Res.* (2018) 122:624–38. doi: 10.1161/CIRCRESAHA.117.311586
22. Burke SJ, Batdorf HM, Burk DH, Noland RC, Eder AE, Boulos MS, et al. db/db mice exhibit features of human type 2 diabetes that are not present in weight-matched C57BL/6J Mice Fed a Western Diet. *J Diabetes Res.* (2017) 2017:8503754. doi: 10.1155/2017/8503754
23. Szklarczyk D, Gable AL, Nastou KC, Lyon D, Kirsch R, Pyysalo S, et al. The STRING database in 2021: customizable protein-protein networks, and functional characterization of user-uploaded gene/measurement sets. *Nucleic Acids Res.* (2021) 49:D605–D12. doi: 10.1093/nar/gkab835
24. Chen Y, Hua Y, Li X, Arslan IM, Zhang W, Meng G. Distinct types of cell death and the implication in diabetic cardiomyopathy. *Front Pharmacol.* (2020) 11:42. doi: 10.3389/fphar.2020.00042
25. Chun-Jun Li LL, Hui Li, De-min Yu. Cardiac fibrosis and dysfunction in experimental diabetic cardiomyopathy are ameliorated by alpha-lipoic acid. *Cardiovascular Diabetol.* (2012) 11:110. doi: 10.1186/1475-2840-11-73
26. Frustaci A, Kajstura J, Chimenti C, Jakoniuk I, Leri A, Maseri A, et al. Myocardial cell death in human diabetes. *Circ Res.* (2000) 87:1123–32. doi: 10.1161/01.RES.87.12.1123
27. Sarbassov DD, Ali SM, Kim DH, Guertin DA, Latek RR, Erdjument-Bromage H, et al. Rictor, a novel binding partner of mTOR, defines a rapamycin-insensitive and raptor-independent pathway that regulates the cytoskeleton. *Curr Biol.* (2004) 14:1296–302. doi: 10.1016/j.cub.2004.06.054
28. Betz C, Stracka D, Prescianotto-Baschong C, Frieden M, Demaurex N, Hall MN. Feature Article: mTOR complex 2-Akt signaling at mitochondria-associated endoplasmic reticulum membranes. (MAM) regulates mitochondrial physiology. *Proc Natl Acad Sci U S A.* (2013) 110:1252634. doi: 10.1073/pnas.1302455110
29. Cunningham JT, Rodgers JT, Arlow DH, Vazquez F, Mootha VK, Puigserver P. mTOR controls mitochondrial oxidative function through a YY1-PGC-1alpha transcriptional complex. *Nature.* (2007) 450:736–40. doi: 10.1038/nature06322
30. Hagan GN, Lin Y, Magnuson MA, Avruch J, Czech MP. A Rictor-Myo1c complex participates in dynamic cortical actin events in 3T3-L1 adipocytes. *Mol Cell Biol.* (2008) 28:4215–26. doi: 10.1128/MCB.00867-07
31. Hirth S, Buhler A, Buhrdel JB, Rudeck S, Dahme T, Rottbauer W, et al. Paxillin and focal adhesion kinase. (FAK) Regulate Cardiac Contractility in the Zebrafish Heart. *PLoS One.* (2016) 11:e0150323. doi: 10.1371/journal.pone.0150323
32. Nouri K, Feng Y, Schimmer AD. Mitochondrial ClpP serine protease-biological function and emerging target for cancer therapy. *Cell Death Dis.* (2020) 11:841. doi: 10.1038/s41419-020-03062-z
33. De Rosa S, Arcidiacono B, Chiefari E, Brunetti A, Indolfi C, Foti DP. Type 2 diabetes mellitus and cardiovascular disease: genetic and epigenetic links. *Front Endocrinol. (Lausanne).* (2018) 9:2. doi: 10.3389/fendo.2018.00002
34. Hu X, Bai T, Xu Z, Liu Q, Zheng Y, Cai L. Pathophysiological fundamentals of diabetic cardiomyopathy. *Compr Physiol.* (2017) 7:693–711. doi: 10.1002/cphy.c160021
35. Wong C, Marwick TH. Obesity cardiomyopathy: pathogenesis and pathophysiology. *Nat Clin Pract Cardiovasc Med.* (2007) 4:436–43. doi: 10.1038/ncpcardio0943
36. Jia G, DeMarco VG, Sowers JR. Insulin resistance and hyperinsulinaemia in diabetic cardiomyopathy. *Nat Rev Endocrinol.* (2016) 12:144–53. doi: 10.1038/nrendo.2015.216
37. Tate M, Deo M, Cao AH, Hood SG, Huynh K, Kiriazis H, et al. Insulin replacement limits progression of diabetic cardiomyopathy in the low-dose streptozotocin-induced diabetic rat. *Diab Vasc Dis Res.* (2017) 14:423–33. doi: 10.1177/1479164117710390

38. Bahrami H, Bluemke DA, Kronmal R, Bertoni AG, Lloyd-Jones DM, Shahar E, et al. Novel metabolic risk factors for incident heart failure and their relationship with obesity: the MESA. (Multi-Ethnic Study of Atherosclerosis) study. *J Am Coll Cardiol.* (2008) 51:1775–83. doi: 10.1016/j.jacc.2007.12.048
39. Bugger H, Abel ED. Molecular mechanisms of diabetic cardiomyopathy. *Diabetologia.* (2014) 57:660–71. doi: 10.1007/s00125-014-3171-6
40. Huynh K, Bernardo BC, McMullen JR, Ritchie RH. Diabetic cardiomyopathy: mechanisms and new treatment strategies targeting antioxidant signaling pathways. *Pharmacol Ther.* (2014) 142:375–415. doi: 10.1016/j.pharmthera.2014.01.003
41. Darley-Usmar VM, Ball LE, Chatham JC. Protein O-linked beta-N-acetylglucosamine: a novel effector of cardiomyocyte metabolism and function. *J Mol Cell Cardiol.* (2012) 52:538–49. doi: 10.1016/j.yjmcc.2011.08.009
42. Montaigne D, Marechal X, Coisne A, Debry N, Modine T, Fayad G, et al. Myocardial contractile dysfunction is associated with impaired mitochondrial function and dynamics in type 2 diabetic but not in obese patients. *Circulation.* (2014) 130:554–64. doi: 10.1161/CIRCULATIONAHA.113.008476
43. Niemann B, Chen Y, Teschner M, Li L, Silber RE, Rohrbach S. Obesity induces signs of premature cardiac aging in younger patients: the role of mitochondria. *J Am Coll Cardiol.* (2011) 57:577–85. doi: 10.1016/j.jacc.2010.09.040
44. Walker JE, Dickson VK. The peripheral stalk of the mitochondrial ATP synthase. *Biochim Biophys Acta.* (2006) 1757:286–96. doi: 10.1016/j.bbabio.2006.01.001
45. Maneg O, Malatesta F, Ludwig B, Drosou V. Interaction of cytochrome c with cytochrome oxidase: two different docking scenarios. *Biochim Biophys Acta.* (2004) 1655:274–81. doi: 10.1016/j.bbabio.2003.10.010
46. Ow YP, Green DR, Hao Z, Mak TW. Cytochrome c: functions beyond respiration. *Nat Rev Mol Cell Biol.* (2008) 9:532–42. doi: 10.1038/nrm2434
47. Sánchez-Alcázar JA AJ, Khodjakov A, Schneider E. Increased mitochondrial cytochrome c levels and mitochondrial hyperpolarization precede camptothecin-induced apoptosis in Jurkat cells. *Cell Death Differentiat.* (2000) 7:1090–100. doi: 10.1038/sj.cdd.4400740
48. Rodenburg RJ. Mitochondrial complex I-linked disease. *Biochim Biophys Acta.* (2016) 1857:938–45. doi: 10.1016/j.bbabio.2016.02.012
49. Stefely JA, Reidenbach AG, Ulbrich A, Oruganty K, Floyd BJ, Jochem A, et al. Mitochondrial ADCK3 employs an atypical protein kinase-like fold to enable coenzyme Q biosynthesis. *Mol Cell.* (2015) 57:83–94. doi: 10.1016/j.molcel.2014.11.002
50. Bugger H, Abel ED. Mitochondria in the diabetic heart. *Cardiovasc Res.* (2010) 88:229–40. doi: 10.1093/cvr/cvq239
51. Gambardella J, Lombardi A, Santulli G. Metabolic flexibility of mitochondria plays a key role in balancing glucose and fatty acid metabolism in the diabetic heart. *Diabetes.* (2020) 69:2054–7. doi: 10.2337/dbi20-0024
52. Taegtmeyer H, McNulty P, Young ME. Adaptation and maladaptation of the heart in diabetes: Part I: general concepts. *Circulation.* (2002) 105:1727–33. doi: 10.1161/01.CIR.0000012466.50373.E8
53. Stanley WC, Lopaschuk GD, McCormack JG. Regulation of energy substrate metabolism in the diabetic heart. *Cardiovasc Res.* (1997) 34:25–33. doi: 10.1016/S0008-6363(97)00047-3
54. Boehm EA, Jones BE, Radda GK, Veech RL, Clarke K. Increased uncoupling proteins and decreased efficiency in palmitate-perfused hyperthyroid rat heart. *Am J Physiol Heart Circ Physiol.* (2001) 280:H977–83. doi: 10.1152/ajpheart.2001.280.3.H977
55. Boudina S, Sena S, Theobald H, Sheng X, Wright JJ, Hu XX, et al. Mitochondrial energetics in the heart in obesity-related diabetes: direct evidence for increased uncoupled respiration and activation of uncoupling proteins. *Diabetes.* (2007) 56:2457–66. doi: 10.2337/db07-0481
56. Thedieck K, Polak P, Kim ML, Molle KD, Cohen A, Jeno P, et al. PRAS40 and PRR5-like protein are new mTOR interactors that regulate apoptosis. *PLoS One.* (2007) 2:e1217. doi: 10.1371/journal.pone.0001217
57. Sarbassov DD, Guertin DA, Ali SM, Sabatini DM. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science.* (2005) 307:1098–101. doi: 10.1126/science.1106148
58. Shende P, Xu L, Morandi C, Pentassuglia L, Heim P, Lebboukh S, et al. Cardiac mTOR complex 2 preserves ventricular function in pressure-overload hypertrophy. *Cardiovasc Res.* (2016) 109:103–14. doi: 10.1093/cvr/cvv252
59. Kanamori H, Takemura G, Goto K, Tsujimoto A, Mikami A, Ogino A, et al. Autophagic adaptations in diabetic cardiomyopathy differ between type 1 and type 2 diabetes. *Autophagy.* (2015) 11:1146–60. doi: 10.1080/15548627.2015.1051295
60. Volkers M, Doroudgar S, Nguyen N, Konstandin MH, Quijada P, Din S, et al. PRAS40 prevents development of diabetic cardiomyopathy and improves hepatic insulin sensitivity in obesity. *EMBO Mol Med.* (2014) 6:57–65. doi: 10.1002/emmm.201303183
61. Quiros PM, Langer T, Lopez-Otin C. New roles for mitochondrial proteases in health, ageing and disease. *Nat Rev Mol Cell Biol.* (2015) 16:345–59. doi: 10.1038/nrm3984
62. Jacinto E, Loewith R, Schmidt A, Lin S, Ruegg MA, Hall A, et al. Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive. *Nat Cell Biol.* (2004) 6:1122–8. doi: 10.1038/ncb1183
63. Volkers M, Konstandin MH, Doroudgar S, Toko H, Quijada P, Din S, et al. Mechanistic target of rapamycin complex 2 protects the heart from ischemic damage. *Circulation.* (2013) 128:2132–44. doi: 10.1161/CIRCULATIONAHA.113.003638
64. Zhou Y, Wang D, Gao X, Lew K, Richards AM, Wang P. mTORC2 phosphorylation of Akt1: a possible mechanism for hydrogen sulfide-induced cardioprotection. *PLoS One.* (2014) 9:e99665. doi: 10.1371/journal.pone.0099665
65. Seiferling D, Szczepanowska K, Becker C, Senft K, Hermans S, Maiti P, et al. Loss of CLPP alleviates mitochondrial cardiomyopathy without affecting the mammalian UPRmt. *EMBO Rep.* (2016) 17:953–64. doi: 10.15252/embr.201642077
66. Gispert S, Parganlija D, Klinkenberg M, Drose S, Wittig I, Mittelbronn M, et al. Loss of mitochondrial peptidase Clpp leads to infertility, hearing loss plus growth retardation *via* accumulation of CLPX, mtDNA and inflammatory factors. *Hum Mol Genet.* (2013) 22:4871–87. doi: 10.1093/hmg/ddt338
67. Hannigan GE, Leung-Hagesteijn C, Fitz-Gibbon L, Coppolino MG, Radeva G, Filmus J, et al. Regulation of cell adhesion and anchorage-dependent growth by a new beta 1-integrin-linked protein kinase. *Nature.* (1996) 379:91–6. doi: 10.1038/379091a0
68. Hannigan G, Troussard AA, Dedhar S. Integrin-linked kinase: a cancer therapeutic target unique among its ILK. *Nat Rev Cancer.* (2005) 5:51–63. doi: 10.1038/nrc1524

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frontiers in Cardiovascular Medicine

ORIGINAL RESEARCH
published: 18 March 2022
doi: 10.3389/fcvm.2022.820483

Seasonal Variation: A Non-negligible Factor Associated With Blood Pressure in Patients Undergoing Hemodialysis

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Objective: To investigate a seasonal variation in blood pressure (BP) for patients undergoing hemodialysis (HD).

Methods: In this retrospective study, we exported all BP measurements from the information system to investigate a seasonal variation of BP. We also investigated a seasonal variation in BP for patients of different gender types, of different age groups, with diabetic nephropathy (DN), and with non-DN having HD. Multiple linear regression models were used to explore the associations between BP and climatic parameters.

Results: In 2019, a total of 367 patients had received HD therapy in the Longwen HD unit. We included nearly 40,000 pre-dialysis BP measurements. The result of our study demonstrated a clear seasonal variation in pre-dialysis BP in general patients with HD, in male and female patients, and patients with DN and non-DN. December seemed to be a peak in the values of pre-dialysis systolic BP (SBP) and diastolic BP (DBP). The nadir values of pre-dialysis SBP and DBP were observed in June and July, respectively. A difference between peak and nadir values of BP is 3.81/2.20 mmHg in patients undergoing HD. Maximal seasonal variation in BP is 9.03/5.08 mmHg for patients with DN. A significant association of SBP and DBP with climatic parameters was found in this study. Pre-dialysis BP was inversely correlated with outdoor temperature, daytime length, and relative humidity.

Conclusion: A clear seasonal variation in BP is observed for patients with HD. Pre-dialysis SBP and DBP are inversely associated with outdoor temperature, daytime length, and relative humidity. The magnitude of a seasonal variation in BP increases in patients with DN.

Keywords: seasonal variation, blood pressure, hemodialysis, hypertension, pre-dialysis, DN

OPEN ACCESS

Edited by:
Modar Kassan, University of Tennessee Health Science Center (UTHSC), United States

Reviewed by:
Costantino Mancusi, Federico II University Hospital, Italy
Charalampos Loutradis, Evangelismos Dialysis Unit, Greece

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Specialty section:
This article was submitted to Hypertension, a section of the journal Frontiers in Cardiovascular Medicine

Received: *23 November 2021*
Accepted: *11 February 2022*
Published: *18 March 2022*

Citation:
Wu Z, Lan S, Chen C, Zhang X, Zhang Y and Chen S (2022) Seasonal Variation: A Non-negligible Factor Associated With Blood Pressure in Patients Undergoing Hemodialysis. Front. Cardiovasc. Med. 9:820483. doi: 10.3389/fcvm.2022.820483

INTRODUCTION

Although renal replacement therapy can effectively prolong the survival rate of patients with end-stage kidney disease (ESRD), the 5-year mortality of the maintenance of patients with hemodialysis (HD) is still reached up to 32.3–25.9% (1). Blood pressure (BP) status is considered a key mediator of outcomes in patients with dialysis (2). Cardiovascular disease is the leading cause of death in patients with dialysis (2, 3).

Uncontrolled hypertension contributes to an increase in the mortality rate of patients with dialysis (2, 3). The pathogenesis of hypertension in patients with dialysis is complex and multifactorial (2, 4, 5). It was first documented that a seasonal variation in outdoor temperature was associated with BP in 1961 (6). Thereafter, a considerable number of studies indicated the impact of a seasonal variation on BP in the general population, in the male and female population, and all age group individuals (7). Nevertheless, a seasonal BP variation is often neglected in clinical practice and clinical studies (7, 8). In 1998, Argilés reported that BP varies seasonally, with higher values in the winter and lower values in the summer in patients with HD (9). Fourteen studies investigated a seasonal BP variation in patients with HD, which were summarized in a previous consensus document (7). With the exception of Adrian Fine's study (10), other studies reported a substantial seasonal systolic BP (SBP) variation in patients with HD (7). In Adrian Fine's study, the result indicated that seasonal changes in BP may be nonclimatic-related (10). A possible explanation for such a divergence included different climate types, older adult patients, and different prevalences of hypertension (10). Data from the Dialysis Outcomes and Practice Patterns Study (DOPPS) on years 2005–2011 indicated that not only a seasonal variation but also a geographical gradient influence the BP in European patients with HD (11). A seasonal variation in BP may also be affected by different geographical gradients and climate types (11). Both seasonal variation and geographical gradient are nonnegligible factors for BP control. As we know, a seasonal BP variation in patients with HD has not been investigated in the Chinese population. In consideration of numerous studies on hypertension in patients with HD, seasonal factors are obviously neglected in clinical practice. Our study attempts to fill the gap in this dilemma. In the present retrospective study, we included a great quantity of data on BP in our HD unit. The aim of the present study is to investigate a seasonal variation in BP for patients who are undergoing HD. We also investigate a seasonal variation of BP in different gender types, different age groups, diabetic nephropathy (DN), and non-DN patients with HD. Data on BP included pre-dialysis BP, post-dialysis BP, and intradialytic BP.

METHODS

Ethics Statement

The present study has been approved by the Ethics Committee of Zhangzhou Affiliated Hospital of Fujian Medical University (2021LWB165). The declaration of Helsinki of the World Medical Association was followed in this study.

Data Collection

Our study period spanned from January 1, 2019, to December 31, 2019. In our previous study, we have described our dialysis center and therapy method (12). Different from our previous study, we only used the data on one of our HD units (Longwen HD unit) in this study. We directly output the data on BP measurements, ultrafiltration volume (UV), and other relevant information from our information system. We used the outpatient information system to collect the use of antihypertensive drugs. These drugs included calcium antagonists (CCBs), angiotensin II receptor blockers (ARB), angiotensin-converting enzyme inhibitors (ACEI), β-blockers, clonidine, and an α-receptor antagonist.

Data on BP Measurements

During HD therapy, the patients received BP measurements at least five times, including pre-dialysis BP, post-dialysis BP, and intradialytic BP measurements at least once an hour. The dialysis machine (Dialog+710200R & Dialog+710207T, B. Braun, Germany) has a device, which was used for automatically measuring BP. The device is an electronic sphygmomanometer module composed of an inflation pump, a solenoid valve, a pipeline, a filter screen, a circuit IC, and a display module. The method of BP measurement was presented as follows: (1) the BP cuff was wrapped around the patients' upper arm; (2) automatically pressurize the cuff to block the brachial artery blood; and (3) slowly reduce the pressure. During BP measurement, the arm sent out sound and pressure, which were recognized by an HD machine. The receiver converted them into electrical signals. Finally, it was converted into digital signals (BP values). When BP was measured, patients were in the supine position. BP measurements were automatically output and stored in the information system.

We exported all BP measurements from the information system. Inclusion criteria included (1) patients diagnosed as ESRD by nephrologists and (2) patients receiving HD in the Longwen HD unit from January 1, 2019, to December 31, 2019. We excluded BP measurements when patients initiated dialysis therapy $<$90 days. We also excluded BP measurements if pre-dialysis SBP was $<$60 mmHg or diastolic BP (DBP) was $<$30 mmHg because these were probably the measurement errors.

Definition

Target values of pre-dialysis and post-dialysis BP in patients with HD were $\leq$140/90 and $\leq$130/80 mmHg, respectively (13). Intradialytic hypertension was defined as an SBP increase of $>$10 mmHg from pre-dialysis to post-dialysis (14).

Climatic Parameters

Climatic parameters were obtained from the Zhangzhou City Weather Website[1,2] and a meteorological website[3]. Daily maximal temperature, minimum temperature, and daytime length were obtained from the Zhangzhou City Weather Website[1,2]. We used month as a unit and monthly calculated the means of daily maximal temperature, daily minimum temperature, and day length. The means of monthly outdoor temperature and relative humidity were obtained from the meteorological website[3]. We used the geographical location of Zhangzhou (117.65° east longitude and 24.55° north latitude) to query information.

During HD therapy, the indoor temperature was adjusted by the air conditioners in the winter and summer. The temperature of the air conditioner in the winter was set as 25°C and 20°C in

[1]http://lishi.tianqi.com/zhangzhou/201902.html
[2]https://richurimo.bmcx.com/zhangzhou_time_2019_01_richurimo/
[3]http://www.wheata.cn/

TABLE 1 | Pre-dialysis blood pressure (BP), post-dialysis BP, intradialytic hypertension, and hemodialysis (HD) ultrafiltration volume (UV) in patients receiving HD.

Month	*N*	Pre-dialysis SBP (mmHg)	Pre-dialysis DBP (mmHg)	Post-dialysis SBP (mmHg)	Post-dialysis DBP (mmHg)	Intradialytic hypertension (%)	Ultrafiltration volume (ml)
Jan.	3,090	146.79 ± 23.31	85.58 ±16.82	142.99 ± 22.61	84.92 ± 15.48	22.59 (21.11, 24.06)	2941 ± 1034
Feb.	2,787	146.66 ± 23.53	85.34 ±16.77	142.80 ± 23.81	85.17 ± 16.19	21.89 (20.35, 23.42)	3,010 ± 1,028
Mar.	3,075	147.36 ± 22.47	86.43 ± 16.85	143.46 ± 23.01	85.85 ± 16.18	20.88 (19.44, 22.32)	2,957 ± 1,013
Apr.	3,131	145.44 ± 22.73	85.13 ± 16.37	143.90 ± 23.42	85.99 ± 15.74	25.93 (24.40,27.47)	2,872 ± 1,042
May	3,305	145.29 ± 21.71	85.66 ± 16.51	143.88 ± 22.63	86.32 ± 15.32	25.63 (24.14, 27.12)	2,838 ± 1,012
Jun.	3,179	144.48 ± 22.45	85.20 ± 16.25	144.38 ± 23.50	86.80 ± 15.91	27.05 (25.51, 28.60)	2,749 ± 1,006
Jul.	3,492	144.65 ± 23.13	84.79 ±16.12	144.57 ± 23.92	86.34 ± 15.71	28.09 (26.60, 29.58)	2,739 ± 986
Aug.	3,423	144.91 ± 23.41	85.44 ±16.32	143.47 ±23.96	86.53 ± 16.02	25.09 (23.64, 26.55)	2,781 ± 945
Sept.	3,127	145.27 ± 23.04	86.00 ± 16.89	142.42 ± 23.52	86.22 ± 15.95	23.41 (21.92, 24.89)	2,837 ± 975
Oct.	3,484	146.41 ± 23.23	86.56 ±16.84	141.54 ± 23.97	85.74 ± 5.90	20.92 (19.57, 22.28)	2,941 ± 989
Nov.	3,426	147.28 ± 23.66	86.54 ± 16.03	140.75 ± 23.50	84.89 ±15.78	19.06 (17.74, 20.38)	2,948 ± 957
Dec.	3,451	148.29± 23.27	86.99 ± 16.48	140.34 ± 23.24	84.71 ±15.61	17.53 (16.26, 18.80)	2,998 ± 956
Total	3,8970	146.07 ± 23.03	85.82 ±16.53	142.85 ± 23.47	85.79 ± 15.82	23.16 (22.75, 23.59)	2,880 ± 998

the summer, maintaining the indoor temperature of about 25°C. The routine setting temperature of an HD machine was 36–37°C.

Other Data

Data on age, gender, primary diseases, HD vintage, vascular access, and other clinical variables were also collected. UV per HD was collected from the information system.

Data Analysis

Blood pressure measurements, UV, and pulse were derived from the information system. We used Excel software to collect and organize data. *Stata* statistical software (version 12.0) and Excel software were used to perform data analysis and plot figures. We calculated the means of monthly pre-dialytic and post-dialytic SBP and DBP. The variables were presented as means ± SD. The coefficient of variation (CV) was also calculated. The prevalence of intradialytic hypertension was calculated on a monthly basis. Means of daily maximum temperature, daily minimum temperature, daily temperature, daily daytime length, and relative humidity were also calculated on a monthly basis. One-way ANOVA was used to compare differences in BP (pre-dialysis, post-dialysis SBP, and DBP) for each month.

All of the data on BP measurements were grouped by gender, different age groups, and DN/non-DN subgroups. The abovementioned statistical analysis was repeated in men and women, different age groups, and DN and non-DN subgroups. A paired *t*-test was used to compare pre-dialysis and post-dialysis BP. The student's *t*-test was used to compare pre-dialysis BP between the different subgroups (male and female; DN and non-DN).

Multiple linear regression models were used to explore the associations between BP measurements and climatic parameters. In the models, BP measurements (pre-dialysis SBP or DBP) were used as independent variables and climatic parameters were used as dependent variables. The models were adjusted for age, gender, primary diseases, vascular access, HD vintage, and UV. The value of $p < 0.05$ was considered statistically significant.

RESULTS

In 2019, a total of 367 patients had received HD therapy in the Longwen HD unit. By December 31, 2019, 356 patients had received HD ≥ 90 days. The mean age of the 356 patients was 53 years. The ratio of male/female was 202/154. By December 31, 2019, the median HD vintage was 29 months (mean HD vintage: 39 months). Major primary diseases were glomerulonephritis (109/356) and DN (87/356). In total, 330 patients used autologous arteriovenous fistula as vascular access.

In 2019, 41,278 HD therapies were completed in the Longwen HD unit. We excluded 14 BP measurements because pre-dialysis SBP was <60 mmHg or DBP was <30 mmHg. We excluded HD therapies when the patients initiated the dialysis <90 days. Finally, a total of 38,970 cases of HD therapies were included in this study. In 2018–2019, 208 patients received echocardiography examinations. A total of 21 (10.10%) had a reduced ejection fraction (<50%). Nine patients with a reduced ejection fraction had hypertension. The mean pre-dialysis pulse was 80.08 ± 14.53 /min.

Pre-dialysis and Post-dialysis BP

Mean pre-dialysis and post-dialysis BP measurements were calculated on a monthly basis. The results were presented in **Table 1**. Mean pre-dialysis and post-dialysis BPs were 146.07/85.82 and 142.85/85.79 mmHg, respectively. Pre-dialysis SBP was significantly higher than post-dialysis SBP ($p < 0.05$). However, we did not find a significant difference between pre-dialysis and post-dialysis DBP.

Among 38,970 pre-dialysis BP measurements, 22,627 (58.06%) pre-dialysis SBP measurements were higher than 140 mmHg. A total of 13,827 (35.48%) pre-dialysis DBP measurements were higher than 90 mmHg. Totally, 27,574 (70.76%) post-dialysis SBP measurements and 23,914 (61.37%) post-dialysis DBP measurements were >130 and >80 mmHg, respectively. In 38,970 HD therapies, the prevalence of intradialytic hypertension was 23.17% (9,028). Pre-dialysis and

TABLE 2 | Pre-dialysis BP, post-dialysis BP, intradialytic hypertension, and HD UV in men and women.

	N	Pre-dialysis SBP (mmHg)	Pre-dialysis DBP (mmHg)	Post-dialysis SBP (mmHg)	Post-dialysis DBP (mmHg)	Intradialytic hypertension (%)	Ultrafiltration volume (ml)
Male	22,211	146.51 ± 22.79	86.47 ± 15.95	143.13 ± 23.26	86.53 ± 15.35	22.38 (21.83, 22.93)	3,069 ± 1,024
Jan.	1,737	147.63 ± 22.68	86.62 ± 16.22	143.70 ± 22.43	85.86 ± 15.37	21.42 (19.49, 23.35)	3,158 ± 1,041
Feb.	1,588	147.16 ± 23.31	86.12± 16.31	142.72 ± 23.12	85.93 ± 15.71	20.34 (18.36, 22.32)	3,222 ± 1,028
Mar.	1,765	147.78 ± 21.77	87.01 ± 15.76	143.85 ± 22.56	86.56 ± 15.52	20.23 (18.35, 22.10)	3,166 ± 1,041
Apr.	1,773	146.49 ± 22.14	86.15 ± 15.21	145.23 ± 22.64	87.41± 14.94	25.76 (23.74, 27.81)	3,071 ± 1,070
May	1,899	146.50 ± 20.79	87.12± 15.46	145.44 ± 21.38	87.93 ± 14.48	24.91 (22.96, 26.85)	3,063 ± 1,037
Jun.	1,823	145.25± 21.91	86.39 ± 15.83	145.35 ± 22.63	88.10 ± 15.20	27.43 (25.38, 29.48)	2,945 ± 1,008
Jul.	1,962	144.93± 23.17	85.37± 15.72	144.88 ± 24.05	87.28 ± 15.14	27.78 (25.69, 29.76)	2,925 ± 979
Aug.	1,942	144.87 ± 23.41	85.58 ± 15.61	143.15± 24.36	86.80± 15.43	23.33 (21.44, 25.21)	2,956 ± 967
Sept.	1,789	145.77 ± 23.47	86.67 ± 16.24	142.67 ± 24.10	87.02 ± 15.63	22.14 (20.21, 24.06)	2,988 ± 1,032
Oct.	1,973	146.48 ± 23.44	86.64 ± 16.49	140.68 ± 23.81	85.63± 15.13	19.51 (17.76, 21.26)	3,123 ± 1,040
Nov.	1,972	147.29± 23.77	86.61 ± 16.29	140.49 ± 23.31	85.08 ± 15.46	19.17 (17.43, 20.91)	3,111 ± 997
Dec.	1,988	148.13 ± 23.08	87.13 ± 16.18	139.92 ± 23.44	84.89 ± 15.79	16.70 (15.06, 18.34)	3,157 ± 1,014
Female	1,6759	145.48± 23.33	84.95± 17.22	142.48 ± 23.75	84.82 ± 16.38	24.21 (23.56, 24.86)	2,637 ± 907
Jan.	1,353	145.71 ± 24.06	84.24± 17.47	142.07 ± 22.80	83.72 ± 15.55	24.09 (21.81, 26.38)	2,685 ± 967
Feb.	1,199	146.00 ± 23.81	84.30± 17.30	142.90 ± 24.71	84.16 ± 16.76	23.94 (21.52, 26.36)	2,734 ± 962
Mar.	1,310	146.79 ± 23.38	85.64± 18.20	142.92 ± 23.60	84.89 ± 16.99	21.76 (19.52, 23.99)	2,689 ± 910
Apr.	1,358	144.08 ± 23.40	83.80± 17.70	142.18 ± 24.30	84.14 ± 16.56	26.14 (23.80, 28.48)	2,629 ± 952
May	1,406	143.66± 22.81	83.69± 17.65	141.78 ± 24.07	84.16 ± 16.13	26.60 (24.29, 28.91)	2,547 ± 900
Jun.	1,356	143.44 ± 23.12	83.60 ± 16.67	143.07 ± 24.57	85.05 ± 16.67	26.55 (24.20, 28.90)	2,497 ± 946
Jul.	1,530	144.29 ± 23.08	84.05 ± 16.58	144.17 ± 23.75	85.14± 16.33	28.50 (26.23, 30.76)	2,505 ± 944
Aug.	1,481	144.94 ± 23.41	85.26 ± 17.22	143.90 ± 23.42	86.18± 16.76	27.42 (25.14, 29.69)	2,549 ± 862
Sept.	1,338	144.61 ± 22.44	85.10 ± 17.68	142.09 ± 22.73	85.15 ± 16.32	25.11 (22.79, 27.44)	2,632 ± 852
Oct.	1,511	146.32 ± 22.96	86.44 ± 17.30	142.66 ± 24.15	85.88± 16.78	22.77 (20.65, 24.88)	2,706 ± 865
Nov.	1,454	147.26 ± 23.51	86.17 ± 15.67	141.09 ± 23.76	84.64± 16.19	18.91 (16.90, 20.93)	2,733 ± 856
Dec.	1,463	148.51 ± 23.54	86.80 ± 16.88	140.91 ± 22.96	84.45±15.47	18.66 (16.66, 20.66)	2,776 ± 820

post-dialysis BP measurements in men and women, different age groups, and DN/non-DN subgroups were also presented in **Tables 2**–**4**. CVs of pre-dialysis SBP and DBP were 15.77% and 19.26%, respectively (**Supplementary Material 1**).

The Use of Antihypertensive Drugs

The use of antihypertensive drugs was listed on a monthly basis (**Supplementary Material 2**). In 2019, 132 patients experienced a decrease in doses of antihypertensive drugs, and 88 patients experienced an increase in doses of antihypertensive drugs. The number of patients for which antihypertensive drug doses began to decrease or increase was presented in **Supplementary Material 3**. We did not find significant differences in antihypertensive doses among 12 months. However, compared with June, the doses of CCB and clonidine were higher in December.

Seasonal Variations in Pre-dialysis SBP and DBP

The mean daily maximum temperature, daily minimum temperature, daily outdoor temperature, daily daytime length, and relative humidity were calculated on a monthly basis and presented in **Figures 1**, **2**. January, February, and December were the coldest months in 2019. From June to September, the outdoor temperature was at the highest level. One-way ANOVA indicated that the mean monthly pre-dialysis SBP and DBP were significantly different ($p < 0.05$).

A clear seasonal variation of pre-dialysis BP was observed (**Figure 3**). The change was that BP decreased with increasing temperature. The mean level of pre-SBP was the highest in December (148.29 ± 23.27 mmHg). From June to August, the mean level of pre-SBP was lower than 145 mmHg. The difference between the peak and the nadir values of mean pre-dialysis SBP was 3.81 mmHg. A similar seasonal variation of pre-dialysis DBP was also observed. The difference between the peak and the nadir values of mean pre-dialysis DBP was 2.20 mmHg.

HD Ultrafiltration Volume

The mean value of HD UV among 38,970 HD therapies was 2,880 ± 998 ml/HD. A similar seasonal variation was observed in HD UV. In June, July, and August, the mean UV was <2,800 ml. February had the highest UV (**Table 1**).

Seasonal Variations in Pre-dialysis SBP and DBP in Men and Women

Male patients with HD had a higher level of pre-dialysis SBP and DBP than female patients ($p < 0.05$). A clear seasonal variation of pre-dialysis SBP and DBP was observed in both

TABLE 3 | Pre-dialysis BP, post-dialysis BP, intradialytic hypertension, and HD UV in patients with DN and non-DN.

	N	Pre-dialysis SBP (mmHg)	Pre-dialysis DBP (mmHg)	Post-dialysis SBP (mmHg)	Post-dialysis DBP (mmHg)	Intradialytic hypertension (%)	Ultrafiltration volume (ml)
DN	9,704	155.24 ± 22.98	83.06± 16.23	148.89± 23.03	81.86 ± 15.23	22.62 (21.79, 23.45)	3,156 ± 974
Jan.	747	155.31 ± 23.10	83.72± 16.90	150.76 ± 22.74	81.45 ± 14.12	26.24 (23.08, 29.40)	3,226 ± 1,065
Feb.	680	155.97 ± 24.76	83.14± 16.94	150.58 ± 25.29	82.83 ± 17.11	22.06 (18.93, 25.18)	3,332 ± 1,069
Mar.	704	156.67 ± 23.17	83.77± 17.20	150.15 ± 23.57	81.78 ± 16.01	20.60 (17.60, 23.59)	3,289 ± 998
Apr.	734	155.18 ± 23.20	82.03± 15.49	150.43 ± 23.63	81.72 ± 16.03	24.52 (21.40, 27.64)	3,222 ± 1,009
May	825	153.80 ± 20.26	82.12 ± 16.40	149.28 ± 22.37	81.60 ± 15.16	24.85 (21.89, 27.80)	3,119 ± 974
Jun.	792	151.21 ± 21.87	80.36± 14.15	148.86 ± 22.43	81.10 ± 15.35	28.41 (25.26, 31.56)	3,020 ± 999
Jul.	887	153.21± 22.90	81.26 ± 14.96	150.30 ± 22.63	82.42 ± 15.33	27.28 (24.35, 30.22)	3,053 ± 1,001
Aug.	889	155.08 ± 23.21	82.80 ± 16.59	150.43 ± 23.31	83.10± 15.74	24.41 (21.58, 27.24)	3,056 ± 926
Sept.	816	153.22 ± 22.41	82.63 ± 16.59	147.22 ± 22.10	81.93 ± 14.87	22.18 (19.32, 25.04)	3,094 ± 929
Oct.	896	155.16 ± 22.34	84.10 ±15.62	147.00 ± 22.53	81.95 ± 14.17	19.42 (16.82, 22.01)	3,171 ± 932
Nov.	876	157.80 ± 23.93	85.13 ± 16.76	146.46 ± 22.70	81.53 ± 14.91	17.35 (14.84, 19.86)	3,163 ± 887
Dec.	858	160.24 ± 23.38	85.44 ± 16.94	146.00 ± 22.77	80.91± 14.20	14.92 (12.53, 17.31)	3,220 ± 901
Non-DN	29,266	143.02 ± 22.23	86.73± 16.52	140.85 ± 23.27	87.10 ± 15.80	23.34 (22.86, 23.83)	2,789 ± 989
Jan.	2,343	144.08 ± 22.72	86.17 ± 16.76	140.51 ± 22.00	86.03 ± 15.73	21.43 (19.76, 23.09)	2,853 ± 1,009
Feb.	2,107	143.66 ± 22.31	86.05 ± 16.65	140.29± 22.76	85.92 ± 15.81	21.83 (20.07, 23.60)	2,906 ± 993
Mar.	2,371	144.59 ± 21.50	87.21 ± 16.67	141.47 ± 22.47	87.05 ± 16.03	20.96 (19.32, 22.60)	2,858 ± 997
Apr.	2,397	142.46 ± 21.73	86.08 ± 16.52	141.81 ± 22.96	87.30 ± 15.42	26.37 (24.60, 28.13)	2,768 ± 1,029
May	2,480	142.46 ± 21.44	86.84 ± 16.39	142.09 ± 22.43	87.90± 15.05	25.89 (24.16, 27.61)	2,748 ± 1,008
Jun.	2,387	142.25 ± 22.19	86.81 ± 16.59	142.89 ± 23.66	88.69 ± 15.64	26.60 (24.83, 28.38)	2,658 ± 992
Jul.	2,605	141.74 ± 22.48	85.99 ± 16.32	142.62 ± 24.04	87.68 ±15.62	28.37 (26.64, 30.10)	2,634 ± 958
Aug.	2,534	141.34 ± 22.41	86.36 ± 16.31	141.03 ± 23.71	87.73± 15.94	25.34 (23.64, 27.03)	2,685 ± 933
Sept.	2,311	142.47 ± 22.60	87.18± 16.83	140.72 ± 23.77	87.73 ± 16.05	23.84 (22.10, 25.58)	2,744± 975
Oct.	2,588	143.38 ± 22.76	87.41 ± 17.17	139.65 ± 24.17	87.06± 16.20	21.45 (19.86, 23.03)	2,858 ± 996
Nov.	2,550	143.67 ± 22.46	87.02 ± 15.75	138.78 ± 23.46	86.04 ± 15.90	19.65 (18.10, 21.19)	2,871 ± 969
Dec.	2,593	144.34 ± 21.85	87.51 ± 16.30	138.46 ± 23.09	85.96± 15.85	18.40 (16.90, 19.89)	2,920 ± 963

TABLE 4 | Pre-dialysis BP, post-dialysis BP, intradialytic hypertension, and HD UV according to different age groups.

Age (years)	N	Pre-dialysis SBP (mmHg)	Pre-dialysis DBP (mmHg)	Post-dialysis SBP (mmHg)	Post-dialysis DBP (mmHg)	Intradialytic hypertension (%)	Ultrafiltration volume (ml)
<20	328	134.58 ± 15.17	88.01 ± 12.05	139.93 ± 17.03	92.92± 11.82	25.61 (20.86, 30.36)	2,121 ± 807
20–29	969	139.10± 18.63	85.95 ± 15.22	134.50 ± 20.80	86.22 ± 16.11	14.55 (12.33, 16.78)	2,815 ± 987
30–39	7,522	144.00 ± 22.08	91.38 ± 17.02	138.77 ± 23.66	89.88 ± 17.13	16.91 (16.06, 17.76)	3,105 ± 852
40–49	7,587	144.44 ± 22.63	87.91± 15.27	142.22 ± 24.19	87.66 ± 15.22	23.12 (22.17, 24.07)	3,176 ± 1,080
50–59	11,218	150.25 ± 21.69	87.44 ± 15.14	145.64 ± 22.19	86.97± 14.23	22.27 (21.50, 23.04)	2,946 ± 1,038
60–69	7,223	146.65 ± 24.80	79.25 ± 14.86	142.57 ± 23.14	79.77± 14.11	24.59 (23.59, 25.58)	2,681 ± 889
≥70	4,123	142.96 ± 25.12	78.67± 18.66	146.55 ± 24.99	81.60 ± 17.51	36.45 (34.98, 37.92)	2,256 ± 764

sexes (**Figures 4A,B**). The greatest difference in mean monthly pre-SBP was 3.26 mmHg in men and 5.07 mmHg in women, respectively. The greatest difference in mean monthly DBP was 1.76 mmHg in men and 3.20 mmHg in women.

Seasonal Variations in Pre-dialysis SBP and DBP in DN and Non-DN Subgroups

Patients with DN had a significantly higher level of pre-dialysis SBP and a lower level of DBP than patients with non-DN ($p < 0.05$). The result indicated that the pulse pressure significantly increased in patients with DN. Although a similar seasonal variation of pre-dialysis BP was observed in both patients with DN and non-DN, the magnitude of a seasonal effect on pre-dialysis BP was greater in patients with DN (**Figures 5A,B**). The difference between peak and nadir values of pre-dialysis BP was 9.03/5.08 mmHg in patients with DN.

Seasonal Variations in Pre-dialysis SBP and DBP in Different Age Subgroups

Pre-dialysis SBP and DBP in different age subgroups are indicated in **Figure 6**. Seasonal variations in pre-dialysis SBP and DBP in different age subgroups are presented in **Figure 7**. The results of one-way ANOVA indicated significant differences in pre-dialysis SBP in different months among different

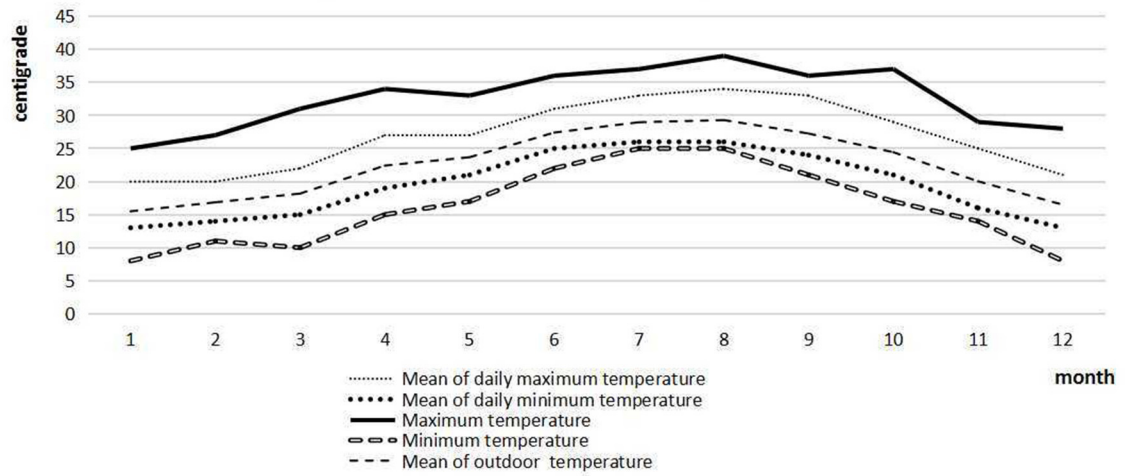

FIGURE 1 | Temperature parameters of Zhangzhou in 2019.

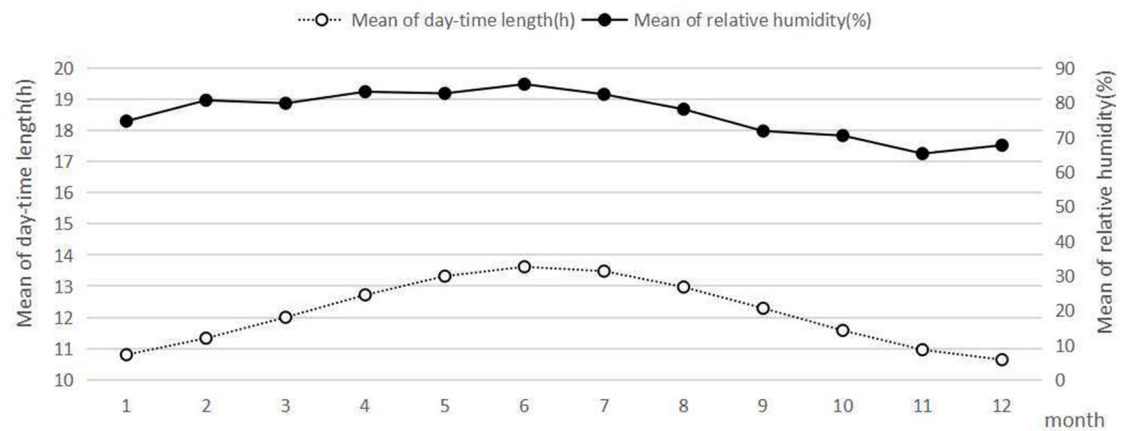

FIGURE 2 | Climatic parameters of Zhangzhou in 2019.

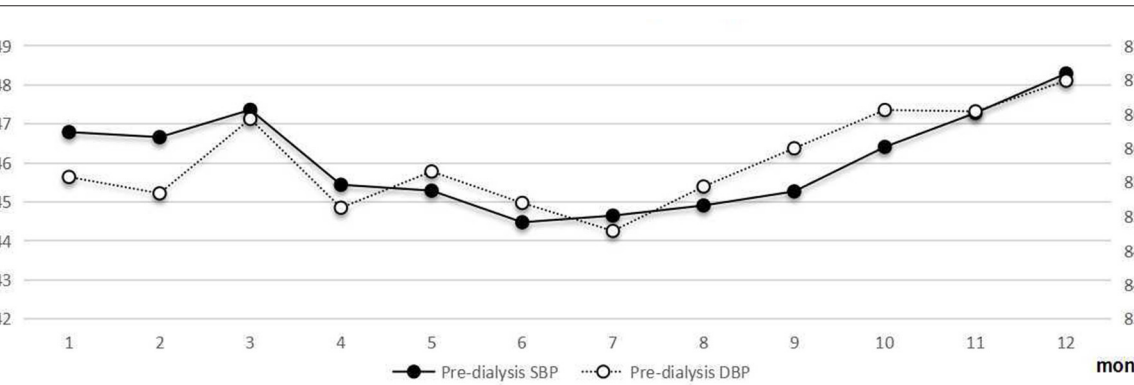

FIGURE 3 | Pre-dialysis systolic and diastolic blood pressure of patients with hemodialysis.

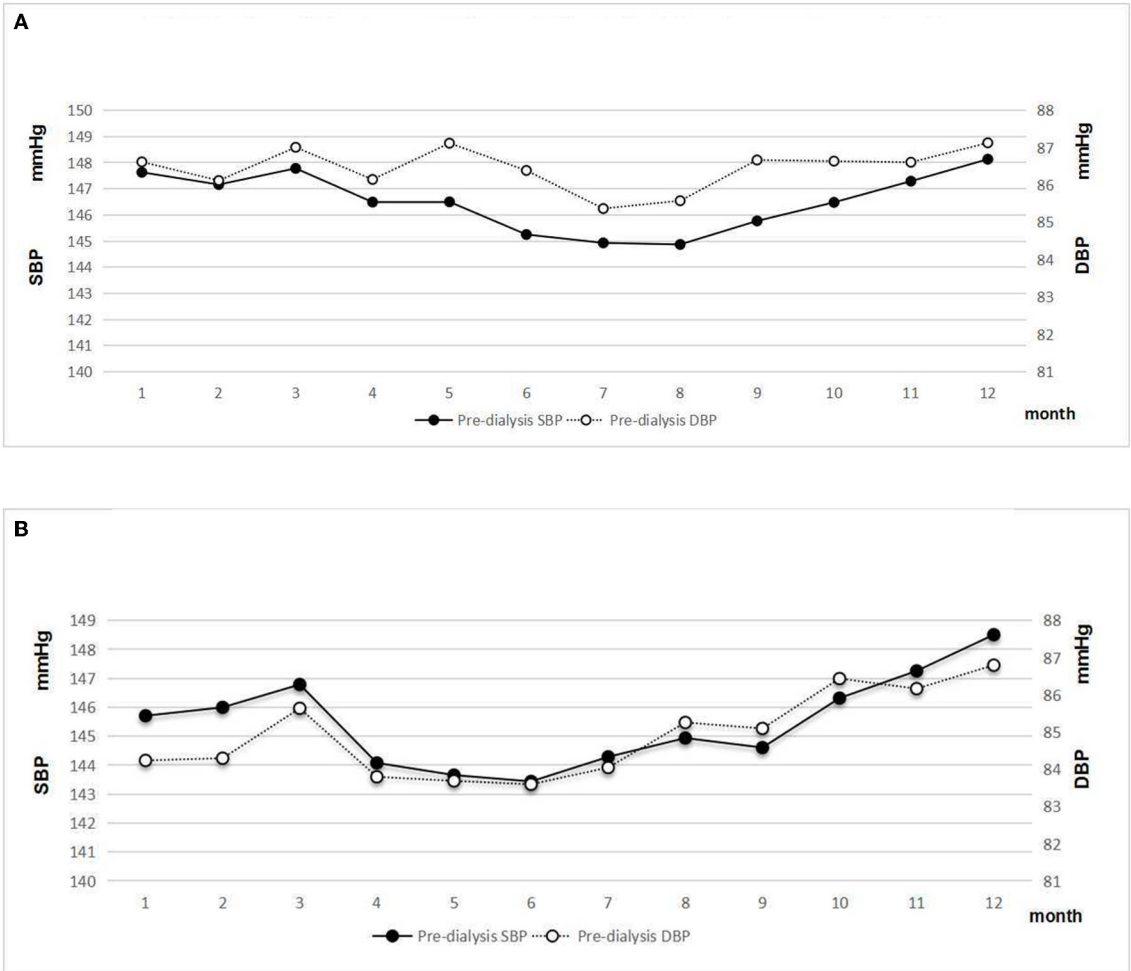

FIGURE 4 | (A) Pre-dialysis systolic and diastolic blood pressure of male patients. **(B)** Pre-dialysis systolic and diastolic blood pressure of female patients.

age subgroups, except for the age subgroup of 30–39-years. Significant differences in pre-dialysis DBP in different months were also found in different age subgroups, except for the age subgroup of 40–49 years and the age subgroup ≥ 70.

Associations Between Pre-dialysis BP and Climatic Parameters

The adjusted models indicated that associations of pre-dialysis SBP and DBP with climatic parameters (the mean of daily maximum temperature, the mean of daily minimum temperature, the mean of daily temperature, the mean of daytime length, and the mean of relative humidity) were significant ($p < 0.05$) (**Table 5**). These associations were independent of age, gender, primary diseases, HD vintage, and UV. In the adjusted models (adjusted for age, male, vascular access, UV, primary disease, HD vintage, and the mean of daily temperature), primary disease and HD vintage were also significantly associated with pre-dialysis SBP and DBP.

The Prevalence of Intradialytic Hypertension

Among 38,970 cases of HD therapy, intradialytic hypertension occurred in 23.17% of HD sessions. Older adult patients with HD had a significantly higher prevalence of intradialytic hypertension (36.45% in patients ≥ 70 years old).

DISCUSSION

In this retrospective study, we included nearly 40,000 pre-dialysis BP measurements in a single HD center. Zhangzhou City, located in southern China, is at the latitude near the Tropic of Cancer. The results from our study demonstrated a clear seasonal variation of pre-dialysis BP in general patients with HD, in men and women, and patients with DN and non-DN. It is consistent with the results of most previous studies based on patients with HD (9, 11, 15). A significant association of pre-dialysis SBP and DBP with climatic parameters was found in this study. The pre-dialysis BP was inversely correlated with outdoor temperature,

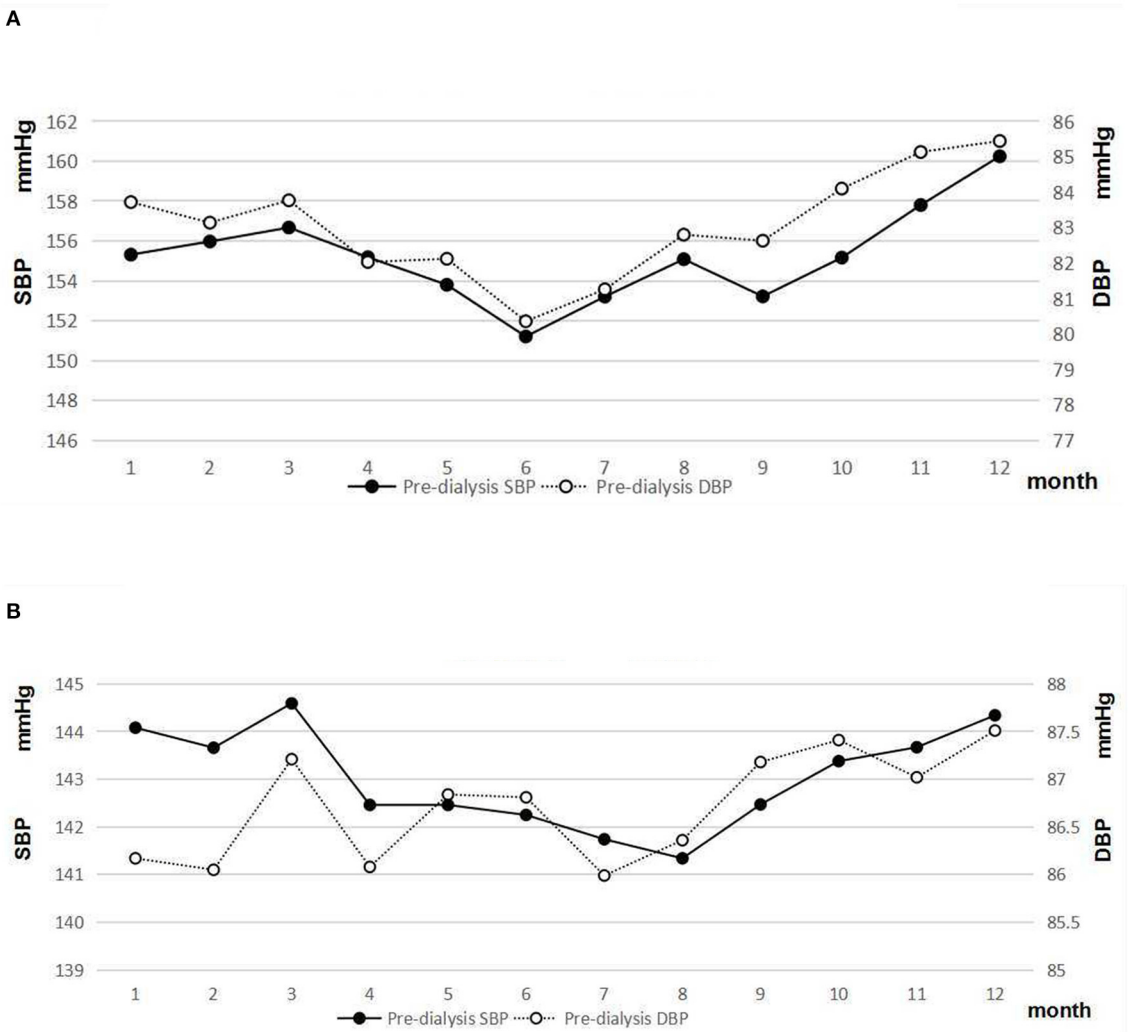

FIGURE 5 | (A) Pre-dialysis systolic and diastolic blood pressure of patients with DN. **(B)** Pre-dialysis systolic and diastolic blood pressure of patients with non-DN.

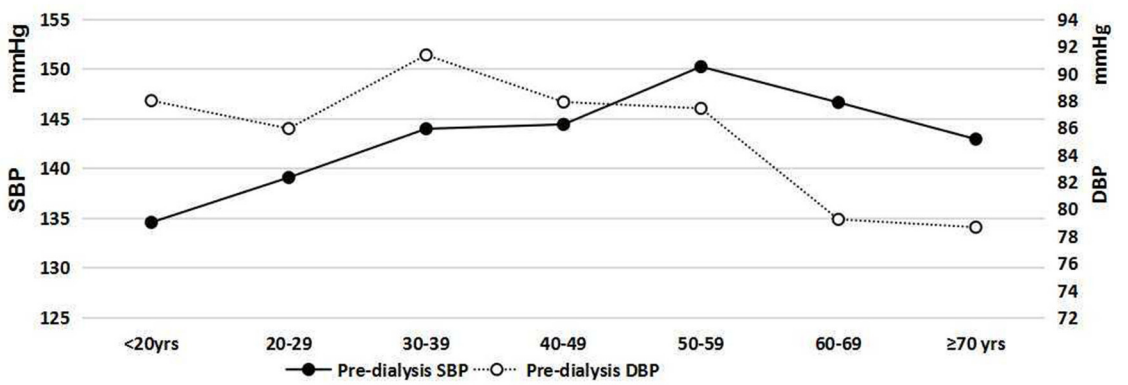

FIGURE 6 | Pre-dialysis blood pressure in different age groups.

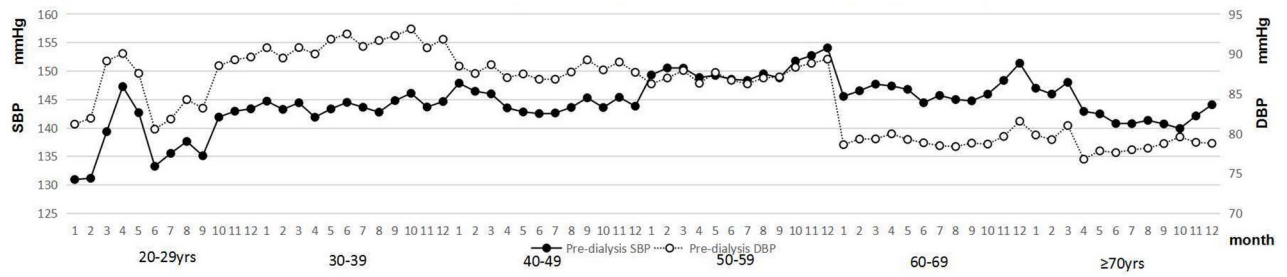

FIGURE 7 | Pre-dialysis blood pressure in different ages on a monthly basis.

TABLE 5 | Association between pre-dialysis BP and climatic parameters.

	Unadjusted model	*P* value	Adjusted model	*P* value
Pre-dialysis SBP				
Mean of daily maximum temperature	−0.20(−0.24, −0.15)	<0.001	−0.15(−0.21, −0.10)	<0.001
Mean of daily minimum temperature	−0.22(−0.27, −0.17)	<0.001	−0.18(−0.23, −0.12)	<0.001
Mean of temperature	−0.21 (−0. 26, −0.17)	<0.001	−0.17 (−0. 22, −0. 12)	<0.001
Mean of daily daytime length	−0.02 (−0. 02, −0.01)	<0.001	−0.015 (−0. 019, −0. 01)	<0.001
Mean of relative humidity	−0.12 (−0. 16, −0.09)	<0.001	−0.12 (−0. 16, −0.08)	<0.001
Pre-dialysis DBP				
Mean of daily maximum temperature	−0. 06 (−0.09, −0.02)	0.001	−0.05(−0.05, −0.09)	0.005
Mean of daily minimum temperature	−0.07(−0.11, −0.04)	<0.001	−0.18(−0.23, −0.12)	<0.001
Mean of temperature	−0.07 (−0. 10, −0.03)	0.001	−0.17(−0. 22, −0. 12)	<0.001
Mean of daily daytime length	−0. 008 (−0. 01, −0. 005)	<0.001	−0.01 (−0. 02, −0. 01)	<0.001
Mean of relative humidity	−0. 08 (−0. 11, −0. 06)	<0.001	−0.12 (−0. 16, −0.08)	<0.001

Adjusted for age, gender, vascular access, ultrafiltration volume, primary disease, hemodialysis vintage.

daytime length, and relative humidity. December seemed to be a peak in the values of pre-dialysis SBP and DBP. The nadir values of pre-dialysis SBP and DBP were observed in June and July, respectively.

Since 1961, considerable studies demonstrate that seasonal variation affects BP levels (7). After 7 years of follow-up, 23,000 individuals were recruited from 10 diverse Chinese regions, concluding that mean SBP was significantly higher in the winter than in the summer (145 vs. 136 mmHg) (16). Above 5°C, every 10°C decrease in outdoor temperature was accompanied by a 6.2-mmHg increase in SBP (16). In another previous study based on over 4,00,000 health screening records in Taiwan, mean monthly values of BP were higher in the winter than in the summer for all age groups (17). Many potential mechanisms were involved in the inverse association between BP and temperature (7). The human thermoregulatory system has the function of rapid self-regulation (7, 18). Cold-induced peripheral vasoconstriction is essential for keeping the body constant temperature (18). Meanwhile, cold-induced peripheral vasoconstriction leads to an increase in peripheral resistance (18). The sympathetic nervous system plays an important role in regulating BP. The activity of the sympathetic nervous system is also responsible for outdoor temperature (7, 18). Winter is accompanied by reducing the amount of sunshine (19). The inverse association of sunlight exposure with BP has been found (19). The reduction of UV light intensity is associated with a decrease in 25 (OH) vitamin D stores and increased parathyroid hormone secretion (19, 20). The change may influence endothelial function, which responds to increasing BP (20). However, Rostand et al.'s study suggested that 25 (OH) vitamin D could not explain the association between greater sunlight exposure and the reduction of BP (19). Other factors, such as decreased sweating, changes in dietary habits, exercise, and various behavioral habits, may contribute to a seasonal variation in BP (7).

The special feature of the present study is that the subjects are patients with HD. The regulation of BP is very complex and multifactorial (21). In clinical studies of relevant BP, frequent and close BP monitoring is required. However, it is difficult to ensure the compliance of family self-monitoring BP for a long time. Therefore, a few large-scale studies on a seasonal variation of BP only took a short-term period monitoring of BP to analyze (22, 23). Patients who are undergoing HD are a special group. HD therapy plays a key role in maintaining patient survival. For this reason, patients must follow the therapeutic schedule. In clinical practice, we have the opportunity to closely monitor patients with HD. Thus, we accumulate a large amount of data of patients with HD. In this study, we analyze the data on 38,970 HD sessions. BP measurements during each HD were included in our data. Even

compared with similar studies based on patients with HD (9, 11), our data are more complete and have better continuity.

An inverse association between pre-dialysis BP of outdoor temperature and relative humidity is observed in our study. Pre-dialysis BP increased in the winter by 3.81/2.20 mmHg (SBP/DBP) in comparison to the summer. The magnitude of a seasonal variation of pre-dialysis BP is relatively small in this study. The change in pre-dialysis BP from the peak to nadir value was 8/7 mmHg (9). The BP seasonal variability is related to a variation in outdoor temperature. Maximal monthly temperatures ranged from 10°C in the winter to 31°C in the summer in the ARGILéS's study (9). In Zhangzhou City, mean monthly maximal temperatures varied from 20°C in January to 34°C in August. A relatively mild change in temperature may be a possible explanation for the smaller seasonal variation in BP.

Volume overload is major pathogenesis of hypertension in patients with dialysis (2, 13). The retention of water interval between the two HD therapies is a unique factor affecting BP in patients undergoing HD (2, 24, 25). In our study, we observed that UV changed with the season (higher in the winter and lower in the summer). Increased sweat in the summer results in increased salt and water loss (7). This change might contribute to BP seasonal variability (7). A strong link between BP variations and interdialytic body weight gain has been established in a previous study (26). Our study also suggested that seasonal factors are independent factors associated with BP in patients with HD.

In this study, we also explored the association with BP of climatic parameters in different subgroups. We found that seasonal BP variations exist in both men and women. Age is another factor linked to BP seasonal variability (7). The association of temperature with BP is stronger in older adults (27). However, in this study, the association between age and a seasonal variation in BP seems to be a little paradoxical. The results indicated a significant seasonal variation of SBP among different age subgroups, except for the age subgroup of 30–39 years. A seasonal variation of DBP was also not found for all different age subgroups. Probably, the association of a season variation with BP is modified in terms of age. When an analysis is performed in different age subgroups, a small sample size is not enough to interpret.

A seasonal variation of BP has also been investigated in patients with DM (28, 29). Seasonal variations of BP in patients with type 1 diabetes and type 2 diabetes were observed (28). In Ushigome's study, the summer-winter difference in morning home BP was 14.0/6.5 mmHg (29). In the current study, we also found a seasonal variation in BP for patients with an increase in DN. A difference between the peak and nadir value of BP was 9.03/5.08 mmHg in patients with DN. In patients with non-DN, this value was only 3.25/1.52 mmHg. It has been observed that seasonal variations of endothelium-dependent flow-mediated vasodilation increased with the presence of type 2 diabetes (30). This may mediate the amplification in seasonal variations of BP in patients with DN. A seasonal variation of BP is also associated with older adult age, which attributes to impaired baroreflex control and enhanced vasoreactivity (7, 27, 29, 31). In this study, patients with DN were significantly older adults than patients with non-DN.

The primary advantage of the present study was that patients with HD were a distinct group. BP seasonal change is associated with mortality and renal outcome in patients with chronic kidney disease (32). BP seasonality varies by a different subgroup (28). To our knowledge, no previous study on BP seasonality was performed in Chinese patients with HD. The second advantage of the present study is a large and complete database. We use the information system to automatically record and store data to ensure the integrity of the data.

The present study also has obvious limitations. The first caveat is that we used pre-dialysis, post-dialysis, and intradialytic BP measurements as the target. During the HD therapy, the accuracy of BP measurements was affected by many factors, including puncture pain, tension, dialysis room environment (2). Patients may be under psychological stress. BP measurements in the HD unit are not standard office BP (2, 4, 13). Even if measured using a standardized protocol, pre-dialysis and post-dialysis may be imprecise (2). Home self-measured BP and ambulatory BP are the better prognostic predictors for the patients who are undergoing HD (2, 4, 13). Ambulatory BP monitoring, the gold standard method for BP evaluation, has a superior risk prediction for mortality (2). Home BP measurement had higher short-term reproducibility, and it improved the prediction of adverse outcomes (2). However, ambulatory BP monitoring and home BP measurements are limited by patient compliance and availability (2). Despite the abovementioned limitations, the values of peridialytic BP measurements are still of clinical importance (2). Considering that the abovementioned BP measurements were obtained under similar conditions, we believe that these BP measurements are still comparable. Second, we must point out that we used all BP measurements from one HD unit. BP measurements for every month were not obtained exactly from the same group. The main causes of change of patients included initiating HD, transferring to other HD units, receiving transplantation, and death. However, age, gender, DN among the BP measurements did not significantly change on a monthly basis (not presented in the result). Third, among 330 patients with an autologous arteriovenous fistula, 18 patients had a right autologous arteriovenous fistula. Because of the arteriovenous fistula, BP was measured on the nonfistula arm. This is also one of the factors affecting BP measurement. We also cannot avoid the disadvantages of a retrospective study.

CONCLUSION

A clear seasonal variation in BP is observed for patients undergoing HD. Pre-dialysis SBP and DBP were inversely associated with outdoor temperature, daytime length, and relative humidity. Peak and nadir values of pre-dialysis SBP and DBP were observed in December and June to July, respectively. A seasonal variation of BP increases in patients with DN. A difference between peak and nadir values of BP is 9.03/5.08 mmHg in patients with DN. Seasonal factors should not be neglected in clinical practice.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The present study has been approved by the Ethics Committee of Zhangzhou Affiliated Hospital of Fujian Medical University (2021LWB165). Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

SC: design research, writing paper, and data analysis. ZW: data collection, processing, analysis, and writing paper. SL: data collection, processing, and analysis. CC: dialysis technician and provide technical support for data collection. YZ and XZ: involving in data collection. All authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.820483/full#supplementary-material

REFERENCES

1. Yao X, Chen S, Lei W, Shi N, Lin W, Du X, et al. The relationship between hemodialysis mortality and the Chinese medical insurance type. *Ren Fail.* (2019) 41:778–85. doi: 10.1080/0886022X.2019.1657893
2. Flythe JE, Chang TI, Gallagher MP, Lindley E, Madero M, Sarafidis PA, et al. Conference Participants. Blood pressure and volume management in dialysis: conclusions from a kidney disease: Improving Global Outcomes (KDIGO) Controversies Conference. *Kidney Int.* (2020) 97:861–76. doi: 10.1016/j.kint.2020.01.046
3. Wang T, Li Y, Wu H, Chen H, Zhang Y, Zhou H, et al. Optimal blood pressure for the minimum all-cause mortality in Chinese ESRD patients on maintenance hemodialysis. *Biosci Rep.* (2020) 40:BSR20200858. doi: 10.1042/BSR20200858
4. Georgianos PI, Agarwal R. Epidemiology, diagnosis and management of hypertension among patients on chronic dialysis. *Nat Rev Nephrol.* (2016) 12:636–47. doi: 10.1038/nrneph.2016.129
5. Sarafidis PA, Persu A, Agarwal R, Burnier M, de Leeuw P, Ferro CJ, et al. Hypertension in dialysis patients: a consensus document by the European Renal and Cardiovascular Medicine (EURECA-m) working group of the European Renal Association-European Dialysis and Transplant Association (ERA-EDTA) and the Hypertension and the Kidney working group of the European Society of Hypertension (ESH). *Nephrol Dial Transplant.* (2017) 32:620–40. doi: 10.1093/ndt/gfw433
6. ROSE G. Seasonal variation in blood pressure in man. *Nature.* (1961) 189:235. doi: 10.1038/189235a0
7. Stergiou GS, Palatini P, Modesti PA, Asayama K, Asmar R, Bilo G, et al. Seasonal variation in blood pressure: Evidence, consensus and recommendations for clinical practice. Consensus statement by the European Society of Hypertension Working Group on Blood Pressure Monitoring and Cardiovascular Variability. *J Hypertens.* (2020) 38:1235–43. doi: 10.1097/HJH.0000000000002341
8. Stergiou GS, Palatini P, Kollias A, Kyriakoulis KG, Myers M, O'Brien E, et al. Seasonal blood pressure variation: a neglected confounder in clinical hypertension research and practice. *Am J Hypertens.* (2020) 33:595–6. doi: 10.1093/ajh/hpaa056
9. Argilés A, Mourad G, Mion C. Seasonal changes in blood pressure in patients with end-stage renal disease treated with hemodialysis. *N Engl J Med.* (1998) 339:1364–70. doi: 10.1056/NEJM199811053391904
10. Fine A. Lack of seasonal variation in blood pressure in patients on hemodialysis in a North American center. *Am J Kidney Dis.* (2000) 36:562–5. doi: 10.1053/ajkd.2000.16194
11. Duranton F, Kramer A, Szwarc I, Bieber B, Gayrard N, Jover B, et al. Geographical variations in blood pressure level and seasonality in hemodialysis patients. *Hypertension.* (2018) 71:289–96. doi: 10.1161/HYPERTENSIONAHA.117.10274
12. Yang W, Wang E, Chen W, Chen C, Chen S. Continuous observation of serum total magnesium level in patients undergoing hemodialysis. *Blood Purif.* (2021) 50:196–204. doi: 10.1159/000509788
13. Kidney Disease Outcomes Quality Initiative (K/DOQI). K/DOQIclinical practice guidelines on hypertension and antihypertensive agents in chronic kidney disease. *Am J Kidney Dis.* (2004) 43:S1–290. doi: 10.1053/j.ajkd.2004.03.003
14. Inrig JK, Oddone EZ, Hasselblad V. Association of intradialytic blood pressure changes with hospitalization and mortality rates in prevalent ESRD patients. *Kidney Int.* (2007) 71:454–61. doi: 10.1038/sj.ki.5002077
15. Wystrychowski G, Wystrychowski W, Zukowska-Szczechowska E, Tomaszewski M, Grzeszczak W. Selected climatic variables and blood pressure in Central European patients with chronic renal failure on haemodialysis treatment. *Blood Press.* (2005) 14:86–92. doi: 10.1080/08037050510008850
16. Yang L, Li L, Lewington S, Guo Y, Sherliker P, Bian Z, et al. China Kadoorie Biobank Study Collaboration. Outdoor temperature, blood pressure, and cardiovascular disease mortality among 23 000 individuals with diagnosed cardiovascular diseases from China. *Eur Heart J.* (2015) 36:1178–85. doi: 10.1093/eurheartj/ehv023
17. Tu YK, Chien KL, Chiu YW, Ellison GT. Seasonal variation in blood pressure is modulated by gender and age but not by BMI in a large Taiwanese population, 1996–2006. *J Am Soc Hypertens.* (2013) 7:216–28. doi: 10.1016/j.jash.2013.01.008
18. Alba BK, Castellani JW, Charkoudian N. Cold-induced cutaneous vasoconstriction in humans: Function, dysfunction and the distinctly counterproductive. *Exp Physiol.* (2019) 104:1202–14. doi: 10.1113/EP087718
19. Rostand SG, McClure LA, Kent ST, Judd SE, Gutiérrez OM. Associations of blood pressure, sunlight, and vitamin D in community-dwelling adults. *J Hypertens.* (2016) 34:1704–10. doi: 10.1097/HJH.0000000000001018
20. Rostand SG. Ultraviolet light may contribute to geographic and racial blood pressure differences. *Hypertension.* (1997) 30:150–6. doi: 10.1161/01.HYP.30.2.150
21. Williams B, Mancia G, Spiering W, Agabiti Rosei E, Azizi M, Burnier M, et al. 2018 ESC/ESH Guidelines for the management of arterial hypertension. *Eur Heart J.* (2018) 39:3021–104. doi: 10.1093/eurheartj/ehy339
22. Hanazawa T, Asayama K, Watabe D, Tanabe A, Satoh M, Inoue R, et al. HOMED-BP (Hypertension Objective Treatment Based on Measurement by Electrical Devices of Blood Pressure) investigators. Association between amplitude of seasonal variation in self-measured home blood pressure and cardiovascular outcomes: HOMED-BP (Hypertension Objective Treatment Based on Measurement By Electrical Devices of Blood Pressure) study. *J Am Heart Assoc.* (2018) 7:e008509. doi: 10.1161/JAHA.117.008509
23. Narita K, Hoshide S, Fujiwara T, Kanegae H, Kario K. Seasonal variation of home blood pressure and its association with target organ damage: The J-HOP study (Japan Morning Surge-Home Blood Pressure). *Am J Hypertens.* (2020) 33:620–8. doi: 10.1093/ajh/hpaa027

24. Tozawa M, Iseki K, Iseki C, Morita O, Yoshi S, Fukiyama K. Seasonal blood pressure and body weight variation in patients on chronic hemodialysis. *Am J Nephrol.* (1999) 19:660–7. doi: 10.1159/000013538
25. Spósito M, Nieto FJ, Ventura JE. Seasonal variations of blood pressure and overhydration in patients on chronic hemodialysis. *Am J Kidney Dis.* (2000) 35:812–8. doi: 10.1016/S0272-6386(00)70249-6
26. Argilés A, Lorho R, Servel MF, Chong G, Kerr PG, Mourad G. Seasonal modifications in blood pressure are mainly related to interdialytic body weight gain in dialysis patients. *Kidney Int.* (2004) 65:1795–801. doi: 10.1111/j.1523-1755.2004.00569.x
27. Alpérovitch A, Lacombe JM, Hanon O, Dartigues JF, Ritchie K, Ducimetière P, et al. Relationship between blood pressure and outdoor temperature in a large sample of elderly individuals: the three-city study. *Arch Intern Med.* (2009) 169:75–80. doi: 10.1001/archinternmed.2008.512
28. Hermann JM, Rosenbauer J, Dost A, Steigleder-Schweiger C, Kiess W, Schöfl C, et al. DPV Initiative. Seasonal variation in blood pressure in 162,135 patients with type 1 or type 2 diabetes mellitus. *J Clin Hypertens (Greenwich).* (2016) 18:270–8. doi: 10.1111/jch.12743
29. Ushigome E, Kitagawa N, Kitae A, Kimura T, Iwai K, Oyabu C, et al. Seasonal variation in home blood pressure and its relationship with room temperature in patients with type 2 diabetes. *Diab Vasc Dis Res.* (2020) 17:1479164119883986. doi: 10.1177/1479164119883986
30. Honda H, Igaki M, Komatsu M, Tanaka SI. Seasonal variations on endothelium-dependent flow-mediated vasodilation in adults with type 2 diabetes and nondiabetic adults with hypertension and/or dyslipidaemia who perform regular exercise. *Endocrinol Diabetes Metab.* (2020) 4:e00168. doi: 10.1002/edm2.168
31. Brennan PJ, Greenberg G, Miall WE, Thompson SG. Seasonal variation in arterial blood pressure. *Br Med J (Clin Res Ed).* (1982) 285:919–23. doi: 10.1136/bmj.285.6346.919
32. See CY, Tseng CT, Lin WR, Chao JY, Kuo TH, Wang MC. Seasonal change in home blood pressure monitoring is associated with renal outcome and mortality in patients with chronic kidney disease. *Front Med (Lausanne).* (2021) 8:672651. doi: 10.3389/fmed.2021.672651

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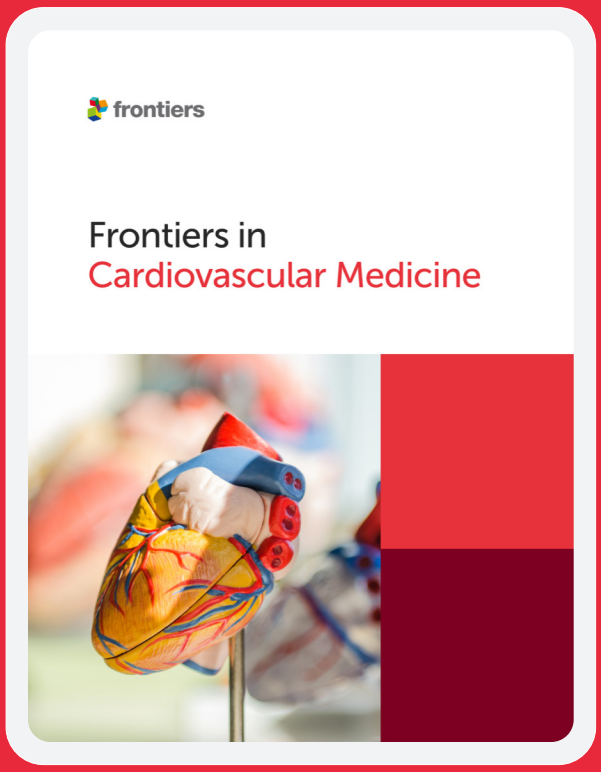

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