ENDOCRINE AND METABOLIC CONSEQUENCES OF CHILDHOOD OBESITY

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ENDOCRINE AND METABOLIC CONSEQUENCES OF CHILDHOOD OBESITY

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Table of Contents

05 ***Editorial: Endocrine and Metabolic Consequences of Childhood Obesity***
Dénes Molnár, Artur Mazur, Aneta Monika Gawlik, Grzegorz Telega, Elpis Vlachopapadopoulou and Malgorzata Wojcik

08 ***Steroid Metabolism in Children and Adolescents With Obesity and Insulin Resistance: Altered SRD5A and 20α/20βHSD Activity***
Marta Sumińska, Rafał Podgórski, Piotr Fichna and Marta Fichna

18 ***Tri-Ponderal Mass Index Reference Values for Screening Metabolic Syndrome in Children and Adolescents: Results From Two National-Representative Cross-Sectional Studies in China and America***
Xijie Wang, Yanjun Chen, Jun Ma, Bin Dong, Yanhui Dong, Zhiyong Zou, Yinghua Ma, Luke Arnold and Wannian Liang

26 ***Obesity-Induced Insulin Resistance Is Mediated by High Uric Acid in Obese Children and Adolescents***
Yang Niu, Qingya Tang, Xuan Zhao, Xuelin Zhao, Xiaomeng Mao, Jinye Sheng, Wei Cai and Yi Feng

33 ***Puberty Status Modifies the Effects of Genetic Variants, Lifestyle Factors and Their Interactions on Adiponectin: The BCAMS Study***
Yunpeng Wu, Ling Zhong, Ge Li, Lanwen Han, Junling Fu, Yu Li, Lujiao Li, Qian Zhang, Yiran Guo, Xinhua Xiao, Lu Qi, Ming Li, Shan Gao and Steven. M. Willi

45 ***Meal-Related Asprosin Serum Levels Are Affected by Insulin Resistance and Impaired Fasting Glucose in Children With Obesity***
Domenico Corica, Giorgia Pepe, Tommaso Aversa, Monica Currò, Selenia Curatola, Alessandra Li Pomi, Angela Alibrandi, Riccardo Ientile and Malgorzata Wasniewska

52 ***Effects of Overweight/Obesity on Motor Performance in Children: A Systematic Review***
Waleska Maria Almeida Barros, Karollainy Gomes da Silva, Roberta Karlize Pereira Silva, Ana Patrícia da Silva Souza, Ana Beatriz Januário da Silva, Mariluce Rodrigues Marques Silva, Matheus Santos de Sousa Fernandes, Sandra Lopes de Souza and Viviane de Oliveira Nogueira Souza

66 ***A Mini-Review of Pediatric Anthropometrics as Predictors of Future Insulin Resistance***
Sean DeLacey and Jami L. Josefson

73 ***Single Point Insulin Sensitivity Estimator in Pediatric Non-Alcoholic Fatty Liver Disease***
Dieter Furthner, Christian-Heinz Anderwald, Peter Bergsten, Anders Forslund, Joel Kullberg, Håkan Ahlström, Hannes Manell, Iris Ciba, Harald Mangge, Katharina Maruszczak, Pia Koren, Sebastian Schütz, Susanne Maria Brunner, Anna Maria Schneider, Daniel Weghuber and Katharina Mörwald

85 ***TG: HDL-C Ratio as Insulin Resistance Marker for Metabolic Syndrome in Children With Obesity***

Ahmad Kamil Nur Zati Iwani, Muhammad Yazid Jalaludin, Abqariyah Yahya, Fazliana Mansor, Fuziah Md Zain, Janet Yeow Hua Hong, Ruziana Mona Wan Mohd Zin and Abdul Halim Mokhtar

97 ***Casual Associations and Shape Between Prepuberty Body Mass Index and Early Onset of Puberty: A Mendelian Randomization and Dose–Response Relationship Analysis***

Jiao Fang, Jingyi Yuan, Dandan Zhang, Wanxu Liu, Puyu Su, Yuhui Wan, Zhihua Zhang, Fangbiao Tao and Ying Sun

106 ***Leptin Does Not Influence TSH Levels in Obese Short Children***

Katarzyna Adamczewska, Zbigniew Adamczewski, Andrzej Lewiński and Renata Stawerska

113 ***High Prevalence of Cardiometabolic Comorbidities Among Children and Adolescents With Severe Obesity From a Large Metropolitan Centre (Hangzhou, China)***

Jinling Wang, Hu Lin, Valentina Chiavaroli, Binghan Jin, Jinna Yuan, Ke Huang, Wei Wu, Guanping Dong, José G. B. Derraik and Junfen Fu

123 ***Lower Circulating Leptin Levels Are Related to Non-Alcoholic Fatty Liver Disease in Children With Obesity***

Stephanie Brandt, Julia von Schnurbein, Christian Denzer, Wolfgang Kratzer and Martin Wabitsch

131 ***Associations Between Body Composition, Leptin, and Vitamin D Varied by the Body Fat Percentage in Adolescents***

Rapheeporn Khwanchuea and Chuchard Punsawad

138 ***Multi-Faceted Influence of Obesity on Type 1 Diabetes in Children – From Disease Pathogenesis to Complications***

Sebastian Ciężki, Emilia Kurpiewska, Artur Bossowski and Barbara Głowińska-Olszewska

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Editorial: Endocrine and metabolic consequences of childhood obesity

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Editorial on the Research Topic
Endocrine and metabolic consequences of childhood obesity

During a very short period of time (approximately four decades), obesity has become a global epidemic and an urgent health and economical burden due to its impact on public health and on the whole society. Obesity is a complex multifactorial disease defined by excessive adiposity and is linked to an increased risk for many noncommunicable diseases (NCDs). Overweight and obesity affect almost 60% of adults and nearly one in three children (29% of boys and 27% of girls) in the European Region according to the latest report of the World Health Organization (1). Childhood obesity is associated with early metabolic sequelae and also linked to increased risk of persistent obesity in adulthood and long-term complications. Obesity increases the risk of the development of the metabolic syndrome, cardiovascular disease, childhood-onset type 2 diabetes mellitus and its associated retinal and renal complications, non-alcoholic fatty liver disease, obstructive sleep apnea, premature menarche, polycystic ovary syndrome, infertility, asthma, orthopedic complications, psychiatric disease, and increased rates of malignancies. Obviously, the present Research Topic could not address all the above listed consequences but rather focused on selected endocrine and metabolic aspects. In this timely Frontiers Research Topic, researchers practicing world wide and having different disciplines contributed reviews (two narrative and one systematic), and novel data on the challenges obesity presents in attempts to stimulate debate on ways forward.

One of the main threads of the papers published in this Research Topic is insulin reistance and metabolic syndrome. Some papers searched probable markers or predictors of insulin resistance. Anthropemtric measures and indexes are cheap, and widely used as screening tools for overweight/obesity. There are further ambitions to use these anthropometric measures and indexes to predict the presence or the future development of metabolic and hormonal complications linked to obesity. DeLacy and Josefson in their mini-review investigated the power of different anthropometric measures and indexes in the prediction of the development of future insulin resistance. The conclusion, as it was expected, is that there is no adequate evidence that the different anthropometric measurements and indexes have sufficient strength to predict the development of future insulin resistance.

Wang et al. investigated the role of tri-ponderal index (TMI) [weight (kg)/ height^3 (m^3)] in the screening of metabolic syndrome. This study included large number of children (57,20 Chinese and 10,441 American), proposed reference values for TMI applying sophisticated statistical analyses. The authors suggested that the TMI could be an accurate and convenient, population-based screening tool for metabolic syndrome and metabolic risk factors in children and adolescents. However, we are not convinced that TMI is superior to BMI or other anthropometric indexes.

Iwani et al. investigated triglyceride/HDL-cholesterol ratio (TG:HDL-C) as a posible marker of insulin resistance in 524 children aged 10-16 years. Since fasting blood glucose level is not a reliablile marker of impared glucose homeostasis in children they recommend the replacement of fasting blood glucose by TG:HDL-C ratio as a better surrogate marker of insulin resistance in children. This is an interesting hypothesis that need further confirmation.

Hyperuricemia is strongly related to obesity and may be linked to insulin resistance, type 2 diabetes and increased cardiovascular risk. Niu et al. investigated the associations between serum uric acid, insulin resistance (defined by homeostasis model assessment-insulin resistance, HOMA-IR) and BMI in 369 Chinese children aged 4-17 years with obesity in a retrospective, cross-sectional design. Due to the design causal relationship between hyperuricemia and insulin resistance could not be established, however, the results suggest that serum uric acid level may have a mediator role in the development of obesity-induced insulin resistance in children and adolescents.

Non-alcoholic fatty liver diesease (NAFLD) is prevalent among children with obesity. The gold standard in diagnosing NAFLD and liver fibrosis is liver biopsy, which is an invasive technique. Several studies have analyzed non-ivasive markers (ultrasound elastography, liver transaminase levels, TG:HDL-C ratio, HOMA-IR, etc.) of liver steatosis and fibrosis. Furthner et al. investigated the possible role of the single point insulin sensitivity estimator (SPISE) as a marker of NAFLD in children with obesity. They used sophisticated, modern methods (magnetic resonace imaging, hyperinsulinemic clamp test) and concluded that SPISE can be a useful surrogate marker of hepatic insulin resitance and NAFLD in obese children.

It is well-known that secondary alterations in steroid metabolism are present in children with obesity. In the paper by Suminska et al. the relationship between obesity, insulin resistance and steroid metabolism was resurrected. The results are not powerful due to the relatively low sample size and the fact that enzyme activities were estimated by the ratios of different metabolites in the collected urine samples. Although, the paper directs our attention towards steroid metabolism in childhood obesity, especially in girls.

Asprosin is a recently identified glucogenic adipokine stimulating hepatic glucose release. Its physiologic role has not yet fully clarified. Asprosin serum levels increase in fasting conditions and decrease after refeeding. Corica et al. reported a paradoxical increase of asprosin in a subroup of children with obesity. The clinical significance of this altered asprosin response to oral glucose load needs further clarification.

There is evidence that metabolic syndrome and cardiometabolic abnormalities, are already present in children and adolescents with obesity. It is especially alarming that the number of abnormal cardiometabolic parameters rose markedly over the study period (2008-2017) in a large cohort of Chinese children and adolescents from the study of Wang et al

Another thread in this Research Topic deals with different aspects of circulating leptin levels (three original research papers). Brandt et al. studied the circulating leptin levels in children with obesity and fatty liver disease. Leptin levels are generally higher in children with obesity as compared with normal weight counterparts. Brandt et al. reported an intriguing observation: children with sonographic steatosis hepatis had significantly lower z-scores of circulating leptin levels compared to children with normal liver ultrasonography. Whether children with NAFLD and low leptin levels (partial leptin deficiency) should be considered as a special subgroup needs to be further explored. Moreover, a crucial question is whether these children could benefit from leptin treatment. An interesting study was published by Adamczewska et al. who investigated the relationship between leptin and TSH levels in obese short children. It is an important finding highlighting that children with idiopathic short stature and obesity may deserve special attention, since in this subgroup leptin does not increase TSH secretion.

Previous studies have revealed that serum 25(OH)D concentrations show a strong inverse correlation with fat mass and serum leptin levels, however, causal relationship has not been confirmed. Nevertheless, the risk of vitamin D insufficiency is 3 times higher in obese children compared to normal weight counterparts. The results of Khwanchuea and Punsawad are more or less in line with previous findings. Due to the cross-sectional design the causal link between body fat, leptin and vitamin D levels could not be established.

There are two papers in the Research Topic investigating the modulating effects of pubertal development and the effect of prepubertal BMI on early onset of puberty Hypoadiponectinemia is well-known phenomenon in obesity, however, the genetic, environmental and lifestyle factors and their crosstalk influencing adiponectin levels are not fully understood. The study of Wu et al. clearly demonstrated that puberty modulates the associations between adiponectin, and genetic variants, lifestyle factors and gene-by-lifestyle interactions. The study included a large sample size and the results and the hypothesis of the authors are exemplarily demonstrated.

A continuous trend toward earlier onset of puberty has been observed around the world. During the same period, the prevalence of obesity has increased worldwide. The question whether prepubertal obesity (BMI) influences the onset of puberty was investigated by Fang et al. in an excellent study.

Children suffering from type 1 diabetes (T1D) usually were considered as lean, however, recent studies have shown that the prevalence of overweight/obesity is increasing in individuals with T1D. The overlap of obesity and T1D may lead to "double diabetes", may hinder effective therapy and result in significant health consequences. The exciting and challenging topic of "double diabetes" was reviewed by Ciezki et al. in a really comprehensive way.

An excellent systematic review by Barros et al. invesigated the effects of overweight/obesity on motor performance in children. The review included 33 rigorously selected studies. The results confirmed and stregthened previous findings that obesity is associated with not only low motor performance but it also linked to increased physical health risk.

As guest editors of this Research Topic, we were rewarded by high quality contributions embracing many aspects of endocrine and metabolic consequences of childhood obesity. We do hope that the published papers will be useful reading for people working in basic and clinical sciences and also for those interested in public health. The aforementioned articles may help in unraveling new biomarkers and in unfolding crucial links between obesity and its consequences. Furthermore, they may stimulate and fertilize future research in this exciting scientific field.

Author contributions

DM drafted this editorial. AM, AG, GT, EV and MW revised and approved the final submitted version.

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.

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Steroid Metabolism in Children and Adolescents With Obesity and Insulin Resistance: Altered SRD5A and 20α/20βHSD Activity

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Alterations in glucocorticoid metabolism may contribute to the development of obesity and insulin resistance (IR). Obesity in turn affects the androgen balance. The peripheral metabolism of steroids is equally an important determinant of their bioavailability and activity. The aim of this study was to evaluate steroid metabolism in obese children and to define which enzyme alterations are associated with IR. Clinical characteristics and anthropometric measurements were determined in 122 obese children and adolescents (72 girls, 50 boys) aged 8 – 18 years. 26 of them (21.3%) were diagnosed with IR (13 boys, 13 girls). Routine laboratory tests were performed and 24h urinary steroid excretion profiles were analyzed by gas chromatography/mass spectrometry. Positive relationship between 5α-reductase (SRD5A) activity and IR was found. According to the androsterone to etiocholanolone (An/Et) ratio the activity of SRD5A was significantly increased in obese children with IR, but the difference remained insignificant once the 5α-dihydrotestosterone to testosterone (5αDHT/T) ratio was considered. Furthermore, this relationship persisted in boys but was not observed in girls. The activity of 20α-hydroxysteroid dehydrogenase (20αHSD) and 20β-hydroxysteroid dehydrogenase (20βHSD) was reduced only in obese girls with IR. Conclude, in the context of obese children and adolescents with IR, we surmise that increased SRD5A represents a compensatory mechanism to reduce local glucocorticoid availability. This phenomenon is probably different in the liver (restriction) and in the adipose tissue (expected increase in activity). We show significant changes in 20αHSD and 20βHSD activity in obese girls with IR, but it is difficult to clearly determine whether the activity of these enzymes is an indicator of the function in their ovaries or adrenal glands.

Keywords: children, adolescents, obesity, insulin resistance, 20α-hydroxysteroid dehydrogenase, 20β-hydroxysteroid dehydrogenase, 5α-reductase, urinary steroid metabolites

INTRODUCTION

In 1974, experts from the World Health Organization (WHO) placed obesity on the infamous list of civilization diseases. Unfortunately, since then, the problem of excess body mass is still increasing among populations worldwide, including children. According to the data from 2016 the number of obese children (aged > 5 years) and adolescents has increased since 1974 from 11 to 124 million nowadays, and a further 213 million are overweight (1). In Poland, the problem of excess body mass, according to various studies (HBSC 2014/2018, COSI, PITNUTS 2016), affects about 28% of young children, 30% of early school-age children and on average some 20% of adolescents up to 15 years of age.

It is well established that steroid hormones play an important role in determining body fat distribution (2–5). Many studies have also proved that obesity is associated with abnormalities in the hypothalamic-pituitary-adrenal (HPA) axis including alterations in diurnal cortisol rhythm and enhanced susceptibility of the HPA axis for activation (4, 6–9). All of the above-mentioned mechanisms may escalate tissue exposure to glucocorticoids. However, circulating cortisol concentrations in obese individuals are reported to be within the reference range (10–14). Adipose tissue dysfunction characterized by increased visceral fat accumulation, larger adipocyte size and more abundant macrophage infiltration of the omental fat is associated with insulin-resistant obesity (15). Klöting et al. demonstrated increased macrophage infiltration into omental compared with subcutaneous adipose tissue, which may provide explanation why many individuals with subcutaneous obesity seem to be spared from insulin resistance (IR) and other adverse metabolic effects (15, 16). The molecular mechanisms determining glucocorticoids impact on diverse adipose tissue subsets are poorly understood. Bidirectional interaction between endogenous glucocorticoids and fat tissue in the coexistence of IR can be explained by elevated GLUT4 expression in the human visceral adipose tissue. Lundgren et al. showed that omental adipocytes, an observation display approximately 2-fold higher glucose uptake rate compared with subcutaneous adipocytes which was additionally supported by the increased number of GLUT4 receptors (17). Furthermore, glucocorticoids exert a marked suppressive action on glucose uptake and the expression of insulin signaling proteins in omental but not in subcutaneous adipocytes (5, 17). There is no doubt that steroids and obesity are linked by several, not fully explained, ways.

So called adrenal androgens, are in fact precursors of the more potent androgens, released from the adrenal cortex in a parallel way to glucocorticoids. Both, adrenal androgens and glucocorticoids are substrates for the same enzymes, however, the effect of their action may be opposite – androgen activation, but loss of glucocorticoid power.

11β-hydroxysteroid dehydrogenases (11βHSD) are enzymes implicated in steroid hormone balance. 11β-hydroxysteroid dehydrogenase type 1 (11βHSD1) predominantly acts as a reductase by recovering cortisol from inactive cortisone and has been localized in the liver, adipose tissue and central nervous system. Tissue-specific expression of 11βHSD1 can enhance local cellular availability of glucocorticoids. 11β-hydroxysteroid dehydrogenase type 2 (11βHSD2) is an enzyme expressed in the epithelial tissues such as kidney, colon, salivary and sweat glands. Its action leads to the oxidation of cortisol to cortisone and prevents excess activation of the mineralocorticoid receptors by cortisol (18, 19).

5α-reductase (SRD5A) is an enzyme widely known for converting testosterone into 5α-dihydrotestosterone (5αDHT), however, it can also exert an effect on other steroids, including several androgen precursors and cortisol (20). SRD5A presents under three isoforms. 5α-reductase type 1 (SRD5A1) is responsible for conversion of androstenedione to androsterone. It occurs mainly in the skin and to a lower extent in the prostate gland. 5α-reductase type 2 (SRD5A2) is involved in the activation of testosterone to the most potent 5αDHT as mentioned above. This enzyme is expressed in the testes, prostate and genital skin. Additionally, both isoenzymes, type 1 and type 2 are found in the liver. The role of 5α-reductase type 3 (SRD5A3) remains unclear, although it appears ubiquitously expressed in human tissues (21). 5α-reductase often co-occurs in duet with 5β-reductase: both enzymes represent a convergence in evolution: they share similar biological functions, but do not have a common ancestor (22).

The 3β-hydroxysteroid dehydrogenase (3βHSD) is a key enzyme in the synthesis of all active steroid hormones, such as glucocorticoids, mineralocorticoids, progesterone, androgens and estrogens (23, 24). No less important function is the hepatic degradation of the androstenone (25). 3βHSD controls critical steroid hormone-related reactions in the adrenal cortex, gonads, placenta, liver, and other peripheral target tissues (24, 26, 27).

Details of the activities of 20α-hydroxysteroid dehydrogenase (20αHSD) and 20β-hydroxysteroid dehydrogenase (20βHSD) remain unclear. The cortols are metabolites of tetrahydrocortisol (THF) and α-tetrahydrocortisol (αTHF) after degradation by 20αHSD or 20βHSD, while the cortolones are metabolites of tetrahydrocortisone (THE) after the action of the same enzymes respectively. The multispecificity of enzymes, especially 20βHSD, appears to imply different physiological roles in various species and alternative effects on steroids metabolism (28–32).

11β-hydroxylase is a steroid enzyme found in the zona glomerulosa and zona fasciculata of the adrenal cortex. There are two isozymes encoded by the CYP11B1 and CYP11B2 genes on human chromosome 8q. The first is involved in the biosynthesis of adrenal corticosteroids, mainly for the conversion of 11-deoxycortisol into cortisol. It is regulated by ACTH (33). Dysfunction of this enzyme is associated with congenital adrenal hyperplasia (34–36). The second isoform is expressed at low levels in the normal adrenal zona glomerulosa, but at higher levels in aldosterone-secreting tumors (33).

17β-hydroxysteroid dehydrogenase (17βHSD) controls the last step in the formation of all androgens and estrogens. It is involved in both: the activation and inactivation of this hormones. Fourteen isoforms of this enzymes have been identified, encoded by HSD17B1 to HSD17B14 genes. Human adipose tissue is capable of active androgen synthesis catalyzed by selected 17βHSD isoforms (37, 38). The intraabdominal

adipose tissue may be substantially androgenic, increasingly so with growing obesity, particularly central obesity.

The peripheral metabolism of steroids is a considerable determinant of their availability and may be responsible for altered activity of those hormones. The aim of this study was to evaluate steroid metabolism in obese children and to investigate which enzymatic alterations can be associated with IR in this population.

RESEARCH DESIGN AND METHODS

The study comprised 122 patients (70 girls, 52 boys) aged between 5 and 18 years (mean 12.0 +/- 3.5) suffering from childhood obesity (**Table 1**). Obesity was defined as BMI values above the 97th percentile of the BMI reference curve based on percentile scales developed for the population of Polish children and adolescents. The group was further stratified according to the presence or absence of IR. Individuals with obesity secondary to an underlying endocrine disorder or genetic syndromes, as well as those under current medication were excluded from the study. All patients underwent routine clinical assessment including general physical examination, basic anthropometric measurements and pubertal stage evaluation based upon Tanner scale. The examination was followed by blood sampling after overnight fast and 24h urine collection according to standard protocol. The study was conducted in line with the Declaration of Helsinki and approved by the Bioethical Committee at Poznan University of Medical Sciences. Informed consent was obtained from the participants aged at least 16 years old and, in case of minors, from their legal representatives.

Biochemical Analyses

All biochemical measurements were performed in laboratory of the university reference hospital. Glucose, insulin levels and lipids profile were assessed after overnight fast (**Table 1**). Standard colorimetric method was used for determination of plasma glucose level (Clinical Chemistry Analyzer AU680, Beckman Coulter). Serum insulin was measured by the chemiluminescence method (Alinity i, Abbott). Insulin resistance was evaluated based upon fasting plasma glucose (FPG) and insulin (FPI) with homeostasis model assessment for IR index (HOMA-IR) (39, 40). For our study IR was defined as HOMA-IR > 97th percentile together with concomitant FPI > 15 μU/mL (41–44).

Quantification of Urinary Steroid Metabolites

24-hour urine collections were performed at home to avoid extra stress connected with hospitalization. To ensure compliance children and parents were primed in the collection procedure and also received written instructions.

Samples were stored at -20˚C until analyzed by the in-house adapted gas chromatography-mass spectrometry (GC-MS) method as previously described (45–47). All measurements were performed in the same laboratory in the Centre for Innovative Research in Medical and Natural Sciences at the University of Rzeszow, Poland. A list of measured urinary steroid metabolites is presented in **Table 2**.

All steroids standards including medroxyprogesterone, cholesteryl butyrate and stigmasterol were obtained from Steraloids (Newport RI, USA), the Sep-Pak C18 column from Waters (Milford, MA, USA), the Lipidex 5000 from Perkin Elmer (Waltham MA, USA), while β-glucuronidase/arylsulfatase liquid enzyme, the powdered sulfatase type H-1 enzyme from Helix pomatia, the derivative agents methoxyamine hydrochloride and trimethylsilylimidazole (TMSI), pyridine, sodium acetate and acetic acid were provided by Sigma - Aldrich (Darmstadt, Germany).

In brief, the method comprises a pre-extraction of a urine sample on a Sep-Pak C18 cartridge with the recovery standard medroxyprogesterone, enzymatic hydrolysis with sulfatase and β-glucuronidase/arylsulfatase, and subsequent extraction of the free steroids again on a Sep-Pak C18 column, derivatization with methoxyamine hydro-chloride 2% in pyridine at 60˚C for 3 hours and next with TMSI at 100˚C for 16 hours after adding the

TABLE 1 | Clinical phenotype of the non-insulin resistant (non-IR) and insulin resistant (IR) groups.

	Non – IR (n = 96)		IR (n = 26)		*p* value
Sex (male/female)	39/57		13/13		0.464
Tanner 1/2/3/4/5 (%)	32/15/11/13/29		30/17/17/17/17		0.620
	Mean + SD	Median (IQR)	Mean + SD	Median (IQR)	
Age (years)	12.2 ± 3.6	13 (9-15)	11.4 ± 3.4	12 (9-14)	0.242
Duration of obesity (years)	6.6 ± 3.6	5 (4-9)	7.6 ± 3.5	7 (5-9)	0.122
BMI (kg/m^2)	28.6 ± 5.4	28.0 (25.0-31.2)	33.4 ± 7.4	32.1 (27.1-37.4)	**0.002**
Z-score BMI	2.1 ± 0.4	2.1 (1.9-2.4)	2.5 ± 0.4	2.5 (2.2-2.7)	**<0.001**
WHR	1.0 ± 0.8	0.9 (0.8-0.9)	0.9 ± 0.0	0.9 (0.9-1.0)	0.285
CHOL (mg/dl)	175.5 ± 31.4	169.0 (157.0-196.0)	179.4 ± 31.3	176.0 (157.0-204.0)	0.719
LDL (mg/dl)	107.2 ± 27.5	102.5 (90.7-120.2)	108.1 ± 24.8	104.0 (94.0-127.0)	0.977
HDL (mg/dl)	45.8 ± 8.7	45.0 (40.0-51.2)	43.6 ± 10.4	43.0 (35.0-52.0)	0.331
TG (mg/dl)	109.7 ± 57.3	96.0 (72.5-128.0)	138.8 ± 83.4	107.0 (87.0-171.0)	0.117
Fasting glucose (mg/dl)	87.7 ± 9.1	87.0 (83.0-91.0)	92.5 ± 6.7	93.0 (88.5-97.5)	**0.005**
Fasting insulin (mU/ml)	11.2 ± 4.3	10.0 (7.8-13.9)	25.8 ± 6.7	23.9 (22.1-27.6)	**<0.001**

Statistically significant differences were bolded.

two internal standards Stigmasterol and Cholesteryl butyrate. Eventually, the sample is purified on a Lipidex 5000 column.

GC-MS was performed with a Shimadzu 2010 Plus gas chromatograph (Kyoto, Japan) interfaced with a single-quadrupole Shimadzu QP-2010 Ultra mass spectrometer. Data were acquired at 70 eV, the ion source temperature was 230°C and the interface temperature was 250°C. The samples (2 µl) were injected using Shimadzu AOC-20i au-to-injector in spitless mode at 260°C and separated through a ZB-1ms (15 m × 0.25 mm I.D., 0.25 µm film thickness - Pheanomenex, Torrance, USA) cross-linked dimethylpolysiloxane capillary column.

The injection was performed at 50°C which was held for 3 minutes, raised to 210°C at 30°C/min, next to 265°C at 2°C/min and finally increased to 320°C using a 20°C/min ramping program over a 48-min period. Helium was used as the carrier gas in linear velocity flow control mode. The flow of gas through the column was 1.2 ml/min. The eluting steroids were detected by selected ion monitoring (SIM). The specific ions used for the determination of each steroid are listed in **Table 2**. The instrument was calibrated by analyzing standard mixtures containing known amounts of the reference steroids and internal standards. The area of the obtained peaks was measured in SIM mode and a six-level calibration curve was set for each analyte. Recoveries were checked with medroxyprogesterone and corrections were made for the losses occurring during sample preparation. **Table 1** from **Supplementary Material** provides method-validation information for all quantified steroids. For all measured steroids, specific peaks in the chromato-grams had to be at least three times higher as the noise above the baseline to be count-ed as valid measurements (signal-to-noise-ratio 3). Urine samples of the same healthy volunteer were measured in all series and the results were compared with the standard values derived from 10 former measurements of this volunteer. For quality control, quantitative results had to be within ± 30% of the individual reference intervals.

Proportion of Urinary Corticosteroid Metabolites

In order to evaluate enzyme activity, we applied proportions of the excreted steroid metabolites (32, 48, 49). The ratios of cortisol (F) to cortisone (E) as well as tetrahydrometabolites of cortisol [THF +5αTHF] to this of cortisone [THE] reflect 11βHSD1 activity. The activities of 5α- and 5β-reductases, located mainly in the liver, can be inferred from the ratio of 5α-dihydrotestosterone (5αDHT) to testosterone (T) and androsterone (An) to etiocholanolone (Et). By analogy, we hypothesize that the 11ß-hydroxyandrosterone (11β-OH-An) to 11ß-hydroxyetiocholanolone (11β-OH-Et) ratio will reflect the 5α-reductase activity and – indirectly – the androgen metabolism after their second crossing through the adrenal cortex. Only 5α-reductase can be evaluated with the androgen ratios, while 5β-reductase predominates for cortisol and cortisone in cooperation with 3αHSD. The activity of the 5β-reductase+3αHSD complex was assessed using the ratios [α-cortol (αC)+β-cortol (βC)+THF]/F and [α-cortolone (αCl)+β-cortolone (βCl)+THE]/E. The activity of 20αHSD was assessed by the ratio of [αC+αCl]/[THF+5αTHF+THE] and the activity of 20βHSD by the [βC+βCl]/[THF+5αTHF+THE] ratio. The proportions of 11β-OH-An/An and 11β-OH-Et/Et were used to determine the activity of 11β-hydroxylase. The last enzyme we evaluated was 17βHSD, the activities of which we assessed using a ratio of androstenetriol (AET) to 16α-hydroxydehydro-androsterone (16α-OH-DHA).

Statistical Analyses

The ratios of the 24h urinary excretion of steroid metabolites were compared using the Statistica 13.3 (StatSoft Inc, Tulsa, OK, USA). Most of the variables did not follow normal distribution hence Mann-Whitney U test was used for comparisons between patients with and without IR. The level of statistical significance was accepted as p-value < 0.05.

TABLE 2 | List of steroid compounds measured in urines.

Trivial name	Abbreviation	Systematic name	M	RT	QIon	Ref. Ion
Cortisol	F	4-pregnen-11β, 17, 21-triol-3, 20-dione	362.5	29.2	606	488
Cortisone	E	4-pregnen-17, 21-diol-3, 11, 20-trione	360.5	27.2	532	515
Tetrahydrocortisol	THF	5β-pregnan-3α, 11β, 17, 21-tetrol-20-one	366.5	23.3	653	472
5α-Tetrahydrocortisol	5αTHF	5α-pregnan-3α, 11β, 17, 21-tetrol-20-one	366.5	23.6	653	551
Tetrahydrocortisone	THE	5β-pregnan-3α, 17, 21-triol-11, 20-dione	364.5	21.9	579	488
Androsterone	An	5α-androstan-3α-ol-17-one	290.4	13.5	270	360
Etiocholanolone	Et	5β-androstan-3α-ol-17-one	290.4	13.7	270	360
5α-Dihydrotestosterone	5αDHT	5α-androstan-17β-ol-3-one	290.4	15.2	391	360
Testosterone	Testosterone	4-androsten-17β-ol-3-one	288.4	15.6	389	268
Androstenetriol	AET	5-androsten-3β, 16α, 17β-triol	306.4	19.3	432	522
16α-Hydroxy-DHA	16α-OH-DHA	5-androsten-3β, 16α-diol-17-one	304.4	17.1	266	446
11ß-Hydroxyandrosterone	11β-OH-An	5α-androstan-3α, 11β-diol-17-one	306.4	16.4	268	448
11ß-Hydroxyetiocholanolone	11β-OH-Et	5β-androstan-3α, 11β-diol-17-one	306.4	16.7	268	448
α-Cortol	αC	5β-pregnan-3α, 11β, 17, 20α, 21-pentol	368.5	25.7	343	563
ß-Cortol	ßC	5β-pregnan-3α, 11β, 17, 20β, 21-pentol	368.5	24.7	343	551
α-Cortolone	αCl	5β-pregnan-3α, 17, 20α, 21-tetrol-11-one	366.5	24.0	449	523
ß-Cortolone	ßCl	5β-pregnan-3α, 17, 20β, 21-tetrol-11-one	366.5	24.8	449	253
Stigmasterol (IS)	SS	5, 22-cholestadien-24β-ethyl-3β-ol	412.7	28.3	394	353
Cholesterol N-butyrate (IS)	CB	5-cholesten-3β-ol n-butyrate	456.8	31.9	368	360
Medroxyprogesteron (IS)	MP	4-pregnen-6α-methyl-17-ol-3, 20-dione	344.5	21.8	443	–

M, molar mass [g/mol]; RT, Retention time [min]; QIon, quantifier ion [m/z]; Ref. Ion, Reference Ion [m/z]. OH, hydroxy; DH, dihydro; TH, tetrahydro.

RESULTS

Of 122 obese children and adolescents 26 (21.3%) were diagnosed with IR (13 boys, 13 girls). Clinical phenotype of non-IR and IR participants is displayed in **Table 1**. The mean age, duration of obesity and WHR were not significantly different between patients with and without IR. The BMI and Z-score BMI were significantly higher in the IR group than in the non-IR group. At the biochemical level, patients with IR versus those without IR presented similar values in lipid profile but significantly higher mean values of fasting glucose and insulin level. No significant differences were found in steroid metabolites excretion between obese subjects with and without IR (**Table 2** from **Supplementary Material**).

In order to assess enzyme activities, typical metabolite ratios were calculated and compared between IR and non-IR cohorts of obese children (**Table 3**). Furthermore, gender-stratified analyses were also performed, comprising 70 girls and 52 boys divided by their insulin sensitivity status (**Tables 4** and **5**). A positive association between SRD5A activity and IR was noticed in our study. The activity of 5α-reductase, measured regardless of gender, was increased in children with IR when based upon the An/Et ratio, but statistical significance was lost when 5αDHT/T and 11β-OH-An/11β-OH-Et ratio were considered. Similar relationship persisted in the group of obese boys, whereas we could not confirm it in obese girls. The activity of 20αHSD, measured by the [αC+αCl]/[THF+5αTHF+THE] ratio and the activity of 20βHSD, measured by the [βC+βCl]/[THF+5αTHF +THE] ratio were both reduced only in the group of girls with IR when compared to non-IR girls, whereas the same ratios remained similar in obese boys with and without IR. We failed to confirm statistically significant differences in the activity of 11βHSD1, 5β-reductase/3αHSD complex, 11β-hydroxylase and 17αHSD between IR and non-IR children (data not shown), therefore no further analyses of these enzymes were performed in our cohort.

DISCUSSION

Childhood obesity is rapidly increasing worldwide (1). Furthermore, insulin resistance seems far more frequent in obese children and adolescents than previously considered (50, 51). However, most data about obesity, IR or other metabolic disorders in relation to steroid imbalance are still derived from the adult populations. Nonetheless, knowledge from adults cannot be directly transferred into paediatric settings, as children are not mere copies of adults. As illustrated in our study cohort, children usually present relatively short duration of obesity, its early onset, and stages of puberty. Objective assessment of IR in children remains difficult due to the developmental changes, which naturally decrease insulin sensitivity during the puberty.

As early as 1974, Savage et al. found significantly higher excretion of 17OH-corticosteroid derivatives and cortisol metabolites in obese children compared to their norm-weight coevals (52). Similar observation was described a few years later by Juricskay and Molnár, who showed increased elimination of cortisol metabolites, pregnenediol and pregnanolone together with increased excretion of adrenal androgens in obese children (53, 54). In the following years, studies were primarily focused on hyperactivity of the HPA axis in children with obesity and metabolic syndrome (55, 56). On the other hand, Vitkin et al. observed a general decrease in the excretion of gluco- and mineralocorticoid metabolites, along with an increase in androgen metabolites and enhanced activity of 17,20-lyase, 17-hydroxylase, 11βHSD1 and a decrease in the activity of 21-hydroxylase in children with obesity (57). At the same time, reports of lack of any correlation between blood cortisol values and body weight emerged - according to Knuttson, individual differences in cortisol concentration represent normal homeostasis rather than pathological phenomenon (11, 13). Another aspect i.e. the relationship between adrenal steroids or androgens and IR in children and adolescents, is even less understood. Adam et al. demonstrated that cortisol may contribute to reduced insulin sensitivity at an early age in Latino children and adolescents (3). In line, serum cortisol was moderately increased in obese children with IR, while weight reduction led to a decrease both in cortisol and IR (58). A recently published study revealed specific steroid metabolomic signature of IR in obese children (with no distinction of gender) characterized by enhanced secretion of steroids from all three adrenocortical pathways (49). This finding may suggest the hypothalamo-pituitary activation and secondary enhancement of early steps of adrenal steroidogenesis.

In our study, we characterized glucocorticoid metabolism and excretion in a cohort of young obese individuals with and without

TABLE 3 | Ratios of steroid metabolites (indicative of enzyme activity): comparison between obese children and adolescents with and without insulin resistance (IR).

	Non – IR (n = 96)		IR (n = 26)		*p* value
	Mean + SD	Median (IQR)	Mean + SD	Median (IQR)	
SRD5A activity					
Androsterone/Etiocholanolone	1.84 ± 0.86	1.67 (0.37-4.75)	2.50 ± 1.11	2.39 (0.81-4.93)	**0.023**
5α-DHT/Testosterone	0.98 ± 1.01	0.63 (0.32-1.22)	0.96 ± 0.78	0.84 (0.42-1.17)	0.600
11β-OH-An/11β-OH-Et	5.65 ± 5.35	3.69 (0.48-24.74)	8.88 ± 13.45	4.55 (0.90-51.74)	0.496
20α-HSD activity					
[αC+αCl]/[THF+αTHF+THE]	0.35 ± 0.12	0.32 (0.13-0.76)	0.35 ± 0.19	0.30 (0.08-1.02)	0.502
20β-HSD activity					
[βC+βCl]/[THF+αTHF+THE]	0.19 ± 0.10	0.17 (0.02-0.68)	0.17 ± 0.09	0.13 (0.08-0.49)	0.126

Statistically significant differences were bolded.

TABLE 4 | Ratios of steroid metabolites (indicative of enzyme activity): comparison between obese boys with and without insulin resistance (IR).

	Non – IR (n = 39)		IR (n = 13)		*p* value
	Mean + SD	**Median (IQR)**	**Mean + SD**	**Median (IQR)**	
Age (years)	11.7 ± 3.4	13 (9-14)	11.7 ± 2.7	12 (11-14)	0.818
BMI (kg/m^2)	29.8 ± 6.1	28.4 (26.0-32.1)	32.8 ± 7.2	32.0 (28.5-36.3)	**0.028**
Z-score BMI	2.2 ± 0.6	2.2 (1.9-2.4)	2.4 ± 0.4	2.3 (2.2-2.6)	0.134
SRD5A activity					
Androsterone/Etiocholanolone	1.90 ± 0.97	1.73 (0.67-4.75)	2.76 ± 0.97	3.11 (1.46-4.17)	**0.035**
5α-DHT/Testosterone	0.93 ± 0.88	0.55 (0.31-1.38)	0.82 ± 0.51	0.84 (0.34-1.21)	0.867
11β-OH-An/11β-OH-Et	5.25 ± 4.45	3.81 (0.64-19.48)	7.59 ± 13.30	4.48 (0.90-49.29)	0.835
20α-HSD activity					
[αC+αCl]/[THF+αTHF+THE]	0.35 ± 0.14	0.32 (0.13-0.76)	0.43 ± 0.23	0.39 (0.18-1.02)	0.368
20β-HSD activity					
[βC+βCl]/[THF+αTHF+THE]	0.20 ± 0.11	0.17 (0.08-0.63)	0.20 ± 0.11	0.17 (0.09-0.49)	0.983

Statistically significant differences were bolded.

IR, also in terms of their gender. Slightly more than 1/5 of the studied group were children and adolescents with IR, which corresponds to commonly observed proportion in general practice. Considering potential differences in individual reactivity to insulin, we were rather interested in activity of particular enzymes than in differences of the concentration of excreted metabolites, which additionally may be affected by the kidney function.

Recent decades have focused on the role of 11βHSD1 in the pathogenesis of obesity and IR through locally increased cortisol levels (59). We were unable to demonstrate difference in activity of 11βHSD1 which is repeatedly mentioned in many studies in obese people with or without additional metabolic disorders (59–66). However, most of these analyses were conducted in adult populations, and compared obese with lean subjects.

As mentioned above, three isotypes of SRD5A are known. The methods used in this study do not allow to clearly distinguish between the enzymes, but we made an attempt to determine organ specificity (liver versus adrenal glands). Clinical data support the hypothesis that insulin acutely enhances ACTH effects on glucocorticoid pathways by stimulation of SRD5A activity and reduced level of the active glucocorticoid - cortisol in different organs (67). In our study we have results from the androgenic part of the steroid panel, not from glucocorticoid metabolism. Nonetheless, it is worth emphasizing that both pathways remain under control of ACTH and there is close relationship between them.

Increased excretion of 5α-reduced steroids was observed in obesity, PCOS and nonalcoholic fatty liver disease (68–70), while decreased excretion was noticed in critical illness (71). In rodents, treatment of obese Zucker rats with insulin sensitizers, decreases SRD5A1 expression in the liver (72). At the same time congenital deficiency of SRD5A1 causes intrahepatic accumulation of glucocorticoids and induces IR, hepatic steatosis and even fibrosis (73). These data further support close relationship between 5α-reductase activity, concentration of glucocorticoids in the liver and metabolic disorders.

SRD5A is a key enzyme implicated in androgen metabolism. It catalyzes the irreversible conversion of testosterone to 5αDHT (classic pathway) in androgen-dependent target tissues (74). The backdoor pathway is an alternative route to 5αDHT synthesis, which circumnavigates androstenedione and testosterone intermediates by 5α-reduction of progesterone or 17-hydroxy-progesterone into pre-androgen metabolites (75–77). In contrast to etiocholanolone, which originates almost exclusively from the classic pathway, androsterone additionally may arise from the backdoor pathway. Therefore, the An/Et ratio can be used as an indicator for the activity of the backdoor pathway (76). Previous reports demonstrated enhanced SRD5A activity in obese individuals with or without other metabolic disorders, an

TABLE 5 | Ratios of steroid metabolites (indicative of enzyme activity): comparison between obese girls with and without insulin resistance (IR).

	Non – IR (n = 57)		IR (n = 13)		*p* value
	Mean + SD	**Median (IQR)**	**Mean + SD**	**Median (IQR)**	
Age (years)	12.2 ± 3.8	13 (9-15)	11.0 ± 4.0	11 (8-14)	0.248
BMI (kg/m^2)	29.5 ± 6.4	28.6 (25.6-33.5)	34.0 ± 7.9	33.0 (27.0-42.3)	**0.028**
Z-score BMI	1.9 ± 0.6	2.0 (1.7-2.2)	2.5 ± 0.4	2.6 (2.2-2.7)	**<0.001**
SRD5A activity					
Androsterone/Etiocholanolone	1.81 ± 0.78	1.64 (0.37-3.81)	2.24 ± 1.22	2.29 (0.81-4.93)	0.402
5α-DHT/Testosterone	1.01 ± 1.10	0.65 (0.34-1.21)	1.09 ± 0.98	0.84 (0.47-1.17)	0.720
11β-OH-An/11β-OH-Et	5.93 ± 5.95	3.66 (0.48-24.74)	10.06 ± 14.02	4.55 (1.59-51.74)	0.560
20α-HSD activity					
[αC+αCl]/[THF+αTHF+THE]	0.35 ± 0.10	0.32 (0.22-0.71)	0.28 ± 0.11	0.27 (0.08-0.51)	**0.029**
20β-HSD activity					
[βC+βCl]/[THF+αTHF+THE]	0.18 ± 0.10	0.17 (0.02-0.68)	0.14 ± 0.05	0.13 (0.08-0.26)	**0.021**

Statistically significant differences were bolded.

observation which we hereby confirm as measured by urinary An/Et ratio (48, 49, 78). Furthermore, our analysis revealed association between SRD5A activity and IR in obese subjects, but once this cohort was gender-stratified, we were only able to detect this relationship in boys. Puberty was underway in a proportion of the studied patients, which may explain the abundance of available androgenic substrates for the enzyme. Sexual dimorphism of the SRD5A activity was formerly described with increased activity in males and also in women with hyperandrogenism in the course of PCOS (32, 79–81).

20αHSD was initially described as a progesterone-metabolizing enzyme of the ovary (82). It is expressed in human endometrium and catalyzes the conversion of progesterone to its inactive form, 20α-dihydroprogesterone. Consequently, it appears to play a role during pregnancy and parturition. High 20αHSD activity has been located in the mouse adrenal cortex and liver, but the enzymatic regulation and functional significance of this tissue-restricted expression pattern remain elusive (83–85). In humans, its expression was detected in liver, brain, and to much smaller extent in adrenals and testes. Over recent years only a few reports have looked at the activity of this enzyme in humans, mostly in obese women or women with PCOS (86–89). Data on the activity of 20βHSD are even less available. In some fish species 20βHSD is a key enzyme in the production of the oocyte maturation-inducing steroid (90, 91). Vazirzadeh et al. suggest a wider metabolic role of 20βHSD than just control of synthesis of the reproduction hormones (92). Experiments on zebrafish show that 20βHSD represents a short pathway to rapidly inactivate and excrete cortisol and hence might be an important enzyme in stress response (28). No reliable data on its activity exist in humans. In our study, we show significant changes in 20αHSD and 20βHSD activity in obese girls with IR compared to those with preserved insulin sensitivity. However, it is difficult to clearly determine whether the activity of these enzymes is an indicator of the function in the ovaries or adrenal glands. In our observation, both enzymes presented diminished activity in obese girls with IR only when assessed by measurements of the ratio of cortols and cortolons to cortisol and cortisone tetrahydro-derivatives. It seems that 20αHSD and 20βHSD dis-play preferential affinity for substrates after 5α/5β-reduction and especially after 3αHSD conversion. Perhaps, insulin resistance itself contributes to enhanced activity of 3αHSD (maybe in complex action with 5β-reductase) and more substrates are available to be metabolized by 20αHSD or 20βHSD. However, there is still open question about plausible reasons of sex dimorphism of such a phenomenon.

The present results are close to the previous thesis that obesity and IR exhibit an increase in activity of SRD5A. Hence, we have added 20αHSD and 20βHSD to disease signature and provide evidence that girls with IR present reduced activity of those frequently overlooked enzymes, with a function which is still not fully understood.

Based on our study, in the context of obese children and adolescents with IR, we hypothesize that increased SRD5A activity (especially in boys) represents a compensatory mechanism to reduce local glucocorticoid availability. This phenomenon is probably different in the liver (restriction) and in adipose tissue (expected increase in activity), but requires further research, especially with regard to the various developmental stages. Increased inactivation of cortisol through enhanced SRD5A activity leads to decreased local glucocorticoid availability and their activation in the liver - in the aim to protect hepatic insulin sensitivity. The statistically irrelevant 11β-OH-An/11β-OH-Et ratio seems to exclude significant role of the local adrenal 5α-reductase activity, because these products were generated by CYP11B1/2. The decrease in activity of 20αHSD and 20βHSD characteristic for obese girls with IR remains unclear and in contrast to the results of studies in women with PCOS (32). The advancement of puberty as a factor influencing the activity of these enzymes, closely related to the reproduction, cannot be ruled out. Some of the girls were pre-pubertal or at early puberty stages, and girls who were menstruating – were evaluated in the first phase of their cycle, therefore they probably had low progesterone levels. Finally, a direct effect of insulin secretion on the activity of each of the tested enzymes cannot be excluded too.

Our observations and interpretation of the results must take into account that in children and adolescents the hormonal status is not as homogeneous and stable as in adults with obesity and IR. Conclusions from the study are mainly hypotheses, which require confirmation in further cohorts. We are aware that our study has some limitation: the small number of subjects in subgroups when the patients were divided according to gender. However, it is important to look for the mechanisms of disorders found in adults, such as obesity and IR, at their early stages, already at the developmental age. This may have an impact on the risk assessment of future complications in the adulthood. Additionally, we revealed some early gender-related differences in steroid metabolism. Indubitably, further studies are warranted to validate our findings and con-firm our assumptions.

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 Bioethical Committee at Poznan University of Medical Sciences. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

Conceptualization – MS, PF, and MF. Patient care and data gathering – MS and PF. Laboratory GC/MS methods application

– RP. Data analysis – MS, RP, and PF. Writing - original draft - MS. Writing - review and editing – RP and MF. All authors have read and agreed to the published version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2021.759971/full#supplementary-material

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Tri-Ponderal Mass Index Reference Values for Screening Metabolic Syndrome in Children and Adolescents: Results From Two National-Representative Cross-Sectional Studies in China and America

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Introduction: To ascertain the possible cut point of tri-ponderal mass index (TMI) in discriminating metabolic syndrome (MetS) and related cardio-metabolic risk factors in Chinese and American children and adolescents.

Methods: A total of 57,201 Chinese children aged 7-18 recruited in 2012 and and 10,441 American children aged 12-18 from National Health and Nutrition Examination Survey (NHANES 2001-2014) were included to fit TMI percentiles. Participants were randomly assigned to a derivation set (75%) and validation set (25%). The cut points of TMI with the lowest misclassification rate under the premise of the highest area under curves (AUC) were selected for each sex, which were additionally examined in the validation set. All of data analysis was conducted between September and December in 2019.

Results: TMI showed good capacity on discriminating MetS, with AUC of 0.7658 (95% CI: 0.7544-0.7770) to 0.8445 (95% CI: 0.8349-0.8537) in Chinese and 0.8871 (95% CI: 0.8663-0.9056) to 0.9329 (95% CI: 0.9166-0.9469) in American children. The optimal cut points were 14.46 kg/m^3 and 13.91 kg/m^3 for Chinese boys and girls, and 17.08 kg/m^3 and 18.89 kg/m^3 for American boys and girls, respectively. The corresponding misclassification rates were 17.1% (95% CI: 16.4-17.8) and 11.2% (95% CI: 9.9-12.6), respectively. Performance of these cut points were also examined in the validation set (sensitivity 67.7%, specificity 82.4% in Chinese; sensitivity 84.4%, specificity 88.7% in American children).

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Conclusions: A sex- and ethnicity- specific single cut point of TMI could be used to distinguish MetS and elevated risk of cardio-metabolic factors in children and adolescents.

Keywords: tri-ponderal mass index, metabolic syndrome, child and adolescent health, health screening, cardiovascular risk

INTRODUCTION

Metabolic syndrome (MetS), specified by the National Cholesterol Education Program Adult Treatment Panel III (ATP III), is a strong and independent predictor of all-cause and cardiovascular disease (CVD) mortality (1, 2). Its prevalence has increased from approximately 2% in the mid-1990s to a current estimate of 10% in American children and adolescents (3–5). It was also estimated that nearly two thirds (63.4%) of American adolescents had at least 1 metabolic abnormality (5). In Chinese children aged 10-16, the prevalence of MetS ranged from 5.5% to 10.9%, depending on the definition used in the study (6, 7). Results from The China Health and Nutrition Survey suggested an overall prevalence of 3.37% in children aged 7-18, using ATP III definition (8). Since childhood MetS is likely to track into adulthood, early identification could help to prevent definitive lesions (9) and target interventions to improve future cardiovascular health (5, 10).

In consideration of the great importance of early identification, an accessible screening tool is needed for daily public health practice (11), especially in the less developed areas where professional pediatricians are deficient. Body mass index (BMI), calculated as weight(kg)/height^2 (m^2) has been the most widely used indicator in screening obesity and related diseases, including MetS (12). In recent decades, given the fact that central obesity is the biggest risk factor of MetS (13), waist circumference has been accepted as another efficient screening tool for MetS in young population (14–16). However, as children's body shape changes dramatically during puberty, both measures required multiple cut-off values to fit age and sex subgroups of children.

Tri-ponderal mass index (TMI), calculated as weight(kg)/height^3 (m^3), has been reported to be a satisfactory adiposity indicator with good age-stability during adolescence (17, 18). Studies have also proven it to be an efficient indicator in screening obesity related cardio-metabolic risks including dyslipidemia, insulin resistance and other cardiovascular diseases (19–21). However, few studies have evaluated TMI cut point for screening of MetS and cardio-metabolic risks in pediatric populations. Although one study conducted in Colombian children and adolescents suggested that TMI could be a satisfying indicator to discriminate MetS, the authors gave age- and sex- specific screening thresholds (22), which didn't take the best advantage of its age-stability.

Using cross-sectional baseline data from the Chinese school-based Health Life Intervention and United States National Health and Nutrition Examination Survey (NHANES 2001-2014), the present study aimed to ascertain the optimal TMI cut point for identifying MetS and cardio-metabolic risk factors in Chinese and American children and adolescents.

METHODS

Study Population

Data for Chinese participants were obtained from baseline data of a Chinese school-based Health Life Intervention study, which was a national school-based multi-centered cluster randomized controlled trial against obesity in Chinese children and adolescents conducted in 2012. The sampling procedure of this study has been published elsewhere in detail (23). Briefly, more than 60,000 children and adolescents from 7 provinces, including Liaoning, Tianjin, Ningxia, Shanghai, Chongqing, Hunan, and Guangdong, participated in the study. In each province, 12-16 primary and secondary schools totaling around 10,000 participants were randomly selected. Of each selected school, two classes were randomly selected from each grade and students in those classes were invited for blood sample collection. Of the 59,916 participants aged 7 to 18 years, 2,715 were excluded due to missing sex, height or weight data. The remaining sample size for TMI derivation and analysis was 57,201, including 15,045 participants with blood samples. The original study was approved by Ethical Committee of the university. All participants and their parents provided signed informed consent.

Data for American participants were obtained from 7 cycles of National Health and Nutrition Examination Survey (NHANES 2001-2014) public release data, for which collection methods had been described in detail elsewhere (24). NHANES is an American national representative and continuous cross-sectional survey conducted every 2 years. In this study, only adolescents aged between 12 and 18 were included, as serum glucose and lipid profiles were unavailable for those younger than 12. Among those aged 12 to 18 years old, 667 participants with missing data of height and weight were excluded. Thus, the sample size for TMI analysis in this study was 10,441, and the sample size for the following analysis was 2,910.

Within each population, participants were randomly allocated to derivation set (75%) and validation set (25%) by a random number table in Stata. Participants with full records of height, weight and age were recruited in fitting TMI percentiles, while only those with complete records of all 5 risk components of MetS were recruited in the following analysis. Basically, the optimal TMI cut points were developed from derivation set and were validated in validation set.

Measurements

All Chinese participants underwent physical examination according to a standard protocol (19). Height was measured using the portable stadiometer (model TZG, China) to the nearest 0.1 cm, with participants standing straight barefoot.

Weight was measured with lever type weight scale (model RGT-140, China) to the nearest 0.1 kg, with participants wearing light underwear. Waist circumference was measured with steel tape at 1 cm above umbilicus to the nearest 1 mm. Height, weight and waist circumference were all measured twice and the mean values were recorded.

Blood pressure was measured according to the recommendation of the National High Blood Pressure Education Program (NHBPEP) Working Group in Children and Adolescents (21). Mercury sphygmomanometers (model XJ11D, China), stethoscopes (model TZ-1, China), and appropriate cuffs were used for blood pressure measurement. Participants were asked to sit quietly for at least 5 minutes prior to the first reading. Systolic blood pressure (SBP) was determined by onset of the first Korotkoff sound and diastolic blood pressure (DBP) was determined by the fifth Korotkoff sound. Blood pressure was measured twice with five minutes' gap between two measurements, and the average of SBP and DBP values were separately calculated. Blood samples were collected after a 12-hour fasting. Fasting plasma glucose (FPG), triglyceride (TG), and high-density lipoprotein cholesterol (HDL-C) were measured using an automatic biochemical analyzer (Roche Modular P800 ISE900; Hoffmann-La Roche Ltd) by a qualified biological testing company. Rigid quality control was enforced in this study. All measurement instruments were calibrated before use, and all examiners were required to pass a standard training course before commencement.

For American participants, anthropometric measurements were collected according to protocols previously published in detail (24). Sex and age were self-reported during home interviews. FPG, TG and HDL-C levels were measured enzymatically at Johns Hopkins University Lipoprotein Analytic Laboratory with the use of a Hitachi 704 Analyzer (Boehringer Mannheim Diagnostics, Indianapolis, IN). Lipid collection and analyses were standardized to Centers for Disease Control and Prevention criteria (25).

TMI for each participant was calculated as weight (kg)/ height^3 (m^3).

Definitions of Metabolic Syndrome

According to the criteria of National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III), children were considered to have MetS if they showed three or more of the following (2, 26): (1) central obesity (waist circumference ≥ 90th sex- and age-specific percentile); (2) high fasting glucose (FPG ≥ 110 mg/dL or 6.1 mmol/L); (3) hypertriglyceridemia (TG ≥ 110 mg/dL or 1.242 mmol/L); (4) low HDL-C (HDL-C ≤ 40 mg/mL or 1.0344 mmol/L); (5) high blood pressure (blood pressure ≥ 90th sex-, age- and height-specific percentile). The definitions were also showed in **Supplementary Table 1**.

As reference values for waist circumference vary across ethnic groups, "High Waist Circumference Screening Threshold Among Chinese Children and Adolescents Aged 7-18 years" (27) and "Anthropometric Reference Data for Children and Adults: United States" (28) were used for Chinese and American populations, respectively. Blood pressure was evaluated with the 2017 version of the Clinical practice guideline for screening and management of high blood pressure in children and adolescents (29).

Statistical Analysis

All of data analysis was conducted between September and December in 2019. The characteristics of participants were given as mean (SD) or number (%). Independent two-tailed t-tests for continuous variables and chi-square (χ^2) tests for categorical variables were used to compare the differences between derivation set and validation set in Chinese and American participants, respectively. LMS curves were generated with LMScharmaker (version 2.5, developed by HARLOW PRINTING LIMITED) to estimate TMI changes with age in both sexes in Chinese and American participants, respectively. Since TMI showed good stability with increasing age, especially within the range from P_5 to P_{90} (results displayed in **Supplementary Figure 1**), the following analyses were carried in the absence of age factors.

The relationships between TMI percentile and prevalence of MetS components were estimated with logistic regression and fractional polynomial regression models by sex. Receiver operating characteristic (ROC) analysis was used to find the possible range of TMI cut-off percentiles, and the alternative points were identified as those with the highest Youden Index (30). Five optimal cut-off percentiles were selected for further analysis based on their capacity to distinguish MetS. The cut-off percentile with the lowest misclassification rate were selected under the premise of the largest area under ROC curves (AUC) (31). The cut-off values obtained from the derivation set were then validated in the validation set, by calculating their AUC and misclassification rate. Sensitivity analyses were conducted between sex groups for participants in both countries, while urban-rural disparities and ethical disparities were estimated for Chinese and American populations, respectively. All analyses were performed using Stata 14.0 (College Station, TX, USA) and associations were considered significant when performed at levels of $P < 0.05$ (two sides).

RESULTS

A total of 57,201 Chinese participants and 10,441 American participants were included in the present study, while 15,045 Chinese and 2,910 American participants had complete records of MetS risk components. The summary of their characteristics, including height, weight, waist circumference and MetS risk factors, are shown in **Table 1**. The prevalence of MetS is 6.6% to 7.0% for Chinese participants and 3.7% to 4.3% for American participants depending on derivation or validation set. Generally, no significant difference in MetS prevalence or other variables was observed between the two sets.

As TMI percentile increased, estimated prevalence of all cardio-metabolic risk factors, including MetS, increased considerably. Notably, between P_{70} and P_{80} in Chinese participants, the estimated prevalence of MetS increased rapidly in both sexes. A similar pattern was also detected in American participants, notably between P_{80} and P_{90} (**Figures 1A–D**).

TABLE 1 | Characteristics of Chinese and American participants, with mean and standard deviation (SD) for continuous variable and number and % for categorical variables.

Characteristics	Chinese population 1		Chinese population 2		*p value*	American population 1		American population 2		*p value*
	N	Data	N	Data		N	Data	N	Data	
Development of TMI percentiles										
Age, mean (SD), year	42,901	11.3 (3.1)	14,300	11.3 (3.1)	0.718	7,830	15.0 (2.0)	2611	14.9 (2.0)	0.004**
Boys, number (%)	42,901	22,117 (51.6)	14,300	7374 (51.6)	0.979	7,830	4009 (51.2)	2611	1305 (50.0)	0.280
Height, mean (SD), cm	42,901	148.2 (16.0)	14,300	148.3 (16.0)	0.818	7,830	164.9 (10.0)	2611	164.5 (10.2)	0.072
Weight, mean (SD), kg	42,901	42.6 (15.1)	14,300	42.8 (15.4)	0.107	7,830	65.0 (19.3)	2611	65.4 (20.3)	0.382
Analysis for metabolic syndrome										
Waist circumference, mean (SD), cm	11,302	65.9 (10.7)	3743	66.1 (10.9)	0.382	2159	80.6 (14.1)	751	81.3 (14.9)	0.309
TG, mean (SD), mmol/L	11,302	1.16 (0.82)	3743	1.18 (0.84)	0.176	2159	0.96 (0.58)	751	0.94 (0.56)	0.398
HDL-C, mean (SD), mmol/L	11,302	1.91 (1.38)	3743	1.92 (1.39)	0.787	2159	1.37 (0.32)	751	1.35 (0.32)	0.283
FPG, mean (SD), mmol/L	11,302	4.12 (1.30)	3743	4.10 (1.33)	0.786	2159	5.11 (0.83)	751	5.13 (1.09)	0.616
SBP, mean (SD), mmHg	11,302	104.6 (12.0)	3743	105.0 (11.9)	0.161	2159	108.9 (10.1)	751	109.1 (10.2)	0.681
DBP, mean (SD), mmHg	11,302	66.5 (9.0)	3743	66.5 (8.8)	0.944	2159	59.6 (11.3)	751	59.6 (10.8)	0.954
Central obesity†, number (%)	11,302	2430 (21.5)	3743	872 (23.3)	0.021*	2159	172 (8.0)	751	76 (10.1)	0.069
Dyslipidemia (TG), number (%)	11,302	3019 (26.7)	3743	1065 (28.5)	0.038*	2159	441 (20.4)	751	142 (18.9)	0.371
Dyslipidemia (HDL-C), number (%)	11,302	1421 (12.6)	3743	512 (13.7)	0.080	2159	309 (14.3)	751	131 (17.4)	0.039*
Glucose intolerance, number (%)	11,302	32 (0.28)	3743	10 (0.27)	0.872	2159	45 (2.1)	751	16 (2.1)	0.939
High blood pressure, number (%)	11,302	2487 (22.0)	3743	828 (22.1)	0.882	2159	158 (7.3)	751	61 (8.1)	0.472
Metabolic syndrome, number (%)	11,302	752 (6.6)	3743	263 (7.0)	0.431	2159	79 (3.7)	751	32 (4.3)	0.458

TG, triglyceride; HDL-C, high density lipoprotein cholesterol; FPG, fasting plasma glucose; SBP, systolic blood pressure; DBP, diastolic blood pressure.
†Central obesity was defined as waist circumference ≥ 90th percentile (age- and sex- specific) for Chinese and American populations, respectively.
**P < 0.05, **P < 0.01.*

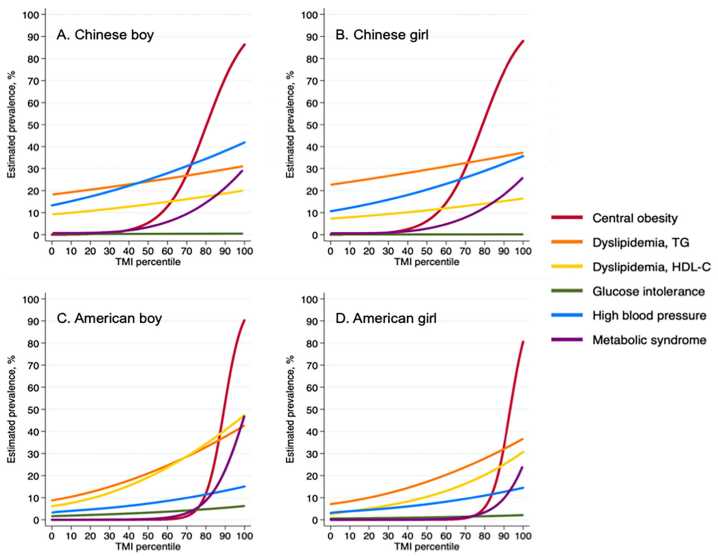

FIGURE 1 | Prevalence of cardiometabolic risk factors according to Tri-ponderal mass index percentiles among Chinese and American children. TMI, tri-ponderal mass index (kg/m^3); TG, triglyceride; HDL-C, high density lipoprotein cholesterol; FPG, fasting plasma glucose. **(A)** Chinese boy; **(B)** Chinese girl; **(C)** American boy; **(D)** American girl.

TMI showed good capacity in diagnosing MetS in both Chinese and American participants. The area under ROC curves for TMI-MetS were 0.8445 (95% CI: 0.8349-0.8537, boys) and 0.7658 (95% CI: 0.7544-0.7770, girls) for Chinese participants and 0.9329 (95% CI: 0.9166-0.9469, boys) and 0.8871 (95% CI: 0.8663-0.9056, girls) for American participants. The optimal cut-off percentiles ranged from 72.9 to 79.9 based on the highest Youden index in Chinese and American participants (**Supplementary Table 2**), therefore P_{70}, P_{75}, P_{80}, P_{85} and P_{90} were selected for further evaluation. The corresponding TMI values of these percentiles are listed in **Supplementary Table 3**.

The results of AUC and misclassification rate analyses are shown in **Figures 2A, B**, demonstrating a reduction in the misclassification rate and AUC as higher percentiles were tested. In Chinese participants, AUC of P_{70}, P_{75} and P_{80} varied from 0.7486 (95% CI: 0.7313-0.7660) to 0.7570 (95% CI: 0.7405-0.7735) with no statistical differences, while the AUC decreased significantly from P_{80} (0.7570, 95% CI: 0.7405-0.7735) to P_{85}

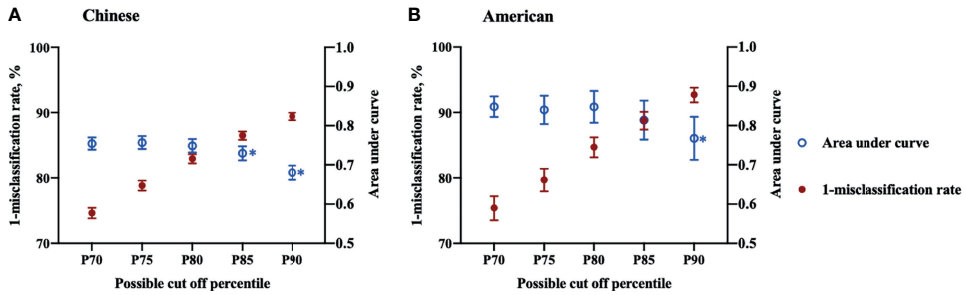

FIGURE 2 | Misclassification rates and area under curves of Tri-ponderal mass index in assessing metabolic syndrome in Chinese and American children. Sex specific P_{70}, P_{75}, P_{80}, P_{85} and P_{90} were used as threshold values for each population.* The area under curve decreased significantly compared to the former cut off percentile. **(A)** Chinese children; **(B)** American children.

(0.7294, 95% CI: 0.7115-0.7474). In American participants, the inflection point of AUC was around P_{90}, where the AUC declined significantly from 0.8140 (95% CI: 0.7645-0.8634) for P_{85} to 0.7674 (95% CI: 0.7127-0.8221). Under the premise of keeping the highest AUC, the points with the lowest misclassification rate were selected for Chinese and American participants, respectively, as the possible thresholds for discriminating MetS (31). The corresponding misclassification rates were 17.1% (95% CI: 16.4-17.8) and 11.2% (95% CI: 9.9-12.6) in Chinese and American populations, respectively. Therefore, the optimal TMI percentile was P_{80} (TMI values of 14.46 kg/m^3 for males and 13.91 kg/m^3) for females, respectively) for Chinese participants and P_{85} (TMI values of 17.08 kg/m^3 for males and 18.89 kg/m^3 for females, respectively) for American participants. The selected optimal TMI values were additionally tested in the validation set, and the results supported what was found in the derivation set (**Table 2**).

The results for sensitivity analyses were displayed in **Supplementary Tables 4, 5** with derivation population. In Chinese population, the capacity of the cut points were generally consistent with those from all population, although 13.93 kg/m^3 (P_{75}) may be optimal for Chinese boys. However, as the sample size of derivations set was rather small, the results should be test in further studies with bigger population. In the American population, P_{85} showed good consistency in all participants except for Mexican American, for whom P_{90} may be more appropriate cut-off percentile, as the AUC was significantly higher than other percentiles.

DISCUSSION

MetS affects approximately 35% of adults in the United States of America and is also a great threat in developing countries, including China (32, 33). Let alone the MetS and related cardiovascular risks originated from childhood has significant influence on future diseases (34), it is of consensus that regular monitoring/management is important for children, especially for those with high MetS risk (7).

The present study found that TMI could be a satisfying index in identifying MetS and other cardio-metabolic risks in young Chinese and American populations. The thresholds established to identify elevated MetS risk were 14.46 kg/m^3 for boys *vs*. 13.91 kg/m^3 for girls in Chinese participants aged between 7 to 18, and 17.08 kg/m^3 for boys *vs*. 18.89 kg/m^3 for girls in American participants aged between 12 to 18. These cut points showed favorable capacity in discriminating MetS and related risks and performed good consistency in subgroup analysis.

TMI as an alternative option for BMI has been paid increasing attention in recent years, especially in pediatric populations where BMI changes with age. Previous studies found that TMI remained steady throughout childhood and showed good consistency to the percent of body fat (17, 18, 20). These

TABLE 2 | Validation of selected cut-off percentile of Tri-ponderal mass index in identifying cardiometabolic risks in Chinese and American validate populations.

Population	Area Under Curve	FPR	FNR	TFR	Sensitivity, %	Specificity, %
Chinese						
P_{75}	0.7624 (0.7355, 0.7893)	23.2 (21.8, 24.6)	24.3 (19.3, 30.0)	23.3 (21.9, 24.7)	75.7 (70.1, 80.5)	76.8 (75.4, 78.2)
P_{80}	0.7502 (0.7212, 0.7792)	17.6 (16.4, 19.0)	32.3 (26.7, 38.3)	18.7 (17.4, 20.0)	67.7 (61.8, 73.0)	82.4 (81.1, 83.6)
P_{85}	0.7322 (0.7177, 0.7463)†	12.5 (11.4, 13.6)	41.1 (35.1, 47.3)	14.5 (13.4, 15.7)	58.9 (52.9, 64.7)	87.5 (86.4, 88.6)
American						
P_{80}	0.8763 (0.8314, 0.9212)	18.5 (15.7, 21.5)	6.7 (0.8, 22.1)	18.0 (15.3, 20.9)	93.8 (79.9, 98.3)	81.5 (78.5, 84.2)
P_{85}	0.8656 (0.839, 0.8891)	11.3 (9.0, 13.8)	15.6 (5.3, 32.8)	11.5 (9.3, 13.9)	84.4 (68.2, 93.1)	88.7 (86.2, 90.8)
P_{90}	0.8048 (0.7226, 0.887)†	7.8 (5.9, 10.0)	31.3 (16.1, 50)	8.8 (6.9, 11.0)	68.8 (51.4, 82.0)	92.2 (90, 94.0)

FPR, false positive rate; FNR, false negative rate; TR, total misclassification rate.
†*Area under curve decreased significantly compared to the former cut off percentile.*

qualities made TMI a satisfactory indicator in daily screening of excessive weight and related cardiometabolic diseases. The present study verified its age-stability and ulterior discussed the possible TMI thresholds to discriminate MetS in both Chinese and American children. Compared to the thresholds derived from a previous study in a Columbian population (22), the present study found higher TMI cut-points in Chinese children, and even higher cut-points in American children. The distinction between ethnic groups had been discovered previously when researchers attempted to set country-specific BMI thresholds, as they found that people from Asia and Pacific regions had a higher percent of body fat and waist-to-hip ratio than Caucasians at a given BMI (35). Further studies also found that even if their anthropometric measurements were within a safe range, people from eastern countries would have higher disease risk than their western counterparts (36, 37). Besides, various cut-off points among different sex, race and ethic subgroups were found, indicating that even if TMI presented good age-stability during childhood, sex and ethic disparities should be considered in setting country-specific cut-points. For more validated TMI cutoff points, more standardized research under global cooperation should be conducted, in order to eliminate any possible deviations resulted from operators and testing examinations. For many years, researchers have been seeking an appropriate screening tool to identify adiposity, as well as obesity-related disease risk in children. Apart from age- and sex- specific BMI used for screening general adiposity, waist circumference and waist-to-height ratio are also becoming popular for central obesity (38). However, as these indicators were normally recommended for further assessment in children with risk of developing other long-term health problems than obesity, their applications in general younger populations were still under debate (39). Although, in our previous research, further combination with waist circumference did not improve the ability of discriminating high risk population of TMI in children and adolescents (20). The present study, with two national-representative surveys, provided a possible choice with both age-stability and good capacity in recognizing cardio-metabolic abnormalities. From a practical point of view, TMI bases only on height and weight measurements, and requires very simple calculation. The number of TMI thresholds for screening adiposity is also much less than those of BMI, and doesn't depend on age- specific percentiles. These qualities make TMI an accessible, easy, and accurate indicator for long-term monitoring of childhood cardio-metabolic abnormalities in primary care settings. And it could also be useful for school-based and community surveillance efforts in early prevention of cardio-metabolic risks (40).

The current study had its limitations. Cross-sectional data was obtained from Chinese and American populations. Although majority of adolescent years were included in the present study, TMI variance at individual levels was still undetected. Meanwhile, it remained undetected that whether the time lag between two populations would make any difference to the primary findings of the present study. In addition, ethnic differences must be taken into consideration in application of TMI. Although this study conducted ethnic-stratified sensitivity analyses in American participants and found good consistency between non-Hispanic white, non-Hispanic black and other ethnic groups, it may be insufficient for making a general cut-point. Ethnic-specific studies may need further consideration, while any differences resulted from operator, testing equipment or definition on specific metabolic disorders should also be taken into consideration. Moreover, the method of waist circumference in Chinese children was not standard. Although it was proved to be similar to the standard method in analyzing abdominal fat (data published in Chinese only), underestimation of actual waist circumference was also possible. This emphasizes the importance of collaborative and standardized cooperation again.

TMI could be an accurate and convenient population-based screening tool for MetS and related cardio-metabolic risk factors in Chinese and American children and adolescents. Corresponding TMI values for sex-specific P_{80} in Chinese and P_{85} in American populations could be utilized as appropriate thresholds in clinical practice.

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 studies involving human participants were reviewed and approved by Ethical Committee of Peking University. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

XW performed all statistical analyses and wrote the manuscript. JM is the principle investigator of the original study. BD and YD are co-investigators and reviewed and edited the manuscript. ZZ and YM are co-investigator of the original study. LA, YC, and WL reviewed and edited the manuscript. XW takes full responsibility for the contents of the article. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2021.739277/full#supplementary-material

Supplementary Figure 1 | Age- and sex- specific Tri-ponderal mass index (TMI) values for Chinese children aged 7 to 18 and for American children aged 12 to 18.

Supplementary Table 1 | Definition of cardiometabolic risk factors. Waist circumference percentiles were determined from "High waist circumference screening threshold among children and adolescents aged 7–18 years" for Chinese children, and from Anthropometric reference data for children and adults: United States, 2011-2014 for American children.

Supplementary Table 2 | Parameters of the receiver operating characteristic (ROC) curves analysis for the diagnostic performance of Tri-ponderal Mass (TMI) in identifying cardiometabolic risks in Chinese and American children. TG, triglycerides; HDL-C, high density lipoprotein cholesterol; CI, confidential interval; PV (+), positive predictive value; PV (-), negative predictive value.

Supplementary Table 3 | Tri-ponderal mass index threshold for the optimal cut-off percentiles.

Supplementary Table 4 | Sensitivity analysis of different subgroups in Chinese validate population. FPR, false positive rate; FNR, false negative rate; TR, total misclassification rate. * Area under curve decreased significantly compared to the former cut off percentile. Sex specific 75^{th}, 80^{th} and 85^{th} percentiles were used as threshold values for each population.

Supplementary Table 5 | Sensitivity analysis of different subgroups in American validate population.

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Obesity-Induced Insulin Resistance Is Mediated by High Uric Acid in Obese Children and Adolescents

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Objective: This study aimed to evaluate whether serum uric acid (SUA) plays a mediating role in the development of insulin resistance (IR) in obese children and adolescents.

Methods: A total of 369 participants aged 4-17 years with obesity who attended the Nutrition Outpatient Clinic for Obesity at Xinhua Hospital from January 2012 to January 2019 were recruited for this retrospective study. We classified participants into two groups on the basis of HOMA-IR values: the low HOMA-IR group (< 3.16) (n = 222) and the high HOMA-IR group (≥ 3.16) (n = 147).

Results: The univariate analysis found that the high HOMA-IR group had higher BMI, SUA, and fasting insulin (FINS) ($P < 0.05$). Multiple linear regression analysis and mediating effect analysis indicated that body mass index (BMI) could directly regulate FINS and HOMA-IR (both $P < 0.05$). The results from the mediating effect analysis found that UA partially played an indirect role in the link between BMI, FINS and HOMA-IR (both $P < 0.05$) but had no effect on fasting blood glucose ($P > 0.05$).

Conclusions: SUA should be investigated in obesity and plays a partial mediating role in insulin resistance induced by obesity in obese children and adolescents.

Keywords: insulin resistance, uric acid, obesity, children, adolescents

INTRODUCTION

Obesity is an increasingly serious clinical and public health problem with more than 100 million obese people worldwide (1). The prevalence of obesity is as high as 20.3% (7-12 y) and 9.6% (13-17 y), respectively, in Chinese children and adolescents (2). Obesity is a driving factor of some endocrine diseases, such as insulin resistance (IR), type 2 diabetes, hypertension, hyperuricemia, and metabolic syndrome (3–6), which places a heavy burden on patients, families, and the public health system (7). Researchers have confirmed that IR is commonly present in obese children and adolescents and is associated with an increased risk of developing type 2 diabetes in adulthood (3, 8). Therefore, identifying and modifying risk factors for IR should be beneficial in preventing diabetes and other metabolic diseases in these high-risk youth.

Obesity diagnosed by body mass index (BMI) has been reported to result in hyperuricemia in more cross-sectional studies (9, 10). Recent studies have found that hyperuricemia not only leads to gout arthritis and nephropathy but may also be related to IR, type 2 diabetes and cardiovascular morbid events (9, 11–13). Thus, SUA interacts with other factors in the modulation of obesity and IR.

However, the mechanism of IR in obese children is still not clear. In addition, to our knowledge, there is no previous epidemiologic study that investigated whether hyperuricemia exerts a mediating effect on the relationship between obesity and IR in children. Therefore, we hypothesized that a BMI-SUA-IR pathway is present in obese children and adolescents. In the current study, we examined the associations among IR, SUA and BMI with multiple linear regression analysis and mediation analysis.

METHODS

Subjects

A retrospective study focused on 369 outpatients with obesity, aged 4-17 years, who attended the Nutrition Outpatient Clinic for Obesity at Xinhua Hospital from January 2012 to January 2019 (**Figure 1**). According to the World Health Organization standards, obesity is defined as BMI ≥ the 95th percentile of children of the same sex and age (14). Participants with severe kidney and liver damage and obesity caused by endocrine diseases or genetic metabolic diseases were excluded from this study. This study was reviewed and approved by the Ethics Committee of Xinhua Hospital, School of Medicine, Shanghai Jiao Tong University (No. XHEC-D-2021-113).

Data Collection and Measurement

All data from the first clinical visit were collected at Xinhua Hospital, located in Shanghai, China. Basic characteristics, including sex, age, height, and weight, were recorded. Accordingly, BMI (kg/m^2) was determined as weight in kg divided by height in meters squared.

Triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), fasting blood glucose (FBG), and fasting insulin (FINS) were tested at the first clinical visit. All of these parameters were measured in the hospital laboratory centre using a Hitachi 7180 automatic biochemical analyzer (Hitachi, Japan). Serum uric acid (SUA) was analyzed using the urase method.

Homeostasis model assessment-β (HOMA-β) and homeostasis model assessment-insulin resistance (HOMA-IR) were calculated to evaluate β-cell function and IR using the following equations: HOMA-β = 20 × FINS (mIU/L)/[FBG (mmol/L)-3.5]%; HOMA-IR = FBG (mmol/L) × FINS (mIU/L)/22.5 (15). At present, there is no determination of the cut-off point of IR in children. Most studies have defined IR with a HOMA-IR value of 3.16 in children and adolescents (16, 17). Thus, we classified participants into two groups on the basis of HOMA-IR values: the low HOMA-IR group (< 3.16) (n = 222) and the high HOMA-IR group (≥ 3.16) (n = 147).

Statistical Analysis

All statistical analyses were carried out using SPSS V.25.0 statistical software. Descriptive participant characteristics are presented as the mean ± SD for continuous variables and median (p25, p75) for non-normally distributed data.

For the categorical variables, the chi-square test and percentage (%) were used. Univariate analysis was performed to analyze the correlation between IR and variables and the correlation between SUA and variables. Furthermore, multiple linear regression analysis was conducted to evaluate the direct impacts of BMI and SUA on IR using different models: Model 1

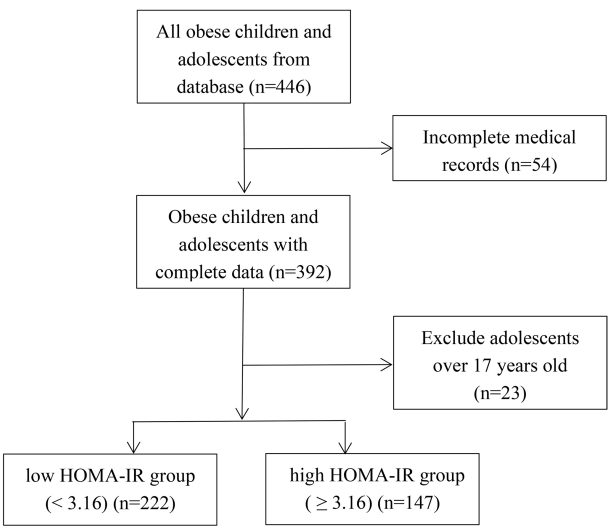

FIGURE 1 | Flowchart of collecting data.

(crude model), Model 2 (adjusted for age and sex), and Model 3 (adjusted for age, sex, TG, TC, HDL-C, and LDL-C) and to evaluate the direct impacts of BMI on SUA using different models: Model 1 (crude model), Model 2 (adjusted for age and sex), and Model 3 (adjusted for age, sex, TG, TC, HDL-C, LDL-C, FBG and FINS).

Advanced analysis of the mediating effect was conducted by constructing two causal pathways, a BMI → SUA → IR pathway (**Figure 2**). For this pathway, BMI was fitted as an independent variable, SUA was the potential mediator, and IR was the outcome of interest. In the pathway, the "total effect" consisted of a "direct effect" (not mediated by SUA) and an "indirect effect" (completely or partly mediated by SUA). If the direct impact was significant [p(c') < 0.05] and the indirect impact was not significant [both p (a) and p(b) > 0.05], then the link between BMI and IR was not mediated by SUA. The association between BMI and IR was fully mediated by SUA, with the indirect effects being significant [both p(a) and p(b) < 0.05] but the direct effects being not significant [p(c') > 0.05]. When both the indirect effects and the direct effects were significant [p(a), p(b) and p(c') < 0.05], SUA played a partial mediating effect on the relationship between BMI and IR. Path analyses were performed using Process v2.16.3 by Andrew F. Hayes (18, 19). All two-sided *P* values < 0.05 were considered statistically significant.

RESULTS

Participants Characteristics and Factors Associated With IR Among the Total Sample

A total of 369 participants, with a mean age of 10.53 ± 2.43 years, were recruited in this study, including 252 (68.29%) boys and 117 (31.71%) girls. Compared with the low HOMA-IR group (< 3.16), the high HOMA-IR group (≥3.16) was older and had higher BMI, SUA, TG, TC, LDL-C, FINS, and HOMA-IR but lower HDL-C (all p < 0.05, **Table 1**).

Correlation Analysis of BMI and SUA With HOMA-IR

The correlation analysis showed that HOMA-IR was closely associated with BMI, SUA, TG, TC, HDL-C, LDL-C and FINS (all p < 0.05, **Table 2**). To further explore the correlations between HOMA-IR and BMI, HOMA-IR and SUA, the results of the multiple linear regression analysis are presented in **Table 3**. The results indicated that both BMI and SUA were risk factors for HOMA-IR with the crude model and when adjusted for age and sex (all p < 0.05, **Table 3**). However, after adjusting for age, sex, TG, TC, HDL-C, LDL-C, and BMI (p < 0.05, **Table 3**), there was no correlation between SUA and HOMA-IR (p > 0.05, **Table 3**).

Correlation Analysis of BMI With SUA

The correlation analysis showed that BMI, TG, TC, HDL-C and FINS were closely associated with SUA (all p < 0.05, **Table 2**). Further multiple linear regression analysis found that BMI was a risk factor for SUA with the crude model adjusted for age and sex and for age, sex, TG, TC, HDL-C, LDL-C, FBG and FINS (all p < 0.05, **Table 4**).

Direct and Indirect Effects of BMI on Markers of Glucose Metabolism With SUA As a Mediator

As displayed in **Table 5**, the mediation analysis based on a causal pathway revealed the mediating role of SUA on the relationship between BMI and glucose metabolism. BMI was directly associated with FINS and HOMA-IR [both p (c')< 0.05, **Table 5**]. In addition, the potential causal effect of BMI on FINS and HOMA-IR mediated by SUA was also presented [All p (a) and p (b)< 0.05, **Table 5**], which implied that SUA could have a mediating impact on the link between BMI and glucose metabolism. However, BMI was not directly associated with FBG and not indirectly associated with FBG with SUA as a mediator (all p > 0.05, **Table 5**).

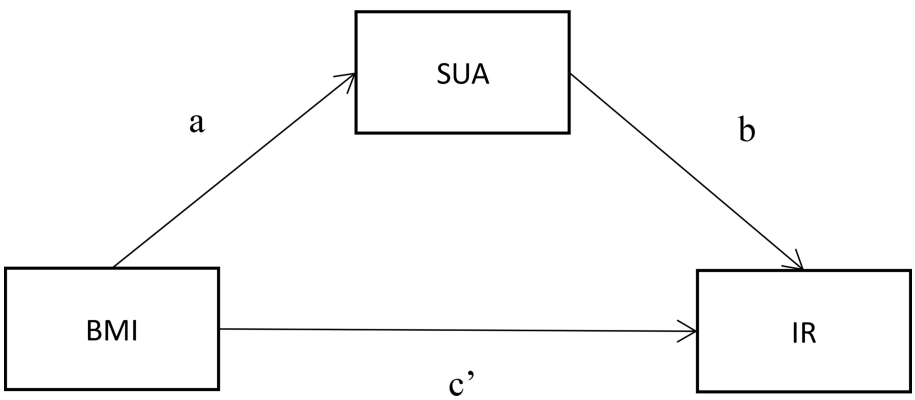

FIGURE 2 | Mediation model for the association between BMI and IR with SUA as a mediator. **(A)** represents the regression coefficients for the association between BMI and SUA; **(B)** represents the regression coefficients for the association between SUA and glucose metabolism; **(C')** represents the total effect between BMI and glucose metabolism.

TABLE 1 | Participants characteristics in different HOMA-IR groups among total sample.

	Total	HOMA-IR		*P*
		<3.16	≥3.16	
n	**369**	**222**	**147**	
Sex				
Boy [n,(%)]	252 (68,29)	157 (70.70)	95 (64.60)	0.309
Girl[n,(%)]	117 (31.71)	65 (29.30)	52 (35.40)	
Age (y)	10.94 ± 2.89	10.19 ± 2.89	12.09 ± 2.50	<0.001
Height (m)	1.52 ± 0.15	1.47 ± 0.15	1.59 ± 0.11	< 0.001
Weight (kg)	64.90 (50.50, 82.00)	56.98 (46.32, 71.40)	76.40 (62.40, 92.30)	<0.001
BMI (kg/m^2)	28.45 ± 4.71	27.15 ± 4.16	30.42 ± 4.83	<0.001
SUA (umol/L)	371.00 (312.50, 454.00)	351.50 (297.50, 406.25)	415.00 (350.00, 497.00)	<0.001
TG (mmol/L)	1.44 ± 0.94	1.33 ± 0.93	1.60 ± 0.92	0.019
TC (mmol/L)	4.22 ± 0.95	4.14 ± 0.96	4.35 ± 0.91	0.009
HDL-C (mmol/L)	1.32 ± 0.29	1.35 ± 0.30	1.26 ± 0.27	0.008
LDL-C (mmol/L)	2.59 ± 0.63	2.52 ± 0.60	2.69 ± 0.66	0.002
FBG (mmol/L)	5.04 ± 0.37	5.02 ± 0.39	5.08 ± 0.35	0.166
FINS (pmol/L)	85.7 (49.51, 123.21)	55.01 (34.24, 77.18)	143.20 (114.11, 185.40)	<0.001
HOMA-β(%)	153.31 (86.73, 257.82)	98.00 (60.39, 144.38)	281.60 (200.32, 357.72)	<0.001
HOMA-IR	2.71 (1.52, 4.04)	1.75 (1.10, 2.49)	4.56 (3.76, 5.86)	<0.001

BMI, body mass index; SUA, serum uric acid; TG, triglyceride, TC, total cholesterol; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; FBG, fasting blood glucose; FINS, fasting insulin; HOMA-β, homeostasis model assessment-β; HOMA-IR, homeostasis model assessment-insulin resistance.

DISCUSSION

This study has demonstrated several unexpected findings with important clinical implications. The results showed that obesity had direct impacts on regulating FINS and HOMA-IR, and the results of mediating effect analysis also highlighted that the link between obesity and IR was partly mediated by SUA in obese children and adolescents. However, the present study did not find that obesity directly or indirectly affects FBG.

Obesity is an ongoing global epidemic that severely affects adults and children. Many risk factors for IR have been identified in obese children and adolescents, and one of them is serum uric acid. However, it remains controversial whether SUA plays a pathogenic role in IR. In our study, it was found that SUA was a risk factor for IR and exerted a mediating role in the process of obesity-induced IR, which implied that SUA may not only directly affect IR but also be the intermediate link leading to IR. That is, a BMI → SUA → IR pathway is present in obese individuals.

It is known that obesity is strongly associated with IR in obese children and adolescents (10, 20), which was in line with our study. However, the mechanisms of obesity leading to IR are not fully understood to date. There has been growing evidence suggesting that IR is induced through chronic low-grade inflammation, which is promoted by adipocytokines (21–23). A previous study suggested that leptin and adiponectin could modulate the relationship between obesity and IR (24). Other studies also showed a positive association between leptin and IR after controlling for potential confounding factors in children and adolescents (22, 25). Thus, these studies could indicate that adipocytokines are the direct pathway of obesity-insulin resistance.

Furthermore, cross-sectional studies showed that an excessive increase in BMI and waist circumference was associated with

TABLE 2 | Univariate analysis examining the association of BMI and SUA with HOMA-IR.

	HOMA-IR		SUA	
	r	*P*	r	*P*
Sex	0.001	0.979	-0.130	0.012
Age	0.310	<0.001	0.497	<0.001
BMI	0.338	<0.001	0.461	<0.001
SUA	0.255	<0.001	–	–
TG	0.243	<0.001	0.108	0.037
TC	0.124	0.017	0.156	0.003
HDL-C	-0.119	0.022	-0.201	<0.001
LDL-C	0.146	0.005	0.004	0.941
FBG	0.097	0.063	0.016	0.756
FINS	0.993	<0.001	0.261	<0.001
HOMA-β	0.834	<0.001	0.259	<0.001
HOMA-IR	–	–	0.255	<0.001

BMI, body mass index; SUA, serum uric acid; TG, triglyceride, TC, total cholesterol; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; FBG, fasting blood glucose; FINS, fasting insulin; HOMA-β, homeostasis model assessment-β; HOMA-IR, homeostasis model assessment-insulin resistance.

TABLE 3 | Multiple linear regression examining the association of BMI and SUA with HOMA-IR.

Dependent variables	BMI		SUA	
	Beta	*P*	Beta	*P*
Model 1				
HOMA-IR	0.338	<0.001	0.255	<0.001
Model 2				
HOMA-IR	0.243	<0.001	0.138	0.016
Model 3				
HOMA-IR	0.211	0.001	0.074	0.188

Model 1: crude model;
Model 2: adjusted for age and sex;
Model 3: adjusted for age, sex, TG, TC, HDL-C, and LDL-C.
BMI, body mass index; SUA, uric acid; HOMA-IR, homeostasis model assessment-insulin resistance.

significant SUA elevation and hyperuricemia (26–28). It has been demonstrated that mature adipocytes and adipose tissue produce and secrete uric acid (29). Thus, obesity could increase the mRNA expression and activity of xanthine oxidoreductase from adipose tissue (29, 30).

Viewed separately, a close relationship between SUA and IR has long been appreciated, which may be another mechanism to explain IR in obese people. Emerging evidence has shown that hyperuricemia is linked with IR and the subsequent promotion and development of T2DM (9, 31, 32). Similar to these studies, we found that the SUA level was higher in the high HOMA-IR group, and SUA was also an independent risk factor for HOMA-IR. Furthermore, another major finding of the present study was that advanced causal mediating analysis supported that IR was partly mediated by SUA, which implies that an obesity → SUA → IR indirect pathway exists in obese children and adolescents.

Regarding the mechanism involved, the causal relationship between hyperuricemia and IR has not been completely illuminated and is still under investigation. In an animal study with eight-week-old male C57BL/6J mice, increased SUA levels might inhibit IRS1 and Akt insulin signaling and induce IR, which indicates a key role of the reactive oxygen species pathway in high SUA-induced IR (29). There are also reports that SUA-induced IR is caused by an increase in tissue NADPH oxidase or hs-CRP levels (33–35).

In the present study, there was no direct or indirect correlation between obesity and FBG. This result is not surprising, as IR precedes the development of diabetes. This hints that obese children oversecrete insulin in response to increasing insulin resistance to maintain the FBG in the normal range. However, this condition is strongly associated with alterations that represent an increased risk for the development of metabolic disorders and the occurrence of diabetes in adulthood (3, 9).

Nevertheless, there are several limitations in this study that deserve comment. First, because of the retrospective study design, other potential mediators could not be fully taken into account. Second, as obesity in our study was evaluated by BMI, which cannot differentiate excess body weight from increased fat mass or fat-free mass, further studies will be conducted to investigate the effects of different body compositions on hyperuricemia and IR.

TABLE 4 | Multiple linear regression examining the association of BMI with SUA.

Dependent variables	Beta	t	*P*	95% CI
Model 1				
SUA	0.461	9.931	<0.001	8.293 to 12.384
Model 2				
SUA	0.246	4.410	<0.001	3.057 to 7.977
Model 3				
SUA	0.206	3.574	<0.001	2.082 to 7.175

Model 1: crude model;
Model 2: adjusted for age and sex;
Model 3: adjusted for age, sex, TG, TC, HDL-C, LDL-C, FBG, and FINS.
BMI, body mass index; SUA, serum uric acid; TG, triglyceride, TC, total cholesterol; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; FBG, fasting blood glucose; FINS, fasting insulin; CI, confidence interval.

TABLE 5 | Direct and indirect effects of BMI on markers of glucose metabolism with SUA as a mediator.

Outcomes	Direct effect (SUA unadjusted)		Indirect effect (SUA adjusted)			
	Estimate[1] (BMI to glucose metabolism)	*P*(c')	Estimate[2] (BMI to SUA)	*P*(a)	Estimate[3] (SUA to glucose metabolism)	*P*(b)
FBG	-0.004	0.310	0.460	<0.001	0.002	0.457
FINS	4.476	<0.001	0.460	<0.001	0.086	0.021
HOMA-IR	0.138	<0.001	0.460	<0.001	0.002	0.022

[1]*Estimate represents the direct effect of BMI on markers of glucose metabolism.*
[2]*Estimate represents the indirect effect of BMI on SUA.*
[3]*Estimate represents the indirect effect of SUA on markers of glucose metabolism.*
BMI, body mass index; SUA, serum uric acid; FBG, fasting blood glucose; FINS, fasting insulin; HOMA-IR, homeostasis model assessment-insulin resistance.

In conclusion, SUA plays a partial mediating role in IR induced by obesity in obese children and adolescents. SUA should be investigated in obesity, and the results of the present study indicate this avenue is worth pursuing. In addition, the long-term direct and indirect effects of obesity and SUA on FBG need to be investigated in the future.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

This study was reviewed and approved by the Ethic Committee of Xinhua Hospital, School of Medicine, Shanghai Jiao Tong University (No. XHEC-D-2021-113). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHORS CONTRIBUTIONS

YN, QT, XuaZ, WC, and YF conceptualized and designed the study, drafted the initial manuscript, and reviewed and revised the manuscript. XueZ, XM, and JS designed the data collection instruments, collected data, carried out the initial analyses, and reviewed and revised the manuscript. All authors contributed to the article and approved the submitted version.

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Puberty Status Modifies the Effects of Genetic Variants, Lifestyle Factors and Their Interactions on Adiponectin: The BCAMS Study

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Background: Hypoadiponectinemia has been associated with various cardiometabolic disease states. Previous studies in adults have shown that adiponectin levels were regulated by specific genetic and behavioral or lifestyle factors. However, little is known about the influence of these factors on adiponectin levels in children, particularly as mitigated by pubertal development.

Methods: We performed a cross-sectional analysis of data from 3,402 children aged 6-18 years from the Beijing Child and Adolescent Metabolic Syndrome (BCAMS) study. Pubertal progress was classified as prepubertal, midpuberty, and postpuberty. Six relevant single nucleotide polymorphisms (SNPs) were selected from previous genome-wide association studies of adiponectin in East Asians. Individual SNPs and two weighted genetic predisposition scores, as well as their interactions with 14 lifestyle factors, were analyzed to investigate their influence on adiponectin levels across puberty. The effect of these factors on adiponectin was analyzed using general linear models adjusted for age, sex, and BMI.

Results: After adjustment for age, sex, and BMI, the associations between adiponectin levels and diet items, and diet score were significant at prepuberty or postpuberty, while the effect of exercise on adiponectin levels was more prominent at mid- and postpuberty. Walking to school was found to be associated with increased adiponectin levels throughout puberty. Meanwhile, the effect of *WDR11-FGFR2*-rs3943077 was stronger at midpuberty ($P = 0.002$), and *ADIPOQ*-rs6773957 was more effective at postpuberty ($P = 0.005$), while *CDH13*-rs4783244 showed the strongest association with adiponectin levels at all pubertal stages (all $P < 3.24 \times 10^{-15}$). We further found that effects of diet score ($P_{interaction} = 0.022$) and exercise ($P_{interaction} = 0.049$) were stronger in children with higher genetic risk of hypoadiponectinemia, while higher diet score and exercise frequency attenuated the differences in adiponectin levels among children with different genetic risks.

Conclusions: Our study confirmed puberty modulates the associations between adiponectin, and genetic variants, lifestyle factors, and gene-by-lifestyle interactions. These findings provide new insight into puberty-specific lifestyle suggestions, especially in genetically susceptible individuals.

Keywords: adiponectin, puberty, diet items, genetic variants, gene-by-lifestyle interaction

INTRODUCTION

Childhood obesity has emerged as a global public health problem, in part due to its association with cardiometabolic disease (1). In China, overweight and obesity have increased rapidly over the past four decades, and the prevalence of overweight and obesity among children aged 6-17 and under 6 is 19% and 10.4% respectively, which has become a major challenge for the country's healthcare system (2, 3). Yet, the mechanisms responsible for obesity's contribution to cardiometabolic risk, like adipocyte metabolic dysregulation, remain unclear (4). Adipose tissue, as an endocrine organ, secretes many peptide hormones, termed "adipokines", that affect systemic metabolism (5). Among the most abundant adipokines, adiponectin is specifically expressed in differentiated adipocytes and exhibits anti-atherogenic, anti-inflammatory and anti-diabetic properties (6). Low adiponectin levels, known as hypoadiponectinemia, and a marker of adipose tissue dysfunction, and the condition that is common in obesity (7), have been robustly associated with an increased risk of insulin resistance, diabetes, cardiovascular diseases, and certain kinds of cancer (8, 9). As a result, adiponectin is regarded as a protective molecule and a potentially novel therapeutic target for diabetes and related diseases (10). Some diabetes drugs, such as rosiglitazone, operate partially by increasing circulating adiponectin levels (11).

Recently, several genetic (12, 13) and environmental factors (14–17) that influence adiponectin levels have been reported in adult populations; however, the understanding of these relationships in children, especially during puberty, is still quite limited (18). Since many adult diseases have their origins in childhood (19), it is important to identify the factors that influence adiponectin levels during pediatric development. As puberty is a period through which the body changes physically, being a physiological process leading to the maturation of children (20) and sex differences of adiponectin seem to develop during the development of puberty (21), we propose that the influences which genetic and environmental factors (as well as gene-environment interactions) exert upon adiponectin levels are mitigated by pubertal stage and that this modulating effect of puberty is mediated through adipose tissue development. Therefore, leveraging the large cohort within the Beijing Child and Adolescent Metabolic Syndrome (BCAMS) study (22), we aimed to examine the effect of pubertal stage upon adiponectin's association with specific gene variants, lifestyle influences, and gene-environment interactions.

Abbreviations: BCAMS, Beijing Child and Adolescent Metabolic Syndrome; GWAS, Genome-wide association study; BMI, Body mass index; MS, Metabolic syndrome; TC, Total cholesterol; TG, Triglycerides; FBG, Fasting blood glucose; HDL, High-density lipoprotein; SNP, Single nucleotide polymorphism; GPS, Genetic predisposition scores; wGPS, Weighted genetic predisposition score; ANOVA, Analysis of variance; ANCOVA, Analysis of covariance; CI, Confidence interval.

MATERIALS AND METHODS

Subjects

The BCAMS study, which has been described in detail elsewhere (22, 23), is an ongoing cohort study of obesity and related metabolic abnormalities in a representative sample of school-age children (n = 19,593, aged 6-18 y, 50% boys) recruited from the Beijing area between April and October 2004. Within this cohort, 4,500 subjects were identified as being at risk of metabolic syndrome (MS), based on at least one of the following criteria: 1) overweight, as defined by body mass index (BMI) percentile; 2) increased total cholesterol (TC) ≥ 5.2 mmol/L; 3) triglycerides (TG) ≥ 1.7 mmol/L; and 4) fasting blood glucose (FBG) ≥ 5.6 mmol/L based on finger capillary blood tests. Next, all children at risk of MS, together with a parallel normal sample of 1,024 schoolchildren, were invited to participate in a further medical examination including venipuncture-based blood tests. In total, 3,506 subjects, including 2,525 subjects with MS risk, ultimately completed the further clinical examination. Thus, the presence of pediatric MS based on clinical examination was defined by the presence of three or more of the following five components (22, 23) (1): central obesity defined as ≥90th percentile for age and gender (2); elevated systolic and/or diastolic blood pressure ≥90th percentile for age, sex and height (3); hypertriglyceridemia defined as TG ≥1.24 mmol/L (4); low high-density lipoprotein (HDL) cholesterol defined as <1.03 mmol/L; and (5) hyperglycemia defined as FBG ≥ 5.6 mmol/L. Accordingly, in the current study, we used the cross-sectional data of 3,402 participants (including 2,112 subjects with MS risk and 1,290 subjects without MS risk based on clinical evaluation), who completed the examination of adiponectin levels, genotype, and lifestyle factors in 2004 (**Supplementary Figure S1** and **Supplementary Table S1**).

Anthropometric Measurements and Pubertal Stages

The subjects' height and weight were measured according to our standard protocol (22, 24). Height in centimeters was measured without shoes to the nearest 0.1 cm. Bodyweight was measured to the nearest 0.1 kg (light indoor clothing, without shoes) using a calibrated electronic scale. BMI was calculated as weight (kg) divided by height squared (m^2). Age- and sex-specific BMI percentiles were used to define overweight (85^{th}) and obesity

(95^{th}) following the Working Group for Obesity in China (25). Puberty stages were assessed by two pediatricians of the same gender, based on Tanner's stages of breast development in girls and testicular volume in boys, in line with Marshall and Tanner (26). The categories of puberty were defined as pre-puberty (Tanner stage I), mid-puberty (Tanner stage II-III), and post-puberty (Tanner stage ≥ IV) (26).

Lifestyle Description

Fourteen lifestyle factors (27), including walking to school, frequency of exercise, duration of habitual sleep, and eleven dietary habits were selected for examination in this study (**Table 1**). And the comparison of lifestyle factors between subjects with risk of MS and without risk of MS was listed in **Supplementary Table S1**. Surveys were conducted retrospectively and participants were asked how often on average they had consumed each food in the previous month. Each dietary item was scored on a 5-point scale according to the frequency categories from "seldom or never" to "> 5 times per week ", with ascending values for favorable foods (1 for "seldom or never" and 5 for "> 5 times per week") and vice versa, according to the direction of their linear associations with adiponectin levels. The diet score was generated by summing all the selected item scores, with a higher diet score indexing predisposition to higher adiponectin levels. Effective exercise was deemed as exercise lasting longer than 30 minutes for extracurricular physical activities such as cycling, running, swimming, dancing, and team sports. Transportation refers to "walking to school" which was recorded and reclassified into two modes, 'walking' and 'non-walking'.

Laboratory Measurements

Venous blood samples were collected after 12 h of fasting. The adiponectin concentration was measured using a monoclonal antibody-based enzyme-linked immunosorbent assay (28). The intra- and interassay coefficients of variation were < 5.4% and 8.5%, respectively.

Single Nucleotide Polymorphism Selection and Genotyping

Genomic DNA was isolated from peripheral white blood cells using QIAamp DNA blood midikits (Qiagen). Genotyping was carried out on a Sequenom Mass Array iPLEX genotyping platform by BioMiao Biological Technology Co., Ltd (29). All these SNPs had genotyping efficiency >0.95 and were in Hardy-Weinberg equilibrium with p-value >0.008 (0.05/6). We selected six SNPs showing strong associations with adiponectin levels in previous genome-wide association studies (GWASs) (12, 13). Among them, five SNPs (*CDH13*-rs4783244, *ADIPOQ*-rs10937273, *PEPD*-rs889140, *CMIP*-rs2925979, and *WDR11-FGFR2*-rs3943077) were identified in an East Asian adult population (12) to have the five strongest associations with adiponectin levels ($P < 10^{-10}$ for each of the five SNPs); while another SNP, *ADIPOQ*-rs6773957, was identified in a European population (13) and was included because it is located at the

TABLE 1 | Comparison of lifestyle factors among the various pubertal stages.

	Entire Population[c]	Prepuberty[cd]	Midpuberty[cd]	Postpuberty[cd]	P-value
N	3402	1092	1076	1234	
Age (years)	13 ± 3	9 ± 2	13 ± 2	15 ± 2	**<0.001**
Sex					
Male	1707	755	649	303	**<0.001**
Female	1695	337	427	931	**<0.001**
BMI (kg/m^2)	21.8 ± 4.9	19.9 ± 4.6	21.9 ± 4.9	23.4 ± 4.7	**<0.001**
Normal weight, %	47.2	45.1	48.7	47.7	**<0.001**
Ln-adiponectin (μg/ml) [a]	1.7 ± 0.6	1.9 ± 0.6	1.6 ± 0.6	1.6 ± 0.6	**<0.001**
Diet[b]					
Breakfast	4.4 ± 1.3	4.7 ± 1	4.4 ± 1.3	4.1 ± 1.3	**<0.001**
Bean	2.7 ± 1.4	2.8 ± 1.4	2.8 ± 1.4	2.5 ± 1.4	**<0.001**
Meat	3.7 ± 1.5	3.7 ± 1.5	3.8 ± 1.4	3.5 ± 1.5	**<0.001**
Sea food	2.2 ± 1.3	2.5 ± 1.3	2.2 ± 1.3	2.0 ± 1.2	**<0.001**
Diary	3.8 ± 1.6	4.2 ± 1.4	4.7 ± 1.6	3.5 ± 1.7	**<0.001**
Vegetable	4.8 ± 0.8	4.7 ± 0.8	4.8 ± 0.8	4.8 ± 0.6	**<0.001**
Fruit	4.1 ± 1.3	4.1 ± 1.3	4.0 ± 1.4	4.1 ± 1.3	0.341
Fast food	1.4 ± 0.8	1.5 ± 0.9	1.4 ± 0.8	1.4 ± 0.7	**<0.001**
Soft drink	2.5 ± 1.5	2.3 ± 1.4	2.6 ± 1.5	2.6 ± 1.5	**<0.001**
Fried food	2.0 ± 1.2	1.9 ± 1.1	2.0 ± 1.2	2.0 ± 1.3	**0.017**
Snacks	2.6 ± 1.5	2.4 ± 1.5	2.6 ± 1.6	2.6 ± 1.5	**0.008**
Exercise[b]	3.4 ± 1.3	3.6 ± 1.2	3.5 ± 1.3	3.1 ± 1.4	**<0.001**
Walking to school, %	57	66	62	45	**<0.001**
Sleep duration (h/day)	8.5 ± 1.2	9.1 ± 0.9	8.6 ± 1.1	8.0 ± 1.2	**<0.001**

[a] *Adiponectin levels were natural logarithmically (ln) transformed.*

[b] *The values of the diet items and exercise were encoded as "seldom or never" = 1; "1 time/2 weeks" = 2; "1-2 times per week" = 3; "3-5 times per week" = 4; "> 5 times per week" = 5.*

[c] *Data are expressed as the mean ± SD or n (%).*

[d] *Prepuberty: Tanner stage I; Midpuberty: Tanner stage II-III; Postpuberty: Tanner stage ≥ IV.*

Boldface type indicates nominally significant values (P < 0.05).

3' UTR of *ADIPOQ*, a very important regulatory area of the gene. Details of the SNPs are listed in **Supplementary Table S2**.

Construction of Genetic Predisposition Scores

As the effects of the SNPs were dramatically different, we used weighted rather than unweighted genetic predisposition scores (wGPSs) to evaluate the genetic structure of the children. wGPSs were calculated by summing the scores of the six SNPs, each of which was weighted using the mean of linear regression β from our study ($wGPS_{all\text{-}BCAMS}$) or that of published GWASs ($wGPS_{all\text{-}GWAS}$) for adiponectin levels (12, 13). *CDH13*-rs4783244 was found to be the strongest modulator of adiponectin levels in the current study, explaining at least 10-fold higher adiponectin levels than the others (**Supplementary Table S2**). We generated two GPSs that excluded *CDH13*-rs4783244, namely, $wGPS_{no\ CDH13\text{-}GWAS}$ and $wGPS_{no\ CDH13\text{-}BCAMS}$, to evaluate the association of genetic structure with adiponectin expression. For each participant j (j = 1, 2,…, 3405), we calculated wGPSs using the following equation:

$$wGPS_n^{(j)} = n \times \frac{\sum_{i=1}^{n} \beta_i^{(j)} \cdot EA_i^{(j)}}{\sum_{i=1}^{n} \beta_i^{(j)}},$$

where n is 6 (i.e., $wGPS_{all}$; including all six SNPs) or 5 (i.e., $wGPS_{no\ CDH13}$; all SNPs but *CDH13*-rs4783244), β_i is the β coefficient of each SNP for adiponectin levels (natural logarithm-transformed) adjusted for age, sex, and BMI, and EA_i is the number of effect alleles (0, 1 or 2) in each SNP. Thus, $wGPS_{all}$ ranged from 0 to 12, while $wGPS_{no\ CDH13}$ ranged from 0 to 10. However, there were no significant differences between the results of $wGPS_{all\text{-}BCAMS}$ and $wGPS_{all\text{-}GWAS}$ or between $wGPS_{no\ CDH13\text{-}GWAS}$ and $wGPS_{no\ CDH13\text{-}BCAMS}$ in the current study. Considering that SNP-adiponectin associations could be different between children and adults, we only report the results for $wGPS_{all\text{-}BCAMS}$ and $wGPS_{no\ CDH13\text{-}BCAMS}$ in the current pediatric study, in the form of $wGPS_{all}$ and $wGPS_{no\ CDH13}$ for brevity.

Statistical Analysis

All analyses were performed using SPSS version 22.0 software for Windows (SPSS Inc.) (30). Adiponectin levels were natural logarithm transformed for analysis. We assigned a score of 1-5 ("seldom or never" = 1; "1 time every 2 weeks" = 2; "1-2 times per week" = 3; "3-5 times per week" = 4; "> 5 times per week" = 5) to each lifestyle factor to facilitate the analyses. The exception was transportation (non-walking = 0, walking = 1). The results are expressed as the mean ± SD or mean (95% CI) unless otherwise stated. We used ANOVA, ANCOVA, and t tests to compare the values of factors between different puberty groups. We performed a linear regression adjusted for confounders including age, sex, and BMI, to evaluate the associations of adiponectin with SNPs, GPSs, and lifestyle factors. Furthermore, gene-lifestyle interactions on adiponectin were tested using linear regression models by including the interaction terms (e.g. diet*genotype) in these models. The β coefficient, which reflects the change in the serum adiponectin concentration, was used to report the effects of genetic variants and lifestyle on adiponectin levels. Associations between SNPs and adiponectin levels were assessed using an additive model in which a score of 0, 1, or 2 was assigned to genotypes according to the number of effect alleles. An SNP association was considered statistically significant if the resulting P-value was less than the Bonferroni-corrected significance threshold of 0.05/6 = 0.008. We only tested the interactions between gene variants and lifestyle factors that were statistically significantly associated with adiponectin levels, and then stratified analyses were conducted to observe effect modification. The gene-by-lifestyle interaction was considered to be significant if the resulting P-value was less than the Bonferroni-corrected significance threshold of 0.05/9 = 0.006 and considered to be "nominally significant" if the P-value between 0.006 and 0.05.

RESULTS

Population Characteristics

Among the 3,402 children in our study, 32% were prepubertal, 32% were midpubertal, and 36% were postpubertal (**Table 1**). Except for fruit intake, the examined lifestyle factors differed significantly among children at different pubertal stages. Children at an advanced pubertal stage generally exhibited a higher BMI and lower adiponectin levels. **Figure 1** highlights the adiponectin levels among the different groups according to sex and puberty status. Compared with prepuberty, adiponectin levels significantly decreased after the onset of puberty, and adiponectin levels were higher in girls after adjusting for age.

Genotypic Influences at Different Pubertal Stages

Supplementary Table S2 shows the genetic information for the six selected SNPs. After controlling for age, sex, and BMI, five of

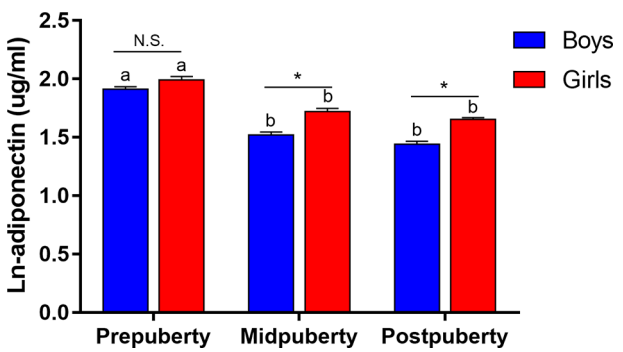

FIGURE 1 | Changes in ln-adiponectin levels during puberty according to sex. Data were shown as mean and SE. Puberty status was reclassified into prepuberty (Tanner stage I), midpuberty (Tanner stage II-III) and postpuberty (Tanner stage ≥ IV). Difference between boys and girls in the same pubertal group was indicated *P < 0.001 and N.S. as non-significant value after adjusted for age. Difference between diverse pubertal groups of the same sex was indicated as different letters with P < 0.05 after adjusted for age, that was, difference between pubertal groups of the same sex with the same letter were not statistically significant.

these loci significantly influenced adiponectin levels: *CDH13*-rs4783244, *ADIPOQ*-rs10937273, *PEPD*-rs889140, *ADIPOQ*-rs6773957, and *WDR11-FGFR2*-rs3943077, explaining 5.2%, 0.7%, 0.5%, 0.2%, and 0.2% of the total variance, respectively. As for the two wGPSs, both $wGPS_{all}$ and $wGPS_{no\ CDH13}$ were strongly positively associated with adiponectin levels and explained 7.1% and 1.9% of the total variance, respectively. **Table 2** outlines the associations between the SNPs and adiponectin levels according to pubertal status in the current study. The changes in the strength of these associations during puberty after adjustment for age, sex and BMI are graphically highlighted in **Figure 2A**. The association of *ADIPOQ*-rs6773957 with adiponectin levels was only significant at postpuberty [β = 0.058 (0.017, 0.099), $P = 0.005$], while *WDR11-FGFR2*-rs3943077 presented a significant effect on adiponectin levels at midpuberty [β = 0.076 (0.027, 0.125), $P = 0.002$]. The other three SNPs, namely, *ADIPOQ*-rs10937273, *CDH13*-rs4783244, and *PEPD*-rs889140, together with $wGPS_{all}$ and $wGPS_{no\ CDH13}$, were associated with adiponectin levels throughout puberty. Most notably, among the six SNPs, *CDH13*-rs4783244 showed the strongest association with adiponectin levels at all pubertal stages.

Impact of Lifestyle at Different Pubertal Stages

Controlling for age and sex, both exercise (P = 0.049) and walking to school ($P = 4.47\times10^{-12}$) was associated with increased adiponectin levels; adjustment for BMI attenuated the association of adiponectin levels with both exercise (P = 0.750) and walking to school ($P = 1.07\times10^{-4}$). Six diet items (breakfast, meat, dairy, soft drink, fried food, and snack) were significantly associated with adiponectin levels in the model adjusted for age and sex (soft drink, fried food, and snack), or in the model further adjusted for BMI (breakfast, meat, dairy, and soft drink) (**Table 3**). To better analyze the association of adiponectin with dietary structure, we used the six dietary items shown to be associated with adiponectin in either adjusted model to construct a new diet score. The diet score showed a stronger association with increased adiponectin levels than any single diet factor in both models with ($P = 1.25 \times 10^{-8}$) or without ($P = 6.32 \times 10^{-6}$) adjustment for BMI (**Supplementary Table S3**). Further analyses of lifestyle factors and gene-by-environment interactions will only focus on nine lifestyle factors that were associated with adiponectin levels in either adjustment model, including walking to school, exercise, diet score, and consumption of breakfast, meat, dairy, soft drink, fried food, and snacks. Further analyses (depicted in **Figure 2B** and summarized in **Supplementary Table S3**) adjusted for age and sex revealed different effects of the nine lifestyle factors on adiponectin levels among the three pubertal stages. However, none of the dietary items nor the diet score was associated with adiponectin levels at midpuberty. Walking to school was the only factor that influenced adiponectin levels throughout puberty [prepuberty: β = 0.114 (0.043, 0.185), $P = 0.002$; midpuberty: β = 0.077 (0.003, 0.151), $P = 0.044$; postpuberty: β = 0.203 (0.142, 0.264), $P < 0.001$]. In addition, exercise frequency was only associated with adiponectin levels after the onset of puberty [midpuberty: β = 0.031 (0.002, 0.060), $P = 0.036$; postpuberty: β = 0.025 (0.001, 0.049), $P = 0.031$]. After further adjustment for BMI, most of the effects of lifestyle factors on adiponectin levels were weakened, except for that of breakfast frequency, which decreased adiponectin levels significantly at postpuberty [β = -0.039 (-0.059 to -0.019), $P = 0.003$] (**Supplementary Table S3**). Additionally, none of the lifestyle factors, including walking to school, significantly affected adiponectin levels at midpuberty independent of BMI.

SNPs-Lifestyle Interactions Across Pubertal Stages

After controlling for age and sex (**Supplementary Table S4**), we found two statistically significant interactions in the entire population: one between *WDR11-FGFR2*-rs3943077 and walking to school [β = -0.079 (-0.132 to -0.026), $P = 0.004$] and the other between *ADIPOQ*-rs6773957 and exercise [β = 0.038 (0.018 to 0.058), $P = 1.71 \times 10^{-4}$], as well as five nominally significant interactions. Regarding the interactions between GPS and lifestyle factors, as depicted in **Figure 3** and **Supplementary Table S5**, we identified nominally significant negative

TABLE 2 | SNPs' effect on adiponectin levels adjusted for age, sex, and BMI.

SNP/GPS	Prepuberty[b]		Midpuberty[b]		Postpuberty[b]	
	β (95%CI) [c]	*P* [c]	β (95%CI) [c]	*P* [c]	β (95%CI) [c]	*P* [c]
ADIPOQ- rs10937273	0.102(0.055 to 0.149)	**2.34×10^{-05}**	0.057(0.010 to 0.104)	**0.028**	0.066(0.023 to 0.109)	**0.002**
ADIPOQ- rs6773957	0.036(-0.011 to 0.083)	0.131	0.007(-0.042 to 0.056)	0.781	0.058(0.017 to 0.099)	**0.005**
CDH13- rs4783244	-0.190(-0.237 to -0.143)	**3.24×10^{-15}**	-0.207(-0.256 to -0.158)	**5.85×10^{-16}**	-0.207(-0.250 to -0.164)	**2.46×10^{-20}**
WDR11-FGFR2- rs3943077	0.021(-0.026 to 0.068)	0.377	0.076(0.027 to 0.125)	**0.002**	0.028(-0.015 to 0.071)	0.193
CMIP- rs2925979	0.002(-0.045 to 0.049)	0.938	-0.022(-0.073 to 0.029)	0.405	-0.031(-0.074 to 0.012)	0.152
PEPD- rs889140	0.064(0.019 to 0.109)	**0.006**	0.088(0.039 to 0.137)	**5.09×10^{-4}**	0.048(0.007 to 0.089)	**0.025**
wGPS-GWAS	0.072 (0.057 to 0.087)	**4.28×10^{-20}**	0.080(0.064 to 0.096)	**3.39×10^{-21}**	0.077(0.063 to 0.091)	**7.14×10^{-26}**
wGPS-BCAMS	0.071(0.056 to 0.085)	**2.53×10^{-21}**	0.076(0.061 to 0.092)	**1.75×10^{-21}**	0.074(0.061 to 0.087)	**3.33×10^{-26}**
wGPS-GWAS(no CDH13)	0.044 (0.024 to 0.064)	**1.01×10^{-5}**	0.046 (0.026 to 0.066)	**1.35×10^{-5}**	0.042 (0.024 to 0.060)	**3.30×10^{-6}**
wGPS-BCAMS(no CDH13)	0.046 (0.028 to 0.064)	**9.40×10^{-7}**	0.045 (0.025 to 0.065)	**6.51×10^{-6}**	0.04 (0.022 to 0.058)	**3.27×10^{-6}**

[a] *Adiponectin levels were natural logarithm transformed for analysis.*

[b] *Prepuberty: Tanner stage I; Midpuberty: Tanner stage II-III; Postpuberty: Tanner stage ≥ IV.*

[c] *Results are adjusted for age, sex, and BMI.*

Boldface type indicates nominally significant values (P < 0.05).

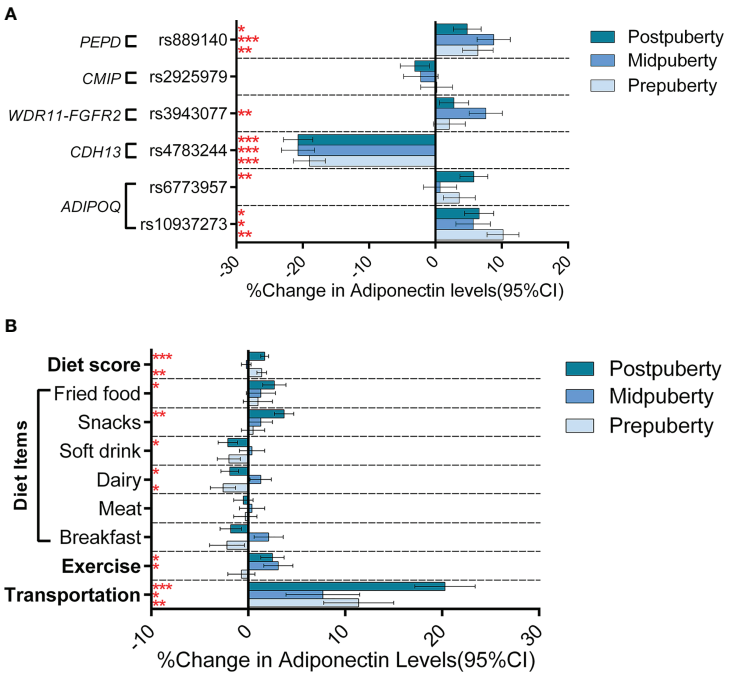

FIGURE 2 | Genetic and lifestyle associations with adiponectin levels according to the different puberty stages. Figure **(A)** shows the effect (histograms) and SEs (error bar) of SNPs on adiponectin levels (% change in adiponectin levels per effect allele) at different puberty stages. Figure **(B)** shows the effect (histograms) and SEs (error bar) of lifestyle factors on adiponectin levels (% change in adiponectin levels when walking to school for the transportation variable and % change in adiponectin levels per assigned score increase for other variables) at different puberty stages. The results were adjusted for age, sex, and BMI. *$P < 0.05$; ** $P < 0.05/6 = 0.008$ (after Bonferroni correction); *** $P < 0.001$.

interactions of $wGPS_{all}$ with diet score [β = -0.003 (-0.005 to 0.000), $P = 0.022$] and $wGPS_{no\ CDH13}$ with exercise [β = -0.009 (-0.017 to 0.000), $P = 0.049$] and snacks [β = -0.006 (-0.012 to 0.000), $P = 0.038$] when age, sex and other lifestyle factors were controlled. The interaction between $wGPS_{all}$ and diet score was more prominent at postpuberty than at other puberty stages.

TABLE 3 | Association of lifestyles factors with adiponectin levels.

Variables	Model 1- unadjusted		Model 2- adjusted for age and sex		Model 3- adjusted for age, sex and BMI.	
	β (95% CI)	P-value	β (95% CI)	P-value	β (95% CI)	P-value
Age	-0.049 (-0.055 to -0.043)	**3.12×10^{-53}**	/	/	/	/
Sex	0.094 (0.055 to 0.133)	**2.69×10^{-6}**	/	/	/	/
BMI (kg/m^2)	-0.048 (-0.052 to 0.044)	**3.23×10^{-132}**	-0.041 (-0.045 to -0.037)	**2.30×10^{-87}**	/	/
Tanner stage	-0.097 (-0.111 to -0.083)	**5.82×10^{-48}**	-0.076 (-0.100 to 0.052)	**6.90×10^{-10}**	-0.025 (-0.049 to -0.001)	**0.040**
Exercise	0.026 (0.010 to 0.042)	**0.001**	0.015 (-0.001 to 0.031)	**0.049**	0.002 (-0.012 to 0.016)	0.75
Walking to school	0.183 (-0.209 to 0.575)	**2.33×10^{-19}**	0.137 (0.098 to 0.176)	**4.47×10^{-12}**	0.074 (0.037 to 0.111)	**1.07×10^{-4}**
Sleep time	0.112 (0.077 to 0.147)	**2.17×10^{-10}**	0.009 (-0.026 to 0.044)	0.641	-0.011 (-0.046 to 0.024)	0.531
Diet factors						
Breakfast	0.013 (-0.003 to 0.029)	0.095	-0.005 (-0.021 to 0.011)	0.479	-0.019 (-0.033 to -0.005)	**0.009**
Bean	0.012 (-0.002 to 0.026)	0.115	0.006 (-0.008 to 0.020)	0.375	-0.004 (-0.018 to 0.010)	0.532
Meat	-0.013 (-0.027 to 0.001)	0.063	-0.009 (-0.023 to 0.005)	0.204	-0.014 (-0.026 to -0.002)	**0.024**
Sea food	0.020 (0.004 to 0.036)	**0.014**	0.008 (-0.008 to 0.024)	0.328	-0.005 (-0.019 to 0.009)	0.537
Diary	0.006 (-0.006 to 0.018)	0.335	-0.010 (-0.022 to 0.002)	0.128	-0.019 (-0.031 to -0.007)	**0.002**
Vegetable	-0.012 (-0.039 to 0.015)	0.387	-0.007 (-0.032 to 0.018)	0.573	-0.003 (-0.027 to 0.021)	0.819
Fruit	0.003 (-0.013 to 0.019)	0.717	-0.004 (-0.018 to 0.010)	0.563	-0.010 (-0.024 to 0.004)	0.147
Fast food	0.016 (-0.009 to 0.041)	0.213	0.004 (-0.020 to 0.028)	0.744	-0.011 (-0.033 to 0.011)	0.352
Soft drink	-0.032 (-0.046 to -0.018)	**5.07×10^{-6}**	-0.016 (-0.030 to -0.002)	**0.017**	-0.018 (-0.030 to -0.006)	**0.004**
Fried food	0.014 (-0.027 to 0.055)	0.523	0.018 (0.002 to 0.034)	**0.026**	0.014 (-0.001 to 0.029)	0.072
Snacks	0.049 (-0.343 to 0.441)	**0.017**	0.021 (0.008 to 0.034)	**0.001**	0.011 (-0.001 to 0.023)	0.072

Adiponectin levels were natural logarithm transformed (ln, e-based) for analysis.
Boldface type indicates nominally significant values (P < 0.05).

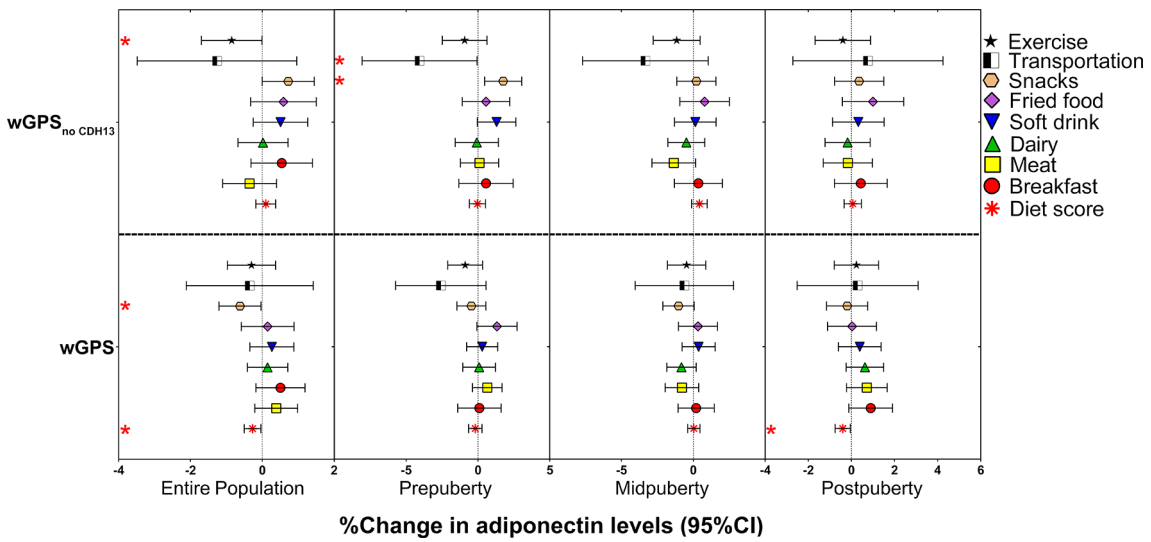

FIGURE 3 | Effects of the interactions of the weighted genetic score with lifestyle factors on the % change in adiponectin levels according to the different puberty stages. The figure shows the effect and 95% CI of the interactions of $wGPS_{all}$ and $wGPS_{no\ CDH13}$ with lifestyle factors on adiponectin levels (% change in adiponectin levels per $wGPS_{all}$ or $wGPS_{no\ CDH13}$ per diet score or per lifestyle factors assigned score increase) in the entire population of children at different puberty stages. The results for the diet items were adjusted for age and sex. The results for the diet score were adjusted for age, sex, and activities (including exercise and transportation type). The results for the activities were adjusted for age, sex, and diet score; * $P < 0.05$.

Further adjustment for BMI did not change the significance of the interactions between $wGPS_{no\ CDH13}$ and exercise and snacks in the entire population and between $wGPS_{all}$ and diet score in children at postpuberty (**Supplementary Tables S4, S5**).

As we identified an interaction between $wGPS_{all}$ and diet score, and an interaction of $wGPS_{no\ CDH13}$ with exercise, further stratified analyses were undertaken to observe effect modification. We firstly analyzed the associations of adiponectin levels with diet score and exercise according to the categories of genetic risk (**Figure 4**). We found that diet score and exercise had greater effects in children with a higher genetic risk for low adiponectin (**Figure 4**).

Second, we compared adiponectin concentrations between different categories of genetic risk and lifestyle levels (**Figure 5**). The difference in adiponectin levels between children at high genetic risk and those at intermediate or low genetic risk was more prominent among children with low and intermediate exercise levels than among those with high exercise levels (**Figure 5A**), whereas no significant difference in adiponectin levels was found among the genetic risk groups when the exercise frequency was high. A similar pattern was observed for diet levels, while an increased diet score significantly attenuated the difference in adiponectin levels between the high genetic risk group and intermediate genetic risk group (**Figure 5B**).

DISCUSSION

In this large cohort of Chinese children, we observed that eight lifestyle factors (breakfast, meat, dairy, soft drink, fried food, and snack consumption, walking to school, and exercise), and reported for the first time that five loci (*ADIPOQ*-rs10937273, *ADIPOQ*-rs6773957, *CDH13*-rs4783244, *WDR11-FGFR2*-rs3943077, and *PEPD*-rs889140) and two weighted polygene scores ($wGPS_{no\ CDH13}$ and $wGPS_{all}$), and some of their interactions were associated with adiponectin levels of children. We noted that the effects of a healthy diet and physical activity were more prominent in children at higher genetic risk of hypoadiponectinemia. We further found that the effects of these factors on adiponectin levels varied between pubertal stages, suggesting a modulating effect.

The value of identifying the associations between SNPs and adiponectin is related not only to the prediction of disease but also to the identification of causal steps on the path from genes to disease that can be targeted to reduce the risk (31). Previous GWASs have identified several genetic variants associated with adiponectin levels in adults (12, 13). Studies have shown that decreased androgen levels (32), decreased adipocyte size growth, better adipose tissue differentiation (5), and less visceral fat accumulation (33, 34), for which dramatic changes are observed during puberty, are associated with increased adiponectin levels (15, 35, 36). However, the relationships between these SNPs and adiponectin in childhood, and especially during puberty, a critical time for adipocyte hypertrophy and hyperplasia (37–39), are unclear. In line with GWASs conducted in adults, we found that *ADIPOQ*-rs10937273, *ADIPOQ*-rs6773957, *CDH13*-rs4783244, *WDR11-FGFR2*-rs3943077, and *PEPD*-rs889140 were associated with adiponectin levels among school children. According to previous studies, ADIPOQ-rs10937273 and ADIPOQ-rs6773957 were two unlinked SNP of adiponectin gene, while CDH13-rs4783244, PEPD-rs889140, WDR11-FGFR2-rs3943077 were associated with the synthesis of adiponectin receptor T-cadherin, adipocyte hypertrophy, and

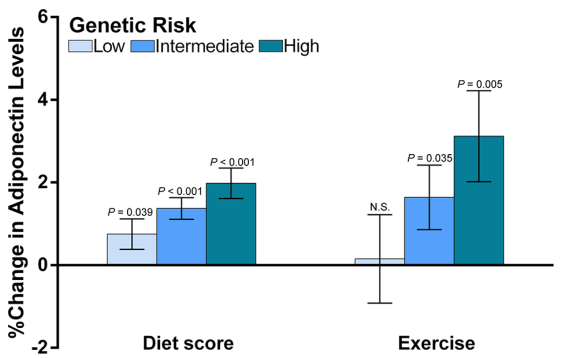

FIGURE 4 | The associations of diet and exercise with adiponectin levels according to the categories of genetic risk. The figure shows the main effects (histograms) and SEs (error bars) of diet score and exercise on adiponectin levels (% change in adiponectin levels per assigned score increase for diet and exercise) according to the genetic risk for decreased adiponectin levels. The data for diet scores were adjusted for age, sex, transportation type and exercise. The data for exercise were adjusted for age, sex and diet score. As we reported an interaction between diet score and wGPSall and an interaction for exercise with wGPSno CDH13, we used wGPSall to identify the genetic modification of diet effect and used wGPSno CDH13 to identify the genetic modification of the exercise effect. Genetic risk was divided into low genetic risk (wGPSno CDH13 or wGPSall > mean +1SD), intermediate genetic risk (wGPSno CDH13 or wGPSall ≥ mean -1SD but ≤ mean+1SD) and high genetic risk (wGPSno CDH13 or wGPSall < mean-1SD). N.S. means the effect of exercise at low genetic risk is not significant.

adipocyte differentiation respectively, which were further related to adiponectin levels. Therefore, our study suggested that the effects of SNPs on adiponectin levels reflected the activation of specific pathways. Different from studies in adults, *CMIP*-rs2925979 (12), an SNP related to lipolysis (40), was not associated with adiponectin levels in this study. One possible explanation is that the major processes of adipose development in children are differentiation and hypertrophy, and the effect of lipolysis might be weak during puberty.

To better understand the role of puberty in the SNP-adiponectin relationship, we divided puberty into three categories based on Tanner's stages. We hypothesized that the modifying effect of puberty reflects the development of adipose tissue in children (**Figure 6**). We found that *PEPD*-rs889140, *ADIPOQ*-rs10937273, and *CDH13*-rs4783244 were significantly associated with adiponectin level both at prepuberty and in later life. Given that *PEPD*-rs889140, *ADIPOQ*-rs10937273, and *CDH13*-rs4783244 were related to collagen synthesis and adipocyte hypertrophy (41, 42), adiponectin, and adiponectin receptor T-cadherin (43, 44) respectively. Therefore, our findings suggested that adipocyte hypertrophy, the expression of both adiponectin and the adiponectin receptor are a continuous process throughout childhood. At midpuberty, the differences between sexes and the effect of *WDR11-FGFR2*-rs3943077, which is related to adipocyte differentiation (12), become significant, which supported that sex hormone levels are elevated and adipocyte differentiation is accelerated (37). At postpuberty, when the process of puberty is near its end, *ADIPOQ*-rs6773957, located in the 3'UTR of the adiponectin gene, is activated by unknown regulators.

Given that low adiponectin levels were associated with increased risk of metabolic disorders and cancers, findings of genetic and environmental factors related to adiponectin at a young age are important for early prevention and detection of these diseases. We found that both $wGPS_{all}$ and $wGPS_{no\ CDH13}$ were associated with increased adiponectin levels throughout

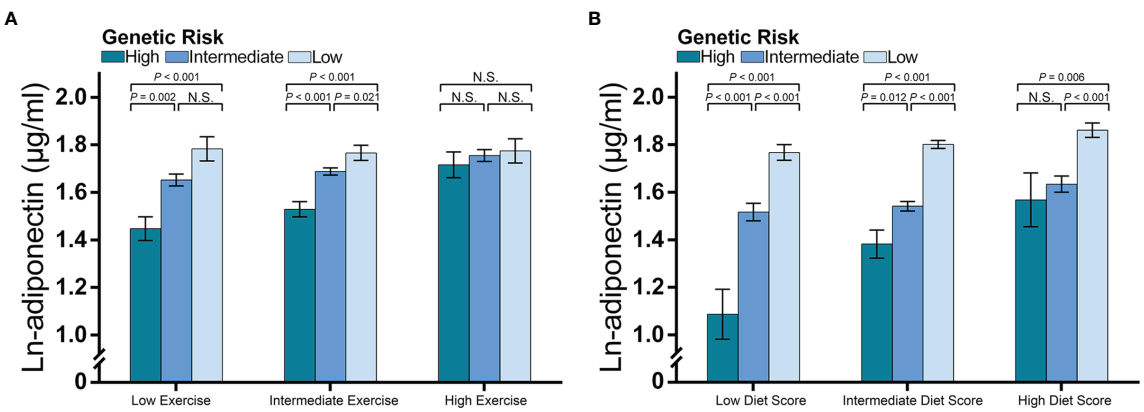

FIGURE 5 | Adiponectin levels according to genetic risk and categories of diet and exercise. The figure shows multivariable-adjusted means (histograms) and SEs (error bar) of the natural logarithm transformed adiponectin levels according to the categories of lifestyle and genetic risk for decreased adiponectin levels. The P-values are the results of an ANCOVA comparing the adiponectin levels among the genetic risk groups. Data for exercise were adjusted for age, sex, and diet score, while data for diet were adjusted for age, sex, and exercise frequency. As we reported an interaction between diet score and wGPSall and an interaction for exercise with wGPSno CDH13, we used **(A)** wGPSall to identify genetic risk categories in the diet subgroups and **(B)** used wGPSno CDH13 to identify genetic risk categories in the exercise subgroups. Genetic risk was divided into low genetic risk (wGPSno CDH13 or wGPSall > mean + 1SD), intermediate genetic risk (wGPSno CDH13 or wGPSall ≥ mean - 1SD but ≤ mean + 1SD) and high genetic risk (wGPSno CDH13 or wGPSall < mean - 1SD). Similarly, diet and exercise were divided into high (diet score> mean + 1SD; exercise frequency ≤ 2 times per week), intermediate (diet score ≥ mean - 1SD but ≤ mean + 1SD; exercise frequency = 3-4 times per week), and low (diet score < mean – 1SD; exercise frequency ≥ 5 times/week). N.S. means the difference is not significant.

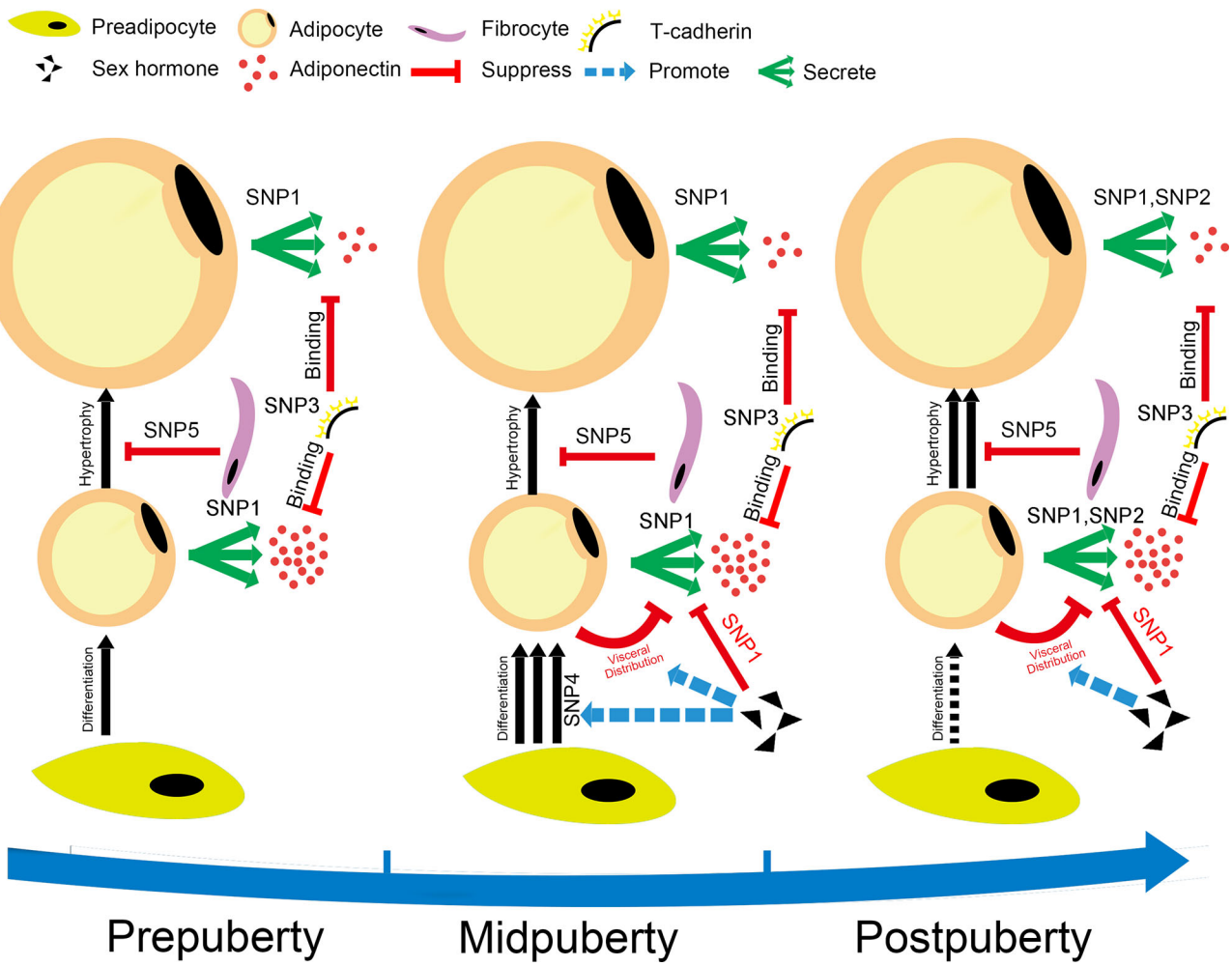

FIGURE 6 | Hypothesis for the changes in adipocyte metabolism during puberty. The figure shows our hypothesis, which is that the modification effect of puberty on the SNP-adiponectin association is based on the development of adipose tissue during puberty. The SNPs shown in this figure are SNP1: *ADIPOQ*-rs10937273; SNP2: *ADIPOQ*-rs6773957; SNP3: *CDH13*-rs4783244; SNP4: *WDR11-FGFR2*-rs3943077; and SNP5: *PEPD*-rs889140. The puberty categories that we used in the current study were as follows: prepuberty (Tanner stage I), midpuberty (Tanner stage II-III), and postpuberty (Tanner stage ≥ IV). Prepuberty is a period during which the processes of puberty have not yet been activated completely. Midpuberty is the phase during which puberty has been activated but is not finished. Postpuberty is the stage at which the processes of puberty are nearly completed, and adolescents at postpuberty are similar to adults. The development of adipose tissue includes hyperplasia, related to *WDR11-FGFR2*-rs3943077, and the hypertrophy of adipocytes. The number of adipocytes increases quickly at midpuberty but remains relatively consistent after postpuberty. The size of adipocytes increases during puberty, which makes them secrete lower amounts of adiponectin. The process of hypertrophy is suppressed by the function of collagen, which might be related to *PEPD*-rs889140. *ADIPOQ*-rs10937273 and *ADIPOQ*-rs6773957 are SNPs located at different regulation sites of the adiponectin gene. They are activated at different puberty stages. Adiponectin is bound by T-cadherin encoded by *CDH13*, which is a high-molecular-weight adiponectin receptor expressed on target cells. Sex steroids decrease adiponectin levels by taking part in the regulation of both the distribution and differentiation of adipocytes.

puberty when adjusted for age, sex, and BMI. Therefore, children with a low $wGPS_{all}$ or $wGPS_{no\ CDH13}$ should pay more attention to their lifestyle to increase the adiponectin concentration in childhood.

In line with previous studies in both adults and children (14–17, 45–48), we found that lifestyle factors, including the consumption of breakfast, meat, dairy, soft drinks, fried food, and snacks and exercise, were associated with adiponectin levels. For the first time, we report that walking to school increases adiponectin levels in school-age children. Moreover, we identified several significant gene-by-environment interactions, suggesting that lifestyle factors affect adiponectin levels by activating a specific regulatory region of a gene containing an SNP. Growing evidence indicates that it is not only energy intake from food consumption but also special components of food that link the adiponectin-diet relationship (49, 50). The negative associations of adiponectin levels with dairy, meat, and breakfast consumption might be explained by the intake of vitamin D (49). Fried food may contribute to increased adiponectin levels by supplying fatty acids for adipocyte differentiation (50). The effect of snacking on

increasing adiponectin levels might also be based on a greater intake of fatty acids. Sugar-sweetened beverage consumption has also been reported to cause an increased BMI and adipokine dysregulation, independent of energy intake (15, 16). Regarding activity factors, previous studies have shown discrepancies regarding the effects of exercise on adiponectin metabolism (48). Some studies indicate that exercise contributes to increased adiponectin levels only by causing weight loss, while others suggest that exercise itself can increase adiponectin levels independent of changes in body composition (48). Additionally, previous studies have indicated that different kinds of exercise might affect adiponectin levels through different mechanisms and that combining resistance exercise with aerobic exercise may be more beneficial (48). Similarly, we found that two activity factors, walking to school and regular exercise, were associated with increased adiponectin levels. However, the effect of exercise on adiponectin levels depended on BMI, while the influence of walking to school did not. Furthermore, we found that exercise and walking to school interacted with different SNPs significantly after adjusting for age, sex, and BMI: exercise interacted with *ADIPOQ*-rs6773957 while walking to school interacted with *WDR11-FGFR2*-rs3943077. Therefore, our results support the idea that different types of exercise affect adiponectin levels through various mechanisms (18).

In the current study, puberty also presented a strong modification effect on adiponectin-lifestyle associations. The modulation effect of puberty on adiponectin-lifestyle might also be explained by adipose tissue development during puberty. Whole-body growth is accelerated during puberty, and the effect of food consumption specifically on adipose tissue metabolism is relatively weak at midpuberty compared with the early and late puberty stage. However, activity factors are still effective methods for controlling weight at the midpuberty stage.

Our study had several strengths. The major advantages of our study include the large sample size of more than 3,400 participants and the completeness of the data, enabling us to analyze the influence of puberty on adiponectin modulation in a novel way. Previous studies addressing puberty and adiponectin have provided limited information regarding the possible mechanisms during this critical life period. The examination of the modifying impact of pubertal development on the effects of other factors led to the generation of several hypotheses regarding metabolic changes in adipocytes that warrant further investigation. Our study also had certain methodologic limitations. Firstly, because the current study was based on the BCAMS study, some detailed lifestyle information was not collected and the lifestyle indicators in this study were a little simple; for example, the components of the children's breakfast were not recorded, which made it challenging to analyze some interactions between lifestyle factors. Besides, we only collected the consumption frequency of diet while not collected the amount of consumption of each diet item, and walking to school was recorded and simply reclassified into only two modes, 'walking' and 'non-walking'. Secondly, although our 3,402-participant study cohort represents one of the largest pediatric cohorts examined to date, even a larger sample size is still required when analyzing data for different puberty subgroups. Thirdly, the ethnic background of human populations plays an important role in both genetic architecture and adiponectin levels; thus, our results cannot be directly generalized without further research in other ethnic groups. Lastly, the current study is a cross-sectional study, and it is therefore impossible to determine how the evaluated lifestyle factors will affect adiponectin levels in the future. Therefore, the examination of a larger prospective cohort with more comprehensive information is warranted to confirm the modulation effect of puberty stages.

CONCLUSIONS

In this large pediatric cohort of a Chinese population, we found that associations of adiponectin with SNPs, lifestyle factors, and gene-by-environment interactions are modified by puberty stages. Children at high genetic risk might benefit more from dietary control, and exercise. The most important periods for diet control were shown to be the early and late stages of puberty, while exercise might be more important after the onset of puberty.

DATA AVAILABILITY STATEMENT

All datasets used in the current investigation are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the ethics committee of the Capital Institute of Pediatrics and is registered at www.clinicaltrails.gov (NCT03421444). The study followed the principles of the Declaration of Helsinki. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

YW analyzed the data, drafted the manuscript. LZ and GL performed the data analyses and edited the manuscript. JF, YL, LL, LH, and QZ contributed to the BCAMS data collection. YG, XX, and LQ contributed to the data interpretation and reviewed the manuscript. SW contributed to the study design, data interpretation, and revised the manuscript. SG was responsible for the BCAMS follow-up study, contributed to the data interpretation and reviewed the manuscript. ML was responsible for the biomarker study in the BCAMS, contributed to the conception and design of the work and the acquisition and the interpretation of the data, and revised the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2021.737459/full#supplementary-material

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frontiers in Endocrinology

BRIEF RESEARCH REPORT
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Meal-Related Asprosin Serum Levels Are Affected by Insulin Resistance and Impaired Fasting Glucose in Children With Obesity

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Asprosin physiologically increases in fasting conditions and decreases with refeeding and has been implicated in glucose homeostasis. An alteration of meal-related circadian oscillation of asprosin has been suggested in adults affected by type 2 diabetes mellitus.

Aims of this study were to test the hypothesis of an alteration in the meal-related variation of asprosin levels in non-diabetic children and adolescents with obesity and to assess which metabolic variables condition this variation in non-diabetic children and adolescents with obesity. This is a cross-sectional study which included 79 children and adolescents with obesity. Children underwent clinical and biochemical assessments, including oral glucose tolerance test (OGTT), and liver ultrasound evaluation. Asprosin serum levels were measured by an enzyme-linked immunosorbent assay at a fasting state and at the 120-minute OGTT timepoint (2h-postprandial asprosin). Fasting and 2h-postprandial asprosin serum levels did not significantly differ in the entire study population (374.28 ± 77.23 vs 375.27 ± 81.26;p=0.837). 55.7% of patients had a significant increase in 2h-postprandial asprosin compared with fasting levels. The asprosin level increase condition was significantly associated with HOMA-IR (OR,1.41; 95%CI,1.005-1.977; p=0.047), fasting glycaemia (OR,1.073; 95%CI,1.009-1.141;p=0.024) and HOMA-B (OR,0.99; 95% CI,0.984-0.999; p=0.035). Moreover, the IFG condition was associated with the increase in asprosin levels (OR, 3.040; 95%CI, 1.095-8.436; p=0.033), even after adjustment for HOMA-IR, BMI SDS, sex and pubertal stage. Insulin resistance and IFG influence meal-related changes of asprosin serum levels in our study population of obese, non-diabetic, children. Alteration of asprosin circadian secretion might be an early biomarker of impaired glucose regulation in obese children with insulin resistance.

Keywords: asprosin, adipokine, glucose homeostasis, childhood obesity, insulin resistance, children

INTRODUCTION

Childhood obesity represents one of the most important health issues worldwide and is associated with an increased risk of metabolic complications, such as insulin resistance (IR) and impaired glucose regulation (IGR), including impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) (1, 2). IR has become a common feature of childhood obesity being directly related to adiposity (3). Adipose tissue is known to play an important role as an endocrine organ secreting several adipokines involved in the pathogenesis of obesity-related IR (1).

Asprosin is a recently identified adipokine, produced mainly by white adipose tissue (4), implicated in the pathophysiology of several conditions, such as obesity, insulin resistance, type 2 diabetes mellitus (T2DM), and cardiovascular diseases, by preclinical and clinical studies (5, 6).

Previous studies described asprosin involvement in glucose homeostasis as consisting of appetite regulation through orexigenic AgRP+ neurons and promotion of hepatic gluconeogenesis under fasting conditions (4, 7). Accordingly, asprosin serum levels increase in fasting conditions and decrease with refeeding in physiological conditions (4).

Several studies have documented a positive correlation between asprosin levels and HOMA-IR (8–10) while others have not confirmed this correlation (6) or have even found a negative correlation (11). Elevated serum asprosin concentrations have been documented in subjects affected by T2DM compared to healthy controls (10, 12, 13). In addition, an alteration of meal-related circadian oscillation of asprosin serum levels has been reported in T2DM patients compared to non-diabetic controls (14).

Based on these findings, although partly contrasting, asprosin seems to play a role in glucose homeostasis regulation, but no data are available on meal-related asprosin level changes in children and adolescents with obesity.

Aims of this single-center cross-sectional study were to test the hypothesis of an alteration in the meal-related variation of asprosin levels in non-diabetic children and adolescents with obesity and to assess which metabolic variables condition this variation.

MATERIALS AND METHODS

This is a single-center, cross-sectional study carried out from October 2020 to May 2021. Inclusion criteria were age between 5 and 16 years; BMI ≥ +2 standard deviation score (SDS), in accordance with definition of obesity by the World Health Organization (WHO) for children from the age of 5 years; Caucasian ethnicity; born as healthy full-term infant adequate for gestational age. Exclusion criteria were genetic and/or endocrine causes of obesity; diabetes; chronic diseases; chronic pharmacological therapies; smoking. All procedures were performed in accordance with the Declaration of Helsinki and were approved by the Ethics Committee of Messina (N.552-17/04/2019). Written informed consent was obtained from all parents or legal tutors.

Clinical and Biochemical Evaluation

At recruitment, physical evaluation was performed according to standardized procedures, including assessment of height, weight, BMI, BMI SDS, waist circumference (WC), WC-to-height ratio (WHtR), systolic and diastolic blood pressure (15). Pubertal stage was determined according to the Tanner classification (15); patients were considered in a pubertal stage from Tanner stages B2 for females and G2 for males. Children underwent fasting biochemical assessment (lipid profile, thyroid, kidney, liver function tests, and oral glucose tolerance test (OGTT)), as previously described (16). OGTT was performed according to a standard procedure (1.75 g/kg body weight, up to a maximum of 75 g) according to the American Diabetes Association (ADA) guidelines (17), with sampling at 0, + 30, +60, +90, +120 minutes for measurements of glucose and insulin. IFG was defined as fasting plasma glucose between 100 and 125 mg/dL and IGT as plasma glucose level 2 hours after a 75-g glucose load (OGTT), which was between 140 and 199 mg/dL (17). Blood samples for the serum asprosin assay were taken after fasting at the beginning of the OGTT, after at least 8 hours of overnight fasting, and at the 120-minute OGTT timepoint (2h postprandial asprosin). Asprosin serum levels were measured using an enzyme-linked immunosorbent assay (ELISA) kit accordingly to manufacturer's instructions (MyBioSource, USA; catalog number:MBS9716571). The detection threshold was 1 pg/mL and no significant cross-reactivity between human asprosin and analogues was reported; the intra-assay and inter-assay coefficient of variation (CV) values were <9% and <11%, respectively. Asprosin concentrations were expressed as pg/mL.

Homeostasis model assessment of insulin resistance (HOMA-IR), homeostasis model assessment of β-cell function (HOMA-B), Matsuda-index, insulinogenic-index (IGI) were calculated, as previously detailed (18). Areas under the curves for glucose (AUCg) and insulin (AUCi) and their ratio were also evaluated. IR was defined as HOMA-IR > 2.5 in prepubertal children and > 4 in pubertal subjects (16). Seventy-one patients underwent hepatic ultrasound (US) assessment. Diagnosis of liver steatosis was made by conventional liver US depending on the presence of at least two of the following abnormal findings: 1) diffusely increased echogenicity of the liver compared with kidney or spleen; 2) US beam attenuation; 3) poor visualization of intrahepatic structures (19, 20).

Statistical Analysis

Numerical data were expressed as mean and SDS and categorical variables as absolute frequency and percentage. The non-parametric approach was used since most numerical variables were not normally distributed, as verified by the Kolmogorov-Smirnov test. The Wilcoxon test for dependent samples was applied to identify possible significant differences between fasting and 2h-postprandial glycaemia, insulin and asprosin, both in the entire study population and in subgroups defined according to sex, pubertal stage, BMI SDS (BMI SDS ≤ or > 2.5) and the presence of IR, IFG and hepatic steatosis. To compare clinical and metabolic parameters between the two groups of patients identified according to meal-related asprosin variation the Mann-Whitney test (for numerical parameters) and the Chi Square test (for categorical variables)

were applied. The Spearman correlation test was used to assess the existence of significant interdependence between asprosin levels (both fasting and 2h-postprandial levels) and clinical and biochemical parameters; in addition, partial correlation was also estimated in order to check for sex, pubertal stage and BMI SDS. A stepwise multiple logistic regression analysis was carried out to identify which metabolic predictors significantly affect the variation of asprosin levels considering the dichotomous variable asprosin increase/non-increase between fasting and 2h-postprandial status, through the estimation of different models (Model 1: age, sex, BMI SDS, HOMA-IR, HOMA-B, IGI, Matsuda index, AUC_i/AUC_g-ratio. Model 2: age, sex, BMI SDS, fasting and 2h-postprandial glycaemia, fasting and 2h- postprandial insulin. Model 3: age, sex, BMI SDS, Triglycerides/HDL-ratio, Total Cholesterol/HDL-ratio. Model 4: age, sex, BMI SDS, Triglycerides, LDL, HDL, Total Cholesterol. Model 5: age, sex, BMI SDS, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), hepatic steatosis). In addition, further logistic regression models were estimated to assess the influence of IFG on the asprosin level increase condition, even after adjustment for HOMA-IR, BMI SDS, sex and pubertal stage (Model 1: crude model; Model 2: HOMA IR; Model 3: HOMA IR, BMI SDS; Model 4: HOMA IR, BMI SDS, sex; Model 5: HOMA IR, BMI SDS, sex, pubertal stage); in this analysis, pubertal stage, rather than age, was chosen as an independent variable because of the well-known significant implications of puberty on metabolic changes.

Statistical analyses were performed using IBM SPSS for Windows, Version 22 (Armonk, NY, IBM Corp.). A p-value < 0.05 was considered to be statistically significant.

RESULTS

Seventy-nine children (aged 11.5 ± 2.6 years) were consecutively recruited, 40 males and 39 females; 64.5% of them were in a pubertal stage. Clinical and biochemical characteristics of the study population are shown in **Table 1**. 67% had IR, 33% had IFG and 8.8% IGT. No patient was diagnosed with T2DM. 39% of patients were diagnosed with hepatic steatosis.

As expected, a significant increase in blood glucose and insulin levels was documented from fasting to 2h-timepoint of OGTT (**Table 2**). Conversely, fasting and 2h-postprandial asprosin serum levels did not significantly differ in the entire study population (**Table 2**). The same trend of asprosin levels was confirmed considering sex and pubertal stage (**Table 2**). Categorizing patients according to the presence or not of IR, no significant change was documented between fasting and 2h-postprandial asprosin levels in either subgroup, as occurred by dividing the population in relation to BMI SDS or the presence of steatosis (**Table 2**). A trend for subjects with IFG to have higher 2h-postprandial asprosin levels was demonstrated, although this finding did not reach statistical significance (**Table 2**).

In particular, contrary to the expected physiological changes in asprosin levels in relation to the meal, the majority of patients in the entire study population (55.7%) had a significant increase in 2h-postprandial asprosin compared with fasting levels (349.38 ± 35.67 *vs* 380.75 ± 83.16; p=0.000), whereas in the remaining 44.3% of patients asprosin levels decreased (405.59 ± 101.30 vs 368.38 ± 79.47; p=0.000). Patients had a significant increase in 2h-postprandial asprosin had significantly higher fasting blood glucose levels and more frequently an IFG condition compared to patients who had a significant decrease in 2h-postprandial asprosin levels (**Table 3**).

Correlation analysis documented a significant positive relation between asprosin and lipid profile parameters. In particular, after adjustment for sex, pubertal stage and BMI SDS, fasting asprosin levels were correlated to triglycerides (r= 0.243, p= 0.036) and triglycerides/HDL-ratio (r= 0.244, p=0.035), while 2h-postprandial asprosin levels were correlated to LDL (r=0.292, p=0.011), triglycerides (r= 0.250, p= 0.031) and triglycerides/HDL-ratio (r= 0.232, p= 0.045). A correlation between asprosin and BMI, BMI SDS, and glucose metabolism parameters (HOMA-IR, HOMA-B, IGI, Matsuda index, AUC_i/AUC_g-ratio, OGTT insulin and blood glucose) was not documented (data not shown).

Considering that 55.7% of patients had an unexpected increase in 2h-postprandial asprosin compared to fasting levels, a stepwise multiple logistic regression analysis was applied to assess which metabolic variables significantly affected the asprosin increase condition. Asprosin level increase condition was significantly influenced by fasting glycaemia, HOMA-IR and HOMA-B. In particular, the asprosin level increase condition was significantly positively associated with HOMA-IR and fasting glycaemia and negatively associated with HOMA-B (**Table 4**). Conversely, no significant associations were found in the models that assessed the increase in asprosin with respect to parameters of lipid metabolism (Model 3), transaminases and the presence of hepatic steatosis (Model 4) (data not shown).

Further logistic regression analysis documented a significant association between IFG and the increase in asprosin levels, even after adjustment for HOMA-IR, BMI SDS, sex, and pubertal stage (**Table 5**).

DISCUSSION

Asprosin is a glucogenic adipokine that stimulates hepatic glucose release *via* the cyclic-AMP (cAMP)-protein kinase A (PKA) pathway (4). Under physiological conditions, asprosin levels decrease after meal intake in both murine models and in humans (4).

For the first time, in our study we have demonstrated, differently than expected, no significant variation between fasting and 2-h postprandial asprosin levels in obese, non-diabetic children, suggesting an alteration in the meal-related regulation of asprosin secretion in these subjects. Furthermore, in the group of patients who had an increase in asprosin between fasting and 2-hour postprandial levels, the IFG condition was significantly more frequent than in patients who had a decrease in asprosin levels. HOMA-IR, fasting glycaemia and IFG

TABLE 1 | Clinical and biochemical features of study population.

	Mean	SDS
Age (years)	11.46	2.61
Weight SDS	2.25	0.58
Height SDS	0.48	1.18
BMI	29.49	4.33
BMI SDS	3.16	0.93
WC (cm)	89.50	10.29
WHtR	0.59	0.05
SBP (mmHg)	114.26	8.50
DBP (mmHg)	69.81	9.68
AST (U/L)	21.03	7.54
ALT (U/L)	23.82	16.28
GGT (U/L)	15.57	14.92
Total cholesterol (mg/dl)	171.92	28.08
LDL-cholesterol (mg/dl)	90.65	29.99
HDL-cholesterol (mg/dl)	53.15	16.95
Triglycerides (mg/dl)	88.24	38.97
Triglycerides/HDL-ratio	1.86	1.17
Total cholesterol/HDL-ratio	3.53	1.29
Uric acid (mg/dl)	5.04	1.19
CRP (mg/dl)	0.28	0.29
HbA1c (%)	5.28	0.33
Fasting glucose (mg/dl)	97.67	8.36
2h-postprandial glucose (mg/dl)	115.09	16.16
Fasting insulin (μUI/ml)	21.11	12.15
2h-postprandial insulin (μUI/ml)	119.98	90.82
HOMA-IR	5.17	3.15
HOMA-B	221.20	120.57
IGI	2.64	1.88
Matsuda-index	0.25	0.13
AUC_g	249.40	29.49
AUC_i	234.19	163.95
AUC_i/AUC_g ratio	0.93	0.62
FT4 (pmol/L)	15.48	2.32
TSH (uUI/ml)	2.68	2.94
Fasting Asprosin (pg/ml)	374.28	77.23
2h-postprandial Asprosin (pg/ml)	375.27	81.26

BMI, Body mass index; SDS, standard deviation score; WC, waist circumference; WHtR, WC-to-height ratio; SBP, ; systolic blood pressure; DBP, diastolic blood pressure; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, Gamma-Glutamyl Transferase; HbA1c, glycated haemoglobin; 2h-postprandial glucose, 120-minutes OGTT glucose levels; 2h-postprandial insulin, 120-minutes OGTT insulin levels; 2h-postprandial asprosin, ; 120-minutes OGTT asprosin levels; HOMA-IR, model assessment of insulin resistance; HOMA-B, homeostasis model assessment for β-cell function; IGI, insulinogenic index; AUCg, area under the curve for glucose and AUCi, insulin; FT3, free triiodothyronine; FT4, free thyroxine; TSH, thyroid stimulating hormone.

significantly influenced the asprosin increase condition in 2-h postprandial evaluation, suggesting that IR and IFG may affect the physiological meal-related variation in asprosin levels.

IR plays a central role in the relationship between obesity and the associated risk of IGR, T2DM, metabolic syndrome and cardiovascular disease (1, 16, 21). Obese children with a more significant alteration in insulin sensitivity are at greater risk of developing T2DM and cardiovascular diseases, compared with peers without IR, given the same BMI (1, 16, 21). Asprosin may be involved in the pathogenesis of IR and IGR. Romere et al. first demonstrated elevated plasma asprosin levels in humans and mice with IR, increased levels of glucose and 2h-insulin in fast mice undergoing continuous or pulsatile overexpression of asprosin, and a significant decrease in glucose and insulin levels, secondary to reduced hepatic glucose release, after immunologic or genetic asprosin action inhibition (4). Several studies have shown higher serum asprosin levels in subjects with IR and/or T2DM and a correlation between these pathological conditions and asprosin serum levels (8–10, 12–14). Wang et al. documented higher plasma asprosin levels in IGR (including IFG and IGT subjects) and newly diagnosed T2DM patients compared to subjects with normal glucose regulation (NGR), showing a significant positive correlation between asprosin and HOMA-IR and a negative correlation between asprosin and HOMA-B (10). Moreover, these authors reported higher asprosin levels in a fasting state and at all intravenous glucose tolerance test (IVGTT) samplings, in adults with IGR compared to NGR and newly diagnosed T2DM, suggesting a role of asprosin as biomarkers to predict prediabetes (10). Interestingly, an alteration of meal-dependent circadian oscillation of asprosin serum levels has been reported in T2DM adult patients compared to non-diabetic controls (14); in particular, Zhang et al. documented a non-significant decrease of 2h-postprandial serum asprosin levels during an OGTT (14).

Our study found that asprosin levels did not significantly decrease at 2h-postprandial assessment in obese non-diabetic children and that this trend was significantly influenced by the presence of IR and IFG. We speculate that in obese children and adolescents with IR and IFG there may be an altered production of asprosin which in turn may promote IR worsening by stimulating hepatic glucose secretion and subsequent hyperinsulinemia. The asprosin increase condition and, in particular, alteration of its circadian secretion, might be an early biomarker of IGR in obese children with IR.

Asprosin may also be implicated in the pathogenesis of the well-known association between IR and lipid profile alteration (22). Zhang et al. demonstrated a strong correlation between asprosin and lipid metabolism (13). Similarly, in our study, fasting and 2h-postprandial asprosin levels significantly correlated with lipid profile parameters even after adjustment for sex, pubertal stage and BMI SDS.

Sample size represented a limitation of our study. For ethical reasons, it was not possible to have healthy children undergo an OGTT to perform the same assessments conducted on the study population. Data on liver ultrasound were available for 71 patients as 8 patients discontinued follow-up before undergoing ultrasound. Due to the cross-sectional design of the study, it is not possible to generalize the findings of the present study. On the other hand, our study has significant strengths. First, our study population consisted in a homogeneous sample of obese Caucasian children with equal distribution according to sex. Second, the assessment of both fasting and meal-related asprosin levels through standardizing the evaluations by performing an OGTT. This type of assessment, compared with a random asprosin measurement, allowed us to verify an alteration of the physiological meal-related fluctuation of asprosin levels in a pediatric population with obesity. The assay standardization is useful in the interpretation of results as it partly compensates for the lack of plasma reference values for asprosin.

In conclusion, this is the first study to demonstrate a non-significant variation between fasting and 2h-postprandial

TABLE 2 | Variations in blood glucose, insulin and asprosin between fasting and 2-h postprandial levels.

Subgroup (N° of patients)	Blood glucose			Insulin			Asprosin		
	T0	T120	p	T0	T120	p	T0	T120	p
Entire study population(79)	97.67 ± 8.36	115.08 ± 16.158	0.000	21.11 ± 12.15	119.97 ± 90.82	0.000	374.28 ± 77.23	375.27 ± 81.26	0.837
Male (40)	97.35 ± 7.52	116.85 ± 16.02	0.000	19.26 ± 11.59	118.97 ± 98.32	0.000	375.03 ± 80.86	370.27 ± 70.11	0.840
Female (39)	98 ± 9.23	113.28 ± 16.3	0.000	23.00 ± 12.56	121.01 ± 83.72	0.000	373.52 ± 74.37	380.41± 91.96	0.548
Prepubertal (28)	96 ± 8.19	119.21± 16.25	0.000	15.26 ± 8.20	119.51 ± 88.41	0.000	408.82 ± 111.14	395.04 ± 84.09	0.374
Pubertal (51)	98.59 ± 8.39	112.82 ± 15.81	0.000	24.32 ± 12.82	120.23 ± 92.99	0.000	355.33 ± 40.0	364.42 ± 78.39	0.265
IR (53)	99.56 ± 7.63	117.39 ± 15.29	0.000	26.26 ± 11.47	138.77 ± 102.37	0.000	382.37 ± 89.01	383.07 ± 94.54	0.968
Non-IR (26)	93.81 ± 8.59	110.38 ± 17.13	0.000	10.58 ± 3.89	81.65 ± 40.75	0.000	357.79 ± 41.34	359.37 ± 40.23	0.568
Steatosis (31)	97.9 ± 7.97	115.9 ± 15.98	0.000	24.65 ± 13.43	131.06 ± 102.88	0.000	368.55 ± 80.95	366.09 ± 68.18	0.984
Non-steatosis (40)	97.5 ± 8.68	116.03 ± 16.88	0.000	17.95 ± 10.68	108.29 ± 75.84	0.000	377 ± 79.92	379.79 ± 90.34	0.819
BMI SDS >2.5 (65)	97.05 ± 8.55	115.52 ± 16.83	0.000	21.16 ± 12.37	118.13 ± 89.86	0.000	378.93 ± 83.74	378.81 ± 86.12	0.959
BMI SDS ≤2.5 (14)	100.57 ± 6.93	113.07 ± 12.89	0.000	20.85 ± 11.48	128.52 ± 98.16	0.000	352.70 ± 25.56	358.81 ± 52.4	0.551
IFG (26)	107.15 ± 5.70	119.34 ± 15.3	0.000	27.38 ± 12.13	136.05 ± 84.23	0.000	361.12 ± 39.23	382.91 ± 101.32	0.078
Non-IFG (53)	93.02 ± 4.71	113 ± 16.29	0.000	18.03 ± 11.01	112.09 ± 93.64	0.000	380.74 ± 89.8	371.52 ± 70.19	0.383

T0, Fasting time point of OGTT; T120, two-hour time point of OGTT. BMI, Body mass index; SDS, standard deviation score; IR, insulin resistance; IFG, impaired fasting glucose.

TABLE 3 | Comparison analysis between groups identified according to meal-related asprosin levels variation.

	Asprosin increase (N = 44 patients)	Asprosin decrease (N = 35 patients)	p value
Age (years)	11.90 ± 2.46	10.91 ± 2.72	0.11
Sex (M/F)	22/22	18/17	0.90
Pubertal stage (prepubertal/pubertal)	13/31	15/20	0.22
BMI	29.91 ± 4.04	28.98 ± 4.67	0.19
BMI SDS	3.08 ± 0.77	3.27 ± 1.09	0.47
WHtR	0.60 ± 0.06	0.59 ± 0.06	0.90
SBP (mmHg)	114.85 ± 8.12	113.48 ± 9.05	0.35
DBP (mmHg)	69.93 ± 9.10	69.65 ± 10.55	0.43
AST (U/L)	21.14 ± 8.70	20.89 ± 5.89	0.63
ALT (U/L)	25.25 ± 18.66	22.03 ± 12.73	0.66
GGT (U/L)	16.59 ± 19.89	14.37 ± 5.06	0.40
Total cholesterol (mg/dl)	171.20 ± 29.08	172.83 ± 27.16	0.89
LDL-cholesterol (mg/dl)	90.59 ± 30.99	90.73 ± 29.17	0.82
HDL-cholesterol (mg/dl)	52.63 ± 18.10	53.80 ± 15.65	0.75
Triglycerides (mg/dl)	88.29 ± 40.56	88.17 ± 37.46	0.77
Triglycerides/HDL-ratio	1.88 ± 1.26	1.82 ± 1.08	0.93
Total cholesterol/HDL-ratio	3.60 ± 1.52	3.43 ± 0.97	0.96
Uric acid (mg/dl)	5.16 ± 1.18	4.89 ± 1.21	0.22
HbA1c (%)	5.28 ± 0.32	5.29 ± 0.34	0.74
Fasting glucose (mg/dl)	99.25 ± 8.78	95.69 ± 7.46	0.04
2h-postprandial glucose (mg/dl)	112.98 ± 15.26	117.74 ± 17.07	0.23
Fasting insulin (μUI/ml)	21.59 ± 11.56	20.49 ± 12.99	0.41
2h-postprandial insulin (μUI/ml)	116.08 ± 81.19	124.88 ± 102.67	0.74
HOMA-IR	5.39 ± 3.18	4.88 ± 3.15	0.34
HOMA-B	215.32 ± 101.10	228.58 ± 142.54	0.85
IGI	2.79 ± 1.68	2.47 ± 2.11	0.24
Matsuda-index	0.24 ± 0.13	0.26 ± 0.14	0.55
AUC_i/AUC_g ratio	0.91 ± 0.44	0.94 ± 0.79	0.50
IR (yes/no)	28/16	25/10	0.46
IFG (yes/no)	19/25	7/28	0.03
Steatosis (yes/no)*	17/23	14/17	0.82

Numerical data are expressed as mean ± standard deviation score. Categorical variables (sex, pubertal stage, IR, IFG, steatosis) are expressed as number of patients.

"Asprosin increase" group identified patients had a significant increase in 2h-postprandial asprosin compared with fasting levels. "Asprosin decrease" group identified patients had not a significant increase in 2h-postprandial asprosin compared with fasting levels.

**The presence of steatosis was evaluated in 71 patients.*

BMI, Body mass index; WHtR, WC-to-height ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, Gamma-Glutamyl Transferase; HbA1c, glycated haemoglobin; 2h-postprandial glucose, 120-minutes OGTT glucose levels; 2h-postprandial insulin, 120-minutes OGTT insulin levels homeostasis; HOMA-IR, model assessment of insulin resistance; HOMA-B, homeostasis model assessment for β-cell function; IGI, insulinogenic index; AUCg, area under the curve for glucose; and AUCi, insulin; IR, insulin resistance; IFG, impaired fasting glucose.

TABLE 4 | Stepwise multiple logistic regression analysis of variables affecting serum asprosin levels.

Model 1: age, sex, BMI SDS, HOMA-IR, HOMA-B, IGI, Matsuda index, AUC_i/AUC_g-ratio					
	B	**P**	**OR**	**95%**	**CI**
HOMA-IR	0.34	0.047	1.41	1.005	1.977
HOMA-B	-0.01	0.035	0.99	0.984	0.999
Model 2: age, sex, BMI SDS, fasting and 2-hpostprandial glycaemia, fasting and 2-h postprandial insulin					
	B	**P**	**OR**	**95%**	**CI**
Fasting glycaemia	0.07	0.02	1.073	1.009	1.141

Dependent variable: asprosin increase/non-increase between 0 and 120 minutes OGTT time point evaluations.
BMI SDS, Body mass index standard deviation score; postprandial glucose, 120-minutes OGTT glucose levels; postprandial insulin, 120-minutes OGTT insulin level homeostasis; HOMA-IR, model assessment of insulin resistance; HOMA-B, homeostasis model assessment for β-cell function; IGI, insulinogenic index; AUCg, area under the curve for glucose; and AUCi, insulin; CI, Confidence interval.

TABLE 5 | OR and 95% Confidence interval (CI) for impaired fasting glucose according to asprosin increase.

Models	OR (95%CI)	*P value*
1	3.040 (1.095 8.436)	0.033
2	3.325 (1.049 10.545)	0.041
3	3.287 (1.033 10.458)	0.044
4	3.286 (1.033 10.457)	0.044
5	3.211 (1.006 10.249)	0.048

Dependent variable: asprosin increase between 0 and 120 minutes OGTT time point evaluations. Model 1: crude model; Model 2: HOMA IR; Model 3: HOMA IR, BMI SDS; Model 4: HOMA IR, BMI SDS, sex; Model 5: HOMA IR, BMI SDS, sex, pubertal stage.

asprosin levels in obese, non-diabetic children and to document an influence of IR, fasting glycaemia and IFG on meal-related changes of asprosin serum levels. The alteration of asprosin circadian secretion might be an early biomarker of IGR in obese children with IR. Further studies on a larger study population are needed to confirm and verify these findings.

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 ethics committee of Messina. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

DC and MW contribute to conception and design of the study. DC, TA, GP, SC, and AL organized the database and prepared the tables. RI and MC performed asprosin measurements. AA performed statistical analysis. DC and MW wrote the first draft of the manuscript. MW and RI supervised the work. DC and MW wrote the final version of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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Effects of Overweight/Obesity on Motor Performance in Children: A Systematic Review

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Childhood obesity is a serious public health problem. Childhood obesity and overweight are associated with the appearance of coordination deficit disorder and can cause impaired motor performance. We searched online databases for all related articles using comprehensive international databases from the Medline PubMed Institute, Web of Science, ScienceDirect, SCOPUS, and PsycINFO up to December 20, 2020. Overall, 33 studies were included in this systematic review. The present review demonstrated that children with higher percentage of body fat had lower levels of moderate to vigorous physical activity, as well as decreased levels of gross motor coordination, as shown by tests for neuromuscular performance. These results corroborate the hypothesis that overweight and obesity in children and adolescents are associated, not only with insufficient performance during gross motor coordination activities, but also with a greater risk to physical health.

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INTRODUCTION

Childhood overweight and obesity are one of the greatest public health challenges worldwide. The World Health Organization estimates that approximately 70 million children will be overweight or obese by 2025, as children below 5 years old have shown a rapid increase in the development of overweight and obesity in recent years (1). Childhood is a critical period for the development of overweight and obesity. Increased consumption of unhealthy sugar, sodium, and fats, in addition to ultra-processed foods, including sugar-sweetened beverages and high-energy, nutrient-poor packaged foods have been strongly associated with weight gain and several nutrition-related non-

communicable diseases (2). The high rate of obesity is associated with an increase in the development of some disease conditions such as systemic arterial hypertension (3), insulin resistance (4), and stroke (5). In addition to these conditions, obesity can affect physical parameters such as motor performance and gross motor coordination, as they seem to be directly related to regular physical activity and body composition in children and adolescents (6).

Motor coordination corresponds to the congruous interactions between the nervous, skeletal, and sensory muscle systems, in order to produce precise motor actions, in addition to quick reactions to everyday situations, which involves proper development of muscle strength and the proper selection of muscles that control the performance of the movement (7). Notably, motor performance in childhood and adolescence may be related to the programming of physiological systems in adult life (8, 9).

Motor competence, on the other hand, is the ability to perform different motor actions, including coordination and gross motor skills (10). Gross motor competence is often defined as proficiency in a range of fundamental movement skills such as throwing, catching, and running, which are normally learned during preschool and early school years (11, 12). These provide a basis for children to develop more in specialized movement sequences, such those required in sports activities (13).

A growing body of studies have investigated the possible relationship between gross motor coordination and the level of adherence to participation in physical activity during adolescence. Most studies found a positive association between better performance in gross motor coordination and participation in physical activities (14, 15).

It is possible that children and adolescents with poor gross motor skills may not want to participate in physical activity, because it can be more challenging for. It is also plausible that among children with poor gross motor skills, sedentary activities (i.e., watching TV and computing games) may be more enjoyable options.

The muscle is characterized by plasticity and, therefore, is more likely to change its structure and function. In animals, accumulation of intramuscular fat caused stiffness in the muscle tissue, which caused less contractility and decreased strength in the gastrocnemius muscle (16). In humans, a longitudinal study carried out on growth and physical fitness related to health and motor competence in elementary school children showed that the pathways for the development of physical and motor fitness are related to the children's body weight. Children who had a low or medium rate of development of physical fitness and motor competence were more likely to develop overweight or obesity at the end of primary school, regardless of sex and body mass index at baseline (17).

In this context, it is necessary to clarify how environmental factors can influence the appearance of overweight and obesity; in addition, it is necessary to understand the relationship between overweight and obesity and motor performance in childhood (18–20). Core motor tasks include bilateral and upper limb coordination, strength, balance, speed, and running agility.

Motor skills are acquired from the physiological maturation of the neuromuscular system and environmental factors (21) and correspond to a group of coordinated movements that children begin to learn during early childhood and involve locomotor skills and object control. Locomotor skills are used to move the body through space, such as running, galloping, and jumping. The object control task is the ability to manipulate and project objects such as throwing, catching, dribbling, kicking, hitting, and rolling (22).

Although the genetic and biological determinants of obesity can interact throughout life, the process that regulates the developmental trajectories of other potentially important behavioral factors linked to the status of body weight has not been investigated.

Another aspect to be noted is that few studies have explored the contribution of current body composition to motor performance of the research participants. Understanding the relationship between overweight and obesity and children's physical activity can guide the development of interventions at different levels that may provide a better chance of increasing the levels of physical activity in the population. Therefore, the objectives of this study were to analyze the influence of overweight and/or obesity on motor performance and gross motor coordination in children and adolescents.

METHODS AND MATERIALS

The protocol for this systematic review been published online (https://www.crd.york.ac.uk/prospero/) in PROSPERO (registration number CRD42020182935) and was reported as per Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) (12).

Search Strategy

This review was conducted in two phases, which included selection of studies followed by data extraction. Studies were selected from the search in the electronic databases Medline/PubMed (National Library of Medicine/Analysis of Medical Literature and Online Recovery System), Web of Science, ScienceDirect, SCOPUS, and PsycINFO, which was carried out in December 20, 2020. The following MeSH terms in Medline, PubMed, and DeCS in other databases were used as search filters: "obesity"; "pediatric obesity"; "metabolic syndrome"; "nutritional and metabolic diseases"; "body mass index"; and "motor skills".

Selection of Studies

Selection of studies was performed independently by WB and RS, according to the following inclusion criteria: (a) original articles addressing metabolic changes related to motor skills; (b) studies assessing individuals aged between 5 years old and 14 years and 11 months old; (c) studies with control and experimental groups (overweight and/or obesity); and (d) articles with a sample size of

less than 30 individuals. No language or period of publication was set. However, a search filter was activated for viewing studies performed only in humans. The following PICOS criteria were established: Population: children and adolescents; Intervention/exposure: motor training; Comparison: between sexes; Results: overweight/obesity, motor coordination; Study design: cross-sectional and longitudinal studies. Initially, the studies were pre-selected according to titles and abstracts. In the next stage of the study selection phase and after excluding duplicate articles, texts considered eligible were read in their entirety.

Data were collected from the selected studies based on the characteristics of the studies, the results, and the components used to assess the intervening factors were verified. For the qualitative synthesis of the data, the following characteristics of the studies were used: author's name, year of publication, country, age variation, sex, nutritional status, total population, analyzed variables, body composition, and motor performance results.

Data Extraction

Selected abstracts were submitted to the second stage of analysis, in which two independent researchers reviewed the articles completely and, by consensus, excluded articles that did not meet the criteria. The following data from eligible articles were extracted: characteristics of the sample (mean age, distribution between sexes, and nutritional status), materials and methods (analyzed variables), and the main results found related to body composition and motor performance. The data extracted from the articles were collected using a standardized method among the authors. It was not possible to perform a meta-analysis in the present study, since there was substantial sample heterogeneity, in addition to the variability in the age range of the population of the studies, which could hinder the reliability of a meta-analysis.

Risk of Bias

The risk of bias was established through of a critical analysis of the studies selected using seven criteria for a methodological judgment supplied by the software Revman 5.3.0 program the Cochrane Handbook 23, developed for systematic reviews and available for free download (https://training.cochrane.org/online-learning/core-software-cochrane-reviews/revman/revman-5-download). Among the criteria that structure the bias assessment are (1) random sequence generation, (2) allocation concealment, (3) blinding of participants and personnel, (4) blinding of outcome assessment, (5) incomplete outcome data, (6) selective reporting, and (7) other bias.

RESULTS

Study Selection

A total of 388 studies were identified in the literature search. Two duplicates were found. Of these 386 studies, 38 met the inclusion criteria based on the title and abstract. Finally, 33 studies (**Figure 1**) were included in this review.

Description of Included Studies

Among the main findings of this review, 1 of the 30 selected articles included children aged between 5 and 7 years old (23), 15 assessed children with ages between 7 and 14 years old (20, 24–40), 6 articles included children between 6 and 10 years old (41–46), and 5 included children in other age groups (47–51). All studies were conducted with children of both sexes (20, 23–50, 52–55) (**Table 1**).

Risk of Assessment

No studies with low risk of bias were excluded. The results are shown in **Figures 2** and **3**.

Nutritional Status and Age Group

The classification of the relationship between nutritional status and age group was heterogeneous among the selected articles. An article that included children between 5 and 7 years old found that most participants had normal nutritional status (23). In children between 7 and 14 years old, two articles reported an inverse association between BMI and motor coordination (27). In another article, children who ate breakfast almost every day had better functional motor skills and a lower BMI than children who did not eat breakfast regularly (38). Overweight was more prevalent in three articles (20, 24, 32), overweight and obesity in three articles (33, 35, 40), normal and overweight in one article (34), normal and obesity in one article (37), and obesity in one article (30), and in most studies, participants were classified as having normal weight (25, 26, 28, 31, 36, 39). In children between 6 and 10 years old, our analysis revealed a higher prevalence of normal weight (41, 44–46), while in two studies, children were classified as overweight and obesity (42, 43). Other articles had a different age range from those already presented. A study of children 5 and 10 years old found that 21.7% of children had obesity at 5 years, and at 10 years old, 22.9% were overweight (47). Another study with children 5–12.8 years old found that the majority of the population studied was eutrophic (50), whereas in another, the majority had overweight and obesity (47); in one study, 1,526 out of 5,138 children evaluated had high BMI (48).

This systematic review investigated the characteristics of body composition and motor performance in children, without orthopedic or neurological changes, notably related to gross motor coordination with or without exposure to physical activity. The results of analysis, specifically the main characteristics of the included studies, were organized according to the correlation between body composition and motor performance (**Table 1**).

Body Composition Related to the Motor Performance of Children and Adolescents

Based on the theory of developmental plasticity, overweight and/or obesity in children and adolescents can interfere with motor performance, alter postural control, and, consequently, modify the state of motor coordination of these individuals. Taking this into account, six included studies assessed the research participants' motor performance using running speed and agility tests such as the six-minute running test, TUDS (timed ascent and descent test), and other explosion tests (28, 34, 35, 37, 43, 54). In one of these studies, the authors found a relationship between an increase in the percentage of body fat and a decrease in the levels of moderate to vigorous physical activity (43).

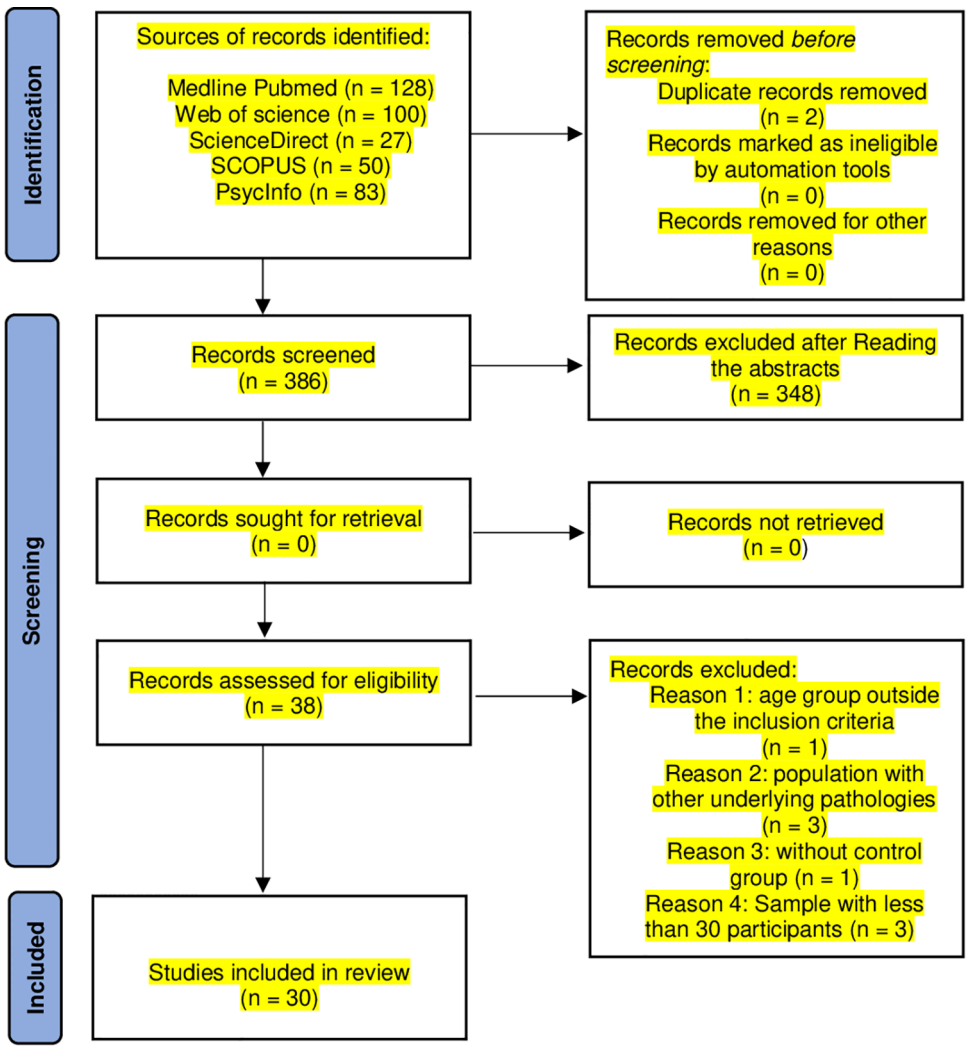

FIGURE 1 | A flow diagram of the selection strategy.

Another study observed a decrease in the levels of static strength and explosive power in girls 7–11 years old with obesity, as well as in boys 10–11 years old with obesity (54). Balance and muscle strength power represent important components related to the ability of physical fitness that have to be sufficiently developed throughout life to perform sports and daily activities to decrease the risk of injuries and falls (56). Furthermore, Tsiros et al. (28) found a decrease in motor performance in children with obesity during the TUG (timed up and go), 6MWT (six-minute walk test), and TUDS tests. Other studies found that using explosion motor performance tests, overweight children had worse performance in the long jump and 10- and 20-m sprints; in addition, individuals with an increased percentage of body fat showed lower indexes in the long jump and repetition during sit-ups, in addition to a deficit in perceived physical capacity (34, 35).

Prevalence of overweight and obesity associated with the levels of physical fitness among primary school age children in Assiut city CPA (Checklist of Psychomotor Activities), KTK (Body coordination test for children: Koërper Koordination Test für Kinder), MABC (Movement Assessment Battery Test for Children), and BOTMP-SF (Bruininks–Oseretsky Test of Motor Proficiency—Short Form) was investigated. Three studies used MABC to assess global motor coordination and balance (26, 31, 50) in a population of 540, 2,029, and 2,057 children, respectively. Another four found a greater propensity to develop deficit of coordination in children with greater accumulation of body fat, BMI, and obesity, successively (32, 42, 47, 53). However, most studies used KTK to assess gross motor coordination.

In this review, worse performance of gross motor coordination in children with obesity was observed (27, 41, 44–46, 48, 49, 55). One study investigated only the participants' balance and found a decrease in balance skills with increasing body mass (52). Furthermore, overweight was negatively associated with lower overall performance of

TABLE 1 | Descriptions of the studies included in the systematic review: age, sex, and nutritional status.

Author, year	Age range	Sex		Nutritional status			
		Female	Male	Malnutrition	Eutrophic	Overweight	Obesity
(23)	5 years	324	370	59 (23 girls) moderate: 41 (22 girls)	410 (200 girls)	92 (45 girls)	60 (17 girls)
(41)	6.5–7.2 years	369	412	8.1% (*n* = 63)	75.9% (*n* = 593)	8.8% (*n* = 69)	7.2% (*n* = 56)
(42)	6–10 years	69	86	–	–	27.1% girls 16.3% boys	12.9% girls 14.0% boys
(47)	Data performed at two different ages: 5 and 10 years	307	361	–	–	5 years: 20.4% 10 years: 22.9%	5 years: 21.7% 10years: 18.1%
(48)	6–14 years	2,787	2,351	–	–	Subjects with high BMI: 1,526	
(43)	6–8 years	204	200	–	–	14.7% girls 11% boys	
(54)	7–11 years	155	178	–	205	72	54
(24)	8–11 years (Three years of intervention)	108 (3rd year) 108 (5th year)	123 (3rd year) 126 (5th year)	–	–	3rd year: 23.4% girls and 23.5% boys 5th year: 21.4% girls and 28.9% boys	3rd year:10.6% girls and 13.9% boys 5th year: 4.1% girls 3.9% boys
(49)	6–14 years	657 (51.5%)	619 (48.5%)	–	–	20.70% girls 17.69% boys	5.02% girls 7.47% boys
(26)	9–12 years	Typical development:456 Disorders of motor coordination and balance: 93 Disorders of motor coordination and balance: 186	631 85 143	– – –	70.0% 61.8% 66.6%	22.4% 23.0% 24.3%	7.5% 15.2% 9.1%
(27)	5–13 years (1st evaluation) 7–13 years (2nd evaluation)	1st evaluation: 1.188 2nd evaluation: 371	1.329 383	Longitudinal study: significant inverse associations within the follow-up subsample participants between *z* scores of BMI and KTK MQ at each point in time (i.e., baseline and follow-up) as well as over the 2-year course			
(20)	10 and 14 years (accompaniment)	318	348	–	10 years: 507 14 years: 486	116 126	43 54
(28)	10–13 years	107	132	–	132 (56 girls)	–	107 (51 girls)
(29)	9–13 years	268	322	Children with coordination disorder: ↑ BMI scores			
(44)	6–10 years	48%	52%	–	50	42	8
(30)	7–10 years	89	64	–	–	35	118 (65 girls)
(31)	9–10 years	951	1078	–	1,154 (577 girls)	434 (230 girls)	441 (144 girls)
(32)	9–11 years	1st wave: 1,120 2nd wave: 1,094 3rd wave: 1,094 4th wave: 1,032 5th wave: 1,032	1,158 1,133 1,133 1,054 1,059	–	–	30.1% 31.2% 29.6% 32.1% 32.3%	9.7% 11.0% 10.0% 10.5% 9.8%
(33)	11– 14 years	120	140	–	103 (49 girls)	86 (40 girls)	71 (31 girls)
(34)	8–10 years	105	105	–	105 (52 girls)	105 (53 girls)	–
(45)	Started: 6.8 ± 0,4 years	301	314	7.5%	77.8%	8.1%	6.6%

(Continued)

TABLE 1 | Continued

Author, year	Age range	Sex		Nutritional status			
		Female	Male	Malnutrition	Eutrophic	Overweight	Obesity
(50)	5–12.8 years	268	272	–	273	202	65
(53)	9–14 years	268	322	–	–	90 (overweight/obese)	
(46)	6.70 ± 0,42 years	278	280	8.1%	78.1%	8.1%	5.7%
(52)	6–11 years	335	341	04.28% (*n* = 29)	68.77% (*n* = 465)	11.24% (*n* = 76)	12.28% (*n* = 83)
(36)	10.4 ± 0.6 years	42.7%	57.3%	–	177	36 (overweight/obese)	
(55)	9–12 years	281	315	BMI only high than> 19.9			
(38)	7–10 years	343	313	Children who eat breakfast almost every day have better functional motor skills and a lower BMI than children who do not regularly eat breakfast			
(39)	7–10 years	198	182	Thinness: 4 High thinness: 2	325	35	Obesity: 10 Severe obesity: 4
(40)	7–14 years	3,294	3,623	–	–	23.2% (overweight/obese)	

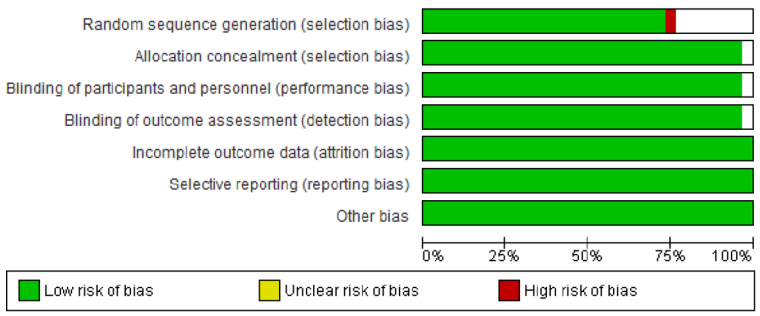

FIGURE 2 | Risk of bias graph: review of authors' judgements about each risk of bias item presented as percentages across all included studies.

movements (24), while children with obesity had mild motor difficulties (20); overweight and obesity were related to less perceived and real physical competence (33), in addition to lower performance in side jumping, standing long jump, 20-m speed back-and-forth running (38), and decreased motor skills (40). Notably, a study including 380 children revealed that the association between nutritional status and motor classification in boys and girls was not significant, which, according to the authors, neutralizes any influence of nutritional status on motor classification (39) (**Table 2**).

DISCUSSION

Overall, the results of this review confirmed the hypothesis that overweight and obesity can negatively affect motor performance and gross motor coordination in children and adolescents, although age, nutritional status, and the measures of motor performance analyzed were different among the investigated studies.

It is well recognized that motor performance in some tests is negatively affected by higher body weight (23, 53). In analyzing the magnitude of the relationships between gross motor coordination, physical activity, and physical conditioning, weight was strongly associated with age and sex in gross motor coordination tests (57, 58). A meta-analysis showed that age was positively associated with locomotion, object control, and stability skills. It is not surprising that the older children are, the better their skills, as long as they continue to participate in activities that develop these skills. Motor development in young children is influenced by biological maturation, and after this period, it depends more on practice and opportunity. Thus, it is conceivable that the relationship between age and gross motor competence may change over the developmental periods of early childhood, preschool, childhood, and adolescence. Notably, although primary evidence confirms age as a positive correlate in most aspects of motor competence, some studies (across all types of motor competence) have not found this relationship (59). One study that found age to be a negative correlate involved

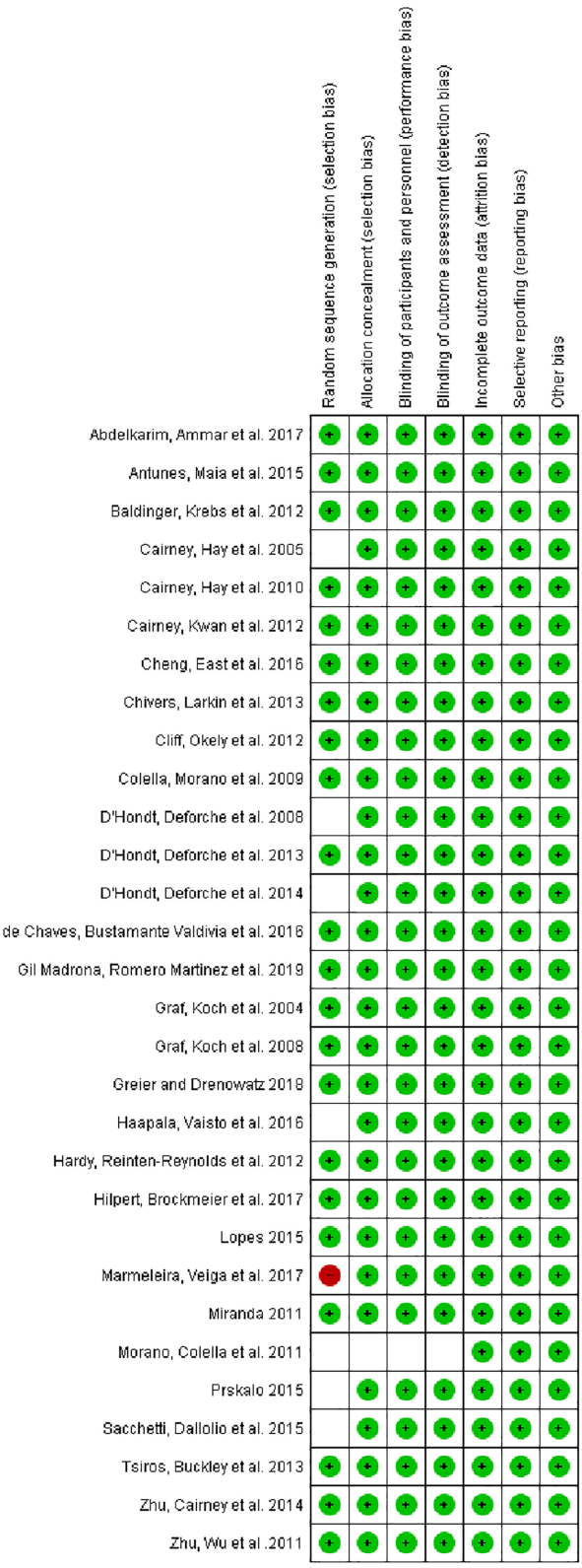

FIGURE 3 | Risk of bias summary: review of authors' judgements about each risk of bias item for each included study.

TABLE 2 | Descriptions of the studies included in the systematic review.

Author, Year	Country	Total population	Variables analyzed (Tests)	Results of body composition and motor performance
(23)	Spain	694 children	BMI (body weight and stature); CPA	GIRLS: Laterality: ↓ Childhood with malnutrition; dynamic coordination: ↓ Obesity Childhood; ↑ BMI worse results in motor execution; tonic postural control: ↓ Childhood with overweight/ obesity; balance: ↑ Childhood with low weight; ↑ BMI ↓ respiratory control; visual-motor coordination between normal weight and Obesity/overweight childhood: ↑ Childhood with normal weight; spatial orientation: ↑ Childhood with normal weight *versus* overweight. BOYS: ↑ BMI ↑ Laterality; respiratory control ↓ and visual-motor coordination ↑ in Obesity/overweight childhood.
(41)	Germany	997 children	Height, body weight, BMI, SES, migratory background, motor skills: KTK and 6-min run, questionnaire on levels of daily and leisure activity, determination of sedentary activities	SES group ↓: ↑% obesity childhood, followed by the medium SES group and the high; obesity childhood group: ↑ migration history; overweight and obesity: ↓ gross motor development and resilience; how much ↑ SES, ↑ gross motor development; ↑ socioeconomic level, ↓ BMI and boys ↑ engine testing performance↑ computer/video game time: ↑ probability highest level of sports activity; history of migration: ↓ probability of participating in organized sports or being physically active at leisure
(42)	Portugal	156 children	%GM e IMC (dobras cutâneas, peso e altura); teste Bruininks-Oseretsky de Proficiência Motora - Forma Curta	↑ Cardiovascular disease risk: 27.5% girls and 24.4% boys excess body weight: 40% girls and 30.3% boys obesity childhood: ↓ gross motor skills and general motor proficiency;
(47)	Chile	668 children	BMI (height and weight); Motor skills: BOTMP-SF test	5 years: 20.4% overweight and 21.7% obesity; 10 years: 22.9% overweight and 18.1% obesity. Boys: ↑ total motor scores. Obesity childhood: ↓ gross and total motor skills (5 and 10 years) 5 obesity childhood years: ↓ performance in fine motor precision task (drawing lines). Childhood with obesity: ↓ motor skills from 5 to 10 years; ↓ motor proficiency at 5 years did not predict obesity or ↑ BMI. Overweight at 5 years was not enough to produce ↓ motor skills from 5 to 10 years; ↓ motor skill was associated with being overweight at 5 years
(48)	Peru	5193 adolescents sea level, *n* = 1299 altitude, *n* = 1292 jungle, *n* = 2602	BMI (height and weight); gross motor coordination: KTK; Physical fitness: Four EUROFIT battery tests (static and explosive muscle strength, flexibility, and speed/agility), abdominal muscle resistance of the Fitness gram battery and cardiorespiratory resistance of the American Alliance for Health, Physical Education, Recreation and Dance test battery; peak growth speed	Height, weight, and all motor performance test: ↑ with age except for sitting and reaching the boys outperform the girls in all tests. Girls: have 5 times + chances of ↓ gross motor coordination ↑ gross motor deficit with ↑ age; more mature girls and children: ↓ prone coordination deficits; ↑ BMI: ↑ prone to gross motor deficit. Children living at sea level or altitude: ↑ prone to gross motor deficit↑ flexible and ↑ strength: ↓ the probability of being diagnosed with deficit of gross motor coordination.
(43)	Finland	512 children	Fat body mass,% body fat, and lean mass; weight and height; physical activity: heart rate and movement sensor, PANIC Physical Activity questionnaire; 50-m shuttle test: running speed and agility; 15-m running test; Martin vigorimeter: handgrip strength; test of standing distance jump: explosive strength of the lower limbs; abdominal test; modified flamingo balance test; box and block test: manual dexterity and speed of movement of the upper limb; sit and reach test: flexibility of the lumbar and hamstring muscles; pubertal status.	Boys: more active, ↓ fat mass and% body fat, ↓ 50-m run time and 15-m run test time, ↑ absolute handgrip power, ↑ jump test standing jump, ↑ test errors balance of the modified flamingo, ↓ cubes moved in the box and block test and ↓ distance achieved in the sit and reach test. Children ↑% body fat and levels ↓ moderate to vigorous physical activity: ↓ neuromuscular performance running and jumping tests. Children ↑ body fat content and ↑ MVPA levels: surpass overweight and ↓ children active in the 15-m sprint and the long jump test. Children ↑% of body fat and levels ↓ of physical activity: ↓ neuromuscular performance
(54)	Croatia	333 children	Motor skills: polygon back - coordination, forward bending on a bench - flexibility, 15 '' manual touch - simple movement speed, long jump - explosive leg strength, flexed and static arm strength, abdominals - repetitive strength and high jump - MMII explosive force; % body fat (sum of subscapular skinfolds and triceps); body fat and fat-free mass; BMI (weight and height).	Obesity girls: between 9% and 13%. Obesity boys: range from 17% with age.7–9 years and 23% 10–11 years. Boys 7–9 years: N/S in motor skills when classified according to body weight. Boys 10–11 years old eutrophic: ↑ coordination, static, explosive and repetitive force. Girls 7–9 years old eutrophic: ↑ static strength and explosive power. Girls 10–11 years old eutrophic: ↑ static strength, explosive power, and coordination

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TABLE 2 | Continued

Author, Year	Country	Total population	Variables analyzed (Tests)	Results of body composition and motor performance
(24)	Italy	231 children	Anthropometric measurements (height, weight, BMI) and motor skills: Sit & Reach test, Forward Roll Test, Forward Throw 2 kg Medicine-ball test, long jump test, 20-m running speed test.	Beginning of the study: 35.8% of children ↑ weight (23.4% overweight childhood; 12.4% obesity childhood); after intervention: ↓ to 29.3% (25.3% overweight childhood; 4% obesity childhood). N/S in the various motor skills. There was an association between BMI and flexibility of the hips and lower back (Sit & Reach Test) or total dynamic body coordination (Advance Test). Overweight childhood: ↑ segmental movements (positive association with BMI), ↓ overall movement performance.
(49)	Portugal	1,276 children	Gross motor coordination (MC): KTK; anthropometry: height and body mass; physical activity: Baecke's questionnaire; and socioeconomic status (SES)	Overweight and obesity: 17.69% and 7.47%, respectively, for boys, and 20.70% and 5.02% for girls. Eutrophic children: overcome childhood with obesity in all tests of gross motor coordination. Gross scores when walking backwards and moving sideways: ↑ with age and performance boys ↑ when moving sideways
(26)	Taiwan	2,057 children	MABC test; anthropometry: height, body weight, waist and hip circumference, BMI	Manual dexterity and ball skills in girls: scores ↑ mastery of manual dexterity; most anthropometric data (weight, waist circumference): ↑ group with developmental coordination disorder and balance deficits; children in the group with developmental coordination disorder and balance deficits: 2× ↑ probability of being obese
(27)	Belgium	2,517 children initially *n* = 754 in the second evaluation	BMI, gross motor coordination: KTK, total physical activity: questionnaire	Performance ↓ in KTK at baseline predicted ↑ BMI *z* score; ↑ baseline BMI *z* score predicted ↓ KTK performance
(20)	Australia	666 children and adolescents Evaluated at 10 and 14 years old	Anthropometric measurements: height, weight, BMI; engine performance: MAND	14 years old eutrophic children group: ↑ general motor performance scores. 14 years: ↑ prevalence obesity childhood with mild motor difficulties. ↓ motor performance and BMI ratio; tasks + affected by BMI: those that involved a change in the center of mass; morphological restrictions of overweight and obesity affect the performance of motor tasks in tasks involving changes in the center of mass, but not static measures of strength
(28)	Australia	239 children Obese *n* = 107 Normal weight *n* = 132	Anthropometry: height, weight, BMI; body composition: dual-energy absorptiometry by x-rays; physical activity: uniaxial accelerometers; demographic/background information; activity capacity restrictions: TUDS; 6MWT, TUG; limitation of participation (performance): Multimedia Activity Recall for Children and Adolescents, QVRS	Obesity childhood: ↓ average accelerometry count, maternal education, and family income. Obesity childhood: ↑ mass, BMI, % fat and fat-free mass; obese group: restrictions on the ability to perform the TUG, the 6MWT and the TUDS; Obesity childhood: ↓ time in self-care activities and without physical difficulty in daily activities; obese: impaired quality of life
(29)	Canada	590 children and adolescents	Height, weight (BMI), and % body fat by bioelectrical impedance analysis; BOTMP-SF; active game participation: participation questionnaire	Youth with Developmental Coordination Disorder: ↑% body fat. Boys with Developmental Coordination Disorder: ↑ BMI of all young people. Boys with Developmental Coordination Disorder: ↑ active play participation associated with ↑ BMI and% body fatBoys with Developmental Coordination Disorder: opposite relationship is observed
(44)	Belgium	108 children	Anthropometry: body height, body weight, BMI, % body fat; level of gross motor coordination: KTK; FPAQ	Progression level of gross motor coordination over a period of 2 years was different, depending on the children's weight status; eutrophic childhood group: ↑ progress; in addition to BMI (negative predictor), participation in sports organized within a sports club (positive predictor) determines the gross performance of motor coordination 2 years later
(30)	Australia	175 children	Anthropometry: height, weight, BMI; fundamental movement skills: TGMD-2 age groups: 7–8 years and 9–10 years; all other SFM: 6 to 7 years and 8 to 10 years	77% obesity childhood; boys: ↑ BMI and performance in object control skills; girls: ↑ proficiency in locomotor skills; all 12 skills in all age groups: domain prevalence was ↓ among overweight/Obesity childhood
(31)	Taiwan	2,029 children	Height, weight, % body fat; coordination: MABC	Boys and girls with obesity: ↓ general motor coordination, mainly in static and dynamic balance; boys: ↑ developmental coordination disorder (DCD) in the obesity group

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TABLE 2 | Continued

Author, Year	Country	Total population	Variables analyzed (Tests)	Results of body composition and motor performance
(32)	Canada	2,278 children 1,979 performed the motor tests	Height, weight, BMI, waist circumference; identification of developmental coordination disorder: BOTMP-SF	Balance and total impairment score: ↑ obesity and overweight; girls: ↑ balance impairment score in obesity and overweight groups
(33)	Italy	260 children	Anthropometry: height, weight, BMI; self-physical description questionnaire: perceived coordination, body fat and sports competence; drawings of Collins Children's Figures: body image; Perceived Physical Capacity Scale: strength, speed and agility and tests involving standing long jump, 2 kg medicinal ball toss, 10 × 5 m shuttle race and 20- and 30-m sprints.	Overweight and obesity girls: ↓ perceived and real physical competence, ↑ perceived body fat and ↑ body dissatisfaction eutrophic children: ↑ standing long jump performance, 20-m shuttle run and 30-m run. Obesity childhood: ↑ pitch performance
(34)	Italy	210 children Normal weight *n* = 105 Overweight *n* = 105	Height, weight, BMI; motor performance tests: 3 explosion tests (standing long jump, medicine ball throw, basketball throw; 2 speed tests: 10- and 20-m sprint; body image: children's drawings of Collins; scale of perception of physical ability for children	Scale of perception of physical ability for children: overweight childhood showed ↑ average body discrepancy; overweight childhood explosion tests: ↑ ball and basketball performance; long jump and 10- and 20-m sprint: eutrophic childhood ↑ performance
(45)	Germany	615 children	Antropometria: altura, peso e IMC; testes motores: TC6; coordenação motora: KTK	Intervention schools: Overweight and Obesity childhood: ↓ motor test results on all tasks
(50)	Belgium	540 children	Anthropometry: height, weight, and BMI; fine motor control: MABC in two postural conditions different: sitting and standing in a tandem position on a balance beam (BB)	Tandem position on balance beam: ↓ obese score in seated condition: N/S between overweight and eutrophic scores performance in placing obese pins: ↓ when seated.
(53)	Canada	578 children	BOTMP-SF, % body fat, weight, height, and BMI	Children with coordination and balance deficit disorder: + prone to being overweight and obesity childhood (analyzing% body fat)
(46)	Germany	668 children	Anthropometry; gross motor development: KTK; resistance: TC6; children's leisure assessment questionnaires	Boys with coordination and balance deficit disorder: risk factor for overweight and obesity in childhood and early adolescence
(52)	Egypt	676 children	Anthropometry: body height, body weight and BMI; physical fitness: DMT 6-18	Overweight childhood: 11.24%; obese: 12.28% running and strength skills: negatively + affected by ↓ body weight balance skills affected by ↑ body mass; weight and endurance skills: affected by abnormal ↑ or ↓ body weight
(36)	Áustria	213 classmates	Height, weight, and BMI; DMT 6–18: resistance, power, speed, coordination, and agility; 6MWT questionnaire participation in sports and use of media; migration status	Eutrophic childhood: 83% adolescents overweight/obesity participants: ↓ motor skills development Participants who lost weight or maintained normal weight: ↑ overall motor skill score over 4 years of follow-up + time using media eutrophic adolescents: ↑ performance in various tests of motor skills motor skills during the 4-year observation period: ↑ absolute performance more pronounced in eutrophic adolescents at baseline
(55)	Portugal	596 children	Anthropometry: weight, % fat, height, waist circumference and BMI; motor coordination: KTK; 20-m shuttle-run test: assess cardiorespiratory fitness	Girls: ↓ CM and ↑% body fat BMI, waist circumference, % body fat and waist/height ratio: related to ↓ CM in both sexes, except for the waist/height ratio after adjustments for girls
(38)	Switzerland	656 children	Coordinating and conditional skills: lateral jump, touch, standing jump, 20 m and shuttle run; weight, height, and BMI; nutritional research	Eutrophic childhood: ↑ running performance, side jump, long jump, and shuttle run. Low weight group: ↑ shuttle race performance. Obesity and overweight group: ↓ performance on 4 items of the motor functional tests (lateral jump, standing long jump, 20 m speed and shuttle run)
(39)	Brazil	380 children	Motor performance: MABC-2—manual dexterity, throw receive skills and static and dynamic balance skills. Antrhropometry: weight, height, and BMI	Male: ↑ movement difficulty. Between ages: association N/S; age ranges by skill compared: significant difference between age range and static and dynamic balance skills (between ages 7 and 9 and between ages 7 and 10) motor classification and nutritional status by sex: N/S, which neutralizes any influence of nutritional status on motor classification

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TABLE 2 | Continued

Author, Year	Country	Total population	Variables analyzed (Tests)	Results of body composition and motor performance
(40)	Australia	6917 children	Demographic information: socioeconomic status (SES); fundamental movement skills: sprinting, vertical jumping side canter and jumping and object control skills (catching, throwing by the arm and kicking); cardiorespiratory endurance fitness: 20-m shuttle race test, parents reports of physical activities organized or not; validated physical activity recovery for adolescents questionnaire	Girls: ↑ low competence skills object control association with functional movement screen and inadequate cardiorespiratory fitness. There was no association between low competence and object control skills and overweight students/obesity. Motor skills: ↑ low overweight. Competence association/obesity; consistent associations for most individual motor skills

BMI, body mass index; CPA, Checklist of Psychomotor Activities; SES, socioeconomic status; KTK, Body coordination test for children, Koërper Koordination Test für Kinder); N/S, not significant; BOTMP-SF, Bruininks–Oseretsky Test of Motor Proficiency—Short Form; MABC, Movement Assessment Battery Test for Children; MAND, McCarron Assessment of Neuromuscular Development; TUDS, timed ascent and descent test; 6MWT, six-minute walk test; TUG, timed up and go; HRQoL, related quality of life; FPAQ, Flemish Physical Activity Questionnaire; TGMD-2, gross motor development test 2; DMT 6–18, German engine test/Deutscher Motorik Test; MLG, Fat-free mass; SLJ, standing long jump; MVPA, moderate to vigorous physical activity; MT, Hand movement time.

adolescents and suggested that the decline in girls' motor competence was due to a reduction in the opportunity to be active (60). It then appears that gross motor coordination improves with age during middle childhood and adolescence, although there is a lack of consensus on sex-related differences between age groups and the gross motor coordination tests used.

In contrast to object control-related skills, which tend to be more static, locomotor activities involve changing or controlling a larger body mass that impedes functional movement and contributes to a higher rate of lower limb orthopedic changes, such as tibia rod and plantar pressure, among children with obesity (61). The negative association between gross motor activity and higher BMI may reflect the composition of assessments where the compound requires better motor coordination while moving and controlling the body, compared to object control skills. Sex, on the other hand, seems to relate differently to various aspects of gross motor competence. Male sex was considered a strong positive correlate of object control and motor coordination tasks, with pre-maturation biological differences being considered for boys and girls, especially in reference to skills such as throwing (62). Research has shown that, compared to girls, boys receive greater encouragement, support, and opportunities to engage in physical activity and sports at home and at school. Thus, girls' opportunities to improve their gross motor skills may be limited (63, 64).

Biological and environmental factors can influence motor coordination, favoring both boys and girls. The activities performed by different sexes facilitate the performance in certain items of motor coordination; therefore, sex can be an intervening factor in motor performance. Regarding overweight and obesity, one of the hypotheses that can explain the interference in the performance related to gross motor coordination tasks is that during the tasks of supporting the body weight, there is a higher proportion of fat mass that must be supported or moved against the action of the force of gravity (65).

Another factor that can interfere with the performance in motor coordination is time, as can be seen in a longitudinal study that investigated the relationship between children's weight and the level of gross motor coordination over time. Baseline measurements were collected from 2,517 children (5 to 13 years old, 52.8% boys). Measurements included the following: height and body weight for the calculation of BMI and gross motor coordination through KTK. After 2 years, 754 participants (7 to 13 years old, 50.8% boys) underwent anthropometric and KTK assessments again. There was a positive relationship between the worst motor performance at KTK at baseline and an increase in BMI. In addition, a higher baseline BMI score also predicted a decrease in KTK performance, suggesting that children's weight negatively influences the level of gross motor coordination in the future and vice versa. Therefore, prevention and intervention initiatives through physical activity must consider this reciprocal causal relationship over the development time (50).

Furthermore, physical activity has a potential protective effect against the development of metabolic diseases during childhood and reduces the prevalence of cardiovascular diseases and diabetes, and morbidity and mortality of adult individuals prematurely (66). Thus, regular physical activity and adequate nutrition during the years of child growth and development increases the possibility of a healthy pattern of physical maturation consistent with a child's genetic potential (67). Dudas et al. (2008) found that overweight children showed lower participation in sports clubs, while even more children with healthy weight were able to ride a bicycle.

In this perspective, this review demonstrated that children with a higher percentage of body fat had lower levels of moderate to vigorous physical activity, as shown by the neuromuscular performance in running and long jump tests (43). In addition, Tsiros et al. (28) performed a study on 239 children, of whom 107 had obesity and 132 had a healthy weight. They observed restrictions in the group with obesity regarding the ability to perform TUG, 6MWT, and TUDS. Morano et al. (33) evaluated 260 students between 11 and 14 years old through the questionnaire of physical self-description: perceived coordination, body fat, and sports competence; Collins Children's Figures Drawings: body image; Perceived Physical Capacity Scale: strength, speed, and agility, and tests involving standing long jump, and 20- and 30-m sprints. Overweight and obese girls

reported less perceived and real physical competence, a higher index of perceived body fat, and body dissatisfaction. Eutrophic childhood, on the other hand, showed better performance in standing long jump, shuttle run, and 20-m and 30-m run.

It is important to note that the mechanisms involving the neuroendocrine and musculoskeletal systems interact with each other and can explain the associations between weight and performance in gross motor coordination tests. Scientific literature demonstrates that stimuli from greater muscle activity are capable of promoting in their microenvironment the synthesis of chemical compounds called myokines. Among these, BDNF (brain-derived neurotrophic factor) and, recently, irisin stand out, because they are able to overcome the blood–brain barrier and can promote a positive outcome in both the cognitive and motor domains (68, 69).

For several years, muscles were considered targets for hormonal action; however, there is growing evidence that muscles, in a retrograde manner, exert unique forms of control over the CNS that affect motor behavior. Therefore, increasing evidence indicates that neural and muscular systems maintain some degree of plasticity throughout life, demonstrating that environmental factors influence the development of the musculoskeletal system and, as a consequence, motor performance.

CONCLUSION

Our results corroborate the hypothesis that overweight and, especially, obesity in children and adolescents are associated not only with insufficient performance during gross motor coordination activities, but also with an increased risk to physical health. It is, therefore, necessary to prevent childhood obesity and reduce the weight of affected children, and promote healthy eating and physical activities in daycare centers, schools, and homes. To be effective, in addition to the educational sector, all sectors of society must be mobilized so that the negative effect of commercial food products on children's diets will be reduced.

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.

AUTHOR CONTRIBUTIONS

WB and MSF contributed to research conception, data collection, interpretation of results, and critical review of the article. RS, KS, ASS, MS, and AS contributed to data analysis and interpretation, drafting, and critical review of the article. SS and VO contributed to data collection and critical review of the article. All authors contributed to the article and approved the submitted version.

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MINI REVIEW
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A Mini-Review of Pediatric Anthropometrics as Predictors of Future Insulin Resistance

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The impact of rising rates of childhood obesity is far reaching. Metabolic syndrome in children is increasing, yet for most children the consequences of excess adiposity will manifest in adulthood. Excess early fat accrual is a risk factor for future insulin resistance. However, certain types of fat and patterns of fat distribution are more relevant than others to metabolic risk. Therefore, adiposity measures are important. The link between childhood obesity and future insulin resistance was initially established with body mass index (BMI), but BMI is an in imperfect measure of adiposity. It is worthwhile to evaluate other anthropometrics as they may more accurately capture metabolic risk. While measures such as waist to height ratio are established as superior screening measures in adulthood - the findings are not as robust in pediatrics. Emerging evidence suggests that alternative anthropometrics may be slightly superior to BMI in identifying those youth most at risk of developing insulin resistance, but the clinical significance of that superiority appears limited. Increasing study is needed in longitudinal and varied cohorts to identify which pediatric anthropometric best predicts adult insulin resistance. We review alternative anthropometrics as predictors of future insulin resistance and identify current gaps in knowledge and potential future directions of inquiry.

Keywords: anthropometrics, adiposity, obesity, insulin resistance, pediatrics

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INTRODUCTION

Rising obesity rates have led to a commensurate unprecedented rise in associated comorbidities including insulin resistance. Insulin resistance is the body's inability to effectively use insulin and a resultant increase in blood glucose levels. In most patients, excess fat is implicated in the development of insulin resistance. Insulin resistance is not just a potential precursor to diabetes mellitus, but is also a critical component of metabolic syndrome. Indeed, insulin resistance is often both a contributor to and a harbinger of other disturbances of metabolism including fatty liver disease and dyslipidemia (1, 2).

Obesity is defined as an accumulation of excess body fat to the extent that it may have an adverse effect on health (3). Obesity has become synonymous with definitions set by health organizations correlating with body mass index (BMI) thresholds. For example, in pediatrics, the World Health Organization (WHO) defines obesity as greater than two standard deviations above the Growth

Reference median for BMI (4). Guidelines for screening and intervention are developed around these definitions. Therefore, it is critically important to assess the clinical impact of these definitions and measurements.

In contrast to obesity, adiposity is typically used as a general term to describe the degree of fat mass accumulation. While the typical population screening measure to define obesity is BMI, more technical/invasive measures such as body composition measurement by dual energy X-ray absorptiometry (DXA) are often used in research to measure adiposity. Indeed, the distribution and type of body fat, not simply the total body fat, has large implications for disease risk.

Anthropometrics are one method to estimate adiposity. Anthropometrics are broadly defined as physical measures of a person's size and form that are physically obtained without the use of advanced equipment. While BMI is the most common anthropometric, other examples include waist circumference and skinfold thicknesses with calipers. Anthropometrics are of particular interest because of the ability to apply them to large populations without high associated costs or medical burden. The challenge is to identify anthropometrics best able to capture the type of adiposity that is predictive of disease.

Measuring adiposity is particularly challenging in children as their body composition and body metrics change physiologically (5). Inherently, proven measures in adults are not directly applicable to pediatric populations. Pubertal status may also change the utility of different anthropometrics because of the changing muscle and fat composition that occurs with sex hormone exposure (6, 7).

Work within the realm of obesity research has followed the progression of disease. While obesity rose in both adults and children in the early 1970s, the rates of obesity and metabolic complications are consistently higher in adults (8). Initial efforts aimed to identify the most clinically salient measures of adult adiposity. Multiple studies have indicated that waist circumference (WC) or waist to height ratio (WtHR) can better predict metabolic disease and insulin resistance than BMI alone in adults (9, 10). Given these findings and the rise of pediatric metabolic syndrome, subsequent efforts aimed to determine which measures of childhood adiposity best predict disease.

Childhood obesity is correlated with concurrent illness and childhood metabolic disease is an increasing phenomenon (11, 12). There is some evidence that alternative anthropometrics such as WtHR may have superiority to BMI z-score in predicting coincident disease such as non-alcoholic fatty liver disease (NAFLD) and insulin resistance (13, 14). However, there is no consensus around superior anthropometrics. Indeed a relatively recent metanalysis did not find significant evidence of superiority of WC or WtHR over BMI for insulin resistance related outcomes, though there was significant heterogenicity in results (11).

However, excess adiposity in childhood has more long-lasting and far-reaching impacts than current health status. Following the philosophy of the developmental origins of disease, insults or exposures in early life can independently influence later disease (15). Childhood obesity is a risk factor for adult insulin resistance even independent of adult obesity or childhood insulin resistance (16). Therefore, it is important to understand childhood adiposity not only as it predicts later adiposity and current insulin resistance, but how it influences future disease risk.

It is critical to be mindful and deliberate about how we measure childhood adiposity to best evaluate disease risk. In this review, we explore different measures of pediatric adiposity, specifically anthropometrics, that may predict insulin resistance. Specifically, we focus on the risk of future adult insulin resistance. We first establish the evidence linking childhood obesity as defined by BMI to adult insulin resistance and then compare different measures of adiposity as predictors. Finally, we identify gaps in the current literature and explore potential future avenues of study.

BMI AS A PREDICTOR OF ADULT INSULIN RESISTANCE

When evaluating childhood adiposity as it predicts future insulin resistance, it is important to first discuss childhood BMI as a measurement. Given that BMI is the most common screening measure of adiposity, many studies have evaluated childhood BMI as a predictor of adult insulin resistance including several metanalyses. Indeed, in a comprehensive meta-analysis of seven cohorts, five cohorts found BMI it to be a statistically significant predictor of adult type 2 diabetes (17). In fact, the authors found diabetes to be the adult comorbidity most closely associated with childhood obesity. However, the associations and predictive value of BMI thresholds was relatively weak. For example, in children 7-11 years old, the odds ratio of diabetes per standard deviation increase of BMI was 1.78 (95% confidence interval of 1.51-2.10). However, the sensitivity of childhood BMI was poor for predicting adult diabetes and insulin resistance- at most, 40% of adults with diabetes would have met the threshold of excess adiposity (BMI>85%tile) in childhood (17, 18). A more recent cohort collaboration also found a significantly increased odds ratio of development of type 2 diabetes in adulthood as childhood BMI increased. Childhood BMI cut-points corresponding to 75th-90th percentile BMI based on CDC growth charts were determined to be at heightened risk for adult type 2 diabetes (19).

BMI has clear drawbacks as a measure of adiposity. BMI measures excess weight but does not differentiate between fat mass and non-fat mass. Additionally, it does not account for distribution of body fat and, in adults, central adiposity is more highly associated with adverse health outcomes than general adiposity (9, 20).

While the relationship between childhood BMI and adult insulin resistance is clearly of statistical significance, the weakness of its predictive value limits the utility of BMI as a screening measure. Therefore, it is worthwhile to explore different measures of adiposity to better capture risk. While the relationship between childhood BMI and adult-onset diabetes is relatively well studied, limited studies have evaluated different

anthropometrics in childhood as they predict adult insulin resistance.

WAIST CIRCUMFERENCE

The most studied BMI alternative is waist circumference (WC). As mentioned previously, it is established that adult WC correlates better with diabetes risk than BMI (10, 21). The stronger association likely exists because of the ability of WC to capture abdominal adiposity. However, the stronger relationship is not as well established in pediatrics.

The literature is particularly limited in evaluating childhood WC as a predictor of future insulin resistance. In general, evidence shows the association of childhood WC with future insulin resistance is stronger than that of BMI. However, the results are heterogenous with some studies showing lack of superiority (22, 23). The predictive ability of childhood WC for adult insulin resistance, as measured by sensitivity or area under the curve (AUC) in a receiver operating curve (ROC), remains low and is either similar to or only slightly superior to BMI (18, 23, 24). Therefore, similar to BMI, though a relationship between WC and adult insulin resistance clearly exists, it is difficult to identify thresholds of WC that reliably identify at risk youth.

SUM OF SKINFOLDS

Sum of skinfolds is a relatively frequently used alternative anthropometric to BMI although still rarely studied in longitudinal cohorts. Similar to WC, the literature suggests that childhood sum of skinfolds is either slightly better than or equivalent to BMI as a predictor of adult insulin resistance. For example, when defining childhood obesity by BMI thresholds alone investigators found no increased risk for adult diabetes, but the risk did exist when defining obesity by left subscapular skinfold (LSSF) thresholds (25). Similarly, a longitudinal study found the odds ratio of adult hyperglycemia higher in those with increased sum of skinfolds when compared to those with increased BMI in childhood (26). Finally, another study found that in a subsection of females, sum of skinfolds had a significantly higher association with fasting insulin levels in adulthood than both BMI and WC. While the study did not specifically evaluate the predictive ability of sum of skinfolds within this population for fasting insulin, it was found to have superior predictive ability for overall adult metabolic syndrome (22).

WRIST CIRCUMFERENCE

Several studies have shown a positive cross sectional relationship between wrist circumference and insulin resistance in children and adults (27, 28). However, the only study that has examined the relationship between pediatric wrist circumference and adult insulin resistance (as measured by euglycemic clamp) did not find that it predicted adult insulin resistance. Importantly, the study also did not find a relationship between childhood BMI and adult insulin resistance. The study was limited by a relatively small sample size and young adult population (29).

OTHER ANTHROPOMETRICS

Other anthropometric measurements include WC adjusted for height, weight adjusted for height, hip circumference, waist-hip-ratio, WtHR, conicity index, abdominal volume index, body adiposity index, and body shape index (**Table 1**). However, there is only one longitudinal study that examined all these measures as predictors of adult insulin resistance. Within the study, abdominal volume index performed the best among these indices at predicting insulin resistance in all three ways in which adult insulin resistance was measured. However, the overall predictive value was still relatively poor (AUC 0.610-0.615). While the predictive ability was superior to BMI in two of the three measures, it did not differ from other anthropometrics in a statistically significant way including: WC, WC adjusted for height, hip circumference, WtHR (23).

FOLLOW UP PERIOD

One important note is that in all studies comparing the predictive ability of alternative anthropometrics, follow up is limited to young adulthood when insulin resistance or diabetes is not as prevalent as in later adulthood. It is reasonable to hypothesize the positive predictive value of anthropometrics may improve with longer follow up. Therefore, an extension of the current longitudinal what? is important to further elucidate the relationship.

ETHNIC AND RACIAL SUBCLASSIFICATION

Absent from most of the literature is a sub-group analysis by race/ethnicity. Previous evidence suggests racial/ethnic differences in thresholds of BMI/obesity at risk for insulin resistance (30, 31). For example, a UK study determined that threshold BMI for equivalent risk of concurrent insulin resistance was markedly lower in South Asian children than White European children (32). Additionally, some cross sectional studies suggest that different types of anthropometrics may better predict insulin resistance depending on the racial group, while others do not find a difference (33, 34).

The specificity of differing anthropometrics based on race is controversial. It is important to consider racial groups as social constructs. While an overlap exists between ancestry/ancestral genetics and race, they are not equivalent. Therefore, the differences in predictive abilities and predictive thresholds of anthropometrics between racial and ethnic subgroups need to be interpreted carefully both for their potential genetic and social etiologies. Many of the longitudinal cohort studies conducted

TABLE 1 | Anthropometric measurement examples with their abbreviations and derived equations.

Anthropometric	Abbreviation	Calculation
Waist Circumference	WC	Not applicable
Wrist Circumference	WrC	Not applicable
Hip Circumference	HC	Not applicable
Waist-to-hip ratio	WHR	WC / HC
Waist-to-stature ratio	WSR	WC / height
Waist-to-height ratio	WtHR	
Body adiposity index	BAI	(HC (cm) / height (m)) - 18
Body shape index	ABSI	WC (m) / (BMI (kg/m2) $^{2/3}$ x height (m) $^{1/2}$)
Body Roundness Index	BRI	$364.2–365.5 \times (1 – ((0.5 \times \text{WC (m)} / \pi)^2 / (0.5 \times \text{Height (m)})^2))^{0.5}$
Abdominal Volume Index	AVI	$[2\ \text{cm (WC (cm))}^2 + 0.7\ \text{cm (WC (cm)} – \text{HC (cm))}^2] / 1{,}000$
Conicity Index	CI	$0.109^{-1} \times \text{WC (m)} \times (\text{weight [kg]} / \text{height [m]})^{-1/2}$
Sum of skinfold thickness	SSI	Triceps + subscapular (mm)

have been in relatively homogenous and non-Hispanic White populations. Increased diversity of the study populations will improve generalizability and uncover potential differences in adiposity risk levels. Whether or not to use universal cut-off points for risk by anthropometrics or use ethnic and racial specific ones is a point of debate. Regardless, inclusion of diverse populations will ensure that risk thresholds are more broadly applicable.

REFERENCE VALUES

One of the difficult aspects of anthropometrics is the establishment of reference ranges and thresholds to define risk. As evidence accumulates around a particular type of adiposity measurement (i.e., BMI) the establishment of risk thresholds becomes clearer. When alternative measurements are not as widely used it is hard to extrapolate similar thresholds. Over time and in different populations, the thresholds will be different and thus it is important to recognize these limitations. Most of the longitudinal studies on prospective insulin resistance risk use thresholds based off each study's sample data (i.e., the upper quartile of WC). Therefore, the findings are difficult to translate to direct clinical decisions. As data in the field grows larger, established population reference ranges may be possible.

ADDRESSING PUBERTY

The predictive value of adiposity can differ by sex and continuing to this difference is important (35–37). As previously mentioned, subtle anthropometric associations with future insulin resistance differ between females and males, but longitudinal cohorts have not completed sub-analysis by sex (22). The sex difference may be less pronounced in prepubertal children. Indeed, a subgroup analysis by pubertal status and age is important as adiposity changes across childhood, particularly in relation to sex hormone exposure. While both sexes increase their total fat stores during puberty, males gain relatively more fat free mass while females gain more fat mass (6). Regional body fat distribution also changes throughout puberty with males exhibiting increasing trunk and waist fat as they progress through puberty compared to females (7). While some of the cohort studies have performed subgroup analyses by pubertal status, most have not. Some evidence suggests there are differences in the predictive ability of anthropometrics by pubertal status, with one study finding that pre-pubertal anthropometrics are more strongly associated with adult diabetes than those in puberty (24). Continuing to explore these differences is crucial in evaluating anthropometrics as potential harbingers of future insulin resistance.

PATTERNS IN ADIPOSITY CHANGE

An increasing field of interest is the study of body composition and adiposity in the context of a trend or pattern of change. Multiple studies have evaluated the trajectory of obesity across childhood and into adulthood as a predictor of insulin resistance. For example, studies have already found those with a greater change in BMI during adolescence have increased future insulin resistance (38). Some have even begun evaluating trends of different adiposity measurements as they relate to short term outcomes (39, 40). As cohorts progress in age, additional studies of trajectories of other measures of adiposity, aside from BMI, on long term disease risk may reveal potential benefits to alternative measurements.

ADVANCED MEASUREMENTS OF ADIPOSITY

As previously discussed, while anthropometrics are one method to estimate adiposity, more advanced and accurate modes of measurement exist. Indeed, evidence exists that these measurements of adiposity better predict insulin resistance than anthropometrics. In a cross-sectional study of adult women, impaired glucose tolerance was better predicted by DXA-measured visceral adiposity than a variety of direct body measurements (41). Additionally, studies in adults and children have found body fat thresholds by air displacement plethysmography to be lower than those established by BMI or WC to define risk (42). In adolescents, visceral adiposity as

measured by DXA was found to be associated with insulin resistance independent of BMI (43). To our knowledge no longitudinal studies exist evaluating the ability of more advanced measures of body fat measurement to predict future insulin resistance. Along with anthropometrics, this would be a valuable addition to the literature though will be limited in sample size.

LIMITATIONS AND FUTURE DIRECTIONS

In the limited existing research, there is consistent evidence that the association between some alternative adiposity measures such as WC or abdominal volume index, and future insulin resistance is slightly higher than for BMI. However, the predictive abilities of other measures remain low and suggests they may not have significant clinical superiority in identifying children at risk of developing insulin resistance. Given the stronger association and relatively stronger predictive power of alternative measures, the field of anthropometrics still deserves attention as a field of inquiry. There is reason to believe that as both diversity of cohorts and length of follow up increases, the differences in predictive ability between other anthropometrics and BMI may increase.

A large limitation to the field of knowledge is the need for large longitudinal cohort studies. While some anthropometrics have been available for some time, other measures of adiposity are relatively new and it will take time to determine their importance in screening as more data accumulates. As mentioned previously, the prevalence of insulin resistance increases with age and thus collecting data into early adulthood will not suffice to establish associations. Current longitudinal studies should be extended into later adulthood to allow for further exploration of the relationship of childhood adiposity and insulin resistance as its prevalence increases with age.

Additionally, the differences between other anthropometrics and BMI may be more significant as subgroup analysis is completed by sex, pubertal status, and racial/ethnic group. Among the few studies that exist on longitudinal risk of insulin resistance, few completed subgroup analysis by sex, pubertal stage or race/ethnicity. Given the evidence within cross sectional research for differences in adiposity between those of differing ancestries and sex hormone exposure, exploration into subgroup analysis should be completed in future research.

Finally, as the study of the adiposity rebound and adiposity trajectories progress, it will be worthwhile to trend alternative anthropometrics over time as well. The small differences in predictive power seen by static measurements may increase as they are studied in this manner.

While anthropometrics are important because of their widespread utility they are proxies for distinguishing different types of fat, for example subcutaneous and visceral fat. As more advanced non-invasive fat measurement methods such as air displacement plethysmography or ultrasound become less expensive and more accessible, the combination of these with anthropometrics in large cohorts will also help advance understanding of the impact of early fat accrual.

Overall, the use of alternative childhood anthropometrics to predict adult insulin resistance is relatively understudied and requires further development. As mentioned, the current literature is limited to a few studies. Most research comparing anthropometrics exists in cross-sectional cohorts where adiposity measures are correlated simultaneously to insulin resistance. However, the link between early adiposity and future insulin resistance is well established and deserves specific attention. We suggest that future prospective longitudinal studies should continue to incorporate anthropometrics in a variety of ways, particularly waist circumference and hip circumference as these are needed in the calculation of a variety of measurements.

AUTHOR CONTRIBUTIONS

SD and JLJ both conceived of the article content. SD prepared the first draft and both authors approved of the final version.

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Single Point Insulin Sensitivity Estimator in Pediatric Non-Alcoholic Fatty Liver Disease

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Background: Attenuated insulin-sensitivity (IS) is a central feature of pediatric non-alcoholic fatty liver disease (NAFLD). We recently developed a new index, single point insulin sensitivity estimator (SPISE), based on triglycerides, high-density-lipoprotein and body-mass-index (BMI), and validated by euglycemic-hyperinsulinemic clamp-test (EHCT) in adolescents. This study aims to assess the performance of SPISE as an estimation of hepatic insulin (in-)sensitivity. Our results introduce SPISE as a novel and inexpensive index of hepatic insulin resistance, superior to established indices in children and adolescents with obesity.

Materials and Methods: Ninety-nine pubertal subjects with obesity (13.5 ± 2.0 years, 59.6% males, overall mean BMI-SDS + 2.8 ± 0.6) were stratified by MRI (magnetic resonance imaging) into a NAFLD (>5% liver-fat-content; male n=41, female n=16) and non-NAFLD (≤5%; male n=18, female n=24) group. Obesity was defined according to WHO criteria (> 2 BMI-SDS). EHCT were used to determine IS in a subgroup (n=17). Receiver-operating-characteristic (ROC)-curve was performed for diagnostic ability of SPISE, HOMA-IR (homeostatic model assessment for insulin resistance), and HIRI (hepatic insulin resistance index), assuming null hypothesis of no difference in area-under-the-curve (AUC) at 0.5.

Results: SPISE was lower in NAFLD (male: 4.8 ± 1.2, female: 4.5 ± 1.1) than in non-NAFLD group (male 6.0 ± 1.6, female 5.6 ± 1.5; P< 0.05 {95% confidence interval [CI]: male NAFLD 4.5, 5.2; male non-NAFLD 5.2, 6.8; female NAFLD 4.0, 5.1, female non-NAFLD 5.0, 6.2}). In males, ROC-AUC was 0.71 for SPISE (P=0.006, 95% CI: 0.54, 0.87), 0.68 for HOMA-IR (P=0.038, 95% CI: 0.48, 0.88), and 0.50 for HIRI (P=0.543, 95% CI:

0.27, 0.74). In females, ROC-AUC was 0.74 for SPISE (P=0.006), 0.59 for HOMA-IR (P=0.214), and 0.68 for HIRI (P=0.072). The optimal cutoff-level for SPISE between NAFLD and non-NAFLD patients was 5.18 overall (Youden-index: 0.35; sensitivity 0.68%, specificity 0.67%).

Conclusion: SPISE is significantly lower in juvenile patients with obesity-associated NAFLD. Our results suggest that SPISE indicates hepatic IR in pediatric NAFLD patients with sensitivity and specificity superior to established indices of hepatic IR.

Keywords: insulin resistance, pediatric obesity, hepatic insulin resistance index, HOMA-IR, receiver-operating-characteristic curve

INTRODUCTION

The prevalence of non-alcoholic fatty liver disease (NAFLD) in children and adolescents is on the rise, hence emerging as one of the crucial healthcare challenges of our time (1–3). A systematic review by Anderson et al. in 2015 reported a prevalence of up to 34% in juveniles with obesity, being more common in males (4).

NAFLD is regarded as the manifestation of the metabolic syndrome in liver (5–7). Although the causative pathophysiological background of NAFLD in pediatric patients still needs further investigation, NAFLD has repeatedly been linked to obesity and insulin resistance (IR) as well as other comorbidities in adults as well as juveniles (8–11). Fang et al. (9) described a "multiple-hit hypothesis" leading to NAFLD, in which fat accumulation and consequently systemic and specifically hepatic insulin resistance play a major role.

Up to date, various mathematically calculable indices of hepatic IR have been developed, among which some indices can be obtained from just a single fasting blood draw (such as the homeostatic assessment index, HOMA-IR), whereas for other indices multiple blood draws are required, possibly representing a more "dynamic" state (such as the Hepatic Insulin Resistance Index, HIRI) (11–18). Recently, Bedogni et al. developed fatty liver prediction models based on Body-Mass-Index (BMI) or waist circumference, alanine aminotransferase, Homeostatic Model Assessment, triglycerides and uric acid to diagnose fatty liver in children with obesity (19). Previously, we developed a simple and inexpensive index, called the "Single Point Insulin Sensitivity Estimator" (SPISE), validated against the gold standard for assessing insulin sensitivity, the hyperinsulinemic-euglycemic clamp test (17), in an adult as well as in a juvenile cohort (20). This index consists of anthropometric as well as laboratory parameters, which enables clinicians to easily diagnose insulin resistance in pediatric patients, whose care calls for non-invasive and broadly accessible tools.

Based upon these considerations, the current study aimed to compare the performance of SPISE to established indices of hepatic IR in pediatric NAFLD-patients with obesity.

MATERIAL AND METHODS

Study Population and Design

Patients (n = 99) were recruited in an obesity specialist clinic in Salzburg (Austria) as part of the BETA JUDO study (BETA cell function in JUvenile Diabetes and Obesity, FP7-HEALTH-2011-two-stage, project number: 279153). Inclusion criteria were age 10-18 years and overweight or obesity according to the WHO criteria (BMI-SDS > 1). Written informed consent was obtained by all caregivers if patients were under the age of 18 years. Exclusion criteria were lack of consent or any chronic liver disease (such as hepatitis B and C). Patients did not report any alcohol intake. Height and bodymass were assessed by means of a standardized, calibrated scale (Seca, Hamburg, Germany). BMI and BMI-SDS were calculated according to the WHO 2006-2007 reference population (21). Waist circumference (cm), hip circumference (cm) and neck circumference (cm) were measured using a flexible tape. Blood pressure was measured twice, using a standardized clinical aneroid sphygmomanometer (Philips patient monitor MP30, Amsterdam, The Netherlands), and the mean value was recorded. Puberty staging was done according to Tanner (by a physician) and all subjects included into this study were staged as pubertal (Tanner II-IV).

Blood Sampling and Biochemical Analyses

After an overnight fast, all patients underwent a standardized oral glucose tolerance test (OGTT, 1.75 g glucose/kg body mass) over 180 minutes as previously described (22, 23). OGTT was performed according to standard procedures by setting an intravenous line in an antecubital vein and subsequent blood draws were performed *via* this line at nine different time points after glucose challenge.

Uric acid, triglycerides, HDL cholesterol, total cholesterol and liver transaminases were measured using an enzymatic photometric test (Modular Analytics System, P-Modul 917, Roche Diagnostics, Vienna, Austria). The evaluation of LDL cholesterol also required an enzymatic photometric test using Integra Manual by Roche Diagnostics. Apolipoprotein (A2) and apolipoprotein (B) as well as high-sensitive CRP were examined by an immunologic turbidimetric test (COBAS- Integra, Roche Diagnostics, Vienna, Austria) and interleukin 6 by an enzyme-linked immunosorbent assay (Modular Analytics System, E-Modul by Roche Diagnostics). Leptin and adiponectin were determined manually using ELISA (Human Leptin ELISA, Biovendor, Brno, Czech Republic; Quantikine ELISA, Human Total Adiponectin/Acrp30 Immunoassay, R&D Systems, Inc., Minneapolis, MN, USA). HbA1c was measured by reversed-phase chromatography and lipoprotein (a) by a turbidimetric test

(COBAS- Integra, Roche Diagnostics, Vienna, Austria). Samples underwent immediate centrifugation at 2500g for 10 minutes at 4°C, subsequently aliquoted and frozen at -80°C. Plasma was consecutively used for analyses of insulin, proinsulin and C-peptide in the central lab in Uppsala. Single-plex ELISA-kits for each analyte were used (Mercodia AB®, Uppsala, Sweden).

Hyperinsulinemic Clamp Test

Euglycemic-hyperinsulinemic clamp tests were used to determine insulin sensitivity within an interval of maximally 3 to 4 weeks after the OGTT and after an overnight fast. The euglycemic clamp glucose target was calculated as the mean value of 3 fasting plasma glucose measurements. The glucose clamp target was set to 80 mg/dL (4.44 mmol/L) in case of a value above 80 mg/dL, and in case of a value above 100 mg/dL (5.55 mmol/L) the clamp goal was 100 mg/dL. Clamp tests were performed for 120 min, with primed-continuous regular insulin infusion [40 mU insulin * min^{-1} * (m^2 total body surface area)$^{-1}$]. Blood samples for the determination of serum insulin and C-peptide were drawn at 0 and 120 minutes and the glucose disposal rate (M-value; milligrams per kilogram per minute) was calculated (20, 24–26).

Magnetic Resonance Imaging (MRI)

MRI-examinations were performed to determine liver fat content (LFC) and volumes of abdominal visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) as previously described (23). All exams were performed using 1.5T clinical MRI-systems from Philips Medical System (Amsterdam, The Netherlands) after a light meal and in close proximity to the OGTT. Water-fat imaging techniques were used throughout. The scans were done over 16 cm along the craniocaudal axis and centered on the L1 vertebra. The adipose tissue volumes were determined using a fully automated segmentation method that uses a filtering technique to separate VAT from SAT. Liver fat image reconstruction was done by a multi-resolution version of a method that employs a whole-image optimization approach (27). A single operator trained by an experienced radiologist performed the measurements by manual segmentation in the axial slices of the water images using the software ImageJ (version 1.42q, http://rsbweb.nih.gov/ij/).

Definition of NAFLD

Patients with NAFLD had a liver fat content >5%, as measured by MRI. This has previously been described and a close relation between histopathological changes and liver fat fraction in MR-imaging has been promoted by various groups (28–34).

Definition of Hepatic Insulin Resistance

Hepatic insulin resistance was analyzed using Homeostatic Model Assessment or HOMA-IR [22.5/fasting insulin * fasting glucose], the Single Point Insulin Sensitivity Estimator or SPISE [600 * HDL-cholesterol^{0,185}/(Triglycerides^{0,2} × BMI^{1,338})], and the Hepatic Insulin Resistance Index [(Glucose AUC_{0-30}) x (Insulin AUC_{0-30})] (11–18, 20).

Statistical Analysis

Descriptive data analysis showed results with mean and standard deviation for continuous variables and number and percentages for categorical variables. Pearson correlation was calculated to show linear dependencies. Receiver operating characteristic (ROC) curves were calculated showing sensitivity (true positive rate) and 1 – specificity (false positive rate) for each threshold of the indicator variable. Graphical representation was combined with area under the curve (AUC) as numerical measure indicating the classification quality. AUC was calculated using trapezoidal rule. The null hypothesis H0: AUC = 0.5 (indicating random classification) was tested using the Wilcoxon Mann Whitney test (H1: AUC > 0.5). Cutoff levels for SPISE were obtained using the maximum of the Youden index (= sensitivity + specificity – 1) (35). All results are presented along with 95%-confidence intervals. Significance was assumed at $p<0.05$. Due to exploratory analysis, p-values are not corrected for multiple testing. All calculations were done with R (The R Project, Version 3.6.0, Linz, Austria).

RESULTS

Descriptive Data of All Patients

A total of 99 patients with obesity were included into this study (male: 59.4%, female: 40.4%). The age of patients was 13.5 ± 2.0 years. Further group characteristics on anthropometric and biochemical parameters are shown in **Table 1**.

Descriptive Data of NAFLD and Non-NAFLD Groups

Patients were further categorized into the ones with and the ones without NAFLD, as defined by MRI-measured liver fat content. They were separated into male and female groups with 41 male and 16 female NAFLD patients as well as 18 male and 24 female non-NAFLD patients. Ages ranged between 12.7 ± 2.2 and 14.3 ± 2.4 years respectively (details see **Table 2**). Liver fat content was highest in the male NAFLD group (15.9 ± 11.9% {confidence interval [CI]: 12.1 - 19.6%}) in comparison to all non-NAFLD patients (male non-NAFLD 3.0 ± 1.0%, P<0.001 {95% confidence interval [CI]: 2.5, 3.5%}; female non-NAFLD 3.1 ± 0.9%, P<0.001 {95% confidence interval [CI]: 2.7, 3.4%}). Liver fat content in female NAFLD patients was 13.5 ± 9.7% (P<0.600 {95% confidence interval [CI]: 8.3, 18.7%}). Further details of anthropometric and biochemical parameters are shown in **Table 2**. SPISE was lower in NAFLD (male: 4.8 ± 1.2, female: 4.5 ± 1.1) than in non-NAFLD group (male 6.0 ± 1.6, female 5.6 ± 1.5; *P*< 0.05 {95% confidence interval [CI]: male NAFLD 4.5, 5.2; male non-NAFLD 5.2, 6.8; female NAFLD 4.0, 5.1; female non-NAFLD 5.0, 6.2}).

In a subgroup analysis in **Table 3**, considering NAFLD according to grade of steatosis as measured *via* MRI, SPISE was significantly lower in patients with higher NAFLD grades respectively more steatosis (non-NAFLD compared to NAFLD grades 1-4: P<0.001). **Figure 1** compared the performance of SPISE, HOMA-IR and HIRI in different steatosis grades. SPISE as well as HOMA-IR and HIRI were not significantly different in higher steatosis grades (2-4).

TABLE 1 | Descriptive data of all patients (n = 99[#]).

	Mean	± SD
Age (years)	13.5	2.0
Gender	male: 59 (59.6%)	
	female: 40 (40.4%)	
Anthropometric data		
Body mass (kg)	86.6	21.0
Height (cm)	164.2	11.2
BMI (kg/m^2)	31.8	5.6
BMI-SDS	2.8	0.6
SBMI (kg/m^2)	34.9	4.2
Waist circumference (cm)	102.4	12.9
Waist/ Hip ratio	1.0	0.1
Systolic blood pressure (mmHg)	121.6	12.0
MRI data		
MRI liver fat content (%)	10.1	10.5
MRI VAT volume (cm^3)	1474.2	558.5
MRI SAT volume (cm^3)	6412.5	2209.7
MRI DSAT volume (cm^3)	3095.1	1228.4
MRI SSAT volume (cm^3)	3085.0	1151.5
Laboratory data		
HbA1c (mmol/mol)	35.0	2.4
Total cholesterol (mmol/L)	4.2	0.8
LDL cholesterol (mmol/L)	2.3	0.7
HDL cholesterol (mmol/L)	1.3	0.4
Triglycerides (mmol/L)	1.2	0.6
AST (µkat/L)	0.5	0.4
ALT (µkat/L)	0.6	0.7
GGT (µkat/L)	0.4	0.3
Uric acid (µmol/L)	351.1	84.0
Adiponectin (µg/mL)	7.7	3.3
Leptin (ng/mL)	36.2	23.9
hs-CrP (mg/L)	3.9	4.3
IL-6 (pg/mL)	7.4	2.4
TNF-alpha (pg/mL)	8.3	1.9
OGTT data		
OGTT fasting glucose (mmol/L)	4.8	0.6
OGTT 120 min. glucose (mmol/L)	6.3	1.4
OGTT fasting insulin (pmol/L)	120.2	64.9
Parameters of insulin resistence		
SPISE	5.2	1.4
HOMA-IR	3.6	2.0
HIRI	55928.4	33615.0

[#]n = 99, except for n = 98 for waist circumference, waist/hip ratio, systolic blood pressure, HbA1c, uric acid, hs-CrP; n = 103 for MRI VAT, MRI SAT, OGTT fasting glucose; n = 96 for MRI DSAT, MRI SSAT; n = 95 for AST; n = 90 for IL-6; n = 89 for TNF-alpha; n = 87 for adiponectin; n = 72 for OGTT fasting insulin; n = 71 for HOMA-IR; n = 72 for leptin; n = 64 for HIRI.

Data are expressed as mean ± standard deviation. All subjects were staged as "pubertal" according to Tanner staging (II-IV).

SD, standard deviation; BMI, body mass index; SDS, standard deviation score; SBMI, smart body mass index; MRI, magnetic resonance imaging; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; DSAT, deep subcutaneous adipose tissue; SSAT, superficial subcutaneous adipose tissue; HbA1c, hemoglobin A1c; LDL, low density lipoprotein; HDL, high density lipoprotein; AST, aspartate transaminase; ALT, alanine transaminase; GGT, gamma-glutamyl transferase; hs-CrP, high-sensitivity C-reactive Protein; IL-6, Interleukin 6; TNF, tumor necrosis factor; OGTT, oral glucose tolerance test; min., minutes; SPISE, Single Point Insulin Sensitivity Estimator; HOMA-IR, homeostatic Model Assessment for Insulin Resistance; HIRI, Hepatic Insulin Resistance Index.

Comparison of Insulin Sensitivity Indices

We performed hyperinsulinemic clamp tests (n=17) and used calculated M-values as excepted means to estimate insulin sensitivity. As shown in **Table 4**, the correlation of M-values and SPISE (r = 0.49) is significantly greater than between M-values and HOMA-IR (r = 0.11) or, respectively, HIRI (r = -0.32).

ROC-Curve Analysis and Optimal Cutoff Levels of SPISE in NAFLD Patients

Finally, we analyzed ROC-curves in male as well as female patients for SPISE, HOMA-IR, and HIRI (**Figures 2**, **3**). In male patients, ROC-curve showed AUC of 0.71 for SPISE (P=0.006, 95% CI: 0.54, 0.87), 0.68 for HOMA-IR (P=0.038, 95% CI: 0.48, 0.88), and 0.50 for HIRI (P=0.543, 95% CI: 0.27, 0.74). In female patients, ROC-AUC was 0.74 for SPISE (P=0.006, 95% CI: 0.58, 0.90), 0.59 for HOMA-IR (P=0.214, 95% CI: 0.32, 0.87), and 0.68 for HIRI (P=0.072, 95% CI: 0.46, 0.90). SPISE seemed to perform better in female patients compared to males (0.74 vs. 0.71 in males), but when comparing ROC-curves the difference was not significant (p=0.814).

The optimal cutoff level for SPISE between NAFLD and non-NAFLD patients was 5.18 overall (Youden index: 0.35; sensitivity 0.68%, specificity 0.67%). When looking at different NAFLD grades, as shown in **Table 3**, the optimal cutoff level was described as following: SPISE > 5.18 between non-NAFLD and NAFLD grades 1-2 (Youden index: 0.36; sensitivity 0.69%, specificity 0.67%), SPISE > 5.79 between non-NAFLD and NAFLD grades 3-4 (Youden index: 0.38; sensitivity 0.86%, specificity 0.52%) respectively.

DISCUSSION

The current study aimed to compare the performance of SPISE as an estimation of hepatic impaired insulin sensitivity in children and adolescents with obesity. The main finding of this study is that SPISE indicates hepatic IR in pediatric patients with sensitivity and specificity superior as compared to established indices of hepatic IR.

Childhood obesity and its comorbidities show a rising prevalence worldwide (1, 2), implicating that an early identification of these diseases is of utmost importance in order to achieve better patient outcomes. Among these comorbidities, NAFLD has been associated with a metabolic deterioration as early as during childhood (8–10). NAFLD predictive risk factors in childhood were demonstrated to include increased waist circumference, elevated waist-to-hip ratio, elevated total cholesterol, triglycerides, fasting insulin, HOMA-IR as well as elevated glucose and insulin concentration in an OGTT (36). Previously, the best independent predictive risk factor for diagnosing NAFLD in non-diabetic children with obesity was suggested to be fasting insulin >18.9 µIU/ml (36). However, fasting insulin and HOMA-IR levels vary considerably depending on the type of insulin assay (37, 38). Hence, multiple surrogate markers of IR have formerly emerged (20, 39, 40).

Among these, the SPISE was developed as an easy and affordable tool for the evaluation of whole-body insulin sensitivity, which is comparable to clamp-derived M-value in sensitivity as well as specificity (19). Several studies have evaluated the SPISE in adult as well as juvenile populations (20, 41–46). Correa-Burrows et al. assessed SPISE for its validity in diagnosing cardiometabolic risks, namely IR and metabolic syndrome, in post-pubertal Hispanic adolescents. SPISE was found to be accurate for the prediction of IR in both groups,

TABLE 2 | Descriptive data of NAFLD (male n = 41[§], female n = 16[+]) and non-NAFLD patients (male n = 18[#], female n = 24[$]). NAFLD was defined as liver fat content > 5% according to MRI).

	Male		Female	
	NAFLD	**non-NAFLD**	**NAFLD**	**non-NAFLD**
Anthropometric data				
Age (years)	13.6 ± 1.9	13.6 ± 1.7	14.3 ± 2.4	12.7 ± 2.2
Body mass (kg)	90.8 ± 21.0	88.4 ± 21.5	88.9 ± 17.3	76.3 ± 20.6
Height (cm)	166.0 ± 11.9	171.5 ± 8.1	161.1 ± 7.5	157.8 ± 10.1
BMI (kg/m^2)	32.7 ± 5.0	29.7 ± 4.8	34.3 ± 6.8	30.3 ± 5.8
BMI-SDS	3.0 ± 0.5	2.6 ± 0.6	2.9 ± 0.8	2.6 ± 0.6
SBMI (kg/m^2)	35.8 ± 3.5	33.2 ± 3.3	36.1 ± 5.5	34.0 ± 4.5
Waist circumference (cm)	106.7 ± 11.4	99.9 ± 14.3	102.3 ± 10.7	96.9 ± 13.8
Waist/ Hip ratio	**1.0 ± 0.1** [0.97, 1.01]	**0.9 ± 0.1** [0.89, 0.96]	0.9 ± 0.1	0.9 ± 0.1
Systolic blood pressure (mmHg)	123.6 ± 13.2	122.0 ± 11.9	120.0 ± 9.3	119.0 ± 11.5
MRI data				
MRI liver fat content (%)	**15.9 ± 11.9** [12.12, 19.64]	**3.0 ± 1.0** [2.54, 3.49]	**13.5 ± 9.7** [8.35, 18.71]	**3.1 ± 0.9** [2.71, 3.44]
MRI VAT volume (cm^3)	**1722.3 ± 654.1** [1515.83, 1928.73]	**1244.4 ± 337.8** [1076.43, 1412.44]	**1572.1 ± 370.2** [1374.82, 1769.35]	**1158.3 ± 388.6** [994.16, 1322.34]
MRI SAT volume (cm^3)	6601.5 ± 2021.3	5518.4 ± 2397.2	7658.2 ± 2134.6	5900.1 ± 2116.0
MRI DSAT volume (cm^3)	3256.9 ± 1196.3	2589.8 ± 1303.4	3660.7 ± 886.3	2874.5 ± 1271.1
MRI SSAT volume (cm^3)	3134.7 ± 950.2	2628.8 ± 1084.2	3821.2 ± 1636.6	2914.6 ± 1017.8
Laboratory data				
HbA1c (mmol/mol)	35.3 ± 3.0	35.4 ± 2.1	34.8 ± 1.8	34.5 ± 1.8
Total cholesterol (mmol/L)	4.2 ± 0.9	4.0 ± 0.8	4.2 ± 0.7	4.3 ± 0.6
LDL cholesterol (mmol/L)	2.4 ± 0.8	2.1 ± 0.5	2.2 ± 0.5	2.4 ± 0.6
HDL cholesterol (mmol/L)	1.3 ± 0.2	1.5 ± 0.5	1.4 ± 0.3	1.4 ± 0.4
Triglycerides (mmol/L)	1.2 ± 0.7	0.9 ± 0.5	1.3 ± 0.4	1.1 ± 0.5
AST (µkat/L)	0.7 ± 0.5	0.5 ± 0.1	0.4 ± 0.1	0.4 ± 0.2
ALT (µkat/L)	**0.9 ± 1.0** [0.56, 1.16]	**0.4 ± 0.2** [0.35, 0.54]	0.4 ± 0.1	0.3 ± 0.1
GGT (µkat/L)	0.5 ± 0.4	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
Uric acid (µmol/L)	360.8 ± 99.9	384.5 ± 84.3	339.8 ± 48.7	317.2 ± 61.0
Adiponectin (µg/mL)	6.6 ± 2.4	9.2 ± 4.6	7.5 ± 3.1	8.5 ± 3.2
Leptin (ng/mL)	32.6 ± 18.4	26.3 ± 33.3	45.3 ± 19.0	43.7 ± 24.9
hs-CrP (mg/L)	4.3 ± 4.9	3.2 ± 3.5	4.5 ± 3.8	3.1 ± 4.0
IL-6 (pg/mL)	7.9 ± 3.7	7.0 ± 0.1	7.2 ± 0.6	7.2 ± 1.0
TNF-alpha (pg/mL)	8.6 ± 1.4	8.1 ± 2.0	7.4 ± 2.1	8.5 ± 2.2
OGTT data				
OGTT fasting glucose (mmol/L)	4.9 ± 0.6	4.9 ± 0.6	4.7 ± 0.7	4.8 ± 0.6
OGTT 120 min. glucose (mmol/L)	6.6 ± 1.4	6.0 ± 1.4	6.1 ± 1.7	6.4 ± 1.2
OGTT fasting insulin (pmol/L)	137.7 ± 74.9	94.5 ± 48.0	131.2 ± 79.4	100.2 ± 31.0
Parameters of insulin resistance				
SPISE	4.8 ± 1.2	6.0 ± 1.6	4.5 ± 1.1	5.6 ± 1.5
HOMA-IR	4.2 ± 2.2	2.9 ± 1.6	4.0 ± 2.6	2.9 ± 0.9
HIRI	56543.0 ± 31614.9	54967.8 ± 32057.1	66954.9 ± 40217.0	48134.5 ± 35071.6

Data are expressed as mean ± standard deviation. All subjects were staged as "pubertal" according to Tanner staging (II-IV).

[§]n = 41 except of n = 40 for waist circumference, waist/hip ratio, systolic blood pressure, HbA1c, AST, uric acid, OGTT fasting glucose, VAT, SAT, DSAT, SSAT; n = 36 for IL-6, TNF-alpha; n = 33 for adiponectin; n = 32 for OGTT fasting insulin; n = 31 for HOMA-IR; n = 30 for leptin; n = 28 for HIRI.

[+]n = 16 except of n = 15 for OGTT fasting glucose; n = 14 for AST, DSAT, SSAT; n = 13 for leptin; n = 10 for OGTT fasting insulin, HOMA-IR, HIRI.

[#]n = 18 except of n = 17 for adiponectin, IL-6, TNF-alpha, VAT, SAT; n = 13 for leptin; n = 12 for OGTT fasting insulin, HOMA-IR; n = 11 for HIRI.

[$]n = 24 except of n = 23 for AST, hs-CrP; n = 21 for adiponectin, IL-6; n = 20 for TNF-alpha; n = 18 for OGTT fasting insulin, HOMA-IR; n = 16 for leptin; n = 15 for HIRI.

NAFLD, non-alcoholic fatty liver disease; MRI, magnetic resonance imaging; SD, standard deviation; BMI, body mass index; SDS, standard deviation score; SBMI, smart body mass index; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; DSAT, deep subcutaneous adipose tissue; SSAT, superficial subcutaneous adipose tissue; HbA1c, hemoglobin A1c; LDL, low density lipoprotein; HDL, high density lipoprotein; AST, aspartate transaminase; ALT, alanine transaminase; GGT, gamma-glutamyl transferase; hs-CrP, high-sensitivity C-reactive Protein; IL-6, Interleukin 6; TNF, tumor necrosis factor; OGTT, oral glucose tolerance test; min., minutes; SPISE, Single Point Insulin Sensitivity Estimator; HOMA-IR, homeostatic Model Assessment for Insulin Resistance; HIRI, Hepatic Insulin Resistance Index.

Confidence intervals were calculated and significant differences between NAFLD and non-NAFLD groups were marked in bold letters and the 95% confidence interval (CI) added in brackets.

with cutoff values of 5.0 (males) and 6.0 (females) indicating IR (41). Similarly, a cutoff value of 5.82 for prediction of IR in metabolic syndrome was determined by Dudi et al. in a north Indian adult population. SPISE was thereby shown to discriminate well between cases and controls (42). More recently, a study analysed data from 909 Italian children with overweight and obesity and normal weight controls undergoing metabolic evaluations. Two-hundred children who were overweight or

TABLE 3 | Descriptive data of NAFLD and non-NAFLD subjects according to grades of steatosis: non-NAFLD[#]: liver fat content (LFC) < 2.6%; grade 0[+]: LFC 2.6 - ≤5%; grade 1[§]: LFC >5 - ≤9.2%; grade 2[%]: LFC >9.2 - ≤15.1%; grade 3 [°]: LFC >15.1 - ≤26.8%; grade 4[◆]: LFC >26.8%. Non-NAFLD in this study was defined as a LFC ≤5%, therefore it comprises of following groups in the table: non-NAFLD and NAFLD grade 0.

	non-NAFLD	NAFLD grade 0	NAFLD grade 1	NAFLD grade 2	NAFLD grade 3	NAFLD grade 4
Anthropometric data						
Age (years)	13.1 ± 2.1	13.1 ± 2.0	14.4 ± 2.1	13.5 ± 1.7	13.7 ± 2.2	12.9 ± 1.8
Body mass (kg)	82.6 ± 26.3	81.0 ± 19.6	90.9 ± 19.0	88.3 ± 17.1	92.8 ± 23.0	88.0 ± 24.4
Height (cm)	165.2 ± 12.9	162.9 ± 11.0	166.5 ± 9.6	160.7 ± 12.3	165.1 ± 11.1	164.1 ± 12.8
BMI (kg/m^2)	29.6 ± 5.7	30.2 ± 5.3	32.8 ± 6.5	34.1 ± 4.8	33.7 ± 5.1	32.3 ± 4.5
BMI-SDS	2.6 ± 0.6	**2.6 ± 0.6** [2.42, 2.87]	2.8 ± 0.7	**3.1 ± 0.4** [2.88, 3.73]	3.1 ± 0.5	3.0 ± 0.6
SBMI (kg/m^2)	33.1 ± 3.7	33.9 ± 4.2	35.1 ± 5.0	36.6 ± 2,9	36.6 ± 3.3	36.1 ± 3.9
Waist circumference (cm)	99.9 ± 17.1	97.5 ± 12.5	104.6 ± 12.6	107.0 ± 9.8	105.1 ± 10.9	106.3 ± 11.8
Waist/ Hip ratio	1.0 ± 0.1	**0.9 ± 0.1** [0.89, 0.94]	1.0 ± 0.1	**1.0 ± 0.0** [0.95, 1.00]	1.0 ± 0.1	**1.0 ± 0.1** [0.96, 1.05]
Systolic blood pressure (mmHg)	120.1 ± 11.3	120.3 ± 12.0	121.1 ± 10.0	120.2 ± 12.1	124.5 ± 13.5	126.9 ± 16.4
MRI data						
MRI VAT volume (cm^3)	**1114.7 ± 350.4** [902.98, 1326.50]	**1226.8 ± 374.0** [1084.53, 1369.03]	1465.5 ± 419.3	**1801.8 ± 512.2** [1476.33, 2127.18]	1763.3 ± 800.5	**2011.5 ± 629.6** [1527.55, 2495.49]
MRI SAT volume (cm^3)	5769.0 ± 2911.1	5730.6 ± 1919.2	7038.8 ± 2428.1	7082.6 ± 1752.9	6666.2 ± 1742.7	6584.3 ± 2246.2
MRI DSAT volume (cm^3)	2540.8 ± 1491.8	2847.4 ± 1185.1	3270.2 ± 1159.4	3647.4 ± 1090.0	3262.7 ± 942.6	3341.3 ± 1528.3
MRI SSAT volume (cm^3)	2648.6 ± 1341.0	2856.5 ± 900.1	3521.3 ± 1478.7	3156.6 ± 921.1	3170.1 ± 865.0	3139.4 ± 1118.0
MRI liver fat content (%)	**1.9 ± 0.4** [1.70, 2.19]	**3.6 ± 0.5** [3.35, 3.74]	**6.3 ± 1.1** [5.81, 6.76]	**11.8 ± 1.4** [10.87, 12.69]	**20.2 ± 3.1** [18.16, 22.14]	**37.1 ± 6.7** [31.94, 42.16]
Laboratory data						
HbA1c (mmol/mol)	**33.5 ± 1.6** [32.56, 34.51]	**35.5 ± 1.9** [34.77, 36.19]	35.0 ± 2.2	35.9 ± 2.4	34.9 ± 4.2	34.6 ± 2.2
Total cholesterol (mmol/L)	3.9 ± 0.8	4.3 ± 0.6	4.2 ± 0.9	4.2 ± 0.6	4.4 ± 0.8	3.9 ± 1.1
LDL cholesterol (mmol/L)	2.3 ± 0.7	2.3 ± 0.6	2.4 ± 0.8	2.4 ± 0.5	2.5 ± 0.8	2.0 ± 0.9
HDL cholesterol (mmol/L)	1.2 ± 0.2	1.5 ± 0.5	1.3 ± 0.2	1.3 ± 0.3	1.2 ± 0.3	1.2 ± 0.2
Triglycerides (mmol/L)	0.9 ± 0.3	1.1 ± 0.6	1.1 ± 0.5	1.1 ± 0.5	1.4 ± 0.7	1.5 ± 1.0
AST (µkat/L)	0.5 ± 0.2	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.7 ± 0.5	1.0 ± 0.8
ALT (µkat/L)	**0.3 ± 0.1** [0.29, 0.40]	0.4 ± 0.2	**0.4 ± 0.1** [0.34, 0.42]	0.5 ± 0.2	**0.9 ± 0.8** [0.43, 1.41]	**1.7 ± 1.5** [0.57, 2.90]
GGT (µkat/L)	**0.2 ± 0.1** [0.21, 0.28]	0.3 ± 0.1	0.3 ± 0.1	**0.3 ± 0.1** [0.28, 0.40]	0.4 ± 0.2	**0.9 ± 0.8** [0.29, 1.51]
Uric acid (µmol/L)	357.9 ± 74.5	340.7 ± 81.0	338.6 ± 87.0	333.1 ± 87.1	380.0 ± 59.4	391.2 ± 117.2
Adiponectin (µg/mL)	7.7 ± 4.7	9.1 ± 3.6	7.3 ± 1.9	8.4 ± 3.9	5.5 ± 2.2	6.0 ± 2.9
Leptin (ng/mL)	22.1 ± 26.4	40.2 ± 30.0	34.9 ± 20.1	44.3 ± 15.0	35.0 ± 20.7	33.7 ± 21.9
hs-CrP (mg/L)	4.7 ± 5.4	2.4 ± 2.5	4.3 ± 4.3	5.5 ± 5.6	2.6 ± 2.0	5.4 ± 6.1
IL-6 (pg/mL)	7.1 ± 0.2	7.2 ± 0.9	7.6 ± 2.6	7.0 ± 0.1	7.0 ± 0.0	9.4 ± 6.6
TNF-alpha (pg/mL)	8.0 ± 1.7	8.4 ± 2.2	8.2 ± 1.6	8.1 ± 1.7	8.2 ± 2.1	8.5 ± 1.5
OGTT data						
OGTT fasting glucose (mmol/L)	4.9 ± 0.7	4.9 ± 0.5	4.6 ± 0.5	5.2 ± 0.9	4.8 ± 0.6	5.0 ± 0.5
OGTT 120 min. glucose (mmol/L)	5.9 ± 1.7	**6.4 ± 1.1** [5.21, 6.38]	5.8 ± 1.4	6.8 ± 1.5	6.4 ± 0.8	**7.8 ± 1.5** [6.64, 8.86]
OGTT fasting insulin (pmol/L)	**92.1 ± 46.9** [63.72, 120.45]	**100.4 ± 34.6** [87.28, 113.58]	**103.8 ± 55.1** [80.56, 127.07]	146.6 ± 62.0	135.9 ± 54.4	**217.8 ± 114.4** [129.87, 305.72]
Parameters of insulin resistance						
SPISE	5.9 ± 1.5	**5.7 ± 1.6** [5.14, 6.35]	5.0 ± 1.3	**4.5 ± 0.8** [4.03, 5.03]	4.5 ± 1.3	4.9 ± 1.2

(Continued)

TABLE 3 | Continued

	non-NAFLD	NAFLD grade 0	NAFLD grade 1	NAFLD grade 2	NAFLD grade 3	NAFLD grade 4
HOMA-IR	**2.7 ± 1.2** [1.95, 3.40]	**3.0 ± 1.2** [2.57, 3.49]	**3.0 ± 1.7** [2.30, 3.72]	**4.7 ± 1.9** [3.49, 5.91]	3.9 ± 1.4	**6.7 ± 3.3** [4.19, 9.24]
HIRI	**29659.4 ± 15652.8** [20200.50, 39118.32]	**57435.4 ± 34798.1** [44198.85, 70671.86]	**59224.3 ± 35461.2** [44250.31, 74198.24]	**55862.6 ± 17165.9** [44955.87, 66769.28]	48151.8 ± 26004.5	**84979.5 ± 53611.3** [43770.26, 126188.80]

Data are expressed as mean ± standard deviation. All subjects were staged as "pubertal" according to Tanner staging (II–IV).

Each group contains male as well as female patients; detailed information on distribution is available on demand.

Exceptions in number of patients: [#]n = 13, except for AST/MRI VAT/MRI SAT (n=12), adiponectin/IL-6/TNF-alpha/OGTT fasting insulin/HOMA-IR (n=9), leptin (n=7), HIRI (n=6); [+]n = 29, except for hs-CrP/TNF-alpha (n=28), leptin (n=22), OGTT fasting insulin/HOMA-IR (n=21), HIRI (n=20); [§]n = 24, except for HbA1c/adiponectin/DSAT/SSAT (n=23), leptin/OGTT fasting glucose (n=22), AST (n=21), OGTT fasting insulin (n=18), HOMA-IR (n=17), HIRI (n=16); [%]n = 12, except for waist circumference/waist-hip-ratio/systolic blood pressure/uric acid (n=11), IL-6/TNF-alpha/OGTT fasting insulin/HOMA-IR (n=9), adiponectin/leptin/HIRI (n=8); [◇]n = 12, except for IL-6/TNF-alpha (n=11), adiponectin (n=10), OGTT fasting insulin/HOMA-IR/HIRI (n=9), leptin (n=7); [♦]n = 9, except for adiponectin/IL-6/TNF-alpha/MRI VAT/MRI SAT (n=8), MRI DSAT/MRI SSAT (n=7), leptin/OGTT fasting insulin/HOMA-IR (n=6), HIRI (n=5).

NAFLD, non-alcoholic fatty liver disease; MRI, magnetic resonance imaging; SD, standard deviation; BMI, body mass index; SDS, standard deviation score; SBMI, smart body mass index; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; DSAT, deep subcutaneous adipose tissue; SSAT, superficial subcutaneous adipose tissue; HbA1c, hemoglobin A1c; LDL, low density lipoprotein; HDL, high density lipoprotein; AST, aspartate transaminase; ALT, alanine transaminase; GGT, gamma-glutamyl transferase; hs-CrP, high-sensitivity C-reactive Protein; IL-6, interleukin 6; TNF, tumor necrosis factor; OGTT, oral glucose tolerance test; min., minutes; SPISE, Single Point Insulin Sensitivity Estimator; HOMA-IR, homeostatic Model Assessment for Insulin Resistance; HIRI, Hepatic Insulin Resistance Index.

Confidence intervals were calculated and significant differences between groups were marked in bold letters and the 95% confidence interval (CI) is added in brackets.

obese were assessed longitudinally for on average of 6.5 years (range 3.5-10). At follow-up, lower basal SPISE strongly predicted the development of abnormal glucose metabolism (AUROC curve: 0.83 [0.72-0.94] regardless of age, sex, fasting/120 mins glucose and insulin at baseline (46). Of interest, SPISE-IR (=10/SPISE) was also a predictor of coronary heart disease and type 2 diabetes in a group of elderly Swedish men (44).

To the best of our knowledge, SPISE has so far not been assessed in children and adolescents with NAFLD. Our results are perfectly in line with data from the Yale Pediatric NAFLD cohort showing that intrahepatic lipid accumulation is associated with reduced insulin clearance and hepatic insulin sensitivity in youths with obesity, irrespective of their ethnic background (11).

Insulin resistance was shown to be indicative of histological severity of liver disease in adults with obesity (47, 48). Additionally, HOMA-IR was an independent predictor of advanced liver fibrosis in nondiabetic Japanese adults with NAFLD (49). Recently, Bedogni et al. developed two multivariable models, using single anthropometric as well as laboratory parameters (BMI or waist circumference, ALT, HOMA-IR, triglycerides and uric acid) (19). Both models were demonstrated to identify fatty liver, as diagnosed *via* ultrasonography (19). However, SPISE may offer an easier and therefore more accessible identification of patients with hepatic insulin resistance. Additionally, a radiologic diagnosis *via* MRI allowed us a much more accurate assessment of liver fat content compared to ultrasonography (28–34, 50).

As described before, SPISE is based on BMI, fasting HDL-cholesterol and triglyceride levels. Hepatic lipid accumulation is closely related to the development of IR (51). Elevated ceramide concentrations, together with their significant correlation with IR parameters in pediatric patients with obesity, were suggested to be associated with molecular pathways involved in insulin signaling impairment strongly linked to the pathogenesis of NAFLD (52). In addition, hepatic expression of genes associated with IR may drive NAFLD development and progression. Thus, genes which can promote intrahepatic fat accumulation, dysregulation of the lipid metabolism, lipotoxicity, and activation of cell survival pathways including activation of cell proliferation and differentiation pathways, were shown to allow classification of adult NASH (Nonalcoholic steatohepatitis)-with-fibrosis patients separately from mild-NAFL (nonalcoholic fatty liver) and NASH patients (53). In agreement with this, TG/HDL-C (triglyceride/HDL-cholesterol) ratio was reported to be useful to identify children and adolescents at high risk of NAFLD (54). This is also in accordance with data demonstrating that the fasting triglyceride-to glucose index was linked to increasing severity of hepatic steatosis and the presence of liver fibrosis in adults with NAFLD and more closely related to NAFLD and liver fibrosis compared to HOMA-IR after adjustment for confounding factors (55).

NAFLD is an exclusion diagnosis and can progress (NASH and fibrosis) if undiagnosed and untreated. A uniform international consent for screening for NAFLD in juvenile obesity does not exist. AASLD Guidance does not recommend screening for NAFLD in children with obesity due to "paucity of evidence" (56). In contrast, NASPGHAN advocates screening by alanine aminotransferase (ALT), but does not recommend ultrasound (US) due to low sensitivity in all children with overweight and obesity and

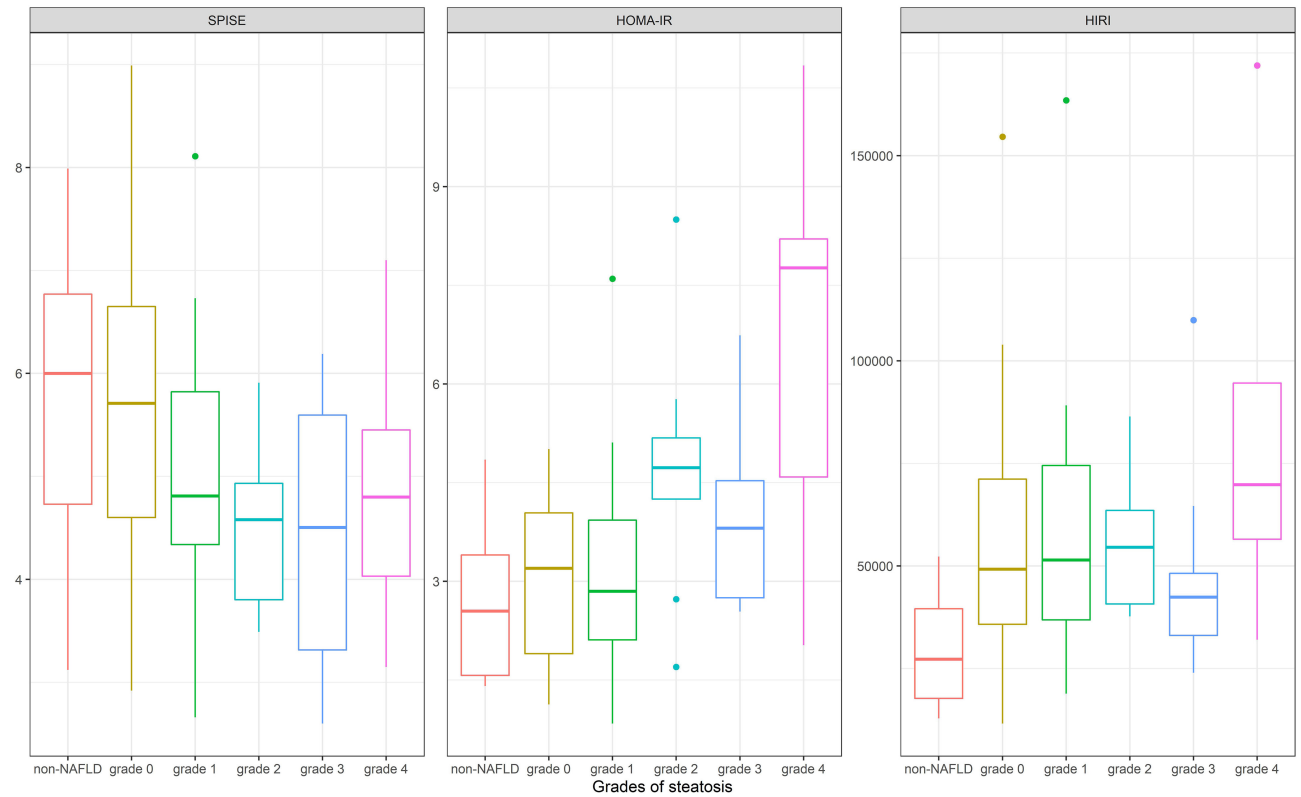

FIGURE 1 | Comparison of the performance of SPISE, HOMA-IR and HIRI according to different steatosis grades [non-NAFLD: liver fat content (LFC) < 2.6%; grade 0: LFC 2.6 - ≤5%; grade 1: LFC >5 - ≤9.2%; grade 2: LFC >9.2 - ≤15.1%; grade 3 : LFC >15.1 - ≤26.8%; grade 4: LFC >26.8%]. SPISE, Single Point Insulin Sensitivity Estimator; HOMA-IR, homeostatic Model Assessment for Insulin Resistance; HIRI, Hepatic Insulin Resistence Index; NAFLD, Non-alcoholic fatty liver disease; LFC, Liver fat content.

TABLE 4 | Pearson correlation coefficients (r) for the relation of M-value (100-120 min.) as derived from euglycemic clamp method and hepatic insulin resistance indices (n = 17).

	r-value	p-value	CI
SPISE	0.489	**0.047***	[0.010 , 0.785]
HOMA-IR	-0.135	0.604	[-0.578 , 0.369]
HIRI	-0.323	0.362	[-0.792 , 0.385]

*$p < 0.05$.

CI, confidential interval; SPISE, Single Point Insulin Sensitivity Estimator; HOMA-IR, homeostatic Model Assessment for Insulin Resistance; HIRI, Hepatic Insulin Resistence Index.

additional risk factors at age 9-11 years (50). Both methods combined seem favorable as ALT might be normal or slightly elevated and US sensitivity diminishes in children where hepatic fat accumulation remains below 30% (57). Up to now, the gold standard in diagnosing fibrosis is liver biopsy, which nevertheless resembles an invasive, complex and time-consuming method (58). Several studies have analyzed non-invasive markers of liver steatosis and fibrosis in order to bypass this method. Kulkarni et al. identified a model of several non-invasive parameters that could predict NAFLD induced fibrosis (59). Above all, it seems that a combination of anthropometric, laboratory as well as radiologic methods might improve the practicability and exactitude of diagnosing NAFLD induced fibrosis in pediatric obesity (60, 61).

Strengths and Limitations

A major strength of this study was the inclusion of both MRI and clamp data in a pediatric cohort. The lack of histological data does not allow us to discriminate between simple steatosis and differing degrees of fibrosis. However, Schwimmer et al. showed a positive correlation between MRI-estimated liver proton density fat fraction and steatosis grades by liver histology (34), which underscores the need to identify patients more readily in clinical practice. In addition, we do not have detailed information on the distribution of ethnicities in our collective, although the majority of our patients is white. This might be important, as differences in IR between ethnicities have been described repeatedly (62, 63). Further, due to its cross-sectional design, our data do not allow us to draw any conclusions on the performance of the SPISE in the evolution of NAFLD longitudinally. However, in order to increase the homogeneity of our cohort, we included data of pubertal patients only (Tanner stages II-IV) and employed robust techniques to assess liver fat content and IR. Due to a limited sample size further studies will be needed in order to validate our findings in larger pediatric cohorts. This would allow

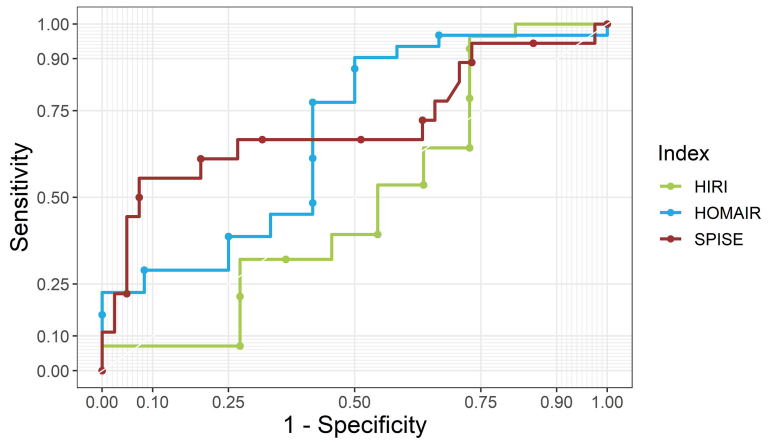

FIGURE 2 | ROC curves for SPISE, HOMA-IR and HIRI for male patients. SPISE, Single Point Insulin Sensitivity Estimator; HOMA-IR, homeostatic Model Assessment for Insulin Resistance; HIRI, Hepatic Insulin Resistence Index.

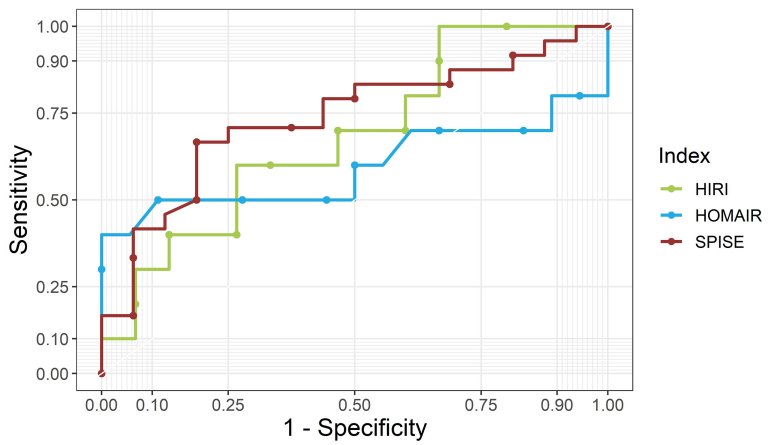

FIGURE 3 | ROC curves for SPISE, HOMA-IR and HIRI for female patients. SPISE, Single Point Insulin Sensitivity Estimator; HOMA-IR, homeostatic Model Assessment for Insulin Resistance; HIRI, Hepatic Insulin Resistence Index.

more detailed analyses of SPISE cutoffs in children with different pubertal stages and degrees of obesity.

In conclusion, in a clinical setting the early diagnosis of NAFLD is of utmost importance, since its progression to fibrosis has substantial impact on overall morbidity in the pediatric population and morbidity and mortality in later life. Thus, additional simple surrogates of hepatic insulin resistance aiding in the clinical diagnosis of NAFLD are needed. SPISE outperformed established indices of hepatic insulin resistance when compared to M-values derived from hyperinsulinemic clamp tests in both males and females. Although neither index (SPISE, HOMA-IR, HIRI) allowed a differentiation of steatosis-grades within the NAFLD group, SPISE may represent an easy surrogate of hepatic insulin resistance in children with overweight or obesity to be used as a screening tool for hepatic risk assessment on a large scale and in longitudinal 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.

ETHICS STATEMENT

The ethical approvals for the study and necessary amendments were obtained from the ethical committees of Uppsala University (Uppsala Regional Ethics Committee, registration numbers 2010/036 and 2012/318) as well as Salzburg University (Ethics Committee Salzburg 2012/1544). The study was carried out according to the Declaration of Helsinki, following an agreement of good clinical practice. The study physician

informed the patients and controls and their families personally and written consent was consequently obtained from children/adolescents and parents separately. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

DF, CHA, HMangge, HManell, PK, SS, DW and KMö conceived and designed the analysis, contributed data, performed the analyses and wrote the paper. KMö, SMB and KMa collected samples and data. PB, AF, JK, HA and AMS collected additional data and contributed to this manuscript.

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ORIGINAL RESEARCH
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TG: HDL-C Ratio as Insulin Resistance Marker for Metabolic Syndrome in Children With Obesity

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Insulin resistance (IR) is an important variable in the diagnosis of metabolic syndrome (MetS). Currently, IR is not part of the existing pediatric definition of MetS, instead elevated fasting blood glucose (FBG) is measured as an indicator of hyperglycemia. Arguably, many obese children with severe IR are still able to regulate their FBG well. Hence, this study aimed to assess the utility of triglyceride-to-high-density lipoprotein cholesterol (TG : HDL-C) ratio as an IR marker in the modeling of pediatric MetS among children with obesity using structural equation modeling (SEM). A total of 524 blood samples from children with obesity (age 10–16 years old) were analyzed for FBG, lipids, insulin, leptin, and adiponectin. Both exploratory (EFA) and confirmatory factor analysis (CFA) were used to examine TG : HDL-C ratio as an IR marker in pediatric MetS. EFA shows that TG: HDL-C ratio (standardized factor loading = 0.904) groups together with homeostasis model assessment-estimated insulin resistance (HOMA-IR) (standardized factor loading = 0.664), indicating a strong correlation to the IR factor. Replacing FBG with TG: HDL-C ratio improved the modeling of MetS structure in children with obesity. Our MetS model of TG: HDL-C ratio as IR component shows comparable model fitness indices (goodness of fit, Akaike's information criterion, and Bayesian information criterion) with leptin: adiponectin ratio (platinum standard for adiposity:IR marker) model. The least model fit was seen when using FBG as an IR surrogate. TG : HDL-C ratio performed better as IR surrogate in MetS structures (standardized factor loading = 0.39) compared to FBG (standardized factor loading = 0.27). TG: HDL-C ratio may be considered as an IR component in pediatric MetS.

Keywords: pediatric, obesity, TG: HDL-C ratio, metabolic syndrome, insulin resistance

1 INTRODUCTION

Metabolic syndrome (MetS) is a cluster of risk factors that includes obesity, dyslipidemia, insulin resistance (IR) or impaired glucose tolerance, and elevated blood pressure (BP). The significance of MetS among pediatrics arises in line with the growth of obesity prevalence among children and the rise of MetS in adults. Early identification and treatment of obese children and adolescents with multiple metabolic derangements, particularly those at higher risk, may curb the risk of developing cardiometabolic diseases such as cardiovascular disease (CVD) and type 2 diabetes mellitus (T2DM). However, identifying those who are affected is rather difficult because clear recommendations about how to diagnose MetS in the young age group are still lacking (1). Among others, one of the limitations is the reliance on elevated fasting blood glucose (FBG) rather than fasting insulin or the homeostasis model assessment-estimated insulin resistance (HOMA-IR) as a measure of impaired glucose regulation whereby many children with severe IR can still regulate their FBG (2). Therefore, there is a need to search for a simple, reliable, and applicable surrogate marker to measure IR among children to improve the existing pediatric MetS definition.

A large multi-ethnic cohort study by Giannini et al. (3) has proposed the use of triglyceride-to-high-density lipoprotein cholesterol (TG: HDL-C) ratio as a cheap and reliable IR surrogate in children with obesity. This study (3) showed some limitations in evaluating the TG : HDL-C ratio as an IR marker, and it was specific to the US population. South Asians are more IR than Caucasians and African-Americans (4), and therefore, TG : HDL-C ratio has more clinical potential in the diagnosis of MetS in this population. Although previous studies have demonstrated the use of TG : HDL-C ratio as an IR marker and identifying children at risk for MetS in the West (5, 6) and Asia (7, 8), none of the studies employed structural equation modeling (SEM) to validate and measure the strength of correlation between TG: HDL-C ratio and IR in the theoretical structure of MetS.

Pediatric MetS diagnosis was based on the grouping of intercorrelated factors and variables that will introduce multicollinearity, which violates one of the conventional regression model assumptions. Factor analysis is a distinctive feature of SEM in which a series of dependence relationships can be examined simultaneously in one technique accounting for measurement error. This type of statistical analysis is not possible in multiple regression analysis. In a typical multiple regression analysis, the association was measured between a single dependent variable and multiple covariates. These covariates are assumed to be measured without measurement error (9). Factor analysis has been used in numerous MetS studies (10, 11) particularly in establishing the parameters used to measure each MetS risk factor such as the use of body mass index (BMI), waist-to-hip ratio, and waist circumference (WC) as measures of obesity (12, 13). Furthermore, the recently emerging MetS scoring was established using factor analysis (14, 15).

To our knowledge, none of the previous pediatric MetS studies has employed SEM in determining the strength of correlation between TG : HDL-C ratio and IR in the theoretical structure of MetS. We hypothesized that TG: HDL-C ratio is highly correlated with measures of IR (insulin and HOMA-IR) and will group together as IR group in the MetS structure. Secondly, we also hypothesized that TG : HDL-C ratio is a better IR surrogate in the MetS structure than FBG. Thirdly, given that children's obesity is homogeneous in this study, the hypothesized model was referenced to a model of an established adiposity-IR marker (leptin:adiponectin ratio). Therefore, the main objective of this study is to examine the utility of TG: HDL-C ratio as an IR marker in the MetS structure of children with obesity.

2 MATERIALS AND METHODS

2.1 Study Design and Participants

This study was performed using a cross-sectional baseline data of children with obesity participating in the My Body is Fit and Fabulous at School (MyBFF@school) programme, a school-based cluster randomized controlled trial (C-RCT) study. Detailed descriptions of the recruitment of MyBFF@school programme have been previously published (16). In general, MyBFF@school was designed to address the rise of childhood obesity among Malaysian schoolchildren. MyBFF@school was conducted for 6 months between February 2016 and August 2016 in the Federal Territory of Kuala Lumpur, Selangor, and Negeri Sembilan of Malaysia. For this study, we randomly selected 524 baseline blood samples from children with obesity older than 10 years but below 16 years old with complete anthropometric measurements, BP measurements, and pubertal staging data (**Figure 1**). Ethical approval was granted by the Medical Research and Ethics Committee (MREC), Ministry of Health Malaysia (NMRR-18-2749-41841).

2.2 Health and Physical Examination

Prior to health and physical examination, children were asked to fast overnight for at least 8 h. Anthropometric measurements were performed by trained personnel, and medical officers and pediatricians performed health examinations. Standing height was measured without shoes to the nearest 0.1 cm using a calibrated stadiometer (Seca 217, Germany). Body weight and body fat mass were measured in light clothing without shoes and socks to the nearest 0.1 kg using a precalibrated body impedance analyzer (InBody 720, Korea). WC was measured twice to the nearest 0.1 cm over the skin midway between the 10th rib and the iliac crest at the end of normal expiration using a non-extensible tape (Seca 201, Germany), and the mean was recorded.

Two BP readings were measured after 5 min of rest using a mercury sphygmomanometer (Accoson, UK) seated with the arm supported at the heart level, and the mean was recorded. Pubertal status was assessed by showing a standardized Tanner staging picture to the child (17, 18). Children were also examined —by pediatricians—for the presence of acanthosis nigricans (AN) over the neck (19). AN was determined based on Burke's quantitative dichotomous score (19).

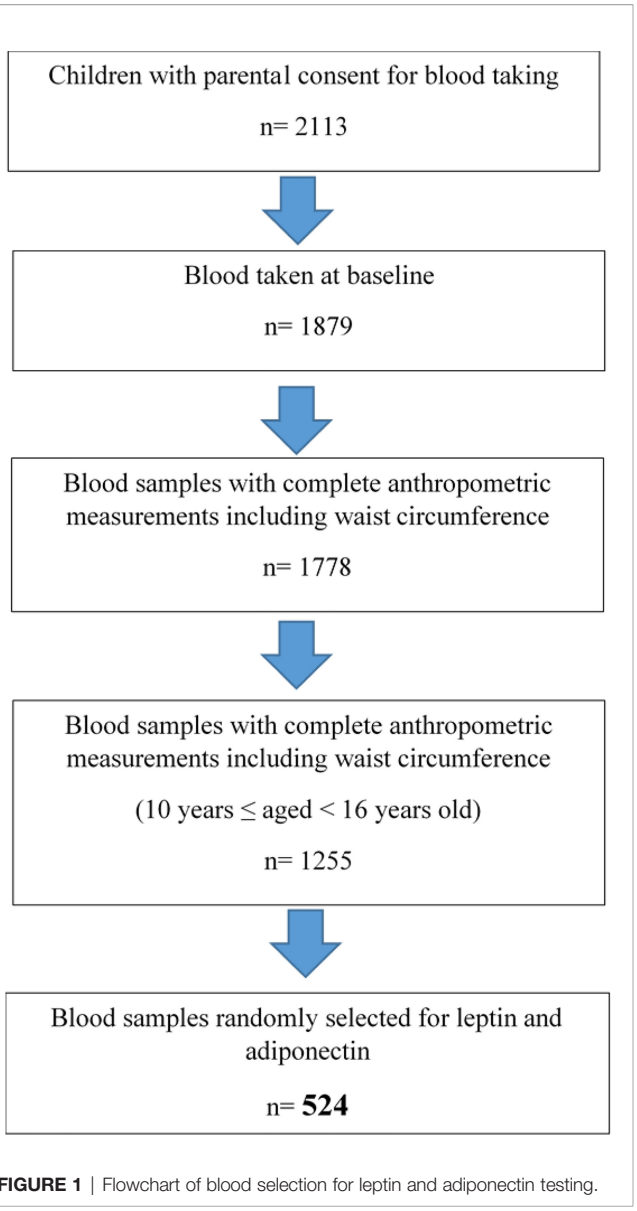

FIGURE 1 | Flowchart of blood selection for leptin and adiponectin testing.

2.3 Blood Sample Collection

Venipuncture was performed by trained nurses and doctors. Blood samples were collected from participating children who consented to blood taking at the participating schools whereby the processing of blood samples was kept to a minimum. Blood samples were transported cold (4°C) in a coolant box with frozen coolant to the central laboratory at the Institute for Medical Research within 2 h of collection and processed on the same day. Aliquots of serum/plasma samples were kept at -20°C and -80°C prior to analysis.

2.4 Biochemical Parameters

HbA1c level was determined by cationic exchanged high-performance liquid chromatography (Adams A1c HA-8160, Arkray Inc., Japan) and followed the National Glycohemoglobin Standardization Programme Guidelines. FBG, TG, total cholesterol, HDL-C, and low-density lipoprotein cholesterol (LDL-C) were analyzed using an automated analyzer (Dirui CS-400, China) with reagents purchased from Randox Laboratories (Antrim, UK).

Fasting insulin concentration was measured using an automated enzyme immunoassay analyzer (TOSOH AIA-360, Japan). Inter-assay coefficient of variability (CV) for insulin at 9.4, 53.7, and 141.8 μU/ml was 5.7%, 3.6%, and 5.2%, respectively. Serum adiponectin was measured using an automated analyzer (Dirui CS-400, China) with reagents purchased from Randox Laboratories (Antrim, UK). The inter-assay CV for adiponectin at 6.1 and 12.4 μg/ml was 9.4% and 5.6%, respectively. Serum leptin was measured by commercial ELISA assay (IBL International, Germany) in two replicates with two controls (Low and High) at each plate. The detection limit of the assay was 0.7–100 ng/ml. The inter-assay CV for low control was <10% and for high control was <15%. In general, immunoassay results are considered reliable when intra-assay CV was <10% and inter-assay CV was <15% (20).

2.5 Operational Definitions of Study Variables

2.5.1 Obesity Status

Overweight and obesity were defined as BMI z-score above 1 and 2 standard deviations for age and sex according to the WHO BMI chart (21).

2.5.2 Pubertal Staging

Stage 1 external genitalia development and breast development for boys and girls were classified as prepubertal, while stage 2 and above were defined as pubertal (17, 18).

2.5.3 Insulin Resistance Index

IR status was defined based on the homeostasis model assessment (HOMA), calculated by multiplying the value of fasting plasma insulin (U/ml) and fasting plasma glucose (mmol/L) and then dividing by 22.5 (22).

2.5.4 Insulin Resistance Status

The pubertal transition from Tanner stage 1 to Tanner stage 3 or 4 is associated with IR (22). For prepubertal children, a score of HOMA-IR ≥2.6 (23) was classified as IR, while a score of less than 2.6 was classified as insulin sensitive. For pubertal children, a score of HOMA-IR ≥4.0 was categorized as IR, while a score of less than 4.0 was categorized as insulin sensitive (24).

2.5.5 Metabolic Syndrome Definition

For children aged 10 to <16 years:

Metabolic syndrome was established based on the International Diabetes Federation (IDF) definition (25). It was considered present if the WC measurement was ≥90th percentile of the Malaysian children WC chart (26) with the presence of at least two of the following criteria: TG ≥1.7 mmol/L, HDL-C <1.03 mmol/L, systolic blood pressure (SBP) ≥130 mmHg and/or diastolic blood pressure (DBP) ≥85 mmHg, or FBG ≥5.6 mmol/L (25).

2.6 Statistical Analysis

The normality test for continuous data was determined using the Kolmogorov–Smirnov test. Means and standard deviations were calculated for continuous variables. In testing the normality

assumption, four variables were found to have a high skewness: TG, TG : HDL-C ratio, FBG, and insulin; these variables were transformed with a natural log function. Comparison of means between two groups was conducted using independent-samples t-test, while for categorical variables, comparisons were made using chi-square test. Statistical significance was set at 0.05.

2.7 Exploratory Factor Analysis

The relationship between TG: HDL-C ratio and leptin:adiponectin ratio (LAR) with MetS components (WC, HOMA-IR, SBP, and DBP) was first examined by exploratory factor analysis (EFA). EFA gathered and divided highly correlated variables in the MetS diagnosis into a specific grouping. This grouping was then confirmed in the confirmatory factor analysis (CFA). Factor extraction was performed using principal component analysis subjected to varimax rotation. Factor extraction produces the minimum number of factors that retain the total variance in the original data as possible. The factor loading of a variable on a factor equals the Pearson correlation coefficient between that variable and the factor. Thus, higher factor loadings represent more correlation between the variable and the factor. Additionally, variables grouped on the same factor is strongly correlated. Hence, represent the factor extracted. For example, the grouping of obesity markers (WC, percentage body fat, BMI z-scores) may be interpreted as the obesity group. Only variables with a factor loading of at least 0.3 (sharing at least 10% of the variance with a factor) were used for interpretation (27). The eigenvalues give information about potential components/factors and their relative explanatory power. In this study, factor extracted is considered valid if the eigenvalues are ≥1.0 (28). The Kaiser–Meyer–Olkin statistic >0.5 was used as a measure of sampling adequacy, and the Bartlett test of sphericity <0.001 was used as a measure of the necessity to perform a factor analysis (29).

EFA was first performed using all traditional variables (WC, DBP, SBP, FBG, TG, and HDL-C) from IDF definition with HOMA-IR and fasting insulin (model 1) as indicators of IR group. Secondly, EFA was performed with all variables from model 1 with the addition of commonly used obesity markers (BMI z-scores and percentage body fat) (model 2) because a minimum of the two variables is later needed to construct each group in the CFA. Additionally, TG : HDL-C ratio and LAR were also included in this EFA. Since factor analysis extracts factors due to the interrelatedness of measured variables (30), in the third EFA (model 3), individual variables of TG : HDL-C ratio and HOMA-IR, that is, TG, HDL-C, fasting insulin, and FBG, were removed from model 2.

2.8 Confirmatory Factor Analysis

CFA was performed to confirm the IR group from EFA and to determine the best model to represent the MetS structures in children with obesity. We used SEM that utilized maximum likelihood estimation in Amos 21.0 (SPSS Inc., Chicago, IL) to develop our CFA models. SEM integrated CFA and path analysis (31). In this approach, MetS structure is visually constructed by correlating the four core risk factors (obesity, lipids, IR, and BP) with specific parameters or indicators measuring each risk factor (**Figure 2**). The analysis provides standardized factor loading that estimates the strength of the relationships between the core risk factors, between risk factors and parameters or indicators measuring each risk factor, and goodness-of-fit indices that indicate the adequacy of the model (32).

Firstly, we tested the basic MetS model from IDF definition using all traditional variables (WC, DBP, SBP, FBG, TG, and HDL-C), with HOMA-IR and fasting insulin (**Figure 2A**) as indicators of the IR group. Secondly, using the same basic IDF MetS model, we replace FBG with TG : HDL-C ratio as IR indicator (**Figure 2B**). Thirdly, using the same basic IDF MetS model, we replace FBG with LAR as IR indicator (**Figure 2C**). This third model is the reference model. The fit of individual model was determined using i) root mean square error of approximation (RMSEA) (threshold, <0.1) (33), ii) the

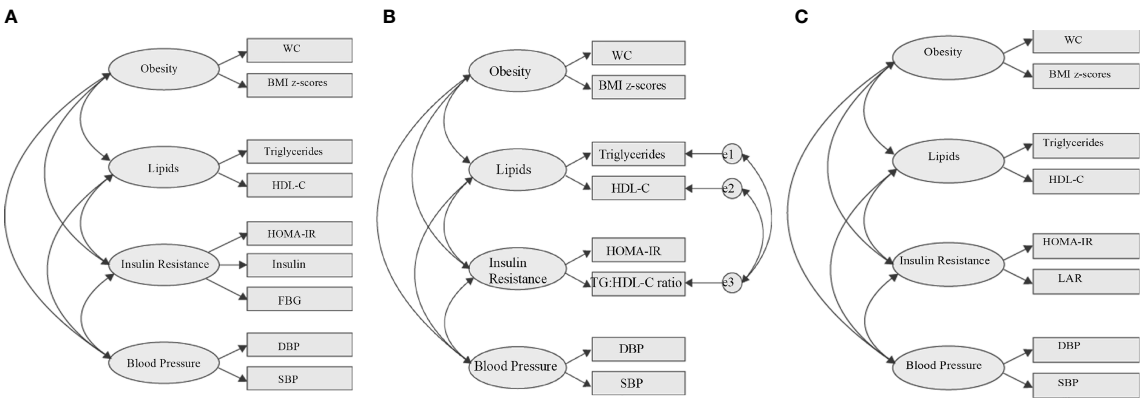

FIGURE 2 | Hypothesized metabolic syndrome factor structure. **(A)** Four-factor correlated model based on the IDF definition. **(B)** Four-factor correlated model replacing fasting blood glucose with TG: HDL-C ratio to the IR component. **(C)** Referenced four-factor correlated model replacing fasting blood glucose with LAR to the IR component. WC, waist circumference; FBG, fasting blood glucose; DBP, diastolic blood pressure; SBP, systolic blood pressure; LAR, leptin:adiponectin ratio; e, residual covariance; HDL-C, high-density lipoproteincholesterol; HOMA-IR, homeostasis model assessment-estimated insulin resistance.

comparative fit index (CFI) (threshold, >0.90) (33), and iii) Tucker–Lewis index (TLI) (threshold, >0.90) (34). Two-sided P values <0.05 were considered to be significant. We compared the goodness of fit of the first two models with the reference model (LAR) using i) goodness of fit (GFI) (threshold, > 0.9) (33), ii) Akaike's information criterion (AIC) (35), iii) Bayesian information criterion (BIC) (35), and iv) Expected Cross-Validation Index (ECVI) (36). The model having smaller AIC, BIC, and ECVI values and closer to the LAR model is considered the preferred and parsimonious (a simple model with great predictive power) model.

All statistical analyses were run using the IBM Corp. Released in 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp. and AMOS software (ver21.0, IBM Corp., Armonk, NY, USA).

3 RESULTS

3.1 General Characteristics of the Children With Obesity

The general characteristics and anthropometric measures of children included in this study are presented in **Table 1**. This study included 524 children with obesity ranging between 10 and 16 years old. More girls have reached puberty (92.4%) compared to boys (56%). The majority of the children (77.7%) were Malay followed by 10.3% of Indian ethnicity, 7.6% of Chinese ethnicity, and 4.4% of other minority ethnicities. Boys were mostly obese (52%) by BMI z-scores compared to the girls. The majority of the children (≈60%) were found to be abdominally obese.

Looking at the biochemical profile (**Table 2**), boys demonstrated higher FBG (4.87 ± 0.7 mmol/L vs. 4.79 ± 0.38 mmol/L) compared to the girls. Whereas girls were found to have higher leptin and LAR with 12.4 ± 8.6 mmol/L and 2.23 ± 1.7 mmol/L, respectively (both P < 0.001) compared to boys. With regard to clinical measures (**Table 3**), 6.9% (n = 36) of the children had MetS, about 40% of the children had IR, and ≈60% had AN.

3.2 Clustering of TG: HDL-C Ratio and Leptin: Adiponectin Ratio With Insulin Resistance Markers

Table 4 displays the grouping of variables by EFA. Each model extracted three or four factors or groups with acceptable total variance of >60%, supporting the multifactorial component of MetS. EFA was performed in this study primarily to see the grouping of TG : HDL-C ratio with IR markers that will indicate the correlation of TG : HDL-C ratio with the IR group in the MetS component. Looking at the IR group in model 1, the IR group (factor 3) is presented by the grouping of HOMA-IR, FBG, and HDL-C with factor loadings of ≥0.3 that may indicate the correlation of lipids with the IR group. Interestingly, in model 2 (addition of percentage body fat, BMI z-scores, TG : HDL-C ratio, and LAR), lipid markers (TG, HDL-C, and TG : HDL-C ratio) were grouped with IR markers (HOMA-IR and fasting insulin) but not FBG (factor 2). Additionally, as expected, the

TABLE 1 | Characteristic and anthropometric measures of children with obesity.

	Boys	Girls	X^2	p-value	All
n (%)	249 (47.5)	275 (52.5)			524
Mean age	12.4 ± 1.9	12.8 ± 1.9		0.02[a]	12.6 ± 1.9
Pubertal status					
n (%)					
Pre-pubertal	110 (44)	21 (7.6)	92.8	<0.001[b]	131 (25)
Pubertal	139 (56)	254 (92.4)			393 (75)
Ethnicity, n (%)					
Malay	185 (74.3)	222 (80.7)			407 (77.7)
Chinese	24 (9.6)	16 (5.8)			40 (7.6)
Indian	30 (12)	24 (8.7)	4.74	0.19[b]	54 (10.3)
Others	10 (4)	13 (4.7)			23 (4.4)
Anthropometric measures					
Obesity status					
BMI z score >1	79 (31.7)	128 (46.5)	12.63	0.002[b]	207 (39.5)
SD					
BMI z score ≥2	129 (51.8)	117 (42.5)			246 (46.9)
SD BMI z score ≥3	41 (16.5)	30 (10.9)			71 (13.5)
SD Abdominal obesity					
WC< 90th centile	97 (39)	96 (34.9)			193 (36.8)
			0.911	0.34[b]	
WC≥ 90th centile	152 (61.0)	179 (65.1)			331 (63.2)
BMI (mean ± SD)	26.7 ± 4.8	27.1 ± 4.9		0.4[b]	26.9 ± 4.9
BMI z score (mean ± SD)	2.36 ± 0.7	2.1 ± 0.7		<0.001[b]	2.2 ± 4.9
WC (cm) (mean ± SD)	83.9 ± 11.6	81 ± 10.6		0.003[b]	82.4 ± 4.9
Percentage body fat (%) (mean ± SD)	37.04 ± 7.2	40.6 ± 6.2		<0.001[b]	38.9 ± 6.9

WC, waist circumference; BMI, body mass index; SD, standard deviation.
[a]*Independent-samples t-test.*
[b]*Pearson chi-square test.*

TABLE 2 | Biochemical measures.

Biochemical profile (mean ± SD)	Boys	Girls	p-value	All
Fasting blood glucose+ (mmol/L)	4.87 ± 0.7	4.79 ± 0.38	0.01	4.8 ± 0.37
Total cholesterol (mmol/L)	4.17 ± 0.67	4.17 ± 0.66	0.99	4.17 ± 0.67
Triglycerides (mmol/L)+	1.03 ± 0.56	0.95 ± 0.41	0.06	0.99 ± 0.49
HDL-C (mmol/L)	1.05 ± 0.21	1.05 ± 0.19	0.97	1.1 ± 0.2
HbA1c (%)	5.15 ± 0.3	5.15 ± 0.3	0.92	5.16 ± 0.3
Insulin (μU/ml)+	16.9 ± 11.4	17.9 ± 9.6	0.24	17.4 ± 10.5
Adiponectin (μg/ml)	6.25 ± 2.7	6.3 ± 2.6	0.8	6.27 ± 2.6
Leptin (ng/ml)	8.2 ± 5.9	12.4 ± 8.6	<0.001	10.39 ± 7.8
LAR	1.53 ± 1.33	2.23 ± 1.7	<0.001	1.89 ± 1.6
TG: HDL-C ratio+	1.04 ± 0.65	0.9 ± 0.46	0.05	0.99 ± 0.56

SD, standard deviation; LAR, leptin:adiponectin ratio.

+ Parameters not normally distributed were log transformed for statistical analysis; however, the actual untransformed values are reported.

TABLE 3 | Clinical measures.

	Boys	Girls	X^2	P value	All
SBP (mmHg) (mean ± SD)	107.7 ± 13	106.6 ± 12.2		0.342	107 ± 12.8
DBP (mmHg) (mean ± SD)	66.61 ± 10.57	67.28 ± 10.7		0.478	66.9 ± 10.6
Metabolic syndrome [n (%)]	16 (6.4)	20 (7.3)	0.147	0.702	36 (6.9)
Non-metabolic syndrome [n (%)]	233 (93.6)	255 (92.7)			
Insulin resistance [n (%)]	100 (40.2)	107 (38.9)	0.09	0.77	207 (39.5)
Insulin sensitive [n (%)]	149 (59.8)	168 (61.1)			317 (60.5)
Presence of acanthosis nigricans [n (%)]	155 (62.2)	153 (55.6)	2.36	0.13	308 (58.9)
Absence of acanthosis nigricans [n (%)]	94 (37.8)	122 (44.4)			216 (41.1)

SBP, systolic blood pressure; DBP, diastolic blood pressure; SD, standard deviation.

TABLE 4 | Factor loadings of traditional variables with or without TG: HDL-C ratio, LAR, PBF, BMI z-scores, and HOMA-IR.

	Model 1 (IDF + HOMA-IR+ Insulin)			Model 2 (Adding PBF, BMI z scores, TG: HDL-C ratio, and LAR)				Model 3 (Removal of TG, HDL-C, Insulin, and Glucose)		
	Factor 1	Factor 2	Factor 3	Factor 1	Factor 2	Factor 3	Factor 4	Factor 1	Factor 2	Factor 3
	Obesity/IR	BP	IR	Obesity/IR	Lipids/IR	BP	IR	Obesity/IR	BP	IR
Triglycerides+	0.609				0.891					
HDL-C	0.472		-0.507		0.51					
HOMA-IR	0.863		0.304	0.464	0.306		0.73	0.407		0.664
Fasting insulin+	0.863			0.519	0.327		0.655			
Fasting blood glucose			0.823				0.771			
TG : HDL-C ratio					0.969					0.904
Leptin:adiponectin				0.609				0.591		
Waist circumference	0.478	0.611		0.773		0.385		0.773	0.381	
Percentage body fat				0.848				0.877		
BMI z scores				0.819				0.832		
Systolic blood pressure		0.905				0.886			0.906	
Diastolic blood pressure		0.894				0.906			0.914	
Variance explained (%)	29.43	26.48	13.83	24.64	18.83	15.3	13.9	32.76	23.51	17.37
Cumulative variance	29.43	55.92	69.75	24.64	43.46	58.77	72.74	32.76	56.27	73.64

BP, blood pressure; IR, insulin resistance; LAR, leptin:adiponectin ratio; PBF, percentage body fat; TG, triglyceride; IDF, International Diabetes Federation; HOMA-IR, homeostasis model assessment-estimated insulin resistance; HDL-C, high-density lipoprotein cholesterol.

+Skewed distributions were logarithmically transformed. Only factor loadings ≥0.3 are shown in the table to improve clarity.

classical IR markers (HOMA-IR, fasting insulin, and FBG) group together (factor 4), as both fasting insulin and FBG were directly correlated with HOMA-IR. Whereas LAR grouped with obesity (WC, PBF, BMI z-scores) and IR markers (HOMA-IR and fasting insulin) rather than only IR markers. This is probably because i) LARs are adipokines and directly related to adipose tissue and ii) previous bivariate correlation analysis has shown that LAR was highly correlated with WC. In the third EFA

(model 3), individual variables of TG : HDL-C ratio and HOMA-IR, that is, TG, HDL-C, fasting insulin, and FBG, were removed. Profoundly, TG : HDL-C ratio and HOMA-IR were distinctly grouped in factor 3 with a strong standardized factor loading that may indicate the IR group. The factor loading for TG : HDL-C ratio and HOMA-IR was 0.904 and 0.664, respectively. Similar to model 2, LAR consistently grouped with the HOMA-IR and obesity markers (WC, PBF, BMI z-scores).

3.3 Evaluation of TG: HDL-C Ratio as Insulin Resistance Component of Metabolic Syndrome

Figure 3 illustrates 2 competing models and 1 reference model of MetS with change variable to the IR risk factor. The first model used traditional variables from the IDF definition with HOMA-IR and fasting insulin (**Figure 3A**). This model was statistically significant, and all the goodness of fit indices have achieved the threshold value with CFI = 0.957, TLI = 0.926, and RMSEA = 0.095. Therefore, this confirms that the proposed construct of MetS is valid. Then, the correlation between the core factors/group (obesity, lipids, IR, and BP) can be scrutinized. Subsequently, the correlation between individual variables and the specific group can also be evaluated. Looking at the correlation between groups, the IR group was strongly correlated with the lipid group (standardized factor loading = 0.75) and moderately correlated with the obesity group (standardized factor loading = 0.46). Additionally, the obesity group also had a moderate correlation with the BP group (standardized loading = 0.51). The BP factor appeared to have weak correlations with the IR group (standardized factor loading = 0.29) and lipid group (standardized factor loading = 0.09). Looking at the IR group, FBG shows a weak standardized factor loading (0.27) indicating a weak correlation to the IR group.

We then tested a model in which FBG was replaced with TG : HDL-C ratio in the IR group. Since TG and HDL-C were directly correlated with TG : HDL-C ratio, we allow the residual errors to covary (**Figure 3B**). In CFA, a minimum of 2 variables is required for each group. However, in the first model, 3 variables (HOMA-IR, fasting insulin, and FBG) were required to achieve the desired goodness of fit. In contrast, in the second model, HOMA-IR and TG : HDL-C ratio were sufficient to construct the modeling. The model was statistically significant, and all the goodness of fit indices achieved the threshold value with higher CFI and TLI compared to the IDF model. The values of CFI and TLI for this model are 0.969 and 0.071, respectively. Additionally, this model has lower RMSEA (0.071) compared to that of the IDF model, indicating that this may be a better MetS model than the current IDF definition. Similar to the IDF model, the IR group was strongly correlated with the lipid group (standardized factor loading = 0.78) and moderately with the obesity group (standardized factor loading = 0.50). The obesity group also had a moderate correlation with the BP group (standardized factor loading = 0.51). The BP group appeared to have weak correlations with the IR (standardized factor loading = 0.29) and lipid (standardized factor loading = 0.24) group. Looking at the IR group, TG: HDL-C ratio shows a better-standardized factor loading (0.39) compared to FBG (0.27) in the IDF model.

Then, we tested our reference model in which LAR was one of the IR measures (**Figure 3C**). The model was statistically significant, and all the goodness of fit indices have achieved the threshold values, with CFI = 0.978, TLI = 0.955, and RMSEA = 0.064. Similar to the previous models, the IR group was strongly

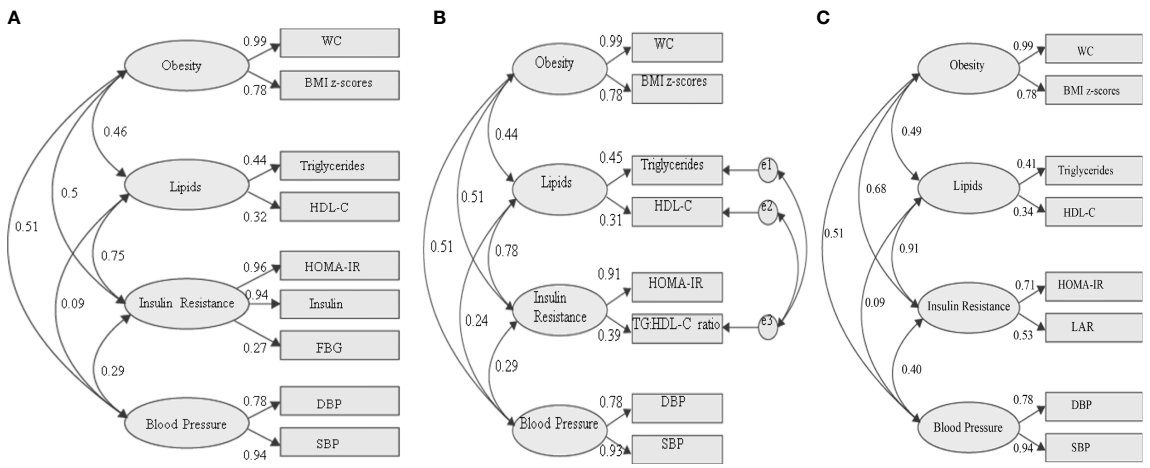

FIGURE 3 | Three different models of metabolic syndrome structures with changed variables in the IR group. **(A)** Fasting blood glucose as measures of IR [fitness indices: P value (<0.05) = <0.001; RMSEA (<0.10) = 0.095; CFI (>0.9) = 0.957; TLI (>0.9) = 0.926]. **(B)** TG: HDL-C ratio as a measure of IR [fitness indices: P value (<0.05) = <0.001; RMSEA (<0.10) = 0.071; CFI (>0.9) = 0.987; TLI (>0.9) = 0.969]. **(C)** LAR as a measure of IR fitness indices: P value (<0.05) = <0.001; RMSEA (<0.10) = 0.064; CFI (>0.9) = 0.978; TLI (>0.9) = 0.955. The standardized factor loadings shown for all models are all statistically significant (P < 0.001). WC, waist circumference; FBG, fasting blood glucose; DBP, diastolic blood pressure; SBP, systolic blood pressure; LAR, leptin:adiponectin ratio; e, residual covariance.

correlated with the lipid group (standardized factor loading = 0.91) and moderately with the obesity factor (standardized factor loading = 0.68). The obesity group also had a moderate correlation with the BP group (standardized loading = 0.51) and IR group (standardized factor loading = 0.4). The BP group appeared to have weak correlations with lipid factor (standardized factor loading = 0.09). Looking at the IR group, LAR shows a higher standardized factor loading (0.53) than FBG and TG: HDL-C ratio.

Finally, we compare the model fit indices of the two competing MetS model with the reference model (**Table 5**). The fit indices will determine the parsimonious model (a simple model with great predictive power). The TG: HDL-C ratio model shows a comparable GFI value to the reference model (LAR) compared to the FBG model. The GFI value was 0.98, 0.979, and 0.952, respectively. Similarly, the TG: HDL-C ratio model shows comparable AIC, BIC, and consistent AIC (CAIC) values to the reference model. Whereas the MetS model with FBG shows about 2-fold higher AIC, BIC, and CAIC values than the model with LAR, indicating the least fit model of the proposed MetS structures. Likewise, ECVI and the 90% ECVI also demonstrate an equal observation with other fit indices.

4 DISCUSSION

We used SEM to examine the usefulness of TG: HDL-C ratio as an IR surrogate in the diagnosis of MetS in children with obesity. Firstly, we have shown by EFA that TG: HDL-C ratio distinctively grouped with HOMA-IR, indicating a strong correlation between TG : HDL-C ratio and HOMA-IR, hence representing the IR group. This finding is not surprising, as numerous studies have shown the correlation between TG : HDL-C ratio and HOMA-IR using Pearson correlation or standard regression analysis (37, 38). However, standard Pearson or regression analysis is a measure of the association between two variables that indicates that the value of one variable changes reliably in response to changes in the value of the other variable. Whereas factor analysis assumes that the relationship (correlation) between variables is due to a set of underlying factors (latent variables) that are being measured by the variables. For example, in this study, the grouping of TG: HDL-C ratio with HOMA-IR may indicate that TG: HDL-C ratio is a measure of IR.

Secondly, CFA was performed to confirm the grouping of TG: HDL-C ratio and HOMA-IR from EFA and to compare whether FBG or TG: HDL-C ratio is a better measure of IR in the diagnosis of MetS in children with obesity. The established and widely used IR surrogate, the HOMA-IR calculations, requires the measurement of fasting insulin and FBG. Due to insulin instability, blood that is collected for insulin measurement must be kept cold and processed immediately, and the plasma is frozen as soon as the blood is withdrawn. Furthermore, measuring insulin is costly (39), and it is not a routine test (40). Therefore, there is a need to search for a simple, reliable, and applicable surrogate marker to measure IR especially among children with obesity. LAR is considered a stable and creditable obesity-IR marker in the pediatric population. The utility of LAR as an IR surrogate in children has been investigated in several studies (41, 42). Adipokines have been recommended as adipose tissue biomarkers by IDF in their "platinum standard" definition of MetS for research (25). Adipokines' inclusion may better reflect adipose tissue function, since abdominal obesity is obligatory for IDF pediatric MetS diagnosis. However, leptin and adiponectin tests are expensive and not routinely tested. Therefore, our model that consists of LAR as an IR surrogate was considered as a reference model.

Our CFA model shows that MetS structure with TG : HDL-C ratio exhibits a better model fit than FBG and is closer to LAR. TG : HDL-C ratio shows higher standardized factor loading to the IR group than FBG, indicating that TG : HDL-C ratio is more correlated to the IR group than FBG (the standardized factor loading was 0.27 and 0.39, respectively). In contrast, when tested on adult males (≥40 years old), Shen et al. (43) demonstrated acceptable standardized factor loading (≥0.3) of FBG and post-challenge glucose in which the IR group was presented by fasting insulin and post-challenge insulin. Although impaired fasting glucose (IFG) was shown to predict diabetes mellitus in adults (44, 45), a similar finding has not yet been proven among children. Hagman et al. (46) reported that at the current IFG cutoff (5.6–6.0 mmol/L) as proposed by the American Diabetes Association, children with obesity show similar acute insulin responsiveness to glucose, insulin sensitivity index, and disposition index to children with normal FBG, suggesting that IFG in children may not be clinically useful as in the adult obese populations. Additionally, another study also reported that impaired insulin sensitivity was not present among youth in the prediabetic range (47, 48), hence signifying that IFG among children with obesity may be a less important driver of morbidity and mortality than that in adults.

Additionally, when comparing two or more competing models with different variables, the one with the smallest AIC and BIC values is the preferred model (35), indicating a preferred

TABLE 5 | Comparison of model fit indices for the proposed models.

Model	GFI	AIC	BIC	CAIC	ECVI	90% CI ECVI
Model 1 (FBG)	0.952	167.72	269.99	293.996	0.321	0.262, 0.394
Model 2 (TG: HDL-C ratio)	0.980	91.88	194.15	218.15	0.176	0.144, 0.222
Model 3 (LAR)	0.970	88.4	182.15	204.16	0.169	0.138, 0.215

FBG, fasting blood glucose; LAR, leptin:adiponectin ratio; GFI, goodness of fit; AIC, Akaike's inclusion criterion; BIC, Bayesian information criterion; CAIC, consistent AIC; ECVI, Expected Cross-Validation Index.

Parsimonious model is indicated by relatively higher GFI and relatively smaller AIC, CAIC, and ECVI. Model 2 shows comparable fitness with the reference model (model 3).

and parsimonious model (a simple model with great predictive power). Additionally, the ECVI measures the likelihood that a model would cross-validate across similar samples from the population (36). Models having smaller ECVI values are considered to have greater potential for replication. In our study, replacing TG: HDL-C ratio as an IR surrogate shows comparable GFI, AIC, and BIC with LAR. Whereas the least model fit was seen when using FBG as an IR surrogate. Moreover, a strong association was seen between the IR and lipid components in all of our hypothesized models, supporting the use of lipids as IR surrogate markers. Therefore, TG : HDL-C ratio can be considered as an IR surrogate marker in MetS components among children with obesity.

In addition, we found that about 60% of our children with obesity were abdominally obese. Thus, in agreement with previous studies, we also found a high prevalence of IR and AN (49). The association between abdominal obesity with IR is common and has been published in numerous studies (50, 51). Despite the high prevalence of abdominal obesity, less than 10% of the children had MetS. In contrast, a higher prevalence was reported among children with obesity in previous studies using the same MetS definition (52, 53). Very recently, using IDF definition, Bitew et al. (54) reported a pooled prevalence of MetS in low- and middle-income countries of 24.1% (95% CI: 16.90, 31.29) among children and adolescents with obesity. Similar to our study, almost 70% of the study subjects had abdominal obesity. Lower MetS prevalence was reported in our study probably due to the binary nature of the MetS diagnosis. Hence, even when the diagnostic criteria are increased, or even borderline, but still below the reference values of MetS diagnosis, the children will not be considered as having MetS. Likely, an individual with measurements in the MetS components just below the threshold for all five components may be at higher risk than someone who just exceeds the cutoffs in three components but has low or normal levels for the other two (55).

Realizing this, the IDEFICS (Identification and prevention of dietary and lifestyle-induced health effects in children and infants) research study proposed a continuous score combining the MetS components for children below 10 years old (56), which were considered at risk of MetS using the pediatric IDF definition. MetS scoring has the advantage of giving about equal chances for each of the components to contribute to the overall prevalence of the MetS. Although IDEFICS has a large sample population, the cohort was specifically targeted to pre-adolescent children aged below 10 years old. In contrast, our study population included children above 10 years old who are physiologically different from the IDEFICS study. Nevertheless, the study of MetS in Malaysian children is relatively new, and cutoff validation and continuous MetS scoring are beyond the scope of this paper. However, we agree that there is a need for future studies to verify the cutoff for the risk factors used in the diagnosis of pediatric MetS, thus improving the prognosis of MetS diagnosis.

In our study, all MetS models were determined to be valid and fit the data well, thus proving appropriateness to examine the potential of the TG : HDL-C ratio as an IR surrogate in the structure of MetS. Of note, percentage body fat was excluded from our CFA model because adding percentage body fat does not provide a good fit for our children's data with obesity. The lack of fit is probably due to the established association between abdominal obesity (visceral fat) and MetS, which is best measured by WC (57). In comparison, percentage body fat is a weight percentage that results from total body fat, which consists of both subcutaneous and visceral fat. Thus, it does not support our factor structure of MetS.

In this study, we have provided the conceptual framework of MetS with the use of the TG: HDL-C ratio as IR surrogate using SEM in children with obesity. Although TG: HDL-C ratio has been extensively studied as an IR surrogate, we must emphasize that our employed measurement technique is novel compared with the existing literature. Our approach provides greater validity to the conclusion of TG : HDL-C ratio as an IR surrogate beyond standard correlation testing such as Pearson or standard regression analysis. Furthermore, we provide evidence on using TG: HDL-C ratio as an IR measure in the diagnosis of pediatric MetS. Additionally, due to the homogeneity of our study population (obesity), we have referred to the conceptual framework of MetS with the inclusion of LAR as obesity IR surrogate marker in addition to HOMA-IR, the general/universal IR surrogate marker. Furthermore, data from only children 10 years old and above were used in this study in parallel with the IDF definition for the diagnosis of pediatric MetS.

One of the limitations of this study was the cutoff value for HOMA-IR. Currently, there is no consensus on the HOMA-IR cutoff value. The common cutoff being used was between 1.14 and 5.56 (58, 59). This wide range may cause a variation in IR prevalence. Thus, it will affect the generalizability of the population. However, this was not the case in this study, as IR status was according to pubertal status, considering the influence of pubertal transition to IR. This study has validated the potential use of the TG: HDL-C ratio as an IR surrogate marker in the diagnosis of pediatric MetS, which may elevate future research of TG: HDL-C ratio for clinical application such as the validation of our proposed MetS definition across ethnicity. Importantly, future studies should apply the cutoff point of the TG : HDL-C ratio to the proposed pediatric MetS modeling, which may then be applied clinically.

In conclusion, IR is more prevalent than MetS in our study population of children with obesity. Thus, it is essential to assess the usefulness of the TG: HDL-C ratio as an IR component in the pediatric MetS structure. We confirmed that the TG: HDL-C ratio may replace FBG as IR surrogate marker in the MetS structure of children with obesity. We proposed targeted intervention for the individual at higher risk for future cardiometabolic risk in a situation where the resources are limited. This target group may be selected by utilizing our proposed MetS structures using TG: HDL-C ratio.

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 Medical Research and Ethics Committee (MREC), Ministry of Health Malaysia (NMRR-18-2749-41841). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

AHM was the principal researcher and was responsible for the overall conception and design of project, coordinating with Ministry of Education and schools for data collection. AKNZI and RMWMZ were responsible for the logistics, collection, biochemical analysis of samples and computerization of all data. MYJ, FMZ, and JYHH were responsible for conceptualizing the clinical data collection and conducted the clinical examinations on the study subjects. FM was responsible for the logistics and collection and directed and oversaw the biological laboratory analysis. AY was responsible for sample size calculation and statistical analysis. All authors contributed to the article and approved the submitted version.

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Casual Associations and Shape Between Prepuberty Body Mass Index and Early Onset of Puberty: A Mendelian Randomization and Dose–Response Relationship Analysis

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Fang J, Yuan J, Zhang D, Liu W, Su P, Wan Y, Zhang Z, Tao F and Sun Y (2022) Casual Associations and Shape Between Prepuberty Body Mass Index and Early Onset of Puberty: A Mendelian Randomization and Dose–Response Relationship Analysis. Front. Endocrinol. 13:853494. doi: 10.3389/fendo.2022.853494

Background: There is an ongoing controversial issue regarding whether onset of puberty is related to childhood BMI.

Objectives: This study aims at investigating the causal association and its shape between prepuberty BMI and early puberty onset.

Methods: Breast development and testicular volume were assessed annually from a population-based prospective cohort of 997 children for consecutive years by professional endocrinologists. Seventeen puberty- and BMI-related SNPs were selected to calculate the polygenic risk score. The two-stage least square method was used to assess and confirm causal effects. A dose–response association between prepuberty BMI and early puberty onset was conducted by using restricted cubic spline Cox regression.

Results: After adjusting for covariates, prepuberty BMI was positively associated with early thelarche among girls (coefficients = 0.18, 95% *CI*: 0.01, 0.29). A non-linear model suggested an inverted U-shaped relationship between prepuberty BMI and risk for early thelarche (χ^2 = 276.3, $p < 0.001$). The risk for early thelarche increased rapidly from prepuberty BMI at 15.70 kg/m^2 (P_{25}) to 20.75 kg/m^2 (P_{85}) and gradually decreased afterward. Compared with the P_{25} of prepuberty BMI, the HRs (95% *CI*) for early thelarche were 5.08 (1.15, 8.55), 4.48 (1.02, 7.74), 10.15 (3.93, 17.50), and 8.43 (1.91, 13.71) for percentiles P_{25}–P_{50}, P_{50}–P_{75}, P_{75}–P_{85}, and ≥P_{85} of BMI categories, respectively. In boys, compared with the P_{25} of prepuberty BMI, boys with BMI between P_{25} and P_{50} showed an increased risk of early puberty (HR: 3.94, 95% *CI*: 1.44, 6.80).

Conclusions: Prepuberty BMI may serve the purpose of identifying the girls at higher risk of early thelarche, which could assist in the adaptation of prevention and intervention strategies targeting childhood obesity. The findings emphasize a non-linear correlation between prepuberty BMI and early puberty onset.

Keywords: body mass index, puberty, Mendelian randomization, causal effects, restricted cubic spline

INTRODUCTION

Puberty is a milestone of life phase, with growth and development of all psychological and physiological systems, especially sexual maturation occurrence and reproductive capacity (1). From the middle of the 20th century until now, a temporal trend toward earlier puberty has been observed around the world (2, 3), although it is less certain for gonadarche in boys (4–6). This is a common concern, as early timing of puberty has a wide range of serious health complications, including increased risk of cardiometabolic diseases, depression, cancers, and possible obstetrical and gynecological problems (7, 8). During the same period, the prevalence of obesity in childhood and adolescence has increased substantially worldwide and become a major public health problem (9). Imperial London and the World Health Organization (WHO) reported a ten-fold increase in the number of adolescents and children with obesity aged 5 to 19 years (10). In developed countries during the years of recent economic crises (last decade), the rising trend in body mass index (BMI) or obesity in children and teenagers has been observed to plateau in high-income countries (11), and there is even a small amount of evidence suggesting a statistically significant reduction in overweight and obesity in Greek schoolchildren aged 6–16 years in both sexes (12) but which continues to increase in low- and middle-income countries (LMICs), especially in populous nations like China (11).

Some researchers have suggested the long-term trend of early puberty to be partly attributed to the rise in childhood obesity (13–15). Longitudinal epidemiological studies show that high BMI is related to earlier pubertal maturation in girls (16, 17), specifically that girls with obesity have earlier age at menarche and timing of thelarche than girls with normal weight (14, 15, 18, 19), while a few studies have not found this association (20). In boys, there have been fewer studies; some evidence reported a positive correlation between BMI or obesity and pubertal development in boys (5, 21–23), while some studies indicate a negative association or fail to find any associations (24, 25).

Mendelian randomization (MR) is an alternative means of assessing the causal effect of childhood BMI on early puberty, designed as a quasi-experimental study that is less susceptible to confounding effects. However, to date, applications of MR have been limited to linear models for the associations between exposure and outcome. A clear determination of the shape in the casual relation between childhood BMI and early onset of puberty would elucidate the comparative relevance of higher and lower BMI values on risk for early onset of puberty.

Based on a 4-year prospective cohort with annual objective assessment of pubertal development, the present study aims at validating the causal association and its shape between prepuberty BMI and early onset of puberty in both sexes, by using single-sample MR as well as restricted cubic spline Cox regression.

METHODS

Study Population

As illustrated in **Supplementary Figure A1**, the perspective puberty cohort is a database containing questionnaires and physical examination information on nearly a thousand school-age children from 2 elementary schools, which was established since March 2016 by clustering convenience sampling in Bengbu, Anhui province, China.

All children underwent a questionnaire survey and physical and pubertal development assessment annually. In 2017, buccal cheek swabs were collected from all the children for DNA extraction and genotyping. The final analysis sample of children who had complete and effective data on BMI status, breast Tanner stage, testicular volume, and genetic susceptibility included 997 children (579 girls and 418 boys) aged 9.33 to 12.17 years in the last follow-up (for detailed information, see Sun et al., 2019) (26). The exclusion criteria were as follows: children with organic or chronic diseases that could affect puberty, or those taking oral or inhaled glucocorticoids or human growth hormone, were excluded in this cohort. Ethical approval for this study was obtained from the Institutional Review Board at Anhui Medical University (No. 20180082). Informed consent for all collected data was obtained from parents and schoolteachers, as well as children.

Measurements

Early Onset of Puberty

Annual breast Tanner assessments were classified between 1 (prepubescent state) and 5 (full sexual maturity state) (27, 28) through both observation and palpation in primary school while testicular volume was estimated by using a Prader orchidometer by trained pediatric endocrinologists. Pubertal onset was defined as attaining breast Tanner stage 2 (B2, thelarche), or testicular volume more than 3 ml (TV4). The 25th percentile was adopted as the cutoff point (P_{25} age) for early onset of thelarche and testicular development in Chinese children based on data from the "China Puberty Collaboration Study (29, 30)," which was age at B2 < 8.0 y for girls and age at TV4 ml < 9.7 y (TV3 ml < 8.67 y) for boys.

Body Mass Index and Classification

In the annual follow-up survey, height and weight were measured by trained and certified medical staff. Body height

was examined with light clothing to the nearest 0.1 cm by using a portable stadiometer, and weight with an electronic scale (Tanita TI1618) to the nearest 0.1 kg. BMI is a measurement of height-adjusted measure of weight, calculated as weight (in kilograms) divided by the square of height (in meters) (kg/m^2). Childhood BMI was derived from all paired height and weight measurements in the age of 6.5 to 12.5 years and age adjusted to 9 years by using a linear regression model. Moreover, it was classified according to percentiles ($< P_{10}$, P_{25}–P_{50}, P_{50}–P_{75}, P_{75}–P_{85}, and $\geq P_{85}$).

Genotyping and Single-Nucleotide Polymorphism Selection

Genomic DNA for all children had been extracted from buccal cheek swabs following a standard protocol. PCR-RFLP and real-time PCR were used to extract DNA and then genotyped in the Sequenom MassARRAY. The mean concordance rates of the genotyping system were 98.8%.

The present study identified 11 and 21 single-nucleotide polymorphisms (SNPs) associated with obesity and early puberty at genome-wide significance in the GWAS datasets from 87,802 women and 35,668 children of European ancestry, respectively (31–33). After excluding five SNPs (rs35327298, rs142058842, and rs4237264 for early puberty; s7550711 and rs13387838 for obesity) with MAFs < 5%, three SNPs (rs5932886 for early puberty; rs1310484 and rs987237 for obesity) had a genotyping rate <10%. Additionally, we have imputed genotype data based on the CHB HapMap data (Phase 2 and Phase 3) and further verified that loci were not in a significant linkage disequilibrium (LD) with each other ($r^2 < 0.3$) in the SNP server and Han Chinese (CHB) data. Moreover, 17 puberty-related SNPs and 7 BMI-related SNPs were retained in the final analysis and the polygenic risk score (PRS) was calculated, as shown in the following formula, respectively:

$$\text{Puberty} - \text{related PRS} = SNP_1 + SNP_2 + SNP_3 + \ldots + SNP_{17},$$

$$\text{BMI} - \text{related PRS} = SNP_1 + SNP_2 + SNP_3 + \ldots + SNP_7$$

Each SNP was recorded as 0, 1, and 2 according to number of effect alleles (e.g., if the effect allele is T, then TT = 2, CT = 1, CC = 0).

Covariates

For assessment of potential confounding factors, the current analysis included delivery mode (vaginal or caesarean section), gestational age (>37 weeks or ≤37 weeks), birthweight, and infancy feeding mode (included exclusive breastfeeding, mixed feeding, and formula feeding), as well as parental BMI, education, and household monthly income (obtained from the parents' questionnaire at baseline).

Statistical Analysis

Statistical calculations were performed with Stata (version 14.0) and R version 3.6.2. We used instrumental variable methods to estimate the causal effect between BMI and early onset of puberty through polygenic risk using the Mendelian randomization design. Instrumental variable regression with two-stage least-square (2SLS) methods was performed using the BMI-related PRS as the instrument (34). MR–Egger is often used in sensitivity analysis, i.e., the causality between obesity and early onset of puberty. The shape of the relationship between prepuberty BMI and early onset of puberty was established by using restricted cubic spline (RCS) Cox regression (35) based on five knots (P_5, P_{25}, P_{50}, P_{75}, and P_{95}) of prepuberty BMI and reference BMI of 15.70 kg/m^2 (P_{25}) and 16.10 kg/m^2 (P_{25}) in girls and boys, respectively, generating hazard ratios (HR) [95% confidence intervals (CI)]. Furthermore, we analyzed the association between prepuberty BMI and early puberty by using categorical BMI (BMI < P_{10}; P_{25}–P_{50}, P_{50}–P_{75}, P_{75}–P_{85}, and $\geq P_{85}$ kg/m^2) with BMI at P_{10}–P_{25} as reference.

Final models were adjusted for age, BMI- and puberty-related PRS, parental BMI, parental education, family income, birthweight, delivery mode, infant feeding, and gestational age. 0.05 was defined as a significance threshold of two-tailed p values.

RESULTS

Participants' Characteristics

The average cohort follow-up rate from Wave 2 to Wave 4 was more than 90%. Among the 997 children in this cohort, 58% were female, and the mean age was 8.01 (SD, 0.85) years at baseline. The average BMI genetic score (7 SNPs) was 4.61 ± 1.40, 15.32 ± 2.47 for puberty genetic score (17 SNPs), as presented in **Supplementary Table A1**.

Approximately one-fifth of children were classified as overweight and obese at each wave, respectively. **Table 1** shows adiposity and pubertal development in boys and girls during the 4-year follow-up. At wave 1 to wave 4, approximately 9.8%, 14.5%, 22.7%, and 24.5% of girls presented early onset of thelarche, and 0.7%, 1.0%, 6.3%, and 9.9% of boys had early onset of testicular development, respectively.

Casual Association Between Prepuberty BMI and Early Onset of Puberty

The coefficients of the bidirectional MR analysis are presented in **Table 2**. The BMI-PRS created from 7 SNPs showed a positive association with prepuberty BMI (coefficient = 0.17, 95% CI: 0.02, 0.33; p = 0.031). The BMI-PRS served as a strong instrument for adiposity, with F statistics of 2.424. The results of 2SLS analysis revealed that prepuberty BMI was associated with early thelarche onset in girls (coefficient = 0.18, 95% CI: 0.01, 0.29; p = 0.005). In MR sensitivity analyses, the IVW, Egger, and Median methods provided results similar to those of the 2SLS analysis of early puberty based on TV4 assessment (**Supplementary Table A2**). No causal association was observed between prepuberty BMI and early onset of testicular development in boys (coefficient = 0.07, 95% CI: -0.08, 1.92; p = 0.113). Furthermore, we also performed an MR sensitivity analysis based on early puberty in boys assessed by TV3, and again no significant association was observed (coefficient = 0.08, 95% CI: -0.07, 1.88; p = 0.365) (**Supplementary Table A3**).

TABLE 1 | Adiposity and pubertal development changes during the 4-year follow-up among all children.

Characteristics	Wave Ⅰ (2016)		Wave Ⅱ (2017)		Wave Ⅲ (2018)		Wave Ⅳ (2019)	
	n	Distribution	n	Distribution	n	Distribution	n	Distribution
Age, years	997	8.01 ± 0.85	997	8.97 ± 0.85	997	10.01 ± 0.81	997	11.01 ± 0.82
Adiposity measurements								
Overweight	997	220 (22.1)	997	191 (19.2)	997	178 (17.9)	997	200 (20.1)
Obesity	997	267 (26.8)	997	270 (27.1)	997	223 (22.4)	997	225 (22.6)
BMI (kg/m^2)	997	17.96 ± 2.96	997	18.60 ± 3.26	997	18.81 ± 3.57	997	19.78 ± 3.89
Pubertal status (boys)								
Early puberty	418	3 (0.7)	418	4 (1.0)	414	27 (6.5)	405	30 (7.2)
Tanner stage 1 (<3 ml)	418	415 (99.3)	418	405 (96.9)	414	269 (65.0)	405	189 (46.7)
Tanner stage 2 (4–8 ml)	418	3 (0.7)	418	13 (3.1)	414	127 (30.7)	405	165 (40.7)
Tanner stage 3 (9–12 ml)	418	0 (0.0)	418	0 (0.0)	414	18 (4.3)	405	34 (8.4)
Tanner stage 4 (15–20 ml)	418	0 (0.0)	418	0 (0.0)	414	0 (0.0)	405	14 (3.5)
Tanner stage 5 (>20 ml)	418	0 (0.0)	418	0 (0.0)	414	0 (0.0)	405	3 (0.7)
Pubertal status (girls)								
Early puberty	579	48 (8.3)	579	76 (13.1)	578	76 (13.1)	572	76 (13.1)
Tanner stage 1 (B1)	579	364 (62.9)	579	206 (35.6)	578	131 (22.7)	572	41 (7.2)
Tanner stage 2 (B2)	579	203 (35.1)	579	254 (43.9)	578	220 (38.1)	572	119 (20.8)
Tanner stage 3 (B3)	579	12 (2.1)	579	99 (17.1)	578	141 (24.4)	572	136 (23.8)
Tanner stage 4 (B4)	579	0 (0.0)	579	20 (3.5)	578	75 (13.0)	572	145 (25.3)
Tanner stage 5 (B5)	579	0 (0.0)	579	0 (0.0)	578	11 (1.9)	572	131 (22.9)

TABLE 2 | Summary of coefficients used for bidirectional Mendelian randomization analysis.

Instrumental variables	Genetic score with intermediate trait			Genetic score with outcomes			Two-stage IV analysis (early puberty or Z-BMI)	
	Coefficient (95% CI)	*p*-value	*F*-value	Coefficient (95% CI)	*p*-value	*F*-value	Coefficient (95% CI)	*p*-value
BMI genetic score (7 SNPs)								
Boys	0.14 (-0.12, 0.40)	0.305	0.649	0.01 (-0.01, 0.03)	0.112	3.167	0.07 (-0.08, 1.92)	0.113
Girls	**0.17 (0.02, 0.33)**	**0.031**	**2.424**	**0.03 (0.01, 0.05)**	**0.004**	8.245	**0.18 (0.01, 0.29)**	**0.005**

Adjusted for age, puberty-related PRS, birthweight, delivery mode, infant feeding, family income, parental BMI, and education.
The values in bold indicate statistical significance under the model.

Non-Linear Relationship Between Prepuberty BMI and Early Puberty

As illustrated in **Figure 1**, the restricted cubic spline shows that compared to reference groups (prepuberty BMI = 15.70 kg/m^2, P_{25}), girls with elevated percentile of prepuberty BMI before the peak risk point (prepuberty BMI = 20.75 kg/m^2) are at increased risk for earlier onset of thelarche. After that, the risk for earlier onset of thelarche decreases with a continued increase in prepuberty BMI ($p_{\text{non-linear}} < 0.001$).

For boys, a higher risk of earlier onset of testicular development with prepuberty BMI was observed in the range of 16.10 kg/m^2 (P_{25}) to 17.35 kg/m^2 (P_{40}), compared to the reference group (prepuberty BMI = 16.10 kg/m^2, $p_{\text{non-linear}} < 0.001$) (**Figure 2**).

Table 3 presents the relationship between categories of prepuberty BMI and risk of early onset of puberty in both sexes. Compared with the reference group (P_{10}–P_{25}), the HRs (95% CI) for early thelarche were 5.08 (1.15, 8.55), 4.48 (1.02, 7.74), 10.15 (3.93, 17.50), and 8.43 (1.91, 13.71) for BMI categories at P_{25}–P_{50}, P_{50}–P_{75}, P_{75}–P_{85}, and ≥P_{85}, respectively, but not in the BMI < P_{10} group. In boys, compared with the reference (P_{10}–P_{25}), only boys at P_{25}–P_{50} of BMI categories showed an increased risk of early onset of testicular development [3.94 (1.44, 6.80)].

DISCUSSION

To our knowledge, this study is the first prospective longitudinal cohort study simultaneously evaluating the causal relationship and its shape between prepuberty BMI with early puberty in both genders. By using Mendelian randomization analysis, we provided robust evidence to support the causal association between increased prepubertal BMI with early onset of thelarche in girls. However, a similar association was not confirmed in boys. For non-linear relations, an inverted U-shaped curve was observed between prepuberty BMI and earlier onset of thelarche. Specifically, girls with elevated percentile of prepuberty BMI before the peak risk point (20.75 kg/m^2) are at increased risk for earlier onset of thelarche. After that, the risk for early thelarche decreases with a continued increase in BMI. In contrast, for boys, the risk for early onset of testicular development was observed with the prepuberty BMI in the range of 25th to 40th percentile, equal to 16.10 kg/m^2 to 17.35 kg/m^2, respectively.

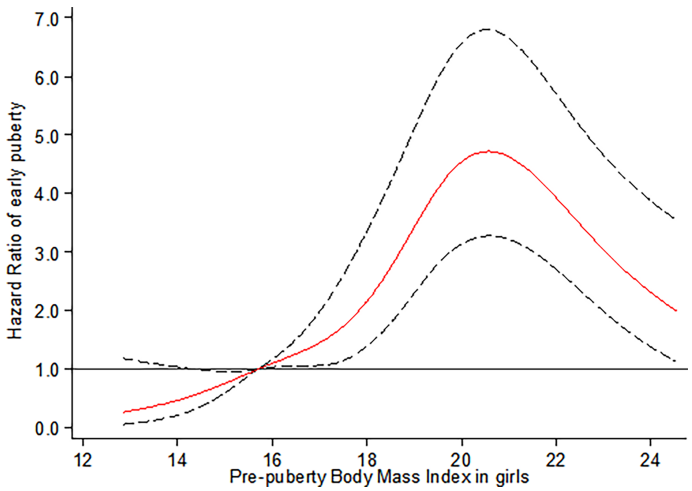

FIGURE 1 | Restricted cubic spline for the association between prepuberty BMI and the HR for early breast development among girls. The curves are based on restricted cubic spline Cox regression with five knots of BMI and a reference BMI of 15.70 kg/m^2 (P_{25}). Individuals with BMI below the 1st or above the 99th percentiles were excluded. Analyses were adjusted for age, BMI- and puberty-related PRS, birthweight, delivery mode, infant feeding, family income, parental BMI, and education.

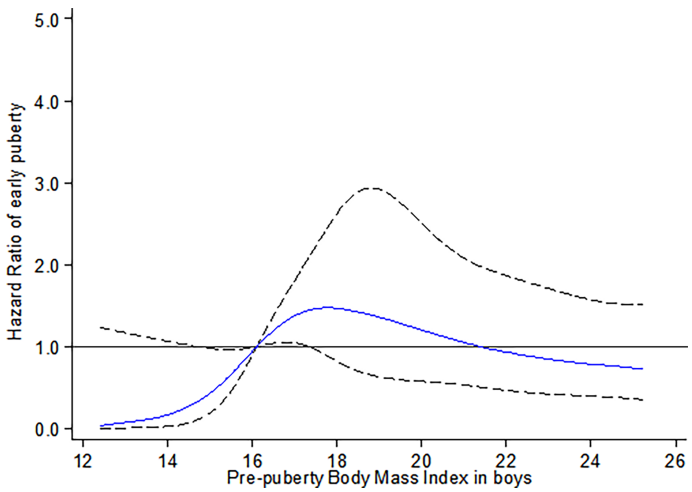

FIGURE 2 | Restricted cubic spline for the association between prepuberty BMI and the HR for early testicular development among boys. The curves are based on restricted cubic spline Cox regression with five knots of BMI and a reference BMI of 16.10 kg/m^2 (P_{25}). Individuals with BMI below the 1st or above the 99th percentiles were excluded. Analyses were adjusted for age, BMI- and puberty-related PRS, birthweight, delivery mode, infant feeding, family income, parental BMI, and education.

Previous longitudinal epidemiological studies have shown that high childhood BMI is related to earlier pubertal maturation in girls (16–19), while potential correlations among boys are controversial (20–25). Additionally, although BMI and timing of puberty are known to be closely linked, the causality and its effects between these traits in boys and girls remain poorly studied (36).

Our finding of MR analysis in girls is consistent with results from the Taiwan Children Health Study (TCHS) (16), supporting the causal effects of higher adiposity accumulation during childhood on earlier onset of puberty. However, TCHS is limited by using a self-reported questionnaire to define breast Tanner stages. Their measurements of pubertal development starting at ages 11 and 12 lead to underestimation of the true age at pubertal onset in girls. In comparison, our study was based on a 4-year cohort with objective annual breast development assessment since childhood (around 6–8 years) and no more than 2% of girls enrolled at baseline initiated puberty, helping to observe and capture the process of pubertal onset.

TABLE 3 | Hazard ratio (95%) of early onset of puberty according to prepuberty BMI in girls and boys.

	Early thelarche in girls (B2)				Early testicular development in boys (TV = 4 ml)			
	No. (%)	Adjusted hazard ratio (95% CI)			No (%)	Adjusted hazard ratio (95% CI)		
		Model 1	Model 2	Model 3		Model 1	Model 2	Model 3
Prepuberty BMI								
<P_{10}	56 (9.7)	**0.79 (0.19, 2.22)**	**0.81 (0.20, 2.30)**	**0.81 (0.24, 2.77)**	39 (9.3)	0.95 (0.21, 2.43)	0.96 (0.21, 2.45)	0.97 (0.24, 1.26)
P_{10}–P_{25}	87 (15.0	Ref.	Ref.	Ref.	65 (15.6)	Ref.	Ref.	Ref.
P_{25}–P_{50}	145 (25.0)	**4.61 (1.05, 7.14)**	**4.95 (1.13, 8.50)**	**5.08 (1.15, 8.55)**	105 (25.1)	1.84 (0.53, 2.87)	1.88 (0.59, 2.94)	**3.94 (1.44, 6.80)**
P_{50}–P_{75}	145 (25.0)	**5.65 (1.31, 9.33)**	**5.02 (1.16, 8.65)**	**4.48 (1.02, 7.74)**	104 (24.9)	1.29 (0.87, 3.89)	1.32 (0.89, 3.97)	2.43 (0.84, 7.00)
P_{75}–P_{85}	58 (10.0)	**9.00 (3.65, 15.70)**	**9.61 (3.64, 15.94)**	**10.15 (3.93, 17.50)**	43 (10.3)	1.59 (0.63, 4.02)	1.59 (0.63, 4.04)	2.52 (0.75, 8.46)
≥P_{85}	88 (15.2)	**7.65 (2.07, 12.78)**	**7.72 (1.78, 13.48)**	**8.43 (1.91, 13.71)**	62 (14.8)	0.74 (0.27, 2.04)	0.74 (0.27, 2.03)	1.09 (0.28, 4.21)
***p* value for trend**		<0.001	<0.001	<0.001		0.406	0.245	<0.001

BMI, body mass index; B, breast; TV, testicular volume.

Model 1 adjusted for age and BMI-related PRS.

Model 2 adjusted for age, BMI- and puberty-related PRS.

Model 3 adjusted for age, BMI- and puberty-related PRS, birthweight, delivery mode, infant feeding, family income, parental BMI, and education.

The values in bold indicate statistical significance under the model.

The results of the present MR analysis indicated no casual association between prepuberty BMI and early pubertal onset in boys, which is inconsistent with two previous MR studies in boys. The Copenhagen Puberty Study (2006–2014) using a mixed longitudinal cohort (n = 93) and cross-sectional study (n = 637) of 730 healthy Danish boys determined the possible causal link between higher BMI and earlier timing of voice break in boys (37). A longitudinal analysis from the Taiwan Children Health Study (TCHS) also revealed that prepuberty BMI (overweight/obesity) predicted early onset of self-reported pubertal development among male adolescents (17). The heterogeneities could be largely explained by the differences in male pubertal assessment. Although voice break, as a measure of puberty, is frequently used in epidemiological studies, it represents a late pubertal milestone and the validity of self-reported voice break remains a question. As male pubertal onset is manifested by the gradual enlargement of genital and testicular size, direct measurement of testicular volume by palpation is likewise preferable to recalled age at voice break, providing a more accurate estimate of age at attaining the milestones of puberty for boys.

The current study, herein, further extended the non-linear dose–response relationship between prepuberty BMI and onset of puberty by using the restricted cubic spline (RCS) Cox regression model of five knots and indicated a significant non-linear dose–response association of prepubertal BMI with early onset of puberty in both genders. Our finding of an inverted U-shaped correlation with an inflection point in the risk function at 20.75 kg/m^2 (equal to BMI threshold for obesity at 9 years of age) in girls further complements existing evidence of non-linear associations between prepubertal BMI with early onset of puberty. This is in line with conclusions from the Danish National Birth Cohort (DNBC) and sibling-matched study (38). The DNBC study indicated that childhood overweight (between 85th and 95th percentiles of BMI) and obesity (≥ 95th percentile of BMI) were associated with earlier puberty timing (self-reported pubertal milestones) in both sexes in a dose-dependent manner by using restricted cubic splines with three knots. A confirmatory analysis of the association was conducted in a sibling-matched study of 1,700 brothers of DNBC, and it reported that a higher BMI was associated with earlier age at attaining most milestones of puberty among girls, but only a tendency toward earlier timing of puberty was observed in boys. Despite the small sample size of the present study, our results highlight the association between objectively assessed BMI and early breast development, which is more convincing than parental report data in the DNBC study.

Our findings in boys supported the results of Bygdell et al. (39) and a DNBC sibling-matched study (38), suggesting that the risk for early testicular development increased with an increasing prepuberty BMI within the range of 16.10 to 17.35 kg/m^2 (equivalent to the range of normal weight). Bygdell et al. (39) demonstrated that prepubertal BMI associated with early timing of puberty (indicated by age at PHV) in normal-weight but not overweight boys. However, the piecewise linear regression model used in their study cannot observe the shape of the non-linear correlation on both sides of the threshold.

Potential Mechanisms

The possible mechanisms and relevance of our present findings in terms of sex divergence of early pubertal timing by prepubertal BMI merit further investigation. Understanding of the neurobiological basis of puberty in general, especially the underlying mechanisms for its metabolic regulation in particular, has substantially expanded in recent years. Sanchez Garrido et al. (40) evaluated sexually dimorphic responses in a metabolic programming of puberty to nutritional challenges in rats of both gender. Their study found that male puberty is more sensitive to postnatal nutritional stress (overfeeding) and female puberty is more vulnerable to peripubertal nutritional stress (high-fat feeding) (41). On the other hand, overfeeding before and after puberty leads to an increase in hypothalamic Kiss1 expression and advances the onset of puberty in female rats, whereas sustained excess energy and obesity are associated with inhibition of Kiss1 expression and lower expression levels of key limiting factors of testicular steroidogenesis in male rodents (42).

Human studies further note that excessive adiposity, in the absence of substantial sex steroid surge, may partly suppress

hypothalamic–pituitary–gonadal function in girls with earlier puberty, although it is known to accelerate pubertal onset, which indirectly elucidated the decreased risk of early pubertal onset in obese girls than overweight ones, but the mechanism is unclear in boys. Future population-based studies to better characterize the association between body fat and onset of puberty in boys are needed and will require focus on the neuroendocrine basis of the physiological control of puberty and its deviations, as well as epigenetic regulation and metabolic cues, to better understand the mechanisms that trigger pubertal initiation and progression in both sexes.

Strengths and Limitations

Although the present study is based on a rigorous longitudinal design with repeated objective assessment of puberty, there are some limitations that should be acknowledged. First, this study was conducted among Chinese children; differences between race/ethnicities should be considered for generalizing our findings. Secondly, our analysis used BMI as the primary indicator to measure child obesity, which may not fully reflect body fat level as its calculation only considers height and weight. Further studies are recommended using skinfold thickness or other measures such as dual-energy X-ray absorptiometry to examine the association between body fat and timing of puberty. Third, we only considered the prepubertal BMI of children, which can explain the effects of prepubertal weight status on early puberty; it is necessary to clarify the influence of weight changes during follow-up on puberty in future analysis. In addition, due to the small size of sample, the number of our candidate SNPs representing exposure and outcome phenotype were relatively small compared with large GWAS, which may affect the stable and reasonable estimates of the MR model. Therefore, we also did a further sensitivity analysis using MR–Egger. Given the difficulty of conducting population surveys, another limitation of this study would be that there has been no evaluation of the individual growth charts and the children's growth patterns related to their target heights and their bone age of course combined at least with basal LH < 08:00 h. Furthermore, although there seems to be no evidence of a secular trend for gonadarche in boys, such a trend seems to be evident for pubarche, and future studies should consider the effect of obesity on adrenarche, especially in boys (4). Some evidence indicated that the physical changes of puberty require a concerted effort from many organs and that these changes are independent of each other, although adrenal maturation often coincides with HPG axis maturation; it is important to note that pubarche itself is not the best indicator of pubertal development. Finally, nearly half of the boys in the cohort had not reached puberty yet at the last follow-up, which may have reduced the power of studies.

CONCLUSION

The current study provided robust evidence on the casual and inverted U-shaped relationship between prepubertal BMI with early thelarche in girls. For boys, although no similar causal association was observed, our finding identified a non-linear relationship between BMI and early onset of testicular development in normal-weight boys. Further studies are warranted to elucidate the mechanisms behind the observed associations, which might aid future interventional studies targeting the prevention of childhood obesity and precocious puberty.

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.

ETHICS STATEMENT

We secured approval from the Institutional Review Boards at Anhui Medical University (No. 20180082). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

YS, PS, YW, and ZZ conceptualized and designed the study, drafted the initial manuscript, and reviewed and revised the manuscript. JF, JY, DZ, and WL designed the data collection instruments, collected the data, carried out the initial analyses, and reviewed and revised the manuscript. FT conceptualized and designed the study, coordinated and supervised the data collection, and critically reviewed the manuscript for important intellectual content. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.853494/full#supplementary-material

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Leptin Does Not Influence TSH Levels in Obese Short Children

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Introduction: Growth hormone (GH) and thyroid hormones are important for children growing. In some obese children a slightly elevated TSH concentration is observed. This may be an adaptive mechanism: stimulation of pro-TRH biosynthesis in the hypothalamus in response to elevated leptin. The increased TSH may also reflect the necessity of maintaining the resting energy expenditure or may be a result of inappropriate, low FT4 concentration. Thus, we evaluated serum TSH and FT4 concentrations in idiopathic short stature (ISS) children (non GH-deficient) and examined the effect of children's nutritional status and levels of selected adipocytokines on thyroid function, searching for the presence of various forms of subclinical hypothyroidism, which may be the cause of the slow growth rate.

Methods: The study group included 115 children (50 girls and 65 boys) with ISS, aged (mean ± SD) 10.4 ± 3.34 years. In each child, lipids, TSH, FT4, IGF-1, maxGH during the stimulation tests, leptin, adiponectin and resistin concentrations were determined. Based on BMI SDS, 3 subgroups: slim (n=26), obese (n=21) and normal weight (n=68) were distinguished.

Results: There was no correlation between leptin level and TSH, FT4 levels. The levels of leptin, total cholesterol and LDL-cholesterol in obese short children were significantly higher than in children from other subgroups. In turn, the levels of adiponectin, resistin, TSH and FT4 did not differ between subgroups. In 7% of children, an elevated TSH level was found (but less than 10 mIU/L), with a similar frequency across subgroups. The higher the leptin, the lower maxGH in clonidine stimulation test was recorded.

Conclusions: It seems that in obese children with idiopathic short stature leptin does not increase TSH secretion. This may be related to a disruption of the effect of leptin on TSH production and could indicate wide ranging disturbances of hypothalamic signals, and consequently be the cause of inappropriate GH secretion.

Keywords: obesity, children, thyroid stimulating hormone, leptin, idiopathic short stature

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INTRODUCTION

Growth hormone (GH), insulin-like growth factor 1 (IGF-1) and thyroid hormones play a key role in the growth processes in children. They act directly, however free T4 (FT4) and free T3 (FT3) also exert a permissive impact on IGF-1 action (1).

A child's short stature may be due to hormonal causes (growth hormone deficiency - GHD, hypothyroidism or hypercortisolemia), chronic diseases (e.g. gastrointestinal diseases such as celiac disease) or some genetic syndromes (e.g. Turner or Prader-Willi syndrome). However, in many children, despite numerous tests, it is impossible to establish the cause of an inadequate high velocity and short stature. In such cases, idiopathic short stature (ISS) is diagnosed; however, it can be assumed that many of these cases are undiagnosed abnormalities caused by other factors involved in the regulation of the growth process (2).

It is known that many environmental factors and disruptors as well neuropeptides affect the production and secretion of TSH (3, 4). In our previous study, we proved a positive correlation between ghrelin and TSH concentrations in children with ISS. We also demonstrated that the higher the TSH, the lower the nocturnal GH and IGF-1 levels were recorded (5). Now, we have decided to analyse the effect of some selected hormones secreted from the adipose tissue (adipocytokines) on the secretion of TSH and FT4 in ISS children.

Subclinical hypothyroidism is observed in about 2% children (6, 7) and its diagnosis and treatment are a matter of controversy (8, 9); there are some conditions in which TSH elevation is transient, with obesity being one of the well documented examples (10). Many studies report that in children with excess body weight, a slightly elevated TSH level is observed (4-10 mIU/l), although it is not a response to reduced FT4 levels, i.e. not a result of hypothyroidism (10, 11). It has been hypothesized that it may be an adaptive mechanism aimed at increasing energy expenditure and the levels of TSH and FT4 correlate positively with resting energy expenditure (12). Thus, the elevated TSH levels in these cases seem a consequence rather than a cause of obesity, and treatment with levothyroxine in obese children is not recommended (12, 13). However, the causes of increased TSH in obesity are not clear and do not apply to all obese children (14). On the other hand, it was shown that among obese teenagers, the higher the TSH concentration, the higher total cholesterol and blood pressure values, which all are symptoms of hypothyroidism (15).

Therefore, the question arises: is the lack of an increase in TSH concentration in some obese children (without thyroid disease) also a normal phenomenon or, on the contrary, can it be the result of a too weak reaction of the hypothalamus and pituitary gland in TRH production/release in response to the body's needs? In the latter case, abnormal hypothalamic and pituitary responses could explain short stature as an effect of relative FT4 deficiency.

The most popular hypothesis explaining the increase in TSH concentration in obese people is that leptin influences the production of pro-TRH in the hypothalamus (16, 17). Serum leptin concentration is strongly positively correlated with body weight and triggers a lot of actions connected with energy expenditure: at the level of the hypothalamus, it also inhibits appetite and increases hepatic gluconeogenesis and muscle fatty acids oxidation in peripheral tissues (18–20). The results of studies concerning leptin concentrations in children with GHD are divergent (21–24). It is possible that in some of them, the relative leptin resistance is observed. On the other hand, it was shown that leptin levels did not differ between children with hypothyroidism and hyperthyroidism, while significant differences were observed for adiponectin and resistin (respectively, higher and lower concentrations in untreated Graves' disease than in hypothyroidism) (25).

Thus, it seems that the abovementioned adipocytokines (leptin, adiponectin and resistin) produced by adipose tissue may be involved in the cross-talk between adipocytes and hypothalamus, with the aim of increasing the release of TRH and TSH, and - in consequence – the production and secretion of FT4.

The aim of the study was to evaluate TSH and FT4 in idiopathic short stature (ISS) children, and to examine the effect of children's nutritional status and levels of selected adipocytokines on thyroid function, in the search for various forms of subclinical hypothyroidism, which may be the cause of the slow growth rate.

Thus, the primary endpoint was the answer to the question how many children with short stature and obesity had elevated TSH levels co-occuring with elevated leptin levels. And - in those who do not have elevated TSH - is it associated with symptoms of hypothyroidism other than short stature, e.g. elevated cholesterol concentration?

MATERIALS AND METHODS

The study included consecutive children admitted to the Department of Endocrinology and Metabolic Diseases of Polish Mother's Memorial Hospital - Research Institute in Lodz over the period of one year for the diagnostics of their short stature, who met the following criteria:

1. height standard deviation score (HSDS) below -2.0 from the mean value for child's age and sex (children's height was measured using a stadiometer) (26);

2. excluding genetic reasons of the short stature (i.e. Turner syndrome, Prader-Willi syndrome) (assessed on the basis of a karyotype);

3. excluding treated hypothyroidism, chronic diseases or undiagnosed gastrointestinal tract complaints (assessed on the basis of a negative history of chronic diseases, as well as normal tests results of tissue transglutaminase antibodies class IgA).

Out of the initially analyzed group of 170 children: 10 did not meet the criterion of height below 3 centile, 7 did not meet the criterion of low height velocity, 4 had treated hypothyroidism, and 1 - celiac disease. Ultimately, 148 short children were qualified for further analyses.

The body mass was assessed in all patients, and that was followed by a calculation of the body mass index standard deviation score for chronological age (BMI SDS for CA).

We used Polish references (26). Next, in all the children, considering the child's current position on centile charts, the height age (HA) was calculated (as the age ascribed to the 50th percentile for a given child's height) and BMI values reffered to HA and expressed as BMI SDS for HA (we adjusted the results to the height age of a child to avoid false results for short children). Based on this value, the analyzed group of short children was divided into three subgroups (according to WHO recomendatios): obese, normal and slim. The obesity and overweight group includes children aged 5–19 with BMI for HA above +1.0 SD (above 90 percentile) and children under 5 years of age with BMI for HA above +2.0 SD (above 97 percentile). Into the slim group, we qualified children with thinness: BMI SDS for HA <-2.0 SD (below the 3rd percentile), regardless of their chronological age.

The stage of puberty was assessed in each child, using the Tanner's scale. Most of the analyzed children were prepubertal (83%).

In all of them, GH secretion was assessed during a 3-hour nocturnal profile and during two (2) stimulation tests: the first one after clonidine administered orally (with the dose of 0.15 mg/m^2 of the body surface) and GH concentration measurements at time 0 and at the 30th, 60th, 90th and 120th minute of the test, and the second one – after intramuscular administration of glucagon (in the dose of 30 μg/kg of body weight, not exceeding 1 mg), with GH concentration measurements at time 0 and at the 90th, 120th, 150th and 180th minute. Based on the results of GHmax values in these tests, we diagnosed:

1. ISS – correct results in - at least - one of the stimulation tests (GHmax values ≥ 10 ng/ml) in 115 children (50 girls and 65 boys),

2. GHD – decreased GH secretion (GHmax values < 10 ng/ml) in 33 children.

In each child, the morning serum cortisol and ACTH levels were routinely assessed to rule out secondary adrenal insufficiency, while in obese children, also the cortisol profile (or a dexamethasone test) was performed to rule out hypercortisolemia. These disorders were not found in any of the children. We also routinely assessed the levels of anti thyroglobulin (a-Tg) and anti thyroid peroxidase (a-TPO) antibodies, they were normal in every child. In each child, the concentration of IGF-1, IGFBP-3, lipids, TSH, FT4, leptin, adiponectin and resistin was assessed in the fasting state on the first day of hospitalisation, just before the first stimulating test. Next, IGF-1 concentrations were calculated as IGF-1 SDS, according to the reference data (27). For the calculation of IGF-1/IGFBP-3 molar ratio, the following molecular masses were used: 7.5 kDa for IGF-1 and 42.0 kDa for IGFBP-3. For IGF-1/IGFBP-3 molar ratio, the cutoff point was established at the median values.

Growth hormone levels were measured using the immunometric method. The measurements were performed by Immulite, DPC assay kits, calibrated to the WHO IRP 98/574 standard set, of the following sensitivity level: 0.01 ng/ml, range: up to 40 ng/ml, the conversion index: ng/ml x 2.6 = mIU/l, the intra-assay CV: 5.3-6.5% and inter-assay CV: 5.5-6.2%.

Both IGF-1 and IGFBP-3 concentrations were assessed by Immulite, DPC assays; WHO NIBSC 1st IRR 87/518 standard was applied, with the analytical sensitivity of 20 ng/ml, calibration range up to 1600 ng/ml, the intra-assay CV: 3.1-4.3% and inter-assay CV: 5.8-8.4%. The assay for IGFBP-3 assessment was calibrated to WHO NIBSC Reagent 93/560 standard, with analytical sensitivity 0.02 μg/ml, the calibration range up to 426 μg/ml, the intra-assay CV: 3.5-5.6% and the total CV: 7.5 9.9%.

The leptin, resistin and adiponectin concentrations were measured using the Millipore Elisa kit (Linco Research, USA). The sensitivity level, intra-assay CV and inter-assay CV were: 0.5-100 ng/ml, 1.4-4.9% and 1.3-8.6% for leptin; from 0.16 ng/ml, 3.2-7.0% and 7.1-7.7% for resistin and from 0.78 ng/ml, 7.4% and 2.4-8.4% for adiponectin, respectively.

Concentrations of TSH and FT4 were measured by the electrochemiluminescent immunoassays (ECLIA) method with commercially available appropriate kits (Roche Diagnostic, Mannheim, Germany). Normal range values were as follows: for TSH: age-dependent ranges - 1–7 years-0.7–5.97 mIU/l; 7–12 years-0.6–4.84 mIU/l; 12–18 years-0.51–4.4 mIU/l with inter-assay coefficients of variation (CVs) 1.3–1.8% and for FT4: age-dependent ranges-1–6 years-0.96–1.77 ng/dl; 6–11 years-0.97–1.67 ng/dl; 11–18 years-0.98–1.63 ng/dl with CVs 2.0–2.4%.

The data were analyzed using Statistica 11.0 software (StatSoft, Inc., Tulsa, OK, USA). The continuous variables were expressed as mean ± standard deviation for normally distributed variables. Shapiro-Wilk's test was used to test the distribution of the variables. Correlations were evaluated using the Pearson's test. A one-way ANOVA was applied for statistical analysis with the subsequent use of a *post-hoc* test, in order to statistically assess differences between groups; Tukey's test was selected because of the uneven amount of data in individual groups. $p < 0.05$ was accepted as significant value.

RESULTS

Among 115 children (50 girls and 65 boys), aged 3.66 to 16.52 yrs; the mean age ± SD: 10.43 ± 3.34 years with ISS, we found: 26 slim children, 68 – with normal body weight and 21 - overweight or obese. The results of the auxological parameters and the laboratory tests results for individual subgroups (divided by BMI values) are presented in **Table 1**.

TSH levels were slightly elevated in 8 children: including 2 out of 26 slim children (7.7%), 2 out of 21 obese children (9.5%) and 4 out of 68 normal weight children (5.9%); FT4 level was in normal range in each of these cases.

As expected, the levels of leptin in obese children were significantly higher than in the other groups, but the levels of adiponectin and resistin did not differ between groups.

The degree of growth deficiency and the other (except leptin) test results did not differ between subgroups.

Mean FT4 concentration was the lowest in the subgroup of obese children, but the values did not reach statistical significance (**Table 1**).

In the whole group of ISS children, we observed a positive significant correlation between adiponectin and FT4

TABLE 1 | The results of analysed parameters in individual subgroups (slim, normal and obese) of ISS children.

	Slim	Normal	Obese	Variance analysis
n = (girls/boys)	26 (14/12)	68 (25/43)	21 (11/10)	
Chronological age (CA) (years)	9.60 ± 3.12	10.56 ± 3.12	10.26 ± 3.31	ns
Height age (HA) (years)	7.18 ± 2.38	8.29 ± 2.62	7.59 ± 2.70	ns
HSDS	-2.71 ± 0.97	-2.53 ± 0.82	-2.53 ± 0.88	ns
BMI (kg/m^2)	13.41 ± 0.80^{a,b}	16.33 ± 1.44^{a,c}	20.40 ± 2.94^{b,c}	<0.0005
BMI SDS for CA	-1.28 ± 0.42^{a,b}	-0.46 ± 0.48^{a,c}	1.43 ± 0.95^{b,c}	<0.0005
BMI SDS for HA	-1.27 ± 0.34^{a,b}	-0.03 ± 0.41^{a,c}	2.05 ± 1.01^{b,c}	<0.0005
TSH (mIU/l)	2.71 ± 1.30	2.53 ± 1.14	2.69 ± 1.52	ns
FT4 (ng/ml)	1.36 ± 0.12	1.31 ± 0.20	1.3 ± 0.14	ns
TG (mg/dl)	74.05 ± 27.32	64.85 ± 25.07	74.29 ± 31.54	ns
CH (mg/dl)	150.00 ± 34.72	160.00 ± 28.39	175.56 ± 49.46	<0.05
LDL-CH (mg/dl)	83.58 ± 28.25^{a}	85.62 ± 26.62^{b}	108.21 ± 36.71^{a,b}	<0.05
HDL-CH (mg/dl)	55.58 ± 19.26	60.72 ± 16.54	60.79 ± 11.91	ns
HDL/CH	0.36 ± 0.10	0.39 ± 0.09	0.34 ± 0.08	ns
Leptin (ng/ml)	2.44 ± 5.11^{a}	4.59 ± 5.04^{b}	11.86 ± 11.47^{a,b}	<0.0005
Adiponectin (ng/ml)	18.43 ± 6.97	17.77 ± 8.30	19.80 ± 11.43	ns
Resistin (ng/ml)	9.91 ± 4.05	10.48 ± 4.01	9.13 ± 2.31	ns
Leptin/Adiponectin ratio	0.07 ± 0.4^{a}	0.35 ± 0.4^{b}	0.73 ± 0.43^{a,b}	<0.0005
maxGH after clonidine (ng/ml)	19.50 ± 9.59^{a}	17.43 ± 8.70^{b}	11.75 ± 6.63^{a,b}	<0.05
maxGH after glucagon (ng/ml)	10.81 ± 7.80	9.86 ± 7.03	12.61 ± 6.02	ns
maxGH during sleep (ng/ml)	16.47 ± 9.31	13.89 ± 8.95	10.86 ± 5.52	ns
IGF-1 (ng/ml)	120.26 ± 65.57^{a,b}	192.30 ± 116.67^{a}	216.79 ± 120.01^{b}	<0.01
IGFBP-3 (µg/ml)	3.81 ± 1.09	4.47 ± 1.13	4.81 ± 1.84	ns
IGF-1/IGFBP-3 molar ratio	0.16 ± 0.06^{a}	0.23 ± 0.12^{a}	0.24 ± 0.11	<0.05
IGF-1 SDS	-1.22 ± 1.19	-1.10 ± 1.18	-0.53 ± 0.78	ns

^{a,b,c}values in the same row with different superscripts are significantly differed (p<0.05); BMI – body mass index, SDS – standard deviation score, TG - triglycerides, CH – cholesterol, IGF-1 – insulin-like growth factor 1, IGFBP-3 – insulin-like growth factor binding proteins 3, maxGH – maximal GH concentration during stimulation tests or during sleep.

concentration (**Table 2**). There was no significant correlations of the body mass index (i.e. BMI SDS for CA or for HA), leptin, adiponectin or resistin concentration with TSH and FT4 concentrations. However, we noticed significant positive correlations between leptin/adiponectin ratio and: cholesterol, LDL-fraction of cholesterol and triglicerides (**Table 2**).

DISCUSSION

In the group of children with ISS included in our study, no increase in TSH serum level was observed with increasing children's BMI and leptin concentration. In the subgroup of obese short children, TSH levels were not higher than in other subgroups. Although the group we studied was small (which is a limitation of our work), it seems that our results are worth showing. Many studies on both children and adults, conducted on large groups of patients, have shown that TSH levels correlate positively with BMI and leptin (28, 29). However, an interesting aspect that distinguishes our study is that it concerned children with short stature. Higher leptin concentrations may partially explain the effect of obesity on thyroid function, perhaps through the effect of leptin on TSH secretion, as this increase has been shown to be correlated with leptin regardless of BMI (28, 29). Thus, it is surprising that we did not find such a relationship in our group. Although the thesis concerning the increase in TSH in obesity due to the increased production of pro-TRH through the stimulation of the hypothalamus by leptin is plausible, there are certainly other mechanisms that influence (modulate) this relationship. One of them may be the excessive concentration of ghrelin, which we wrote about in the previous work (10). It is also possible that inflammation drives the changes in TSH and thyroid hormone levels in obesity. Weight reduction is likely to be associated with a reduction in inflammation and may explain

TABLE 2 | The correlation of TSH, FT4 and lipids concentration with BMISDS and selected adipocytokines.

	BMI SDS for HA	Leptin	Adiponectin	Leptin/adiponectin ratio	Resistin
TSH	0.06	-0.09	+0.04	-0.13	+0.07
FT4	-0.02	-0.02	+ 0.2*	-0.18	+0.1
Cholesterol	+0.21	+0.2	+0.03	+0.29*	-0.19
LDL-Cholesterol	+0.24*	+0.14	-0.03	+0.26*	-0.12
HDL-Cholesterol	+0.14*	+0.07	+0.21	0.05	-0.15
Triglicerides	-0.04	+0.26*	-0.05	+0.28*	+0.11

**p < 0.05; BMI SDS for HA - body mass index standard deviation score for height age*

It was observed that the higher the leptin the lower the GH secretion during the test with clonidine (r=-0.32, p < 0.05), as well as the negative correlation between leptin/adiponectin ratio and GH secretion during the test with clonidine (r=-0.39, p < 0.05) and during sleep (r=-0.2, p < 0.05).

the observed correlation between weight loss and a reduction in TSH (12). We have also recently analysed the prevalence of elevated TSH in children with acute respiratory infection and found elevated TSH in 10% of the cases, which returned to normal in all children shortly after recovery (30).

The slightly elevated TSH levels are seen in obese individuals, but not in all of them (12, 31–33). In a study by Habib et al. (5), TSH and FT4 levels were assessed in 850 children aged 2 to 18 years, and it was found that elevated TSH levels are observed in 17.2% of overweight and 20.5% of obese children; in contrast to 9.9% of slim and only 3.8% of normal weight children. In turn, in the study by Wolters et al. (12), elevated TSH was observed in 39% of 477 obese children, however the authors set the cut-off point for the elevated TSH concentration at a lower level, i.e. 3.0 mIU/l.

Thus, the prevalence of hyperthyrotropinemia among obese patients differs in individual analyses.

Meanwhile, in 2020, Wang et al. (11) found that increased TSH levels are more often observed in girls with generalized obesity compared to those with central obesity. We did not analyse the type of obesity in our research. It should also be taken into account that among our patients there were no patients with extreme obesity. The highest value of BMI was +3.9 SD.

In 2019, Ruszała et al. (14) assessed the influence of the thyroid axis dysfunction on the occurrence of metabolic obesity complications. They analysed a group of 100 obese children, where 25 children had features of the metabolic syndrome and 75 did not. The authors found no case of overt thyroid disease within the whole analyzed group. There were no significant differences in mean TSH, FT4, and FT3 levels in patients with and without the metabolic syndrome. Moreover, an elevated TSH level was found in 8% of obese patients with the metabolic syndrome and 24% of obese patients without it. The authors concluded that an isolated increased TSH level is not common in obese adolescents and there is no correlation between TSH, FT3, FT4 levels and BMI SDS value. Moreover, isolated increased TSH levels were not associated with the occurrence of the metabolic syndrome in obese adolescents (14).

In the present study, we also analysed lipids profile. We found a significantly higher concentration of total cholesterol and LDL-cholesterol in the subgroup of children with obesity. However, we did not find any significant correlation between lipids and each of the analysed hormones (i.e. TSH or FT4). In particular, a positive correlation between proatherogenic lipids (cholesterol, LDL-cholesterol and triglycerides) concentrations and leptin/adiponectin ratio was found. It may suggest that the metabolic disorders which we observed were the result of too weak stimulation of pro-TRH and TSH by leptin (e.g, in certain disorders at the hypothalamic level) and, in consequence, the relative hypothyroidism. It may also be a possible explanation of an additional observation we made, namely a negative correlation between leptin concentration (or leptin/adiponectin ratio) and GH secretion during the stimulation test with clonidine. This phenomenon, observed in obese children, can be explained - among others - by the blocking effect of lipid disorders on GHRH and GH secretion (34, 35). Therefore, it is possible that some obese children experience a weaker action exerted by TRH and GHRH jointed on the level of the pituitary gland.

In turn, Bossowski et al. (25) explored other aspects of these issues. They analysed leptin, adiponectin and resistin in children with untreated Graves' disease and hypothyroidism in Hashimoto's thyroiditis. The authors showed higher adiponectin and lower resistin levels in hyperthyroidism than in hypothyroidism. The analysis of leptin levels revealed no significant differences between children with subclinical hypothyroidism and untreated Graves' disease. Thus, their research also supports the idea that leptin and TSH levels are in a poor cause-and-effect relationship. However, they suggested that disturbances in thyroid hormones in thyroid diseases have a significant effect on the levels of adiponectin and resistin released by adipose tissue (25). We also observed the same relationship between FT4 and adiponectin concentrations in the analysed group of children. It is to be noted that a higher FT4 concentration should be beneficial for the decreased leptin/adiponectin ratio. However, in the analysed group of short children we did not find that relationship. Thus, the observed phenomenon of increased TSH in some obese children is probably multifactorial, and in children without thyroid disease could trigger protective effects, but it does not seem to apply to all obese children and especially to obese children with idiopathic short stature.

The lack of leptin influence on TSH concentration could indicate wide ranging disturbances of hypothalamic signals, and consequently be the cause of inappropriate GH secretion.

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.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Bioethical Committee at the Polish Mother's Memorial Hospital-Research Institute (PMMH-RI) in Lodz. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

Conceptualization, KA and RS. Methodology, RS and KA. Resources, RS. Writing—original draft preparation, KA and ZA. Writing—review and editing, AL. Supervision, AL and RS. All authors have read and agreed to the published version of the manuscript.

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High Prevalence of Cardiometabolic Comorbidities Among Children and Adolescents With Severe Obesity From a Large Metropolitan Centre (Hangzhou, China)

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Objective: This study aimed to describe the clinical characteristics of children and adolescents with obesity, and the prevalence of cardiometabolic comorbidities over 10 years in this population from a large metropolitan centre in China.

Methods: This was a cross-sectional study (2008–2017) of patients aged <18 years with obesity [body mass index (BMI) ≥ 95th percentile for age and sex] enrolled at the Department of Endocrinology, Children's Hospital of Zhejiang University School of Medicine (Hangzhou, Zhejiang Province). Clinical assessments included anthropometry, blood pressure, liver ultrasound, lipid profile, oral glucose tolerance test, and uric acid. For examination of outcomes, our study cohort was stratified by sex and age bands (<10 vs. ≥10 years), with the study period also split into two strata (2008–2012 and 2013–2017).

Results: A total of 2,916 patients (1,954 boys and 962 girls) were assessed at a mean age of 10.5 years. Patients almost invariably presented severe obesity (median BMI SDS = 2.98; Q1 = 2.60, Q3 = 3.39). Obesity-related comorbidities were common among boys and girls, including type 2 diabetes mellitus (2.6% and 3.6%, respectively), abnormal glycaemia (33.6% and 35.5%, respectively), hypertension (33.9% and 32.0%, respectively), dyslipidaemia (35.2% and 39.6%, respectively), hyperuricaemia (16.2% and 8.3%, respectively), acanthosis nigricans (71.9% and 64.0%, respectively), abnormal liver function (66.9% and 47.0%, respectively), and non-alcoholic fatty liver disease (NAFLD) (63.8% and 45.1%, respectively); 38.7% of boys and 44.4% of girls aged ≥10 years had metabolic syndrome. Notably, the incidence of many cardiometabolic comorbidities was in 2013–2017 compared to 2008–2012. For example, rates of hypertension among boys aged <10 years and aged ≥10 years rose from 28.4% and 26.5% to 48.0% and 35.8%, respectively, and in girls from 20.3% and 20.8% to 41.7%

and 39.6%, respectively. In 2013–2017, 9.5% of girls in the older group had metabolic syndrome compared to 2.2% in 2008–2013.

Conclusions: We observed a high incidence of obesity-related cardiometabolic comorbidities among Chinese children and adolescents with severe obesity over 10 years. It was particularly concerning that rates of several comorbidities rose markedly over the study period, highlighting the need to address the obesity epidemic early in life (in China and elsewhere) to prevent the development of obesity-related comorbidities and, subsequently, of overt disease.

Keywords: abnormal liver function, acanthosis nigricans, blood pressure, China, glucose metabolism, hypertension, insulin sensitivity, NAFLD

INTRODUCTION

Worldwide, obesity is a major public health issue (1). The number of children and adolescents aged 5 to 19 years with obesity has risen 10-fold over the last four decades, reaching 124 million in 2016, with the global prevalence increasing from 0.7% to 5.6% in girls and from 0.9% to 7.8% in boys between 1975 and 2016, respectively (2). Notably, the mean body mass index (BMI) among children and adolescents has increased steadily, including in China (3). In 2015–2019, the prevalence of overweight and obesity in China was 6.8% and 3.6%, respectively, for children aged less than 6 years, and 11.1% and 7.9%, respectively, for those aged 6–17 years (4), with higher obesity rates reported in urban areas (5).

Children with obesity are at increased risk for cardiometabolic comorbidities, including hypertension, dyslipidaemia, hyperglycaemia, non-alcoholic fatty liver disease (NAFLD), and metabolic syndrome, which often track into adulthood with an increased risk of cardiovascular morbidity and mortality (6, 7). Of note, several paediatric definitions of metabolic syndrome agree on its components but differ in diagnostic criteria, and the International Diabetes Federation (IDF) definition seems to be more easily adopted in clinical practice (8). Moreover, the hazard ratio for type 2 diabetes mellitus is markedly elevated among adolescents with severe obesity (9). China is currently experiencing an accelerating diabetes epidemic as a result of a combination of factors (many of which interact), including increasing rates of obesity, changes in dietary habits (e.g., high in fat) and lifestyle (i.e., sedentary), ageing, and genetic and epigenetic factors (10). Of interest, a higher risk of diabetes at a lower BMI has been observed in the Chinese population compared to Europeans, likely resulting from the former's greater visceral adiposity (11) and lower insulin response (12). Additionally, childhood obesity may favour early pubertal development or skeletal maturation and adverse psychosocial outcomes, including depression, anxiety, and eating disorder (13–15).

Therefore, childhood obesity and related cardiometabolic comorbidities are important issues that must be addressed. However, the incidence and severity of cardiometabolic comorbidities among Chinese children and adolescents with obesity have been overlooked in China over the last decades. Thus, our primary aim was to describe the clinical features of children and adolescents with obesity, particularly obesity-related cardiometabolic comorbidities. In addition, we also examined possible changes in the incidence of these comorbidities in boys and girls over the 10-year study period.

METHODS

Study Participants

This was a cross-sectional study of children and adolescents voluntarily brought to our hospital by their parents who were concerned about excessive weight gain. These patients were then referred to the Department of Endocrinology at the Children's Hospital of Zhejiang University School of Medicine, National Clinical Research Center for Child Health in Hangzhou, between January 1, 2008, and December 31, 2017. Hangzhou is the capital of Zhejiang Province, with a population of 6.77 million in 2008 and 7.53 million in 2017, including 1.05 million and 1.25 million children and adolescents aged ≤17 years, respectively (16, 17). The Children's Hospital is one of only two National Clinical Research Centers for Child Health in China, recording approximately 81,000 inpatient and 3.5 million outpatient visits per year. Patients were only included once in this study, corresponding to their first visit to our clinic during the 10-year study period.

The main inclusion criterion was obesity at presentation, defined as a BMI SD score [SDS; derived as per the WHO standards (18) for age and sex] ≥1.645 (i.e., ≥95th percentile). At admission, none of our patients was on therapy with medications known to affect energy metabolism; had an overt chronic heart, lung, or kidney disease; or had been previously with diagnosed endocrine or metabolic dysfunction (e.g., Wilson's disease), genetic disorders (e.g., Prader–Willi syndrome), or any severe chronic illness.

Clinical Assessments

Participants were admitted to our inpatient clinic and underwent comprehensive clinical assessments over 24 h performed by nurses. Demographic characteristics were recorded or obtained from clinical records.

Anthropometry

Standing height was measured to the nearest 0.1 cm using a wall-mounted Harpenden stadiometer while patients were barefoot.

Weight was measured with the participant in light clothing using a digital scale to the nearest 0.1 kg; BMI was subsequently derived. Height, weight, and BMI were transformed into SDS (18). Waist circumference was measured to the nearest 1 mm with a tape measure around the participant's body in the horizontal plane, at the midpoint level between the lowest rib and the iliac crest, on bare skin, and at the end of normal expiration. The waist-to-height ratio was then calculated. Maternal and paternal anthropometry data (i.e., height and weight) were obtained by self-report, and their BMI was calculated.

Cardiometabolic Parameters

Systolic (SBP) and diastolic blood pressures (DBP) were measured using a sphygmomanometer on the right upper arm while patients seated and after a 5-min rest. Venous blood samples were taken on the morning of the assessment after an overnight fast. Parameters measured included glucose, insulin, glycated haemoglobin (HbA1c), triglycerides, low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL), total cholesterol, aspartate transaminase (AST), alanine transaminase (ALT), and uric acid.

All participants underwent a 75-g oral glucose tolerance test (OGTT; 1.75 g per kg, maximum 75 g), with blood samples drawn at 0, 30, 60, 90, and 120 min for glucose, insulin, and C-peptide measurements. Insulin sensitivity was assessed using the Matsuda index, which is strongly correlated with the hyperinsulinaemic–euglycaemic clamp and has high reproducibility during multiple measures (19).

Cardiometabolic comorbidities assessed included the following: impaired fasting glucose, impaired glucose tolerance, type 2 diabetes, and abnormal glycaemia (20); hypertension (21); dyslipidaemia (22); metabolic syndrome (22); NAFLD (23); and hyperuricaemia (24) (**Table 1**). Acanthosis nigricans was also recorded, as it is a clinical sign of hyperinsulinaemia and insulin resistance (26, 27). Central obesity was defined as per Chinese criteria for children and adolescents based on the waist-to-height ratio (21) (**Table 1**).

Statistical Analyses

Descriptive data for demographic and anthropometric characteristics of our overall study population are provided as means ± SDs or frequency (n) and percentages (%). The incidence of central obesity and obesity-related cardiometabolic comorbidities is provided as n (%).

Patients were then stratified according to the year of admission (into two 5-year study periods: 2008–2012 and 2013–2017), sex (male and female), and age (<10 and ≥10 years). Within each age group, the incidence of comorbidities was compared between sexes and between study periods using Fisher's exact tests.

Continuous outcomes for anthropometry, blood pressure, glucose metabolism, and lipid profile were also compared between the groups mentioned above. Potential differences were assessed using general linear regression models adjusting for the participant's age, with the latter replaced with height for blood pressure outcomes. The data distribution of each outcome was examined, and, where appropriate, data were log-transformed to approximate a normal distribution. For continuous variables, differences between sexes or study periods are reported in the text as the estimated marginal means (adjusted means) and 95% CIs.

Analyses were performed in SPSS v25 (IBM Corp., Armonk, NY, USA) and SAS v9.4 (SAS Institute, Cary, NC, USA). All tests were two-tailed, with statistical significance maintained at $p < 0.05$, and without adjustment for multiple comparisons as per Rothman (1990) (28).

RESULTS

Our study population consisted of 2,916 children and adolescents with obesity, assessed at a mean age of 10.5 ± 2.6 years, including 1,954 boys and 962 girls (**Table 2**). Participants had a median BMI SDS of 2.98 (Q1 = 2.60, Q3 = 3.39; range 1.65–9.51), and the vast majority had severe obesity (**Figure 1**). Among caregivers, 1 in 3 mothers (33.2%) and more than half of fathers (54.7%) had overweight or obesity (**Table 2**).

As shown in **Table 3**, there was a high incidence of cardiometabolic comorbidities among children and adolescents with obesity in China over the 10-year period covered by this study. Among boys and girls, these included type 2 diabetes (2.6% and 3.6%, respectively), abnormal glycaemia (33.6% and 35.5%), hypertension (33.9% and 32.0%), and dyslipidaemia (35.2% and 39.6%), with almost all boys (99.6%) and girls (98.5%) having central obesity (**Table 3**).

There was a greater proportion of boys than girls with hyperuricaemia (16.2% vs. 8.3%, respectively; $p < 0.0001$), acanthosis nigricans (71.9% vs. 64.0%; $p < 0.0001$), abnormal liver function (66.9% vs. 47.0%; $p < 0.0001$), and NAFLD (63.8% vs. 45.1%; $p < 0.0001$), with these sex differences largely observed in the two age groups (**Table 3**). Among children aged <10 years, there was a greater incidence of hypertension in boys than girls (38.6% vs. 31.9%; $p = 0.021$) (**Table 3**). In addition, the incidence of obesity-related comorbidities was greater among patients aged ≥10 years than in the younger group (**Table 3**). Notably, 38.7% of boys and 44.4% of girls aged ≥10 years had metabolic syndrome (**Table 3**).

When the two 5-year periods (2008–2012 vs. 2013–2017) were compared, among the younger boys, there was a higher incidence of impaired fasting glucose, abnormal glycaemia, acanthosis nigricans, and, in particular, hypertension (**Table 4**). These differences were underpinned by higher SBP (+6.2 mmHg; 95% CI 4.3, 8.0 mmHg), DBP (+2.3 mmHg; 95% CI 1.0, 3.7 mmHg), fasting glucose (+0.20 mmol/L; 95% CI 0.13, 0.27 mmol/L), and a Matsuda index that was 18% lower (95% CI −1.3%, −31.6%) in 2013–2017 (**Table 5**). Conversely, the incidence of dyslipidaemia was lower in the later period, likely associated with HDL +7.3% higher (+0.09 mmol/L; 95% CI 0.05, 0.14 mmol/L) and possibly slightly lower triglycerides (**Table 5**).

In the older group of boys, in 2013–2017, there was a higher incidence of hypertension, abnormal liver function, and abnormal glycaemia, including type 2 diabetes (4.9% vs. 1.5%; **Table 4**). As seen among the younger boys, these comorbidities were more frequent in the later period in association with higher SBP (+4.3 mmHg; 95% CI 2.9, 5.8 mmHg), DBP (+2.3 mmHg; 95% CI 1.3, 3.4 mmHg), fasting glucose (+0.13 mmol/L; 95% CI 0.07, 0.19 mmol/L), fasting insulin (+14%; 95% CI 5%, 24%), and

TABLE 1 | Diagnostic criteria for central adiposity and obesity-related cardiometabolic comorbidities assessed.

Condition	Age	Minimum diagnostic criteria	Reference
Impaired fasting glucose	All	Fasting plasma glucose ≥5.6 and <7.0 mmol/L	Arslanian et al. (20)
Impaired glucose tolerance	All	a) Fasting plasma glucose <7.0 mmol/L; AND b) 2-h plasma glucose ≥7.8 and <11.1 mmol/L[#]	Arslanian et al. (20)
Type 2 diabetes	All	a) Fasting plasma glucose ≥7.0 mmol/L; OR b) 2-h plasma glucose ≥11.1 mmol/L[#]	Arslanian et al. (20)
Abnormal glycaemia	All	a) Impaired fasting glucose; OR b) Impaired glucose tolerance; OR c) Type 2 diabetes	Arslanian et al. (20)
Hypertension	<10 years	a) Systolic blood pressure ≥120 mmHg; OR b) Diastolic blood pressure ≥80 mmHg	Chinese Medical Association (21)
	≥10 years	a) Systolic blood pressure ≥130 mmHg; OR b) Diastolic blood pressure ≥85 mmHg	Chinese Medical Association (21)
Dyslipidaemia	All	a) Triglycerides ≥1.7 mmol/L; OR b) HDL <1.03 mmol/L	Zimmet et al. (22)
Metabolic syndrome	≥10 years	a) Obesity; AND b) any two of: • Abnormal glycaemia (as defined above; • Hypertension (as defined above); • Dyslipidaemia (as defined above);	Zimmet et al. (22)
Hyperuricaemia	All	Plasma uric acid ≥5.5 mg/dl	Loeffler et al. (24)
NAFLD	All	"A diffusely echogenic change in liver B-ultrasonography, with or without elevated serum aminotransferase levels and other factors that can cause liver fatty infiltration or aminotransferase elevation, such as hepatitis virus infection, drug-induced injury, and other metabolic diseases, such as Wilson's disease, were excluded."	Chinese Liver Disease Assoc (23).
Central obesity	≥6 and <10 years	Waist-to-height ratio ≥0.48	Chinese Medical Association (21)
	≥10 and <16 years	Boys: Waist-to-height ratio ≥0.48 Girls: Waist-to-height ratio ≥0.46	Chinese Medical Association (21)

Adapted from Jin et al. (25).
HDL, high-density lipoprotein cholesterol; NAFLD, non-alcoholic fatty liver disease.
[#]*Parameter measured after a 75-g glucose load from an oral glucose tolerance test.*

lower Matsuda index (−15%; 95% CI −5%, −25%) in 2013–2017 (**Table 5**).

Among the younger girls, there were proportionally fewer patients with abnormal liver function in 2013–2017 (**Table 6**), but the rate of hypertension was 2-fold higher (41.7% vs. 20.3%), which was associated with higher SBP (+9.2 mmHg; 95% CI 7.2, 11.3 mmHg) and DBP (+2.6 mmHg; 95% CI 1.0, 4.2 mmHg) (**Table 7**). In addition, girls in the second period had fasting glucose concentrations 0.15 mmol/L higher (95% CI 0.04, 0.26 mmol/L) and Matsuda index 28% lower (95% CI −10%, −42%) (**Table 7**).

For girls aged 10 years or older, there was a higher incidence of acanthosis nigricans, hypertension, and a 4.5-fold higher incidence of type 2 diabetes affecting nearly 10% of girls in 2013–2017 (9.5% vs. 2.2%) (**Table 6**). In this older group of girls, there were other clinical parameters worse in 2013–2017 than in 2008–2012, including higher waist-to-height ratio (+0.011; 95% CI 0.000, 0.022), SBP (+4.8 mmHg; 95% CI 2.4, 7.2 mmHg), DBP (+4.6 mmHg; 95% CI 2.8, 6.3 mmHg), LDL (+0.26 mmol/L; 95% CI 0.13, 0.39 mmol/L), and fasting insulin (+28%; 95% CI 9%, 50%) but lower Matsuda index (−42%; 95% CI −26%, −54%) (**Table 7**). Conversely, HDL was higher (+0.07 mmol/L; 95% CI 0.02, 0.11 mmol/L) and the cholesterol:HDL ratio lower (−0.21; 95% CI −0.41, −0.02) (**Table 7**).

DISCUSSION

To our knowledge, this was the largest single-centre cross-sectional study (n = 2,916) to comprehensively assess obesity-related comorbidities among children and adolescents with severe obesity in China. There was a high incidence of a range of cardiometabolic comorbidities, including abnormal glycaemia, hypertension, dyslipidaemia, hyperuricaemia, acanthosis nigricans, abnormal liver function, and NAFLD. These findings are not surprising given the strong association between higher BMI during adolescence and increased risk for cardiometabolic complications (15, 29). Our paediatric population characterised by very high BMI SDS illustrates the extent of the potential impacts of childhood obesity (and related comorbidities) on public health in China and elsewhere, a growing challenge that has been previously underestimated (30, 31).

TABLE 2 | Demographic, anthropometric, and clinical characteristics of our study population of children and adolescents with obesity from Hangzhou (Zhejiang Province, China) in 2008–2017.

n		2,916
Demography	**Age (years)**	10.5 ± 2.6
	Female	962 (33.0%)
Anthropometry	**Height SDS**	1.02 ± 1.18
	Weight SDS	3.39 ± 1.02
	BMI SDS	3.08 ± 0.82
	Waist-to-height ratio	0.604 ± 0.057
Parental characteristics [1]	**Maternal BMI (kg/m^2)**	24.00 ± 3.52
	Maternal obesity	166 (5.9%)
	Maternal overweight or obesity	969 (33.2%)
	Paternal BMI (kg/m^2)	25.76 ± 3.50
	Paternal obesity	295 (10.6%)
	Paternal overweight or obesity	1,596 (54.7%)

Data are the mean ± SD or n (%), as appropriate.
BMI, body mass index; SDS, SD scores.
[1]*The total n for maternal and paternal BMI was 2,799 and 2,793, respectively.*

In 2013, the prevalence of metabolic syndrome in Chinese children with obesity was 28.8% (32), in contrast to our observed rates of 40.1% and 46.2% for boys and girls, respectively, in 2013–2017. The much higher incidence in our study population is not surprising given that most of our patients had severe obesity. Other common comorbidities in our study included abnormal liver function, NAFLD, and acanthosis nigricans. The latter, in particular, is highly prevalent and a specific clinical sign of insulin resistance, a key component of the metabolic syndrome (33, 34). Our patients also had insulin sensitivity assessed with the Matsuda index, a robust measure to examine abnormalities in glucose metabolism in children and adolescents with obesity (35). Previous studies have also shown high rates of disorders in glucose metabolism among asymptomatic children and adolescents with obesity (36). Here, we also observed an apparent reduction in insulin sensitivity and an associated increase in fasting glucose levels between the two 5-year periods (2008–2012 vs. 2013–2017); these findings suggest that children with severe obesity are displaying increasing levels of impairment in glucose metabolism and possibly increased risk of progressing to type 2 diabetes.

The growing problem of childhood obesity seems to be underpinning the increasing burden of NAFLD. In our study, 2 out of 3 boys aged 10 years or older displayed liver function abnormalities and/or were affected by NAFLD. In a study on ≈15,000 children and adolescents from 6 centres throughout China, we identified elevated liver enzymes associated with

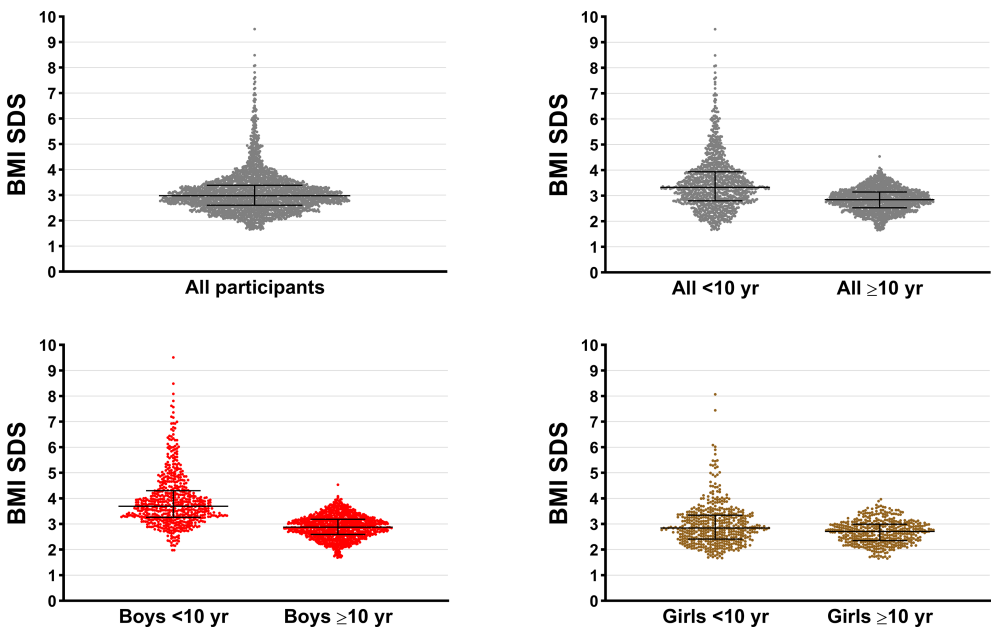

FIGURE 1 | Distribution of body mass index SD scores (BMI SDS) among our study population of children and adolescents with obesity assessed in 2008–2017 in Hangzhou (Zhejiang Providence, China). Horizontal bars represent the median and the interquartile range.

TABLE 3 | Incidence of cardiometabolic comorbidities among children and adolescents with obesity assessed between 2008 and 2017 in Hangzhou (Zhejiang Province, China).

	All ages			Aged <10 years			Aged ≥10 years		
	All	**Boys**	**Girls**	**All**	**Boys**	**Girls**	**All**	**Boys**	**Girls**
N	2,916	1,954	962	1,132	623	509	1,784	1,331	453
IFG	722 (24.8%)	481 (24.7%)	241 (25.1%)	275 (24.4%)	154 (24.9%)	121 (23.8%)	447 (25.1%)	327 (24.6%)	120 (26.5%)
IGT	432 (14.9%)	282 (14.5%)	150 (15.6%)	137 (12.2%)	82 (13.2%)	55 (10.8%)	295 (16.6%)	200 (15.1%)	95 (21.0%)**
Abnormal glycaemia	996 (34.3%)	655 (33.6%)	341 (35.5%)	361 (32.0%)	205 (33.1%)	156 (30.7%)	635 (35.7%)	450 (33.9%)	185 (40.8%)**
Type 2 diabetes	85 (2.9%)	50 (2.6%)	35 (3.6%)	10 (0.9%)	5 (0.8%)	5 (1.0%)	75 (4.2%)	45 (3.4%)	30 (6.6%)**
Hypertension	968 (33.3%)	660 (33.9%)	308 (32.0%)	402 (35.6%)	240 (38.6%)	162 (31.9%)*	566 (31.8%)	420 (31.6%)	147 (32.2%)
Dyslipidaemia	1,058 (36.7%)	684 (35.2%)	374 (39.6%)*	348 (31.1%)	184 (29.7%)	164 (32.7%)	710 (40.2%)	500 (37.8%)	210 (47.3%)***
Hyperuricaemia	391 (13.7%)	313 (16.2%)	78 (8.3%)****	56 (5.1%)	32 (5.2%)	24 (4.9%)	335 (19.1%)	281 (21.4%)	54 (12.2%)****
Acanthosis nigricans	1,980 (69.3%)	1,378 (71.9%)	602 (64.0%)****	673 (60.9%)	412 (67.5%)	261 (52.7%)****	1,307 (74.6%)	966 (73.9%)	341 (76.5%)
Abnormal liver function	1,733 (60.4%)	1,292 (66.9%)	441 (47.0%)****	551 (49.7%)	358 (58.3%)	193 (39.1%)****	1,182 (67.1%)	934 (70.9%)	248 (55.9%)****
NAFLD	1,678 (57.6%)	1,245 (63.8%)	433 (45.1%)****	477 (42.2%)	313 (50.3%)	164 (32.3%)****	1,201 (67.4%)	932 (70.2%)	269 (59.4%)****
Metabolic syndrome	–	–	–	–	–	–	716 (40.1%)	515 (38.7%)	201 (44.4%)*
Central obesity[1]	2,736 (99.2%)	1,853 (99.6%)	883 (98.5%)	962 (98.9%)	528 (99.6%)	434 (98.0%)	1,774 (99.4%)	1,325 (99.5%)	449 (99.1%)

IFG, Impaired fasting glucose; IGT, Impaired glucose tolerance; NAFLD, non-alcoholic fatty liver disease.

[1]Central obesity as per Chinese standards is not diagnosed in children aged less than 6 years (21); therefore, 93 boys and 56 girls were excluded from the calculation of these rates. Data are n (%).

**p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001 for comparisons in incidence of a given comorbidity within a particular group.*

metabolic syndrome features, highlighting the role of chronic insulin resistance and metabolic syndrome in the aetiology of liver injury in Chinese youth (37). While we have no data on the clinical history of our patients (particularly in regard to weight gain over time), our findings are not surprising given that, after 2 years of age, obesity progressively increases the risk of developing NAFLD in adolescence (38). Therefore, it is important to monitor liver function and the potential development of NAFLD over time among children and adolescents with obesity, specifically liver function tests and liver ultrasound (39, 40).

The link between obesity and hypertension is well established (41, 42). The reported prevalence of hypertension in schoolchildren with obesity was approximately 11% in the United States (43). In Greece, a study on 2,655 schoolchildren aged 9–13 years showed higher rates of

TABLE 4 | Incidence of cardiometabolic comorbidities among Chinese boys with obesity assessed between 2008 and 2017 in Hangzhou (Zhejiang Province, China).

Boys	Aged <10 years			Aged ≥10 years		
	2008–2012	**2013–2017**	**p-Value**	**2008–2012**	**2013–2017**	**p-Value**
N	298	325		600	731	
Impaired fasting glucose	59 (20.1%)	95 (29.2%)	**0.009**	132 (22.1%)	195 (26.7%)	0.055
Impaired glucose tolerance	38 (12.9%)	44 (13.5%)	0.91	76 (12.8%)	124 (17.0%)	**0.037**
Abnormal glycaemia	78 (26.5%)	127 (39.1%)	**0.001**	174 (29.1%)	276 (37.8%)	**0.001**
Type 2 diabetes	1 (0.3%)	4 (1.2%)	0.38	9 (1.5%)	36 (4.9%)	**0.001**
Hypertension	84 (28.4%)	156 (48.0%)	**<0.0001**	158 (26.5%)	262 (35.8%)	**<0.0001**
Dyslipidaemia	105 (35.4%)	79 (24.5%)	**0.004**	253 (42.4%)	247 (34.2%)	**0.002**
Hyperuricaemia	14 (4.7%)	18 (5.6%)	0.72	125 (21.2%)	156 (21.5%)	0.95
Acanthosis nigricans	176 (61.5%)	236 (72.8%)	**0.003**	414 (69.3%)	518 (70.9%)	0.59
Abnormal liver function	191 (65.0%)	167 (52.2%)	**0.001**	393 (68.0%)	573 (78.6%)	**<0.0001**
NAFLD	155 (52.2%)	158 (48.6%)	0.38	434 (73.4%)	500 (68.8%)	0.07
Metabolic syndrome	–	–	–	222 (37.0%)	293 (40.1%)	0.26

Data are n (%). p-Values are derived from Fisher's exact tests, and correspond to comparisons in the incidence of a particular comorbidity between time periods within a given age group. Statistically significant p-values (<0.05) are shown in bold.

NAFLD, non-alcoholic fatty liver disease.

TABLE 5 | Cardiometabolic parameters among Chinese boys with obesity assessed between 2008 and 2017 in Hangzhou (Zhejiang Province, China).

Boys		Aged <10 years			Aged ≥10 years		
		2008–2012	2013–2017	p-Value	2008–2012	2013–2017	p-Value
n		298	325		600	731	
Age		7.9 ± 1.7	8.0 ± 1.7	0.36	11.9 ± 1.4	12.1 ± 1.4	0.15
Anthropometry	**Height SDS**	1.38 ± 1.16	1.44 ± 1.13	0.51	0.85 ± 1.19	0.95 ± 1.17	**0.031**
	BMI SDS	3.90 ± 1.05	3.92 ± 1.02	0.17	2.88 ± 0.44	2.90 ± 0.44	0.17
	Waist-to-height ratio	0.624 ± 0.059	0.627 ± 0.059	0.33	0.604 ± 0.048	0.609 ± 0.053	**0.042**
Blood pressure	**Systolic (mmHg)**	112.4 ± 12.7	118.8 ± 11.9	**<0.0001**	119.6 ± 14.7	124.5 ± 13.3	**<0.0001**
	Diastolic (mmHg)	67.4 ± 8.2	69.9 ± 9.1	**<0.001**	69.3 ± 8.6	71.7 ± 10.0	**<0.0001**
Glucose metabolism	**Matsuda index**	75.9 (62.2, 92.8)	60.9 (56.3, 66.0)	**0.036**	49.4 (43.8, 55.7)	41.7 (39.6, 43.4)	**0.005**
	HbA1c (%)	5.85 ± 0.57	5.76 ± 0.71	0.08	5.85 ± 0.54	5.82 ± 0.63	0.43
	Fasting glucose (mmol/L)	5.21 ± 0.53	5.41 ± 0.38	**<0.0001**	5.25 ± 0.61	5.38 ± 0.45	**<0.0001**
	Fasting insulin (μIU/ml)	10.7 (9.7, 11.9)	12.0 (10.9, 13.2)	0.11	15.8 (14.9, 16.9)	18.1 (17.1, 19.2)	**0.002**
Lipid profile	**Total cholesterol (mmol/L)**	4.28 ± 0.73	4.33 ± 0.81	0.47	4.41 ± 0.82	4.34 ± 0.90	0.16
	Triglycerides (mmol/L)	1.15 (1.10, 1.21)	1.08 (1.03, 1.14)	0.08	1.25 (1.21, 1.30)	1.26 (1.22, 1.30)	0.87
	LDL (mmol/L)	2.32 ± 0.54	2.68 ± 0.61	**<0.0001**	2.51 ± 0.66	2.74 ± 0.68	**<0.0001**
	HDL (mmol/L)	1.23 ± 0.28	1.32 ± 0.26	**<0.0001**	1.23 ± 0.31	1.25 ± 0.26	0.11
	Total cholesterol/HDL	3.62 ± 0.94	3.36 ± 0.80	**<0.0001**	3.80 ± 1.17	3.59 ± 1.02	**<0.0001**
Inflammatory marker	**Uric acid (μmol/ml)**	3.89 ± 0.93	3.97 ± 0.86	0.31	4.57 ± 1.13	4.68 ± 1.12	0.17

For Matsuda index, fasting insulin, and triglycerides the back-transformed data are reported as means and respective 95% CIs; all other data are means ± SDs. Statistically significant p-values (<0.05) are shown in bold.

BMI, body mass index; HbA1c, glycated haemoglobin; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; SDS, SD score.

hypertension of 25.3% and 20.8% for girls and boys, respectively (44). Among Chinese youth, Cao (2009) reported rates of hypertension of 11.5% in girls and 21.7% in boys with obesity aged 12 to 17 years (45). The higher rates of hypertension among our patients (33.3%) would be expected given their obesity severity, as alluded to earlier. For example, Lo et al. showed that the odds of hypertension were 2.7 times greater in children with severe obesity compared to those with moderate obesity (46). Notably, the incidence of hypertension increased between 2008–2012 and 2013–2017 among our patients, irrespective of sex or age, reflecting corresponding increases in SBP and DBP over time. The reasons for the changing incidence of hypertension over time are unclear, particularly in the absence of differences in obesity levels. It is possible that changes in dietary habits and physical activity levels could explain, at least in part, the worsening rates of hypertension in the latest period. However, this cannot be ascertained in our study population, as such information was not recorded. Nonetheless, independent of the underlying causes of our observed trend, blood pressure should be monitored in the long-term (47) among paediatric patients with obesity, as a longer duration of hypertension increases the cardiovascular risk and end-organ damage (48).

Of note, the ratio of boys to girls in our study was approximately 2:1. It is plausible that this could reflect some bias among parents, who would be more likely to identify weight issues in boys than in girls. Previously, in a study of more than 20,000 children and adolescents from 6 centres across China, we reported that parents were more likely to overestimate the BMI status of girls compared to boys (49). However, the child's sex was not associated with the parents' ability to correctly identify an obesity issue or, most importantly in the context of the present study, of seeking treatment for their child if weight issues were identified (49). Most likely, the overrepresentation of boys in our

TABLE 6 | Incidence of cardiometabolic comorbidities among Chinese girls with obesity assessed between 2008 and 2017 in Hangzhou (Zhejiang Province, China).

Girls	Aged <10 years			Aged ≥10 years		
	2008–2012	2013–2017	p-Value	2008–2012	2013–2017	p-Value
N	232	276		178	275	
Impaired fasting glucose	56 (24.1%)	65 (23.6%)	0.92	42 (23.6%)	78 (28.4%)	0.28
Impaired glucose tolerance	24 (10.3%)	31 (11.2%)	0.78	39 (21.9%)	56 (20.4%)	0.72
Abnormal glycaemia	73 (31.5%)	83 (30.1%)	0.77	66 (37.1%)	119 (43.3%)	0.20
Type 2 diabetes	nil	5 (1.8%)	0.07	4 (2.2%)	26 (9.5%)	**0.002**
Hypertension	47 (20.3%)	115 (41.7%)	**<0.0001**	37 (20.8%)	109 (39.6%)	**<0.0001**
Dyslipidaemia	84 (36.5%)	80 (29.5%)	0.10	93 (52.5%)	117 (43.8%)	0.08
Hyperuricaemia	12 (5.3%)	12 (4.5%)	0.68	15 (8.7%)	39 (14.5%)	0.08
Acanthosis nigricans	106 (48.0%)	155 (56.6%)	0.058	118 (68.2%)	223 (81.7%)	**0.001**
Abnormal liver function	104 (46.0%)	89 (33.2%)	**0.004**	88 (50.6%)	160 (59.3%)	0.08
NAFLD	84 (36.2%)	80 (29.0%)	0.09	103 (57.9%)	166 (60.4%)	0.63
Metabolic syndrome	–	–	–	74 (41.6%)	127 (46.2%)	0.38

Data are n (%). p-Values are derived from chi-square tests or Fisher's exact tests; they correspond to comparisons in the incidence of particular comorbidity between time periods within a given age group. Statistically significant p-values (<0.05) are shown in bold.

NAFLD, non-alcoholic fatty liver disease.

TABLE 7 | Cardiometabolic parameters among girls with obesity assessed between 2008 and 2017 in Hangzhou (Zhejiang Province, China).

Girls		Aged <10 years			Aged ≥10 years		
		2008–2012	2013–2017	p-Value	2008–2012	2013–2017	p-Value
n		232	276		178	275	
Age		7.9 ± 1.4	7.9 ± 1.3	0.88	12.3 ± 1.7	12.6 ± 1.7	0.13
Anthropometry	Height SDS	1.26 ± 1.08	1.35 ± 1.02	0.36	0.50 ± 1.08	0.60 ± 1.15	0.15
	BMI SDS	3.04 ± 0.87	2.91 ± 0.74	**0.015**	2.67 ± 0.43	2.71 ± 0.47	0.68
	Waist-to-height ratio	0.587 ± 0.061	0.577 ± 0.052	**0.026**	0.587 ± 0.057	0.601 ± 0.058	**0.046**
Blood pressure	Systolic (mmHg)	108.0 ± 12.4	117.4 ± 12.1	**<0.0001**	118.5 ± 14.1	124.0 ± 12.0	**<0.0001**
	Diastolic (mmHg)	65.5 ± 8.1	68.2 ± 9.9	**0.002**	68.3 ± 8.5	73.1 ± 9.9	**<0.0001**
Glucose metabolism	Matsuda index	90.0 (71.5, 114.4)	63.4 (58.0, 69.4)	**0.004**	56.3 (41.7, 76.7)	32.5 (30.3, 35.2)	**<0.0001**
	HbA1c (%)	5.74 ± 0.57	5.66 ± 0.55	0.07	5.87 ± 0.56	5.78 ± 0.71	0.21
	Fasting glucose (mmol/L)	5.24 ± 0.57	5.39 ± 0.67	**0.007**	5.27 ± 0.78	5.38 ± 0.71	0.07
	Fasting insulin (μIU/ml)	11.6 (10.3, 13.1)	12.1 (10.9, 13.5)	0.62	18.1 (16.0, 20.5)	23.2 (21.0, 25.6)	**0.003**
Lipid profile	Total cholesterol (mmol/L)	4.29 ± 0.92	4.11 ± 0.84	**0.033**	4.25 ± 0.88	4.30 ± 0.94	0.53
	Triglycerides (mmol/L)	1.51 ± 1.67	1.28 ± 0.73	0.07	1.47 ± 0.76	1.52 ± 0.77	0.45
	LDL (mmol/L)	2.43 ± 0.71	2.55 ± 0.58	**0.028**	2.48 ± 0.70	2.74 ± 0.67	**<0.0001**
	HDL (mmol/L)	1.22 ± 0.31	1.28 ± 0.28	**<0.0001**	1.11 ± 0.25	1.17 ± 0.24	**0.004**
	Total cholesterol/HDL	3.74 ± 1.27	3.35 ± 0.91	**<0.0001**	3.97 ± 1.08	3.77 ± 0.97	**0.033**
Inflammatory marker	Uric acid (μmol/ml)	3.88 ± 0.89	3.92 ± 0.90	0.65	4.42 ± 0.84	4.54 ± 1.01	0.34

For Matsuda index, fasting insulin, and triglycerides the back-transformed data are reported as means and respective 95% CIs; all other data are means ± SDs. Statistically significant p-values (<0.05) are shown in bold.

BMI, body mass index; HbA1c, glycated haemoglobin; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; SDS, SD score.

study is simply a reflection of the greater prevalence of obesity in boys in the general population: in 2013, the prevalence of obesity in children and adolescents in China was 6.9% among boys and 2.8% among girls (50).

The main limitation of our study was the lack of complete data on pubertal development, which was only recorded on approximately 80% of our study population; thus, our study cohort was stratified using a relatively arbitrary age threshold, as used for the classification of the metabolic syndrome (22). While there was no selection bias by study investigators since all patients were self-reported (by their parents), the BMI SDS 10th percentile among our patients was 2.27, illustrating that our study population was skewed towards the upper end of the BMI SDS spectrum, consisting primarily of individuals with severe obesity. This is not surprising, as we have shown that Chinese caregivers are more likely to identify problems with excess weight in children with severe obesity (49). Nonetheless, this means that our observed incidence of obesity-related comorbidities cannot be readily extrapolated to children and adolescents with more moderate levels of obesity. Another limitation of our study was a potential regional bias; our patients were assessed in east China, and the prevalence of obesity-related comorbidities may differ in other regions of the country. Lastly, this was a cross-sectional study, as longitudinal data were not available; since comparisons between the two study periods were made between two different groups of patients, we cannot ascertain the progression of obesity-related comorbidities in individual patients over time. Nevertheless, our study is particularly valuable due to the comprehensive range of clinical assessments performed (e.g., blood pressure, OGTT, and liver ultrasound) and the large number of children and adolescents assessed.

In conclusion, our study shows a high incidence of obesity-related cardiometabolic comorbidities among Chinese children and adolescents with severe obesity over 10 years, including hypertension, NAFLD, and abnormalities in glucose metabolism. It was particularly concerning that rates of several comorbidities rose markedly over the study period, highlighting the need to address the obesity epidemic early in life in China and elsewhere to prevent the development of obesity-related comorbidities and, subsequently, of overt disease.

DATA AVAILABILITY STATEMENT

The data presented in this article are not readily available because of the conditions of the ethics approval. The anonymized data on which this article was based could be made available to other investigators upon bona fide request, and following all the necessary approvals (including ethics) of the detailed study proposal and statistical analyses plan. Requests to access the dataset should be directed to Prof. Junfen Fu, fjf68@zju.edu.cn.

ETHICS STATEMENT

This study was approved by the Medical Ethics Committee of the Children's Hospital of Zhejiang University School of Medicine (No. 2020-IRB-098). Written informed consent was obtained from parents (or caregivers) and verbal or written consent from each child as appropriate to their age. This study was performed following all applicable institutional and international guidelines and regulations for medical research, in line with the principles of the Declaration of Helsinki (51).

AUTHOR CONTRIBUTIONS

JF was responsible for funding acquisition. JF, JD, JW, HL, BJ, and JY contributed to the study design. JW, HL, JY, KH, WW, and GD carried out the clinical assessments. JW, HL, JY, BJ, and

JD were responsible for data curation and analyses, with results critically reviewed by JF, VC, KH, WW, and GD. JW, VC, and JD wrote the manuscript with critical input from all other authors. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Lower Circulating Leptin Levels Are Related to Non-Alcoholic Fatty Liver Disease in Children With Obesity

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Background: While for individuals with obesity an association between hyperleptinemia and an increased risk of non-alcoholic fatty liver disease (NAFLD) is assumed, a leptin deficiency is also related to the development of NAFLD early in life in *ob/ob* mice, in patients with leptin deficiency due to biallelic likely pathogenic variants in the leptin gene, and in patients with lipodystrophy.

Objectives: To investigate the association of circulating leptin levels in pre-pubertal children with obesity and steatosis hepatis.

Methods: The cross-sectional study consisted data of n=97 (n_{male}=76) pre-pubertal children (11.8 ± 1.5 years) with obesity (BMIz: 2.4 ± 0.4). Fasting concentrations of cardiometabolic parameters were measured: insulin, c-peptide, glucose, triglyceride, cholesterol, HDL, LDL, AST, ALT, GGT, leptin. Steatosis hepatis was diagnosed by an ultrasound examination (mild, moderate or severe). Patients were categorized into two groups: low z-score of circulating leptin levels (≤25th percentile) vs. normal z-score of circulating leptin levels.

Results: One-third of the children with obesity were diagnosed with steatosis hepatis (I°: 63.6%, II°/III°: 36.4%). Children with steatosis hepatis had significantly lower z-scores of circulating leptin levels compared to children with an unremarkable liver ultrasonography (-2.1 ± 0.8 vs. -0.7 ± 0.6). Z-scores of circulating leptin levels correlate negatively with degree of steatosis hepatis. Children with low z-scores of circulating leptin levels had significantly higher triglyceride, fasting insulin and c-peptide levels compared to children with normal z-scores of circulating leptin levels.

Conclusion: Prepubertal children with NAFLD and obesity and partial leptin deficiency might be defined as a clinical subgroup.

Keywords: obesity, children, NAFLD, leptin, partial leptin deficiency

INTRODUCTION

Children with obesity are at risk for numerous health problems, including nonalcoholic fatty liver disease (NAFLD). The estimated prevalence of NAFLD is 3–10% of children worldwide, and ranged between 26.4% and 34.2% in children with obesity in epidemiological studies (1–3). Studies have demonstrated that there is a high risk for a progression of pediatric NAFLD to end-stage liver disease in adulthood. This emphasizes the importance of research in effective prevention and intervention strategies. As no pharmacological agents for the treatment of pediatric NAFLD exist, the cornerstone therapeutic strategy is lifestyle intervention. A recently published review demonstrated, that the positive effects of a lifestyle intervention on liver-associated parameters are weak and are strongly associated with body weight loss (4). Since lifestyle interventions are largely unsuccessful in achieving and maintaining clinically meaningful weight loss (5, 6), the search for new pharmacological interventions in adults and children with NAFLD is on focus. One possible approach would be to further investigate the role of the adipokine leptin in relation to the development of NAFLD (7, 8). Hyperleptinemia is thought to be associated with an increased risk of NAFLD in children, adolescents, and adults with obesity (9). In contrast, there are observations from *ob/ob* mice and from patients with leptin deficiency due to biallelic likely pathogenic variants in the leptin gene, in which liver steatosis has also been described (10, 11). Metreleptin substitution in patients with leptin deficiency results in a reduction of liver fat content prior to any detectable weight reduction (11). The treatment of obesity by Metreleptin was already studied in the 2000s. In several intervention studies in subjects with obesity, high doses of Metreleptin failed to show an effect on body weight loss (12). However, there was a subgroup of patients with low circulating leptin levels at baseline who benefited from the intervention with Metreleptin in terms of greater body weight loss (13). In a recently published open-label therapy intervention study, Akinci et al. investigated, whether Metreleptin administration for 12 months has an impact on the global NASH score in adults with obesity and with relative leptin deficiency (individual leptin level <25th percentile for leptin levels related to age and BMI of a reference group). Akinici et al. described that there was significant weight loss and significant reduction in adults with obesity and with relative leptin deficiency in the global NASH score after Metreleptin administration for 12 months (14). A state of relative leptin deficiency could be defined by low circulating leptin levels relative to BMI. Since there is no uniform, widely accepted definition of "relative leptin deficiency" in patients with obesity, the question arises how to identify patients with relatively low leptin levels. During childhood and adolescence, leptin levels depend primarily on sex and BMI (fat mass) and rise with progression of pubertal development. Blum et al. developed a formula for the calculation of the z-score for circulating leptin levels in childhood and adolescence, in which pubertal stage, sex and BMI are considered (15). In the present study, we hypothesize that there is an association between reduced z-scores of circulating leptin levels and diagnosis of steatosis hepatis in pre-pubertal children with obesity.

METHODS

Study Population

The study population consists data of n=97 children with obesity, who were admitted to an inpatient rehabilitation clinic (Murnau, Germany) to participate in a weight loss program. The clinical examination at admission to the clinic included anthropometric measurements, ascertainment of Tanner stage (16, 17), withdrawal of a fasting blood sample, a liver ultrasonography and a questionnaire about medications. Study sample inclusion criteria were prepubertal age and no intake of medications potentially affecting insulin, glucose, aminotransferase or cholesterol levels.

Written informed consent from parents and written assent from children were obtained. The institutional ethical review board of the university of Ulm approved all study proceedings.

Anthropometrics

Weight and height were measured to the nearest 0.1 kg and 0.1 cm. Individual BMI value was calculated (weight divided by the square of height; kg/m^2). Individual BMI values were converted into standard deviation scores (BMI-SDS) using the LMS method (18).

Ultrasound Examinations

An ultrasound examination was performed by a single examiner experienced in sonography (4000 ultrasound examinations per year and accredited instructor of the German Society for Ultrasound in Medicine (DEGUM)). The examinations were performed with the ultrasound device Versa Plus from Siemens, Erlangen, Germany. For imaging of liver, gallbladder, and aorta, a 3.5 MHz convex transducer was used. The ultrasound gel was the product "Ultrasound contact gel" from the company Wasserfuhr (Caesar and Loretz, Hilden, Germany). In order to create the best possible conditions for sonographic assessment of the upper abdomen, patients were called in after a fasting period of at least five hours. Fasting state was defined as: no food, no drinks, no chewing gum, and no cigarettes. The diagnosis of steatosis hepatis was made by one examiner based on a comparison of the hepatic and renal parenchyma, taking into consideration the dorsal attenuation of the diaphragm and ability to assess the liver vessels. According to established diagnostic criteria (19, 20), the degree of fatty infiltration of the liver was classified as "mild" (I°): liver parenchyma more echoic, no dorsal sound reduction; "moderate" (II°): additional dorsal sound reduction, diaphragm still presentable; "severe" (III°): dorsal sound reduction, diaphragm can no longer be visualized. Due to the small number of cases in group III° (severe), these subjects were combined with those in group II° (moderate or severe) for the statistical analysis.

Biochemical Analyses- Fasting Blood Sample

Fasting blood samples were obtained after an overnight fast. Plasma glucose was measured with the GOD-PAP, serum cholesterol concentrations were measured with the CHOD-PAP, triglyceride with the GPO-PAP method on a LP 700

system (Dr. Lange, GmbH, Berlin, Germany). HDL concentrations were measured by the method precipitation with dextran sulfate/magnesium chloride. LDL concentrations were measured by precipitation of LDL with polyanions and calculation of LDL cholesterol from total cholesterol and cholesterol in the precipitation supernatant. Fasting plasma insulin and C-peptide concentrations were measured using immunoassays (Insulin RIA 100; Pharmacia & Upjohn, Kalamazoo, MI; and C-PEPTID EIA-1293, DRG, Instruments, Marburg, Germany). Fasting AST Aspartat-Aminotransferase (AST), Alanin-Aminotransferase (ALT) and Glutamat-Pyruvat-Transaminase (GGT) (U/l) were measured using the Dimension RxL system (Dade Behring, Eschborn, Germany) applying standard methods (DuPont, Billerica, MA). Adiponectin was measured using an enzyme immunoassay (Quantikine1; R&D Systems, Minneapolis, MN) with an intraassay coefficient of variation of 7.2%). Leptin levels were quantified using enzyme-linked immunosorbent assay (Mediagnost, Reutlingen, Germany). To investigate the research question, we calculated the sex, Tanner stage and BMI dependent z-score for circulating leptin levels using the formula published by Blum et al. (15). We used the 25th internal percentile of the z-score of circulating leptin levels to define two groups of children: (A) children with low z-scores of circulating leptin levels: z-score for circulating leptin levels (≤25th internal percentile), (B) children with normal z-scores of circulating leptin levels (>25th internal percentile).

Statistical Analyses

Data are presented as means, standard error of the mean (SE), median and interquartile range (IQR) for continuous variates and as percentages for categorical variates. Box plots are composed of five horizontal lines that display the10th, 25th, 50th, 75th and 90th percentiles of available. To test for group differences the Student´s t-test was performed. Kruskal–Wallis tests and analyses of covariance were used to compare means of z-scores of circulating leptin levels across patient groups with no, mild, moderate or severe liver steatosis. All tests were performed with the Statistical Analyses System version 9.4 (Statistical Analyses System Institute Inc., Cary, North Carolina, USA). Statistical significance was inferred at two-tailed $p<0.05$.

RESULTS

Prevalence of NAFLD

The basic characteristics of the cohort of pre-pubertal children with obesity in total and separated for males and females are shown in **Table 1**. The majority of children in the cohort were male (male: 78.4 vs. female: 21.7%). The BMI z-score was comparable between males and females. One-third of the pre-pubertal children with obesity were diagnosed with hepatic steatosis by ultrasound. The majority of the pre-pubertal children with obesity were affected by mild fatty infiltration of the liver (steatosis hepatis I°: 63.6%) and 36.4% were affected by moderate or severe liver steatosis (steatosis hepatis II° or III°). The percentage of pre-pubertal children with obesity and with steatosis hepatis was higher in males than in females (male: 38.2 vs. female: 19.1%).

Pre-Pubertal Children With Obesity and With NAFLD Had Lower z-Scores of Circulating Leptin Levels Than Those Without NAFLD

Children with steatosis hepatis had significantly lower z-scores of circulating leptin levels compared to children with an unremarkable liver ultrasonography finding (**Figure 1A**). The z-scores of circulating leptin levels declined with increasing severity of steatosis hepatis (**Figure 1B**).

Higher Proportion of Children With NAFLD in the Group of Children With Low z-Scores of Circulating Leptin Levels Than in the Control Group

The percentage of children affected by steatosis hepatis was higher in the group of children with low z-scores of circulating leptin levels than in children with normal z-scores of circulating leptin levels (46.2% vs. 29.6%) (**Figure 2A**). Among the children with steatosis hepatis, those children with low z-scores of circulating leptin levels had more often a moderate or even severe degree of steatosis hepatis compared to children with normal z-scores of circulating leptin levels (58.3% vs. 23.8%) (**Figure 2B**).

Children With Low z-Score of Circulating Leptin Levels Had Higher Triglyceride, Fasting Insulin and c-Peptide Levels Compared to Children of the Control Group

Pre-pubertal children with obesity and with low z-scores of circulating leptin levels show significantly higher liver enzyme levels of AST (17.3 ± 6.8 vs. 14.7 ± 5.5 U/l, $p<0.05$) and ALT (18.4 ± 9.1 vs. 14.2 ± 8.1 U/l, $p<0.05$), higher fasting insulin (16.8 ± 13.4 vs. 13.9 ± 10.0 mU/l, $p<0.05$) and c-peptide levels (2.0 ± 1.1 vs. 1.6 ± 0.7 µg/l, $p<0.05$), higher triglyceride (114.9 ± 76.7 vs. 98.8 ± 58.3, $p<0.05$), higher total cholesterol and lower HDL cholesterol levels (44.7 ± 8.0 vs. 48.8 ± 9.4 mg/dl, $p<0.05$) compared to children with normal z-scores for circulating leptin levels (**Table 2**). Levels of adiponectin, fasting glucose and LDL cholesterol were not significantly different between the two groups of children. BMI z-scores (2.8 ± 0.2 vs. 2.2 ± 0.4, $p<0.05$) were higher in the group of children with low z-scores of circulating leptin levels than in children with normal z-scores for circulating leptin levels.

DISCUSSION

The aim of this work was to investigate the relationship between z-scores of circulating leptin levels and the prevalence and the degree of steatosis hepatis in a well-characterized cohort of pre-pubertal children with obesity. We observed that pre-pubertal children with obesity and with steatosis hepatis have significantly

TABLE 1 | Description of the anthropometric and metabolic parameters (adipokine concentration, concentration of liver enzymes, insulin and lipid metabolism), as well as of the distribution of pre-pubertal children with obesity and with steatosis hepatis in the whole cohort (n=97), as well as separated for males (n=76; 78.4% of cohort) and females (n=21; 21.6% of cohort).

	Whole cohort			Male			Female		
	mean±STD	median	IQR	mean±STD	median	IQR	mean±STD	median	IQR
Age [years]	11.8±1.5	11.8	10.7–12.8	12.0±1.3	12.1	11.0–12.9	11.0±1.8	10.8	9.8–12.0
Anthropometrics									
BMI SDS	2.4±0.4	2.4	2.1–2.7	2.4±0.4	2.4	2.1–2.7	2.4±0.5	2.4	2.2–2.7
BMI [kg/m^2]	29.4±4.0	29.0	26.8–32.0	29.7±3.9	29.1	27.0–32.2	28.2±3.9	27.8	26.1–29.7
Adipokines									
Adiponectin [µg/ml]	6.4±2.7	5.8	4.4–8.2	6.3±2.7	5.7	4.4–7.8	6.9±2.9	5.9	4.4–9.4
Leptin [ng/ml]	27.1±13.8	23.7	17.5–32.9	27.7±13.0	24.9	18.6–33.1	25.1±16.4	20.9	14.7–27.7
Z-score circulating leptin level	-1.2±1.6	-1.2	-2.2–-0.1	-1.1±1.6	-1.1	-2.2–0.0	-1.7±1.4	-1.8	-2.8–-0.8
Liver enzymes									
GGT [U/l]	14.8±11.0	12.7	10.8–15.1	15.7±12.2	12.9	11.3–16.7	11.6±3.7	10.9	8.8–13.7
AST [U/l]	15.4±5.9	14.3	12.2–16.1	15.8±6.5	14.1	12.1–17.5	13.9±2.6	14.6	12.8–15.7
ALT [U/l]	15.3±8.6	12.6	10.7–17.2	15.8±9.2	12.3	10.8–17.3	13.6±5.5	13.0	10.4–14.6
Insulin metabolism									
Fasting glucose [mg/dl]	83.4±8.5	82.4	78.4–88.0	84.4±7.3	83.4	79.7–88.1	79.7±11.3	79.7	73.0–83.8
Fasting insulin [mU/l]	14.7±11.0	11.4	8.8–16.1	15.7±11.8	11.9	9.2–17.4	11.0±7.0	9.1	5.4–14.4
Fasting c-peptide [µg/l]	1.7±0.9	1.5	1.1–1.9	1.8±0.9	1.5	1.2–1.9	1.4±0.7	1.2	0.8–1.8
Lipid metabolism									
HDL [mg/dl]	47.7±9.2	46.7	41.5–52.3	48.3±9.5	47.0	41.3–54.5	45.5±7.6	45.2	41.5–47.6
LDL [mg/dl]	129.0±33.9	127.0	100.0–153.0	130.3±34.6	128.0	100.0–154.5	124.4±31.3	123.0	100.0–141.0
TG [mg/dl]	103.1±63.8	85.7	63.0–120.0	108.5±67.7	91.5	66.6–123.5	83.5±42.8	74.4	61.9–85.7
Cholesterol [mg/dl]	198.3±33.9	197.0	176.6–220.6	200.8±34.0	198.5	177.5–225.0	189.4±32.7	186.1	171.0–206.0
Steatosis hepatis [% (n)]									
No	66.0 (64)			61.8 (47)			81.0 (17)		
Yes	34.0 (33)			38.2 (29)			19.0 (4)		
Steatosis hepatis [% (n)]									
I° (mild)	63.6 (21)			58.6 (17)			100 (4)		
II° or III° (moderate or severe)	36.4 (12)			41.4 (12)			0 (0)		

Parameter values are presented as means±standard deviation (STD), medians and interquartile ranges (IQR).

lower z-scores of circulating leptin levels than children with an unremarkable liver sonographic finding, and z-scores of circulating leptin levels correlate negatively with degree of steatosis hepatis. Furthermore, the percentage of children with all degrees of steatosis hepatis was higher in the group of children with low z-scores of circulating leptin levels compared to children with normal z-scores of circulating leptin levels.

Pre-Pubertal Children With Obesity and With NAFLD Have Low z-Scores of Circulating Leptin Levels

We have shown that pre-pubertal children with obesity and with NAFLD have significantly lower z-scores of circulating leptin levels than children without NAFLD. Few studies have examined the association between circulating leptin levels and steatosis hepatis in children and adolescents with obesity. In the clinical trial "Exercise Training and Hepatic Metabolism in Overweight/Obese Adolescents (HEPAFIT) with steatosis hepatis" (n=122 adolescents (11-17 years)) leptin levels and CAP (indicator of fat deposition in the liver) as well as HOMA-IR and CAP were positively associated, even after adjustment for age, sex and body fat (21). In another study higher circulating leptin levels in n=72 children and adolescents (9-18 years) correlated with more severe degree of steatosis hepatis, ballooning and NAFLD activity score (NAS) (9). In a further analysis of this cohort, the patients were divided into two groups according to their NAS score: ≥5 vs. ≤4. The group of children and adolescents with a NAS score ≥5 showed significantly higher circulating leptin, TNF-alpha, triglyceride, and γGT levels and higher BMI z-scores than the group of children and adolescents with a NAS score ≤4 (22). Taken together, these studies suggest a positive association between circulating leptin levels and NAFLD and also with degree of NAFLD in children and adolescents with obesity. We would like to point out that all of these studies have a limitation, since the circulating leptin levels were analyzed without the consideration of the factors that influence circulating leptin levels during childhood and adolescents: Tanner stage, sex and BMI. In contrast to the studies described above, we calculated z-scores for circulating leptin levels according to references published by Blum et al. (15) and we showed that pre-pubertal children with obesity and with NAFLD had lower z-scores of circulating leptin levels than children without NAFLD.

We used the 25th internal percentile of z-scores of circulating leptin levels as cut-off to identify pre-pubertal children with obesity and with low z-scores of circulating leptin levels. A similar approach was used by Akinci et al, who defined relative leptin deficiency in adults as circulating leptin levels below the 25th percentile of a BMI and sex-matched American population (14). Akinci et al. hypothesized that there are adults with obesity and with relative leptin deficiency and NAFLD in whom

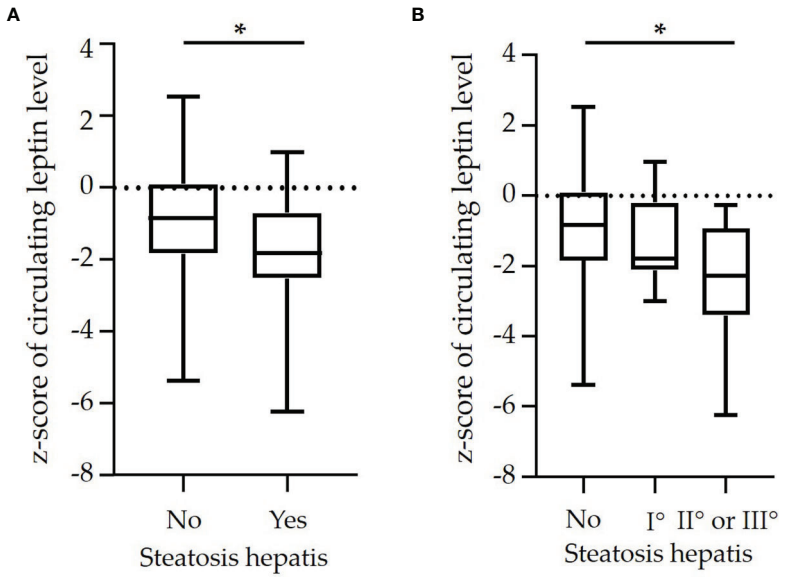

FIGURE 1 | Box plots of z-scores of circulating leptin levels in dependence on **(A)** diagnosis of steatosis hepatis [yes vs. no (n=64 vs. n=33)] and in dependence on **(B)** degree of steatosis hepatis [no steatosis hepatis (n=64] vs. mild (I°: n=21) vs. moderate or severe [II° or III°: n=12)] in a cohort of n=97 pre-pubertal children with obesity. (*p<0.05).

treatment with exogenous Metreleptin administration may improve liver parameters. The assumption, that there are individuals among the population with common obesity who have low circulating leptin levels and the opportunity for leptin to act when its level raised from low (below physiological level) to normal, are strengthened by two studies in adults with obesity treated with Metreleptin (12, 13). While the majority of patients with obesity did not benefit from this treatment, patients with reduced basal leptin levels showed an improved reduction in fat mass (12) or body weight under treatment with Metreleptin (13).

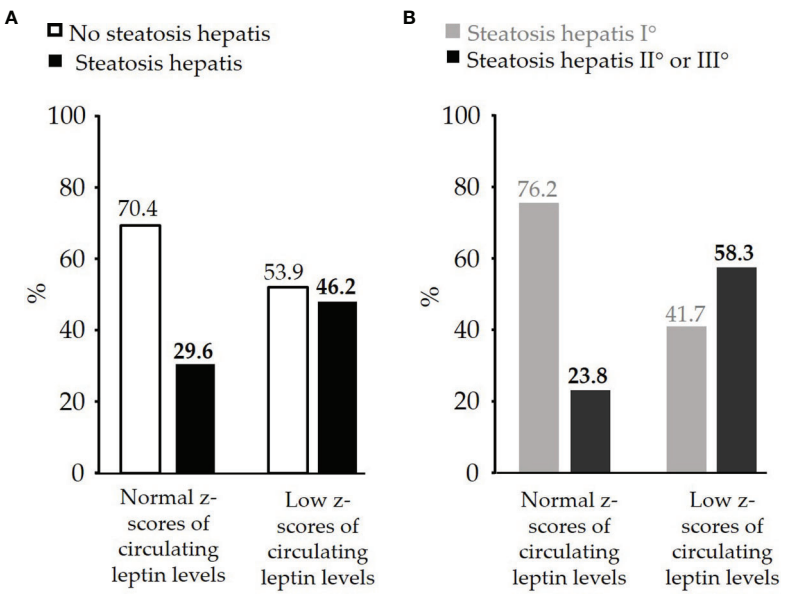

FIGURE 2 | **(A)** Comparison of percentage of pre-pubertal children with obesity and with or without diagnosed steatosis hepatis between the group of children with normal z-scores of circulating leptin levels (n=71) and the group of children with low z-scores of circulating leptin levels (n=26), **(B)** Comparison of percentage of pre-pubertal children with obesity and with steatosis hepatis I° (mild: n=21) or steatosis hepatis II° or III° (moderate or severe: n=12) between the group of children with normal z-scores of circulating leptin levels and the group of children with low z-scores of circulating leptin levels.

TABLE 2 | Comparison of anthropometric and metabolic parameters (adipokine, liver enzymes, insulin and lipid metabolism) as well as of the percentage of pre-pubertal children with obesity and with diagnosed steatosis hepatis between the group of children with normal z-scores of circulating leptin levels (n=71) and the group of children with low z-scores of circulating leptin levels (n=26).

	Normal z-scores of circulating leptin levels [n=71]			Low z-scores of circulating leptin levels [n=26]			
	mean±STD	median	IQR	mean±STD	median	IQR	p-value
Age [years]	11.6±1.5	11.6	10.7–12.7	12.2±1.4	12.6	10.9–13.1	>0.05
Anthropometrics							
BMI z-score	2.2±0.4	2.2	2.0–2.5	2.8±0.2	2.8	2.7–3.0	<0.05
BMI [kg/m^2]	27.6±2.7	27.9	25.8–29.5	34.1±3.0	34.3	32.1–35.7	<0.05
Adipokine							
Adiponectin [µg/ml]	6.6±2.9	6.4	4.4–9.0	5.8±2.1	5.5	4.1–7.3	>0.05
Leptin [ng/ml]	24.2±11.3	22.0	16.3–30.3	35.1±16.7	31.9	21.5–44.8	<0.05
Z-score circulating leptin level	-0.5±1.1	-0.7	-1.5–0.1	-3.2±1.0	-2.9	-3.6–-2.5	<0.05
Liver enzymes							
GGT [U/l]	15.1±12.6	12.7	10.9–14.7	14.0±5.1	12.5	10.6–17.1	>0.05
AST [U/l]	14.7±5.5	13.9	11.9–16.1	17.3±6.8	14.7	14.0–18.7	<0.05
ALT [U/l]	14.2±8.1	12.0	9.6–16.6	18.4±9.1	15.3	12.1–20.4	<0.05
Insulin metabolism							
Fasting glucose [mg/dl]	84.7±8.4	83.6	79.8–88.2	79.9±7.7	79.4	76.1–85.0	>0.05
Fasting insulin [mU/l]	13.9±10.0	11.3	8.9–15.6	16.8±13.4	13.4	8.6–20.4	<**0.05**
Fasting c-peptide [µg/l]	1.6±0.7	1.4	1.1–1.8	2.0±1.1	1.6	1.3–2.5	<0.05
Lipid metabolism							
HDL [mg/dl]	48.8±9.4	47.6	41.5–52.9	44.7±8.0	44.7	38.1–47.3	<0.05
LDL [mg/dl]	131.6±30.9	131.0	107.0–154.0	121.8±40.6	115.5	89.0–152.0	>0.05
TG [mg/dl]	98.8±58.3	85.7	61.9–119.0	114.9±76.7	82.7	65.4–124.0	<0.05
Cholesterol [mg/dl]	202.2±29.6	198.0	185.7–220.6	187.8±42.5	181.0	150.0–223.0	<0.05
Steatosis hepatis [% (n)]							
No	70.4 (50)			53.8 (14)			<0.05
Yes	29.6 (21)			46.2 (12)			
Steatosis hepatis [% (n)]							
No	70.4 (50)			53.8 (14)			
I° (mild)	22.2 (16)			19.2 (5)			
II° or III° (moderate or severe)	7.4 (5)			27.0 (7)			<0.05

Parameter values are presented as means±standard deviation (STD), medians and interquartile ranges (IQR).

Role of the Adipokine Leptin in the Development of the NAFLD

We observed that pre-pubertal children with obesity and with low z-scores of circulating leptin levels had significantly higher triglyceride, fasting insulin and fasting c-peptide levels compared to children with normal z-scores of circulating leptin levels. This may at least partially be explained by relative leptin deficiency. We assume that these pre-pubertal children with obesity have too low circulating leptin levels related to their BMI. The physiological role of leptin and the anti-steatotic effect of leptin cannot be fully mediated. Our hypothesis is supported by observations from animal and clinical studies. In leptin-deficient *ob/ob* mice as well as in patients with congenital leptin deficiency, a striking metabolic phenotype including severe degree of steatosis hepatis has been described. Metreleptin substitution in humans with congenital leptin deficiency reverses steatosis hepatis as shown by our group recently (11). Furthermore, a systematic literature review provided evidence for a higher frequency of metabolic abnormalities in LEP *wt/-* than in *wt/wt* subjects, including hypercholesterinemia, hyperinsulinemia, and hypertriglyceridemia. Animal studies demonstrated lower leptin levels in LEP *wt/-* compared to *wt/wt* animals, especially in relation to fat mass (23). Furthermore, in humans with lipodystrophies, a group of disorders that are characterized by a selective deficiency of subcutaneous adipose tissue and low circulating leptin levels, severe forms of NAFLD have been described together with other metabolic complications including insulin resistance and high triglyceride levels. Leptin replacement therapy in lipodystrophic patients leads to an improvement in ectopic lipid disposition and in fatty liver disease (24). Also, in normal weight adults, 5-8% are diagnosed with NAFLD (lean NAFLD) (25). It has been shown that normal weight adults (Caucasian) with NAFLD (lean NAFLD) had significantly lower leptin levels than obese adults with NAFLD, but circulating leptin levels did not differ between lean healthy and lean NAFLD adults (26). But it should be considered that in this study the circulating leptin levels in lean NAFLD and healthy adults were compared without considering the age, BMI, and gender specific effect on circulating leptin levels. The question remains open whether lean NAFLD adults have lower z-scores for circulating leptin levels compared to healthy lean adults.

Metreleptin – a Treatment Option for NAFLD in Children With Obesity?

As no pharmacological treatment for NAFLD in childhood and adolescence is currently available, the cornerstone of therapeutic strategies for pediatric NAFLD remains lifestyle intervention. A review published in 2020 summed up the results of n=10 RCTs assessing the efficiency of dietary and lifestyle interventions on several

NAFLD-related parameters in children and adolescents with imaging or biopsy-proven NAFLD. All interventions described an improvement in liver outcomes in conjunction with weight loss (4). It is known that it is very difficult to achieve clinically relevant and lasting body weight reduction in children and adolescents with obesity or extreme obesity by lifestyle intervention (5, 6). Since the effective treatment of NAFLD by lifestyle modification has no sustainable effect, the search for pharmacological agents is of high relevance. One approach in adults was to study the treatment of NAFLD with Metreleptin. In adult patients with lipodystrophy and NAFLD it has been shown, that treatment with Metreleptin resulted in a significantly reduced liver volume and steatosis (24). In adults with obesity and with relative leptin deficiency, Akinci et al. showed within an open-label therapy intervention study, that the global NASH score was significantly reduced under Metreleptin administration for 12 months (12). We hypothesize that Metreleptin might be a treatment option in children with obesity, NAFLD and with low z-scores of circulating leptin levels with the aim to improve fatty infiltration and hepatic inflammation.

Limitations

We calculated z-scores for circulating leptin levels, which consider the dependency of circulating leptin levels on sex, Tanner stage and BMI during childhood and adolescence. The reference values were based on a cohort of children and adolescents older than 6 years of age who were primarily normal weight (15). Although the extreme ranges for upper (overweight/obesity) and lower BMI values (underweight) are not present in this reference cohort, we assume that these references can be used to detect discrepancies between given BMI value and circulating leptin levels. Within the presented study, liver ultrasound examination was conducted in pre-pubertal children with obesity at one time point. Liver ultrasound measurement of these patients at the end of lifestyle intervention would be necessary to study if pre-pubertal children with obesity and with low z-scores of circulating leptin levels at baseline showed a stronger improvement in parameters of liver metabolism and liver steatosis under lifestyle intervention than children with normal z-scores of circulating leptin levels.

Conclusion

In conclusion, we showed that pre-pubertal children with obesity and with NAFLD had lower z-scores of circulating leptin levels than children without NAFLD. Furthermore, children with low z-scores of circulating leptin levels had more often a severe degree of steatosis hepatis than children with normal z-scores of circulating leptin levels. In our study cohort, pre-pubertal children with obesity and with low z-scores of circulating leptin levels are characterized by an altered metabolic profile including higher triglyceride, insulin and c-peptide concentrations compared to children with normal z-scores of circulating leptin levels. We hypothesize that the group of pre-pubertal children with obesity and with low z-scores of circulating leptin levels could possibly benefit from treatment with Metreleptin in terms of an improvement in parameters of liver metabolism and potentially also weight loss. Further research is needed, to better characterize the phenotype of relative leptin deficiency in the context of obesity in childhood, clarify the underlying pathophysiology, and understand potential implications for designing targeted therapeutic interventions. One crucial step in this direction will be the development of updated and extended reference values for circulating leptin levels during childhood and adolescence including cohorts of children and adolescents with obesity and with extreme obesity.

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.

ETHICS STATEMENT

The institutional ethical review board of the University of Ulm approved all study proceedings. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

SB and MW researched data and wrote the manuscript. WK collected patient's data, reviewed and edited the manuscript. CD and JvS reviewed and edited the manuscript. All authors had final approval of the submitted and published versions.

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Associations Between Body Composition, Leptin, and Vitamin D Varied by the Body Fat Percentage in Adolescents

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Background: Serum leptin levels reflects one's degree of obesity and can affect vitamin D levels. The relationship between body fat, leptin, and 25-hydroxyvitamin D (25(OH)D) has not been extensively studied in adolescents. This study aimed to investigate the correlations between body composition and leptin and 25(OH)D levels in boys and girls.

Methods: Participants aged 12–14 years (n = 205) were grouped according to sex. After body composition was recorded using bioelectrical impedance analysis, they were classified into three groups according to body fat percentage (%BF) (< 30, ≥ 30 and < 40, and ≥ 40). Serum leptin and 25(OH)D levels were measured using the enzyme-linked immunosorbent assay (ELISA). Correlations between all variables were analyzed according to sex and the percentage of BF groups.

Results: Boys and girls with %BF ≥ 30 showed no difference in body mass index (BMI), % BF, and leptin and 25(OH)D, while other variables of body composition were more common in boys than in girls. The %BF, body fat mass (BFM), and 25(OH)D of both sexes with %BF ≥ 30, and leptin levels of boys with %BF ≥ 40 increased with an increase in %BF. A negative correlation between leptin and 25(OH)D levels was found in boys with %BF < 40 and girls with %BF < 30. In the %BF ≥ 30 and < 40 groups, there were negative correlations between leptin, BFM, free fat mass, and muscle mass (MM); between leptin, 25(OH)D, and height in boys; and between 25(OH)D, body weight, BMI, and MM in girls.

Conclusion: A negative correlation between leptin and 25(OH)D levels varied according to sex, while for body composition, it was evident at 30 and 40% BF.

Keywords: body fat percentage (BF%), leptin, 25-hydroxyvitamin D, adolescents, body composition

INTRODUCTION

Obesity is defined as an excessive proportion of body fat relative to lean body mass due to a chronic imbalance between energy intake and expenditure. Leptin resistance, or its inability to modulate energy intake and expenditure is common in obesity. Leptin is an adipokine secreted primarily by adipocytes, and it inhibits energy intake and regulates energy homeostasis by acting *via* hypothalamic receptors (1, 2). Research shows that leptin is a marker of obesity and reflects the degree of adiposity. Circulating leptin concentrations are determined by body fat mass (BFM), body mass index (BMI), and sex. In fact, leptin concentration is higher in females than in males regardless of BFM (1, 3, 4). In addition, leptin levels change significantly during progressive pubertal stages, with girls having higher serum leptin levels than boys which rise throughout puberty, concomitant with an increase in estrogen levels (4). Furthermore, serum leptin concentrations are higher in early adolescence than in childhood and may play a role in pubertal development (4). Body fat percentage (%BF), basal metabolic rate, muscle mass (MM), bone mass, and serum 25-hydroxyvitamin D (25(OH)D) had an impact on serum leptin (5).

In obesity, there is not only an imbalance of adipokines, but also a decrease in vitamin D bioavailability (6, 7). Adipose tissue is a target for vitamin D and the main storage depot for vitamin D and its metabolites (8, 9). Leptin exerts an autocrine–paracrine lipolytic effect on adipocytes by interacting with the vitamin D receptor, and it inhibits the enzyme that converts 25(OH)D to 1,25 dihydroxyvitamin D (10). Thus, vitamin D depletion might increase appetite and lead to obesity by directly regulating leptin expression (6, 11). Previous studies reported that there was a negative association between %BF and vitamin D and a positive correlation between %BF and leptin that confirmed excess of % BF, leading to decreased vitamin D and raised leptin (12). The distribution of fat in adolescents with BMI of 36 ± 5 and %BF of 40 ± 5 might be associated with vitamin D status with decreased 25(OH)D (13). A recent study in young adults aged 20–21 years found that males and females demonstrated positive relationships between serum leptin and BMI, waist circumference, and %BF; however, males showed inverse correlations between serum leptin, MM, and 25(OH)D (5). In adults, the relationship between total body fat and 25(OH)D levels also varied by sex (14). In addition, the relationship between serum 25(OH)D and leptin is largely explained by the presence of adiposity or the amount of body fat, which disappeared after adjustment for total body fat and waist circumference (15).

Although the inverse relationship between leptin and 25(OH) D has been reported with respect to BMI and %BF, this association has not been fully explored in young adolescents. Moreover, there was a report in Thai school children aged 6–14 years that dietary calcium intake was low, vitamin D status was sufficient, and girls experienced a decline in 25(OH)D levels with increasing age (16). Thus, this study investigated the correlations between body composition and leptin and 25(OH)D levels in boys and girls stratified degree of obesity by %BF.

Abbreviations: BFM, body fat mass; BMI, body fat index; %BF, body fat percentage; MM, muscle mass; 25(OH)D, 25-hydroxyvitamin D; BW, body weight; FFM, free fat mass; ELISA, Enzyme-Linked Immunosorbent Assay.

MATERIALS AND METHODS

Study Participants

Students with ages 12–14 years and BMI-for-age > 50th percentile (17) were selected from high schools in southern Thailand. Those with chronic diseases, other conditions such as asthma, allergies, or gastritis, and those using steroids were excluded. Participants and their parents received the information regarding the purposes and methods of the study, and they were required to sign the informed consent. Participants (n = 205) were grouped by sexes (107 boys and 98 girls), and after body composition measurement, they were classified by %BF into three groups, group 1, %BF < 30 (28 boys and 12 girls); group 2, %BF ≥ 30 and < 40 (38 boys and 43 girls); group 3, %BF ≥ 40 (41 boys and 43 girls), according to the %BF cutoff values at 95th percentile for age, 30% (18, 19) and 40% (20). Participants were asked about their lifestyle habits, and they did not exercise regularly; during the day they spent most of their time sitting in class and ate three meals a day with snacks and no calcium and vitamin D supplementation (21).

This study was reviewed and approved by the Human Research Ethics Committee of Walailak University, Thailand (Approval Number: WUEC-19-102-21). A consent form was obtained from all participants or their legal representative before enrollment.

Anthropometric and Body Composition Measurements

Body composition, including body weight (BW, kg), BMI (kg/m^2), %BF, BFM (kg), free fat mass (FFM, kg), and MM (kg), were analyzed by bioelectrical impedance analysis (22, 23) using a TANITA SC-330ST series body composition analyzer (Tanita Corporation, Tokyo, Japan). To increase measurement accuracy, participants wore light clothes and no shoes. Since the level of hydration, the presence of edema, and the daily weight variability affected the total body weight, 0.5 kg was subtracted from the obtained weight values (23). Standing heights (m) were measured without shoes using a locally made stadiometer and were recorded to the nearest 0.1 cm (21).

Measurements of Leptin and 25-Hydroxyvitamin D by Enzyme-Linked Immunosorbent Assay (ELISA)

After an overnight fast, participants were collected venous blood samples in clotted blood tubes. The blood samples were centrifuged at 2000 revolutions per minute for 10 minutes, and the serum was harvested into 1.5 mL microcentrifuge tubes and stored at -70°C until used to measure leptin and 25(OH)D.

Leptin and 25(OH)D levels were determined by an immunometric sandwich ELISA using commercial ELISA kits leptin (R&D Systems, Inc., Minneapolis, MN, USA, sensitivity to

7.8 pg/mL and intra-assay coefficient of variability < 10%), and 25 (OH)D (DRG Diagnostics, Frauenbergstrasse, Germany, sensitivity to 2.89 ng/mL and intra-assay coefficient of variability < 10%), per the manufacturer's instructions. Standard or diluted serum samples were prepared and incubated in coated microplates at room temperature. Immunoassays were performed in duplicates. After washing, a mixture of capture and detector antibodies was added to the plate and incubated at room temperature. A 3,3′,5,5′-tetramethylbenzidine (TMB) substrate was added to each well and incubated for 10 minutes to detect the antigen-antibody complex reaction. Finally, a stop solution was added to each well, and the optical density (OD) was measured at 450 nm using a microplate reader.

Statistical Analysis

Descriptive data of all variables were presented as mean ± standard deviation (S.D). Differences in variables of body composition, leptin, and 25(OH)D among sexes and %BF groups were compared by independent-samples T-test. The correlations between those variables were calculated by Pearson's correlation coefficient (r). The P values less than 0.05, 0.01, or 0.001 were considered statistically significant. Data analysis was performed using IBM SPSS statistics version 22.0 software license authorization wizard.

RESULTS

Table 1 shows that for all participants, BMI, %BF, BFM, and 25(OH)D levels were not different between boys and girls, whereas boys had BW, height, FFM, and MM greater than girls, and girls had higher leptin levels than boys. When participants were divided into three groups, similar results were obtained for all groups: the BW, height, and MM of boys were higher than those of girls. In group 1, girls had higher %BF and leptin levels, and lower FFM than boys. In group 2, boys had more BFM and FFM than girls. In group 3, the BFM of boys was greater than that of girls. Although, serum 25(OH)D levels were not different between boys and girls in all groups, they appeared to be slightly less than 20 ng/mL in groups 1 and 2 of girls. Furthermore, **Table 2** shows that BW, BMI, %BF, and BFM of both sexes increased with increasing %BF, but their heights did not differ. Boys and girls in group 3 had higher 25(OH)D levels than those in group 1 and 2. The FFM and MM of group 1 were greater than those of groups 2 and 3 in girls, while leptin levels in group 3 were higher than those in group 1 in boys.

Table 3 shows that in the group made up of only boys, BW and height were positively correlated with 25(OH)D as well as all variables of body composition (except between height and %BF), and that BMI was positively correlated with %BF (r = 0.938, p < 0.001), BFM (r = 0.963, p < 0.001), leptin (r = 0.195, p = 0.044), and 25(OH)D (r = 0.252, p = 0.009). Leptin and 25(OH)D levels positively correlated with %BF (r = 0.212, p = 0.029 and r = 0.212, p = 0.028, respectively), leptin levels negatively correlated with 25(OH)D levels (r = -0.35, p < 0.001), and 25(OH)D levels positively correlated with BFM (r = 0.262, p = 0.006) and FFM (r = 0.193, p = 0.046). In group 1, BW and BMI positively correlated with all variables of body composition. Height positively correlated with FFM (r = 0.885, p < 0.001) and MM (r = 0.885, p < 0.001), while leptin negatively correlated with 25(OH)D (r = -0.64, p < 0.001). In group 2, BW, height, and BMI positively correlated with all body composition variables; BW and height negatively correlated with

TABLE 1 | Characteristics of study participants and comparisons[1] of the variables between boy and girl.

Variable[2]	Sex	Total participants (107 boys and 98 girls)		Group 1 (%BF < 30) (28 boys and 12 girls)		Group 2 (%BF ≥ 30 and < 40) (38 boys and 43 girls)		Group 3 (%BF ≥ 40) (41 boys and 43 girls)	
		Mean ± S.D.	*P* value	Mean ± S.D.	*P* value	Mean ± S.D.	*P* value	Mean ± S.D.	*P* value
Age (year)	Boy	13.36 ± 0.61	0.427	13.16 ± 0.69	0.983	13.39 ± 0.55	0.318	13.39 ± 0.63	0.760
	Girl	13.29 ± 0.65		13.17 ± 0.84		13.26 ± 0.63		13.35 ± 0.61	
BW (kg)	Boy	75.18 ± 14.74	0.002	60.35 ± 6.55	< 0.001	72.97 ± 10.66	0.003	87.36 ± 11.45	< 0.001
	Girl	69.35 ± 12.44		51.43 ± 3.84		66.43 ± 8.67		77.27 ± 10.66	
Height (m)	Boy	1.66 ± 0.07	< 0.001	1.66 ± 0.06	< 0.001	1.64 ± 0.07	0.002	1.67 ± 0.07	< 0.001
	Girl	1.59 ± 0.06		1.58 ± 0.06		1.60 ± 0.06		1.59 ± 0.06	
BMI (kg/m^2)	Boy	27.36 ± 4.52	0.850	21.86 ± 1.87	0.066	26.84 ± 1.82	0.103	31.60 ± 2.97	0.050
	Girl	27.25 ± 4.14		20.74 ± 1.29		26.00 ± 2.64		30.31 ± 2.97	
%BF	Boy	36.79 ± 11.91	0.200	21.77 ± 4.42	0.037	35.89 ± 2.32	0.597	47.88 ± 8.55	0.080
	Girl	38.58 ± 7.71		25.39 ± 5.79		35.59 ± 2.69		45.25 ± 4.12	
BFM (g)	Boy	29.12 ± 14.18	0.325	13.29 ± 3.74	0.934	26.27 ± 4.82	0.014	42.58 ± 11.54	0.001
	Girl	27.47 ± 9.52		13.18 ± 3.50		23.74 ± 4.22		35.19 ± 7.41	
FFM (g)	Boy	45.94 ± 6.87	< 0.001	46.95 ± 4.30	< 0.001	45.62 ± 7.53	0.041	45.55 ± 7.69	0.607
	Girl	41.88 ± 4.86		38.24 ± 2.32		42.70 ± 5.01		42.07 ± 4.85	
MM (g)	Boy	43.83 ± 6.01	< 0.001	44.46 ± 4.05	< 0.001	44.15 ± 5.84	0.001	43.11 ± 7.22	0.006
	Girl	39.26 ± 4.46		35.93 ± 2.14		40.02 ± 4.60		39.44 ± 4.45	
Leptin (ng/mL)	Boy	22.32 ± 5.18	0.049	19.95 ± 6.89	0.007	22.59 ± 4.21	0.380	23.70 ± 4.07	0.643
	Girl	23.59 ± 3.97		25.08 ± 4.17		23.45 ± 4.48		23.32 ± 3.32	
25(OH)D (ng/mL)	Boy	22.24 ± 5.69	0.102	21.15 ± 5.70	0.396	21.19 ± 7.06	0.248	23.97 ± 3.57	0.174
	Girl	21.06 ± 4.49		19.49 ± 5.26		19.62 ± 4.68		22.94 ± 3.31	

[1]*Independent-samples T-test was used to compare the differences in mean ± S.D. of variables between sexes.*

[2]*S.D., Standard deviation; BW, body weight; BMI, body mass index; %BF, body fat percentage; BFM, body fat mass; FFM, fat free mass; MM, muscle mass; 25(OH)D, 25-hydroxyvitamin D.*

TABLE 2 | Comparisons[1] of the variables between %BF groups[2] of boys and girls.

Variable[3]	*P* value					
	Boys (n = 107)			Girls (n = 98)		
	Group 1 and 2	Group 1 and 3	Group 2 and 3	Group 1 and 2	Group 1 and 3	Group 2 and 3
BW (kg)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Height (m)	0.326	0.548	0.096	0.267	0.330	0.898
BMI (kg/m^2)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
%BF	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
BFM (g)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
FFM (g)	0.370	0.337	0.964	< 0.001	< 0.001	0.558
MM (g)	0.808	0.374	0.488	< 0.001	< 0.001	0.555
Leptin (ng/mL)	0.080	0.013	0.239	0.262	0.131	0.883
25(OH)D (ng/mL)	0.979	0.025	0.033	0.938	0.008	< 0.001

[1]*Independent-samples T-test was used to compare the differences in mean ± S.D. of variables (**Table 1**) between %BF groups.*
[2]*Group 1, %BF < 30; Group 2, %BF ≥ 30 and < 40; Group 3, %BF ≥ 40.*
[3]*BW, body weight; BMI, body mass index; %BF, body fat percentage; BFM, body fat mass; FFM, fat free mass; MM, muscle mass; 25(OH)D, 25-hydroxyvitamin D.*

TABLE 3 | Correlation coefficient, *r* (*P* value), between BMI parameters, body composition, leptin, and 25(OH)D in boys.

Variable[1]	%BF *r* (*P* value)	BFM (g) *r* (*P* value)	FFM (g) *r* (*P* value)	MM (g) *r* (*P* value)	Leptin (ng/mL) *r* (*P* value)	25(OH)D (ng/mL) *r* (*P* value)
Total Boys (n = 107)						
BW (kg)	0.779 (< 0.001)	0.891 (< 0.001)	0.271 (0.005)	0.289 (0.003)	0.112 (0.249)	0.327 (0.001)
Height (m)	0.001 (0.992)	0.220 (0.023)	0.735 (< 0.001)	0.791 (< 0.001)	-0.090 (0.359)	0.296 (0.002)
BMI (kg/m^2)	0.938 (< 0.001)	0.963 (< 0.001)	-0.027 (0.780)	-0.032 (0.745)	0.195 (0.044)	0.252 (0.009)
Leptin (ng/mL)	0.212 (0.029)	0.179 (0.065)	-0.115 (0.237)	-0.092 (0.347)	1.000	-0.350 (< 0.001)
25(OH)D (ng/mL)	0.212 (0.028)	0.262 (0.006)	0.193 (0.046)	0.187 (0.053)	-0.350 (< 0.001)	1.000
Group 1: Boys with %BF < 30 (n = 28)						
BW (kg)	0.614 (0.001)	0.815 (< 0.001)	0.851 (< 0.001)	0.852 (< 0.001)	-0.024 (0.905)	0.263 (0.176)
Height (m)	-0.102 (0.607)	0.157 (0.424)	0.885 (< 0.001)	0.885 (< 0.001)	-0.073 (0.713)	0.257 (0.186)
BMI (kg/m^2)	0.892 (< 0.001)	0.938 (< 0.001)	0.377 (0.048)	0.378 (0.047)	0.064 (0.747)	0.072 (0.717)
Leptin (ng/mL)	-0.052 (0.793)	-0.027 (0.890)	0.017 (0.930)	0.014 (0.943)	1.000	-0.640 (< 0.001)
25(OH)D (ng/mL)	0.142 (0.470)	0.191 (0.330)	0.220 (0.261)	0.224 (0.252)	-0.640 (< 0.001)	1.000
Group 2: Boys with %BF ≥ 30 and < 40 (n = 38)						
BW (kg)	0.439 (0.006)	0.951 (< 0.001)	0.777 (< 0.001)	0.971 (< 0.001)	-0.340 (0.037)	0.311 (0.057)
Height (m)	0.322 (0.049)	0.871 (< 0.001)	0.755 (< 0.001)	0.952 (< 0.001)	-0.388 (0.016)	0.334 (0.040)
BMI (kg/m^2)	0.540 (< 0.001)	0.920 (< 0.001)	0.680 (< 0.001)	0.860 (< 0.001)	-0.220 (0.184)	0.243 (0.142)
Leptin (ng/mL)	-0.146 (0.382)	-0.326 (0.046)	-0.357 (0.028)	-0.322 (0.049)	1.000	-0.500 (0.001)
25(OH)D (ng/mL)	0.227 (0.170)	0.334 (0.040)	0.243 (0.141)	0.271 (0.100)	-0.500 (0.001)	1.000
Group 3: Boys with %BF ≥ 40 (n = 41)						
BW (kg)	0.450 (0.003)	0.731 (< 0.001)	0.153 (0.338)	0.154 (0.335)	-0.079 (0.625)	0.222 (0.163)
Height (m)	-0.260 (0.100)	0.108 (0.500)	0.719 (< 0.001)	0.719 (< 0.001)	0.121 (0.452)	0.224 (0.160)
BMI (kg/m^2)	0.799 (< 0.001)	0.899 (< 0.001)	-0.328 (0.036)	-0.326 (0.037)	-0.155 (0.334)	0.138 (0.389)
Leptin (ng/mL)	-0.097 (0.544)	-0.067 (0.675)	0.050 (0.755)	0.050 (0.757)	1.000	-0.043 (0.792)
25(OH)D (ng/mL)	0.005 (0.973)	0.093 (0.563)	0.204 (0.202)	0.203 (0.203)	-0.043 (0.792)	1.000

[1]*BW, body weight; BMI, body mass index; %BF, body fat percentage; BFM, body fat mass; FFM, fat free mass; MM, muscle mass; 25(OH)D, 25-hydroxyvitamin D.*

leptin (r = -0.34, p = 0.037 and r = -0.388, p = 0.016, respectively); while height positively correlated with 25(OH)D (r = 0.334, p = 0.04). Leptin levels in group 2 negatively correlated with all variables of body composition (except %BF), and 25(OH)D (r = -0.5, p = 0.001). Additionally, 25(OH)D levels and BFM were positively correlated (r = 0.334, p = 0.04). In group 3, there were positive correlations between BW and %BF (r = 0.45, p = 0.003), BFM (r = 0.731, p < 0.001), as well as height with FFM and MM (r = 0.719, p < 0.001), while BMI was positively correlated with %BF (r = 0.799, p < 0.001) and BFM (r = 0.899, p < 0.001), but negatively correlated with FFM (r = -0.328, p = 0.036) and MM (r = -0.326, p = 0.037). However, there were no correlations between body composition, and leptin and 25(OH)D levels in group 3.

Table 4 shows that in a group of total girls, BW, height, and BMI were positively correlated with all variables of body composition, while %BF and BFM were positively correlated with 25(OH)D levels (r = 0.302, p = 0.002 and r = 0.279, p = 0.005). In group 1, there were positive correlations of BW and height with %BF (r = 0.630, p = 0.028 and r = 0.591, p = 0.043) and BFM (r = 0.804, p = 0.002 and r = 0.661, p = 0.019), and a negative correlation between leptin and 25(OH)D (r = -0.784, p = 0.003). In group 2, BW and BMI were positively correlated with all variables of body composition and negatively correlated with 25(OH)D (r = -0.329, p = 0.031 and r = -0.373, p = 0.014), while height positively correlated with BFM (r = 0.511, p < 0.001), FFM (r = 0.711, p < 0.001) and MM (r = 0.711, p < 0.001). FFM and

TABLE 4 | Correlation coefficient, *r* (*P* value), between BMI parameters, body composition, leptin, and 25(OH)D in girls.

Variable[1]	%BF *r* (*P* value)	BFM (g) *r* (*P* value)	FFM (g) *r* (*P* value)	MM (g) *r* (*P* value)	Leptin (ng/mL) *r* (*P* value)	25(OH)D (ng/mL) *r* (*P* value)
Total Girls (n = 98)						
BW (kg)	0.763 (< 0.001)	0.937 (< 0.001)	0.723 (< 0.001)	0.723 (< 0.001)	-0.004 (0.969)	0.183 (0.072)
Height (m)	0.139 (< 0.001)	0.350 (< 0.001)	0.713 (< 0.001)	0.713 (< 0.001)	0.079 (0.437)	0.060 (0.558)
BMI (kg/m^2)	0.843 (< 0.001)	0.935 (< 0.001)	0.501 (< 0.001)	0.500 (< 0.001)	-0.048 (0.639)	0.179 (0.078)
Leptin (ng/mL)	-0.091 (0.375)	-0.026 (0.798)	0.038 (0.710)	0.039 (0.702)	1.000	-0.193 (0.057)
25(OH)D (ng/mL)	0.302 (0.002)	0.279 (0.005)	-0.076 (0.457)	-0.077 (0.453)	-0.193 (0.057)	1.000
Group 1: Girls with %BF < 30 (n = 12)						
BW (kg)	0.630 (0.028)	0.804 (0.002)	0.442 (0.150)	0.437 (0.155)	0.218 (0.496)	-0.205 (0.524)
Height (m)	0.591 (0.043)	0.661 (0.019)	0.093 (0.775)	0.092 (0.777)	0.050 (0.877)	-0.017 (0.959)
BMI (kg/m^2)	0.015 (0.964)	0.136 (0.674)	0.415 (0.180)	0.411 (0.185)	0.193 (0.547)	-0.206 (0.520)
Leptin (ng/mL)	-0.191 (0.552)	-0.078 (0.810)	0.478 (0.116)	0.484 (0.110)	1.000	-0.784 (0.003)
25(OH)D (ng/mL)	0.056 (0.863)	-0.024 (0.940)	-0.302 (0.341)	-0.306 (0.333)	-0.784 (0.003)	1.000
Group 2: Girls with %BF ≥ 30 and < 40 (n = 43)						
BW (kg)	0.479 (0.001)	0.928 (< 0.001)	0.947 (< 0.001)	0.947 (< 0.001)	-0.062 (0.694)	-0.329 (0.031)
Height (m)	0.095 (0.543)	0.511 (< 0.001)	0.711 (< 0.001)	0.711 (< 0.001)	-0.021 (0.892)	-0.074 (0.636)
BMI (kg/m^2)	0.569 (< 0.001)	0.857 (< 0.001)	0.737 (< 0.001)	0.737 (< 0.001)	-0.050 (0.751)	-0.373 (0.014)
Leptin (ng/mL)	0.113 (0.470)	-0.010 (0.948)	-0.104 (0.507)	-0.103 (0.511)	1.000	-0.248 (0.108)
25(OH)D (ng/mL)	-0.059 (0.707)	-0.247 (0.111)	-0.353 (0.020)	-0.353 (0.020)	-0.248 (0.108)	1.000
Group 3: Girls with %BF ≥ 40 (n = 43)						
BW (kg)	0.541 (< 0.001)	0.920 (< 0.001)	0.795 (< 0.001)	0.794 (< 0.001)	0.294 (0.055)	0.295 (0.055)
Height (m)	0.032 (0.838)	0.489 (0.001)	0.826 (< 0.001)	0.825 (< 0.001)	0.265 (0.086)	0.255 (0.098)
BMI (kg/m^2)	0.721 (< 0.001)	0.900 (< 0.001)	0.477 (0.001)	0.476 (0.001)	0.196 (0.208)	0.204 (0.189)
Leptin (ng/mL)	0.034 (0.831)	0.239 (0.123)	0.281 (0.067)	0.281 (0.068)	1.000	0.247 (0.110)
25(OH)D (ng/mL)	0.096 (0.541)	0.236 (0.128)	0.287 (0.062)	0.287 (0.062)	0.247 (0.110)	1.000

[1]*BW, body weight; BMI, body mass index; %BF, body fat percentage; BFM, body fat mass; FFM, fat free mass; MM, muscle mass; 25(OH)D, 25-hydroxyvitamin D.*

MM were negatively correlated with 25(OH)D (r = -0.353, p = 0.02). In group 3, there were strong positive correlations between BW, height, BMI, and all variables of body composition except between height and %BF, but no correlations between body composition variables, leptin, and 25(OH)D.

DISCUSSION

This study included participants aged 13–14 years, which is the period of age when growth spurt occurs (24). The BMIs for total boys and girls were more than the 97th percentile for age (17), which classified them as obese. Obesity is defined by the WHO as a condition of abnormal or excessive fat accumulation in adipose tissue. However classifying obesity during childhood or adolescence has the added complication of height still increasing, and body composition continually changing (25). In this study, participants in group 1 were defined as having low excess %BF due to %BF < 50th percentile (20), whereas groups 2 and 3 consisted of participants with moderate and high excess % BF, because %BF was above the 95th percentile of %BF-for-age (18, 19). When referring to another percentile curve (20), the mean %BF of girls in groups 2 and 3 was at the 90th and > 95th percentiles, respectively. In each group, BMI did not discern the differences in body composition based on sex (26), and high BMI did not distinguish excess fat mass from lean mass (27). In addition, most variables of body composition varied with the increase in %BF in both sexes, while height, FFM, and MM of boys remained unchanged, which corresponded to growth development during puberty in boys (28). Contrarily, decreases in FFM and MM were observed in girls with the highest excess %BF. However, these characteristics may be related to leptin and 25(OH)D levels which are discussed later.

Furthermore, this study did not find a decrease in 25(OH)D levels due to an increase in %BF as reported in previous studies (13, 29), and 25(OH)D levels appeared to be slightly less than 20 ng/mL in girls with %BF < 40, whereas participants with high excess %BF (≥ 40) had higher serum 25(OH)D levels than the other groups. This finding may be consistent with a previous study reporting that 25(OH)D levels are associated with BMI, sex, puberty, and age (30). The highest 25(OH)D levels were observed in groups with %BF ≥ 40, which might be due to unchanged leptin levels according to the increased %BF, and seemed to be the lowest leptin levels compared to other groups. These results were in contrast to previous findings that serum leptin was increased and that serum vitamin D might be decreased in obese individuals (3, 4, 6, 31). Thus, sex and the amount of %BF are likely the important factors in determining changes in serum leptin and 25(OH)D levels in adolescents.

In this study, boys and girls showed differences in the correlations between leptin, and 25(OH)D levels, likely due to differences in body composition and their relationships in each % BF group. The negative relationship between serum leptin and 25 (OH)D was clearly demonstrated in boys with %BF < 40, which was evident in the positive relationships between BMI and body composition and in the inverse relationships with leptin levels. Conversely, the relationship between serum leptin and 25(OH)D was not observed in boys when BMI and excess %BF (> 40) increased, which was likely explained by the change in the lean proportion and the negative relationships between BMI, FFM, and MM. Thus, in boys, lean mass (FFM and MM) and excess BF were likely key variables that indicated a correlation between

leptin and 25(OH)D. Although, there are a few studies reporting the relationship between leptin and 25(OH)D in adolescents with obesity, previous studies in adults revealed that measures of adiposity largely explained the negative association of serum 25 (OH)D with leptin, and that MM, %BF, and serum 25(OH)D had an impact on serum leptin (5, 32). Furthermore, %BF explained all sex differences in leptin concentrations, and lean body mass was inversely related to leptin concentrations (32).

In girls, whether the inverse relationship between leptin and 25(OH)D was modulated by excess %BF because it appeared in a group with %BF < 30 remained unclear, whereas strong correlations between BMI and body composition were not observed. However, this relationship might be a result of the highest leptin levels and the lowest 25(OH)D levels in girls in this group when compared to other groups, and explained by the excess %BF, thus leading to decreased vitamin D and increased leptin levels (12). In contrast to boys, 25(OH)D levels were inversely related to lean mass (FFM and MM), BW, and BMI in girls with moderately excess %BF. Although, strong correlations between variables of BMI and body composition appeared clearly in girls with high excess %BF, they did not seem to contribute to the relationship between leptin and 25(OH)D. This might be because the 25(OH)D levels in girls did not decrease with increasing %BF, as found in previous studies (12, 13). In addition, the relationship between fat distribution and vitamin D status may be dependent on metabolic factors as well as parathyroid hormone, which is released in response to low 25 (OH)D (13). However, this study suggests that BW, BMI, and MM should be considered when interpreting serum 25(OH)D levels as markers of vitamin D status (33).

This study is limited by the small number of participants; thus, it may not be sufficient in powered to be generalizable to Thai early adolescents and to allow comparisons between sexes and groups of excess %BF. Further research is needed to explore the relationship between height, leptin, and 25(OH)D in boys with approximately 30-40%BF, including the inverse relationship between leptin and 25(OH)D in girls.

CONCLUSIONS

Negative correlations between leptin, 25(OH)D, and body composition appeared clearly in boys and girls when using % BF at 30 and 40 to classify their degrees of obesity. The relationship between leptin and 25(OH)D is sex-specific and differ depending on body composition, and the degree of excess body fat may be a determinant.

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.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The Human Research Ethics Committee of Walailak University, Thailand (Approval Number: WUEC-19-102-21). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

RK conceived and carried out study design, data collection, data analysis, data interpretation, literature search, and writing of the manuscript. CP carried out ELISA measurement, and involved in writing of the manuscript. All authors contributed to the article and approved the submitted version.

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REVIEW
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Multi-Faceted Influence of Obesity on Type 1 Diabetes in Children – From Disease Pathogenesis to Complications

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The prevalence of overweight and obesity among youth patients with diabetes type 1 is increasing. It is estimated, that even up to 35% of young patients with this type of diabetes, considered so far to be characteristic for slim figure, are overweight or even obese. General increase of obesity in children's population complicates differential diagnosis of the type of diabetes in youths. Coexistence of obesity has clinical implications for all stages of diabetes course. It is confirmed that obesity is the risk factor for autoimmune diabetes, and is connected with the earlier onset of diabetes in predisposed patients. Many diabetic patients with obesity present additional risk factors for macroangiopathy, and are recognised to present metabolic syndrome, insulin resistance, and typical for diabetes type 2 - polycystic ovary syndrome, or non-alcoholic fatty liver disease. The prevalence of obesity rises dramatically in adolescence of diabetic child, more often in girls. It has negative impact on metabolic control, glycaemic variability and insulin demand. The risk for microangiopathic complications increases as well. The treatment is difficult and includes not only insulinotherapy and non-pharmacological trials. Recently treatment of insulin resistance with biguanids, and treatment with typical for type 2 new diabetes drugs like GLP-1 analogues, SGLT-2 receptor inhibitors, or even cases of bariatric surgery also has been reported.

Keywords: diabetes type 1, obesity, children, pathogenesis, complications, treatment

INTRODUCTION

Over the past few decades obesity became a worldwide epidemic (1) not only in adults but also in paediatric patients (2). According to WHO, in 2016 over 340 million of youth aged 5-19 were overweight or obese as were 39 million children under the age of 5 in 2020. This pandemic has not spared individuals with type 1 diabetes mellitus. Thus far, patients suffering from T1D usually were

considered as lean, whereas those with type 2 diabetes generally exhibit overweight and obesity, yet growing number of recent studies have shown that the prevalence of the problem of excessive body weight is increasing in individuals with T1D (3–8). This type of disease is caused by progressive, irreversible autoimmune destruction of β-cells, leading to a total insulin deficiency and additionally, is more and more prevalent in the younger age groups. It was estimated that around 108.300 youths under 15 years were diagnosed with T1D in 2021 (9). It follows that both excessive body mass and T1D are increasing problems in population. Furthermore, their overlapping may cause significant health consequences.

Excessive body weight has been linked to T1D from its very beginning, numerous studies presumed its role in autoimmune diabetes pathogenesis (10, 11). Apart from a great number of environmental factors of overweight and obesity in T1D such as dietary mistakes, fear of insulin induced hypoglycaemia resulting in excessive carbohydrates intake and lack of exercise, sedentary lifestyle (12), authors frequently list modern patterns of intensive insulin therapy itself (13–15). Obesity can contribute to the challenges in attaining optimal glycaemic control (16). Excessive body weight in T1D patients predispose to the increased risk of the development of some serious health conditions like metabolic syndrome, cardiovascular or kidney diseases, thus increasing morbidity and mortality, causing the reduced life expectancy. Moreover, increased BMI which increases insulin resistance in combination with T1D may lead to the development of so-called double diabetes (17). General increase of obesity in children's population complicates differential diagnosis of the type of diabetes in youths. The problem of obesity existing among youths with T1D looks definitely more like outbreak than occasional issue as up to 30-40% of young patients with T1D are recognised to be overweight or obese (4, 8, 18).

The aim of the present review is to summarize the most actual knowledge of the aetiology and repercussions of obesity in paediatric patients with T1D from diabetes pathogenesis to its complications, along with treatment directions that can withhold or counteract them.

OBESITY AS A PATHOGENIC FACTOR OF DEVELOPING T1D

Complex Aetiology of T1D

The pathogenesis of T1D is very complex and depends on numerous heterogeneous aspects (19). Commonly mentioned are genetic, environmental, immune and recently added are microbiome factors (**Table 1**). HLA (human leukocyte antigen) located on chromosome 6 within the major histocompatibility complex (MHC) region - especially the HLA-DR3/4-DQ8 alleles are likely to be the main genetic risk factors responsible for the development of T1D by causing β-cell hyper-expression of MHC I presenting β-cell neoepitopes to cytotoxic T cells, hence β-cells destruction (20). Genes implicated in islet inflammation and beta-cell apoptosis play an important role in the onset of T1D. Thus far, genome-wide association studies (GWAS) have identified around 50 susceptibility loci for T1D (21). A large number of these candidate genes for example, IFIH1, PTPN2, CTSH, CLEC16A and also GLIS3 are expressed inside pancreatic islets and β-cells and are seemed to be involved in modulating the β-cells response to the immune system and viral infection and also in insulitis and apoptosis (22).

Immunologic factors include several abnormalities in maturation and differentiation processes of T-cells, which can cause T-lymphocyte escape central or peripheral tolerance induction (23). Some recent studies have demonstrated that patients with T1D present quantitative and qualitative deficit in the subpopulation of T regulatory lymphocytes, which may explain the limitless immune response, which eventually leads to autoimmunization (24). Apoptosis is the most likely way of insulitis and β-cell loss in T1D. One of the theories says that the autoreactive T lymphocytes within the islet microenvironment induce an inflammatory reaction with high levels of the proinflammatory cytokines, which stimulate the caspase cascade. Other hypotheses imply that apoptosis is induced through the perforating system or Fas/Fas ligand interaction directly by contact of autoreactive T lymphocytes with β-cells (24, 25). Autoantibodies against glutamic acid decarboxylase 65 (GAD65), tyrosyl phosphatase (IA-2), insulin (IAA) may prelude the onset of clinical manifestation of T1D for years, furthermore multiple islet autoantibodies are associated with highest risk of type 1 diabetes (26).

Environmental factors involved in the development of T1D include viral infections (27). Some studies have accumulated a lot of evidence supporting that enteroviral persistence in various tissues and its highly cytolytic activity can play an important role in some chronic diseases (28), while other have shown that congenital rubella can increase incidence of T1D in children (29). A hypothesis of molecular mimicry suggests that the immune response is targeted against autoantigens that mirror the viruses' antigens resulting in cellular destruction. It has been showed that molecular mimicry with human cytomegalovirus, Coxsackie virus or rotavirus could promote autoimmunity to some islet antigens leading to autoimmunity in T1D (30–32). On the other hand, some experimental studies have suggested that it can't be excluded that enteroviruses would be protective against T1D in certain conditions (33). There are also some evidences that COVID-19 may be a trigger for the development of diabetes as well as a factor that worsens complications in patients with existing diabetes (34–36). Further studies should take identifying the viruses connected with the occurrence of T1DM and determining their participation to pathogenesis of the disease into careful account.

There were some perturbations that vaccinations might be associated with the following development of chronic autoimmune diseases, T1D inclusive (37, 38). However, studies in a cohort of 584,171 participants have exhibited that recommended immunization schedule had no direct correlation with the incidence of T1D in children (39). Moreover, vaccines are extensively recommended in youths with T1D because it is a disease with an elevated risk of infection due to poor glycaemic

control (40). Nevertheless, future researches are required to thoroughly determine the problems correlated with vaccine administration in patients with T1D.

Another environmental risk factors of the development of T1DM are lifestyle habits. Although, sedentary lifestyle, physical inactivity, high in fats and carbohydrates diet or smoking are factors contributing mainly to the development of type 2 diabetes and in general, they are widely known for increasing BMI (41), there were some studies demonstrated that high energy intake, especially rich in protein, fat, nitrosamine and carbohydrates, particularly disaccharides and sucrose, as well as more rapid growth in childhood or a larger relative body size, in both length and fat mass, were independently associated with the ongoing β-cell destruction and lead to an earlier clinical presentation of type 1 diabetes (42). Moreover, over-nutrition in childhood, hence increased childhood body size may rise T1D risk (43).

Human gut microbiota is another possible factor worthy of attention. Microbes colonizing the human gut feature in metabolic ailments and by extension seem to play an important role in the development of both diabetes and obesity, with autoimmune background. Human microbiota impact many aspects such as gut permeability, inflammatory responses, nutrient absorption, lipid metabolism, polysaccharide breakdown and bile acid modification. Studies have implied that T1D children show the increased Firmicutes to Bacteroidetes ratio, positive correlation of Bacteroides abundance with the presence of autoantibodies and negative correlation of Faecalibacterium abundance with HbA1c (44). Moreover, in the children with diabetes the quantity of essential to maintain gut integrity bacteria was significantly lower compared to their healthy controls (45). Decreased microbial diversity is believed to be associated with the T1D development, reported both in T1D patients (46) and in autoantibody-positive children (47). Changes in nutrition, agricultural production, product preparation, personal hygiene and antibiotic use, especially during the first years of life can change the composition of the gut microbiota (48). Increased risk of T1D in children born by caesarean section was suggested in conjunction with lack of contact with the mother's vaginal microbiome hence, following distinctions in their gut microbiota due to abnormal colonization (49, 50). Recent investigations have proposed that the intestinal microbiome plays a key role in the mechanisms behind a proinflammatory issue that results in the destruction of pancreatic islet β-cells and loss of insulin generation in T1D (51). Nevertheless more studies are required to examine the complex role of human gut microbiota in the pathogenesis of T1D and to develop strategies to control the development of the disease.

The most frequently mentioned risk factors of T1D were briefly discussed, yet many authors suggest that also obesity itself has an impact of the development of T1D (**Table 1**). Obesity results in deficiencies of the human self-tolerance mechanisms by triggering chronic low-grade inflammation, reducing regulatory B as well as T cells, further resulting in increased Th17 and Th1 cells, creating the sublime milieu for the development of autoimmune disorders (10). Corpulent children with new-onset T1D exhibited a paradigm of adipokines and cytokines that suggest a proinflammatory state, which may lead to T1D onset and its complication (52). Studies have shown that genetic susceptibility in combination with a high-fat diet leads to the development of a T1D-like phenotype that is characterized by mononuclear cell infiltration and insulitis, surprisingly only in male examinees (53). A meta-analysis of nine studies has provided comprehensive evidence of a connection between childhood obesity and subsequent risk of diabetes with an OR of 1.25 and 2.03 (54). Higher BMI is believed to be an environmental accelerator which may contribute to the noticeable increase in both type 1 and type 2 of diabetes in childhood and younger age of onset (55). However, within the SEARCH cohort this dependence was seen only in children with fasting C-peptide levels below the median (56). A prospective cross-sectional study in a large cohort of T1D youth has also demonstrated that central obesity is correlated with earlier onset of the disease (57).

Nonetheless, because the aetiology of T1D is complex, it can be expected that the data presented above might be discussed. On the contrary, a case-control study has showed that high birth weight but not alone excessive weight gain prior to manifestation is related to earlier onset of diabetes in childhood (58). In a study of 777 children from the BABYDIAB cohort, insulin sensitivity and BMI-SDS were similar in both autoantibody-positive and

TABLE 1 | Factors contributing to the development of T1D.

Factors contributing to the development of T1D	
Genetic factors	**Alterations in gut microbiota**
HLA-DR3/4-DQ8	nutrition, agricultural production, product preparation, personal hygiene, antibiotic use, caesarean section
Ins-VNTR polymorphisms	
CTLA-4 polymorphisms	
Immunologic factors	**Environmental factors**
disturbed T-cells maturation and differentiation	viral infections
autoreactive T lymphocytes	molecular mimicry
deficit in the Treg subpopulation	unhealthy lifestyle habits
autoantibodies: anti-GAD65, IA-2, IAA, ZnT8	economic indicators
Obesity (accelerator hypothesis)	
chronic low-grade inflammation	
self-tolerance mechanisms deficiency	
glucotoxicity insulin	
resistance	

-negative children (59). In the TrialNet Pathway to Prevention cohort, despite more children with excessive weight were determined to be as a single autoantibody-positive than those in the normal range of weight, they have found no convincing evidence in relation between conversion from single autoantibody to multiple autoantibodies or progression to T1D and BMI (60). The incidence of T1D is parallel to the increase in overweight and obesity, particularly in children with lower risk of developing the disease, i.e. older age children without high-risk HLA haplotypes- HLA DR3-DQ2 or DR4-DQ8 (61). Although evidence of potential epigenetic modifications of gene expression such as DNA methylation, underlines the need to define the role of external factors and their influence on gene expression in autoimmune diseases (62, 63). Attention is drawn to the fact that genes do not evolve so quickly and no other known environmental factor has a similar to obesity increase in the incidence of T1D.

The "Accelerator Hypothesis"

In 2001, Wilkin T.J. published a theory that the divergence between an insulin-dependent (type 1) and a non-insulin-dependent diabetes mellitus (type 2) blurs and considers overlay rather than overlap of these two types. Decreased glucose control and hence rising blood glucose is believed to be a result of weight gain induces an increase in insulin resistance. Glucotoxicity accelerates apoptosis of β-cells by which activates their immunogens, either promotes autoimmunization in genetically predisposed individuals (64). In fact, it differentiates both types of diabetes only by tempo of changes and perceives them as a continuum, where the fluctuations between genetic response and insulin resistance evaluate the age of critical β-cell destruction along with clinical presentation (65).

A large number of studies have been performed to investigate the accelerator hypothesis. The 'obesogenic' environment which promotes insulin resistance could explain rising prevalence of T1D in people with insulin deficiency and/or islet autoimmunity (66). In the retrospective analysis of a cohort of 9,248 German and Austrian children with type 1 diabetes mellitus (67) as well as in Kibirige M. Report (68), BMI was inversely associated with age at diabetes onset. Nonetheless, higher BMI at T1D onset and the observation that pre-diabetic children are heavier and more insulin resistant than their peers may imply convergence of type 1 and type 2 diabetes phenotypes (69). On the other hand, retrospective data from 2020 have demonstrated no evident tendency between increasing of type 1 incidence in the ethnically homogenous paediatric population of Lesser Poland and younger age of diagnosis along with higher BMI-SDS over the study period. Only 2.7% of children were obese, 5.7% exhibited underweight when 91.6% presented BMI-SDS within the normal range at the time of diagnosis (70). The control of weight gain, and by extension insulin resistance, could be the means of minimising both obesity and diabetes. Further studies to determine the role of capability for prevention of type 1 diabetes based on avoidance of extensive body mass are needed.

OBVIOUS AND LESS OBVIOUS CAUSES OF OBESITY IN TYPE 1 DIABETES

Excessive weight in type 1 diabetes results from various complex factors. Insulin replacement therapy is believed to contribute most to weight gain in people with type 1 diabetes (71), but it can also be affected by their behaviour, lifestyle choices, psychosocial factors and fear of hypoglycaemia (72). Independent factors such as age, sex and duration of disease also play a role in development of overweight/obesity (73).

Intensive insulin therapy used in children with type 1 diabetes allows to maintain near-normoglycaemia and thus avoid long-term complications of the disease, what is the primary purpose of treatment (74). In spite of this, it is a key factor that contributes to obesity in patients with T1D (75). Results of Diabetes Control and Complications Trial (DCCT) indicated that during the first year of intensive insulin therapy subjects gained more weight (5.1 ± 4.6 kg) than participants treated conventionally (2.4 ± 3.7 kg) (76), and over the course of 6.5 years of the same study about 25% of subjects who were treated with intensive insulin therapy gained more weight than group treated in conventional way what resulted in occurrence of obesity (77). After that, Epidemiology of Diabetes Interventions and Complications (EDIC), the follow-up to DCCT demonstrated that subjects continued to gain weight. Their waist circumference and insulin dose also increased in the course of the study (78). In contrast, the Kaminsky and Dewey study showed that in spite of the risk of weight gain due to intensified insulin therapy, the BMIs of adolescents with T1D and a control group without chronic disease were similar, but it is worth noting that physical activity levels were also similar in both groups (79).

It is not fully understood how insulin therapy affects the weight gain, but there are several hypotheses. Patients treated with intensive insulin regime have significantly reduced HbA1c comparing to patients treated conventionally. Their blood glucose level falls below the renal threshold and consequently, glucosuria is almost completely eliminated. Their consumed calories are conserved, leading to reduced energy expenditure and weight gain (71).

It is extremely important to emphasize that supply of exogenous insulin does not exactly imitate endogenous one. Physiologically, insulin passes through the portal vein to the liver where it inhibits gluconeogenesis and then about 40-50% of insulin enters the systemic circulation. It acts on adipose tissue and muscle where it enhances glucose uptake and inhibits lipolysis. When exogenous insulin is administered subcutaneously, it enters the systemic circulation first and has a greater effect on adipose tissue and muscle than the liver. A disproportion between peripheral and hepatic insulin is created, which may lead to excess fat accumulation and peripheral hyperinsulinemia (71, 80).

Furthermore, bypassing the liver by exogenous insulin supply also has its consequences in impaired hepatic glycogenolysis and gluconeogenesis regulation during fasting and postprandial inhibition of glucagon secretion. This results in an

inappropriate increase in glucagon levels and a decrease in stored glycogen in the postprandial state (81).

The DCCT study also showed that intensive insulin therapy leads to increased risk of hypoglycaemic episodes. What is already known, fear of hypoglycaemia is one of the factors causing weight gain in patients with type 1 diabetes. In an attempt to defend against the occurrence of hypoglycaemia (which remains the most common acute complication in people with type 1 diabetes), patients snack when they work out and overeat when hypoglycaemia occurs (71, 76). Moreover, they may prefer to wait to exercise until their blood glucose levels are in the proper range. Consequently, fear of hypoglycaemia during physical effort may have a negative impact on its frequency and quality (82).

The authors of The SWEET Registry demonstrated that switching from the multiple daily insulin injection to the continuous subcutaneous insulin infusion is significantly associated with an improvement in glycemic control but also with an increase in BMI in more than 4,000 youths with T1D (15). Interestingly, there are findings suggesting that in cases of co-occurring obesity and type 1 diabetes, making some changes to current therapy and using new long acting analogues as basal insulins, such as detemir, degludec and glar-300 may slightly prevent the weight gain associated with intensive insulin therapy (83). However, the study by Baskaran et al. showed that despite an increase in the use of intensive insulin therapy among paediatric patients with T1D from 52 to 97% between 1999 and 2009, the incidence of obesity/overweight in this group remained similar during these years (18, 82). Therefore, there must be other factors that have contributed to weight gain in these patients. Analysing further the problem, a sedentary lifestyle with lack of exercise, unhealthy eating habits and lack of sleep also play a role in weight gain.

Obesogenic environment with easy availability of processed, high-energy foods, poor dietary habits and sedentary behaviour are the main factors causing obesity in the general population and they contribute significantly to weight gain among patients with type 1 diabetes (83). It is worth noting that patients with T1D treated with intensive insulin therapy focus more on the amount of carbohydrates in a meal rather than on balanced nutrition. Because no food is prohibited, this results in the development of poor eating habits. Excessive amounts of fat (especially high saturated) and calories in their diet leads to weight gain and impaired metabolic control. In addition, some studies demonstrated that other eating behaviours like skipping breakfast and dinner may also contribute to overweight in young patients with T1D (12). Myśliwiec et al. in their study, which evaluated the dietary habits of adolescent males with type 1 diabetes, showed that a significant number of the subjects made many dietary mistakes, their diet was not well balanced and contained an excess of simple carbohydrates. Interestingly, most of the adolescents with type 1 diabetes who participated in the study had normal body fat distribution (84).

Regular physical activity is essential for young patients with T1D, and it has been proven to result in improved BMI, triglyceride, and cholesterol levels in this group. Unfortunately, young patients with T1D have less physical activity than those without the disease, and the previously mentioned fear of hypoglycaemia may contribute to this (12). In addition, Jamiołkowska-Sztabkowska et al. showed that regular physical activity contributes to longer partial remission time in children with newly diagnosed type 1 diabetes, resulting in better metabolic control of the disease in the long term (85).

Moreover, adolescents with T1D declare extended time spent in front of a screen (82). It was demonstrated that children with T1D who watched television during meals consumed more fat than children who did not, and girls with T1D who spent more time watching TV had a higher likelihood of being overweight (12).

Some researchers suggest that sleep deficiency may be related with overweight and obesity in youth with T1D. They sleep shorter than their healthy peers and have more periods of sleep apnoea. Disturbances in sleep architecture or sleep restriction may decrease insulin sensitivity and worsen diabetes compensation, thus inadequate amount of sleep has been linked to weight gain (82, 86).

Psychosocial factors such as depression, poor self-esteem, higher levels of stress, low social support or having a negative body image are believed to be associated with the occurrence of overweight or obesity, but this has not been intensively studied in relation to T1D (12).

It is noteworthy that eating disorders are more frequent in adolescents with type 1 diabetes than in their healthy peers. Anorexia nervosa, bulimia nervosa, binge eating or other specified feeding and eating disorders may be associated with this disease (87). A recent Danish national survey of adolescents with T1D found that about one-third of the subjects presented with symptoms of overeating and binge eating. Binge eating symptoms have been shown to be associated with lower quality of life, emotional problems and higher HbA1c and BMI-SDS (88). Furthermore, the study by De Keukelaere et al. revealed that increased age, being a female, longer time since diagnosis and

TABLE 2 | Causes of obesity in children with type 1 diabetes.

Intensive insulin therapy	Factors related to the disease	Psychosocial factors
exogenous insulin has a greater effect on adipose tissue excess fat accumulation peripheral hyperinsulinemia impaired hepatic glycogenolysis and gluconeogenesis risk of hypoglycaemia	ncreased age at onset longer time since diagnosis diagnosis after puberty focus more on the amount of carbohydrates in a meal rather than on balanced nutrition fear of hypoglycaemia	depression poor self-esteem higher levels of stress low social support negative body image
Eating disorders	**Obesogenic environment**	**Other**
anorexia nervosa bulimia nervosa binge eating other specified feeding and eating disorders	sedentary lifestyle lack of exercise unhealthy eating habits high-energy foods	extended screen time sleep disturbances female sex

diagnosis after puberty are independent factors associated with weight gain in children with T1D (73). The most common reasons of obesity in T1D are summarised in **Table 2**.

OBESITY AS A PROBLEM IN DIFFERENTIAL DIAGNOSIS OF DIABETES TYPES

In the past, diabetes diagnosed in childhood was almost always considered to be type 1. Nowadays, autoimmune-mediated type 1 diabetes still accounts for the majority of diagnoses in children. However, making an accurate diagnosis of the type of diabetes has become difficult, complex and complicated process, which may be challenging. Thus, the diagnostic approach of clinicians to children diabetes requires changes because clinical manifestations of the different subtypes of diabetes can overlap. Children with type 1 diabetes were for a long time considered to be thin at diagnosis, mainly due to the weight loss that usually occurs earlier. However, recent studies indicate an increase in BMI of children with type 1 diabetes. It must be highlighted that increasing prevalence of obesity in children results not only in their growing incidence of type 2 diabetes but also, and more frequently, in the development of a combined type 1 and type 2 diabetes (73, 89). Consequently, the presence of obesity creates new problems in the differential diagnosis of diabetes types.

Severe diabetic symptoms and significantly elevated glucose levels usually occur in individuals with type 1 diabetes. In addition, 40-60% of them are diagnosed with life-threatening diabetic ketoacidosis. Islet cells autoantibodies and autoantibodies to glutamic acid decarboxylase (GAD), the tyrosine phosphatases islet antigen 2 (IA-2) and IA-2β, insulin and zinc transporter 8 are autoimmune markers of this disease. According to the latest American Diabetes Association (ADA) clinical recommendations, the presence of multiple islet antibodies is considered to be a risk factor for clinical diabetes and their detection is recommended for screening for presymptomatic type 1 diabetes (90).

Therefore, type 1 diabetes in children can be predicted by the development of multiple islet autoantibodies. An interesting study by Ziegler et al. found that most children, who were at risk for type 1 diabetes and were diagnosed as having autoantibodies eventually developed the disease over the next 15 years (26). These findings seem to be confirmed by Gorus et al., who demonstrated that individuals with multiple autoantibodies develop symptomatic diabetes within 20 years; and this process is accelerated when IA-2A or ZnT8A are present (regardless of age, HLA-DQ genotype, and number of autoantibodies) (91). Hence, it is clear that the presence of autoantibodies is inseparably associated with type 1 diabetes.

According to ADA, screening for type 2 diabetes should be considered in children and adolescents after the onset of puberty or after age 10 years (depending on which occurs first) who are overweight or obese with one or more risk factors for diabetes (maternal gestational diabetes, a family history of type 2 diabetes in a 1st or 2nd degree relative, a predisposing race, or signs or symptoms of insulin resistance). There are reports of the onset of type 2 diabetes before the age of 10, which can be linked to the presence of multiple risk factors. As recommended, a fasting plasma glucose, oral glucose tolerance test and HbA1c can be used to diagnose pre-diabetes or diabetes in children and adolescents (90).

It is important that MODY (maturity-onset diabetes of the young) should not be omitted in the differential diagnosis of diabetes in children. It belongs to the group of monogenic diabetes, resulting from mutations of a single gene that is involved in the functioning of the pancreatic β-cells. It is inherited in an autosomal dominant manner, responsible for 1-5% of all diabetes cases and is typically diagnosed between the second and fifth decades of life. However, the estimated incidence of MODY in youth under 15 years of age with new-onset diabetes is 2.4%. There are 14 subtypes of MODY depending on the gene affected (92). It is relatively difficult to distinguish MODY from type 1 and type 2 diabetes using clinical characteristics. In contrast to type 1 diabetes, patients with MODY have preserved pancreatic β-cells function and their diabetes is well controlled with no or low doses of insulin for at least 5 years after diagnosis. On the other hand, the clinical features of type 2 diabetes are similar to MODY, but in most cases people with type 2 diabetes are obese or overweight, while MODY diabetes is not associated with increased body weight. However, both type 2 diabetes and MODY patients have a family history of diabetes (93). Interestingly, the American Diabetes Association recommends that children and young adults without typical features of type 1 or type 2 diabetes and with a family history of diabetes in successive generations should undergo genetic testing for MODY (90).

As mentioned, there are many clinical and diagnostic features that can be used to determine the type of diabetes in youth, such as severity of symptoms, age at onset of the disease, family history of diabetes, occurrence of overweight/obesity, C-peptide levels, islet antibodies and markers of insulin resistance. Some mentioned markers are more useful than others, and they may be helpful in identifying the type of diabetes (94, 95). But as mentioned earlier, clinical features at onset can overlap - obesity and ketoacidosis can occur in both type 1 and type 2 diabetes as well as age at diagnosis does not accurately differentiate diabetes types (96).

Patients with type 1 diabetes are usually described as those with low or normal weight, with little tendency to develop metabolic syndrome or insulin resistance (97). The prevalence of obesity and the presence of markers of insulin resistance are typically associated with people with type 2 diabetes (94). Moreover, the presence of islet antibodies can be found in most patients with T1D, while in patients with T2D they are usually absent (94). The presence of positive islet antibodies is recognised as factor determining autoimmune origin of disease, yet there exists the subfamily of type 2 diabetes with positive islet antibodies, being neither typical type 2 nor type 1, or quite opposite being both type 1 and type 2 (98). It must be emphasised that the boundaries between these types are blurring.

The increasing prevalence of obesity and overweight among patients with type 1 diabetes (T1D) has resulted in the emergence

of double diabetes - new term which characterizes individuals with T1D who show clinical signs of type 2 diabetes (T2D) - obesity and insulin resistance (99). We may suspect double diabetes in a paediatric patient who has features typical of both T1D and T2D - when antibodies to β-cells are found in a child with T2D, or when a child with T1D is overweight/obese (96).

Furthermore, ADA in their latest guidelines recommends that overweight or obese children and adolescents who are suspected of having type 2 diabetes should be tested with a pancreatic autoantibody panel test to ensure that they do not suffer from type 1 diabetes (100).

It is extremely important fact that C-peptide, produced in equal amounts with insulin and used in assessing endogenous insulin secretion plays a large role in determining the type of diabetes. The presence of sustained insulin secretion and thus, C-peptide 3-5 years after diagnosis may indicate type 2 diabetes. When levels of C-peptide are low due to insulin deficiency, it may suggest the occurrence of type 1 diabetes. Clinicians must be careful when interpreting higher results, especially in obese individuals or those with signs of insulin resistance, because obese, insulin-resistant patients may have normal or elevated C-peptide levels at disease recognition, even if they have autoimmune-related type 1 diabetes and they will develop complete insulin deficiency in the future (101).

Szypowska et al. indicated a positive correlation between fasting C-peptide levels and BMI-SDS. In this study, 11.3% of the children with newly diagnosed type 1 diabetes were overweight or obese, and the median fasting C-peptide level in this group was higher than in normal- or underweight subjects. Consequently, the C-peptide levels of obese or overweight children with type 1 diabetes at the initial stage were preserved to a large extent (102).

Interestingly, study by Buryk et al. confirmed the association of an autoimmune process with the occurrence of insulin-requiring diabetes in children, but independently of obesity or the presence of autoantibodies. The authors connected obesity and diabetes-related autoimmunity with an enhanced T-cell response particularly in those with the highest levels of body fat and/or insulin resistance. Importantly, there are patients with autoimmune-related diabetes, at a point in their disease, when only T cells and not yet antibodies are measurable. According to the study, when comparing insulin resistant with insulin sensitive subject at the same point in their course of autoimmune progression, such insulin levels that are inadequate to maintain euglycaemia will appear earlier in the insulin resistant individual. The study also revealed that individuals with positive T-cell reactivity, but without the presence of conventional autoantibodies, were the group with the highest BMIz. Consequently, group of patients who may be classified as having type 2 diabetes because of the lack of autoantibodies, actually have diabetes associated with autoimmunity by T cells (103).

In conclusion, the occurrence of obesity in children with diabetes favours the overlap of clinical manifestations of different types of this disease. This creates a significant problem for clinicians, as it is more difficult for them to make a proper diagnosis. Recent studies have shown that clinical and diagnostic features previously used to differentiate between types of diabetes, such as C-peptide levels, weight on admission, presence of markers of insulin resistance or even the presence of islet antibodies, may lose their value in the face of the increasing prevalence of obesity in children.

OBESITY AS A FACTOR THAT ALTERS/ DISRUPTS THE WELL-KNOWN COURSE OF TYPE 1 DIABETES AFTER DIAGNOSIS

Most patients with long-duration T1D continue to secrete low levels of endogenous insulin yet as mentioned before, overweight and obese children exhibit higher levels of C-peptide than their lean peers. Usually, this preserved β-cells function means that patient achieves a good glycaemic control with low insulin demand. Interestingly, it was observed that the degree of metabolic control in individuals with greater weight gain was similar to those who maintained a stable weight, but at the expense of a higher daily insulin dose (78). Across two large registries in the US and Europe, higher BMIz was demonstrated to be significantly related to greater HbA1c levels and more incidents of severe hypoglycaemia (16). This apparent paradox may be due to the fact that healthy adipose tissue is essential for β-cell function. Likewise, while residual C-peptide secretion was proved to be correlated with lower risk of severe hypoglycaemia in T1D,people with higher BMI present this complication more often, presumably due to the fact that excessive weight impacts on impaired awareness of hypoglycaemia. The causes of this phenomenon are not completely understood, so it requires further investigation (104).

Additionally, Lee and co-authors described that patients with higher BMI were characterized by increasing HbA1c together with weight gain (105). A prognostic model that included BMI, immunological markers, and age has predicted exacerbation of T1D defined by diminished residual fasting C-peptide after 1 year of follow-up [AUC of 0.936] (106). It suggests that obesity may be also qualified as a T1D progression predictor.

OBESITY AS A FACTOR OF DEVELOPING T1D VASCULAR COMPLICATIONS

Although type 1 diabetes is a treatable disease and despite advances in its treatment, it still remains a huge burden for patients. It carries the risk of serious complications, beginning with the occurrence of episodes of hypoglycemia or ketoacidosis and ending with long-term micro- and macrovascular complications. Microvascular complications are usually manifested in retino-, neuro- and nephropathy, but can also have an impact on cognitive function, the heart and other organs. In turn, conditions such as atherosclerosis, thrombosis in the heart, peripheral arteries and brain are considered to be

macrovascular complications of type 1 diabetes. It is extremely important that cardiovascular disease remains a main cause of premature morbidity and mortality, resulting in an 8-13-year shorter life expectancy for people with type 1 diabetes compared to people without this condition (107). Atherosclerosis at the endothelial level begins early in patients with T1D even though coronary, cerebrovascular and peripheral artery disease does not manifest until adulthood. Therefore, patients with T1D diagnosed in childhood life present a high risk of premature development of cardiovascular disease thus maintaining good metabolic control throughout childhood and adolescence is essential for their future health and quality of life (108, 109). According to Merger et. al, the occurrence of metabolic syndrome in type 1 diabetes is associated with an increased incidence of micro- and macrovascular complications regardless of glycaemic control (110). Furthermore, youth with T1D and overweight or obesity have a higher likelihood of coexisting hypertension, abnormal lipids and elevated alanine aminotransferase compared to their healthy peers. It was also demonstrated that increased insulin resistance, which affects not only people with increased body weight without diabetes, but also individuals with type 1 diabetes and overweight or obesity, has a negative impact on the development of microvascular complications of type 1 diabetes and may be related to the occurrence of these macrovascular (71, 111).

SEARCH for Diabetes in Youth Study found that obesity, increased LDL cholesterol and triglycerides as well as lower HDL cholesterol are risk factors for diabetic peripheral neuropathy among youth with type 1 diabetes (112). This seems to be confirmed by a recent study by Franceschi et al., which showed that waist/height ratio and lipid disorders are one of the key risk factors not only for diabetic peripheral neuropathy but also for cardiac autonomic neuropathy for young people with type 1 diabetes (113). Furthermore, Price et al. in their study indicated obesity as the predominant risk factor for retinopathy and for cardiovascular disease (114). Some studies also demonstrated a link between kidney disease and obesity in both type 1 and type 2 diabetes and it was showed that high BMI ≥30 kg/m2 along with lipid disorders and elevated blood pressure are associated with an increased risk of albuminuria in adolescents and young adults with type 1 diabetes (115, 116). Interestingly, a group of children with diabetic nephropathy and coexisting type 1 diabetes participating in the Burlaka and Maidannyk study showed higher blood cholesterol levels and higher systolic and diastolic blood pressure values than children with type 1 diabetes without signs of nephropathy. Consequently, lipid disorders may be one of the factors that affect the kidney vasculature and blood pressure (117). In contrast, other studies have observed a reduced prevalence of micro/macroalbuminuria in obese children with T1D compared to those with healthy weight. Therefore, it cannot be ruled out that obesity carries some protective effect from micro/macroalbuminuria and this association requires further investigation (118).

It should be noted here that the American Heart Association identified type 1 diabetes as a high cardiovascular risk factor for paediatric patients, whilst obesity was placed in the "at risk" category. When obesity is severe, then it becomes a moderate risk factor for cardiovascular disease for these patients (119). Redondo et al. showed that there is an increased prevalence of cardiovascular risk factors in children with coexisting type 1 diabetes and overweight or obesity. In their study, obese children were more likely to be diagnosed with hypertension and dyslipidemia compared to those with healthy weight (3.5 and 2.2 times, respectively, after adjusting for age, sex, race/ethnicity, HbA1c and diabetes duration). Additionally, children with T1D and overweight had 1.4 times higher likelihood of having dyslipidemia despite rates of hypertension were similar to their normal weight peers (118). This seems to be confirmed by the recent study of Gomes et al. in which Brazilian adolescents with coexisting T1D and overweight/obesity had a higher prevalence of conventional risk factors for micro- and macrovascular complications of diabetes, such as duration of diabetes, hypertension, high LDL-cholesterol and presence of metabolic syndrome (120).

Although intensive insulin therapy has been proven to reduce the incidence of micro- and macrovascular complications of type 1 diabetes, its use is unfortunately associated with weight gain as a side effect of therapy. Therefore, its positive effects may be partially offset by the presence of obesity-related risk factors for cardiovascular disease. According to Purnell et. al, increased weight gain due to intensive insulin therapy is associated with central obesity, insulin resistance, progressive rise in blood pressure, dyslipidemia and increased measures of intima-media thickness and coronary artery calcium - subclinical markers linked to increased risk of cardiovascular disease (78, 108).

Atherogenic lipid profile connected with excessive body weight and also with unsatisfactory diabetes control in T1D patients are commonly known risk factor of CVD. This lipid profile is characterized by elevated levels of triglycerides, normal or slightly increased levels of LDL cholesterol and reduced levels of HDL cholesterol. The serum presence of dysfunctional HDL and small, dense LDL is prolonged and their binding to the arterial wall increases causing atherosclerotic changes (121). The prospective SEARCH study exhibited that the frequency of dyslipidemia with inadequate glycaemic control (HbA1c ≥ 9%), longer T1D duration, obesity, and hypertension correlated with arterial stiffness index and augmentation index (122). Moreover, dyslipidemia was observed in obese or overweight adolescents with well-controlled diabetes more often than in the group with insufficient diabetes control but normal BMI ranges (123). It shows that maintaining proper body weight in T1D patients is very important to reduce the risk of CVD.

OBESITY IN T1D AS A CAUSE OF ADDITIONAL DISEASES

A large number of studies has demonstrated obesity as one of the prominent causes of negative health outcomes, which impacts physical wellness, by increasing the risk for the development of among others asthma, sleep apnoea, metabolic syndrome,

osteoarticular and cardiovascular diseases, stroke or even certain types of cancers hence, higher total mortality, as well as sanity causing depression, decreased quality of life and poor self-esteem. Similarly to adults, obesity in children is correlated with analogous issues but in youth it may also cause femoral epiphyses or premature puberty. Additionally, poorer cognitive function, brain health, educational attainment may be more marked (124). All of listed above can be more dangerous for individuals with T1D because both obesity and T1D consequences can superimpose on each other leading to exacerbation of patients' health condition (**Table 3**).

Another serious health issue correlated with both obesity and T1D is metabolic syndrome (MS). It has multiplicitous definitions, although is widely considered as a constellation of hyperglycaemia, high insulin resistance, abdominal obesity, hyperlipidemia, and hypertension (125). Metabolic syndrome or several of its components can be associated with chronic complications from diabetes, for example an increased chance for cryptogenic sensory peripheral neuropathy, which impacts small unmyelinated axons early in its course or even autonomic and large neurons later on (126). Even though metabolic syndrome tends to be manifested more frequently in people with diabetes than in general population, a significantly higher prevalence of its criteria was demonstrated in a group of overweight and obese than in normal weight T1D patients (127).

Non-alcoholic fatty liver disease (NAFLD), considered lastly as one of the components of MS, is a common chronic liver disorder that coexists in people with obesity and diabetes. Its prevalence increases with age, progress of metabolic diseases and BMI (128). Forasmuch as adipose tissue dysfunction that occurs in obesity is believed to be a key contributor to the pathogenesis of NAFLD, it is reasonable to anticipate a negative impact on liver health in obese T1D patients. Unfortunately, there have been no published studies that have compared the NAFLD phenotype between T1D individuals with normal and higher BMI ranges. NAFLD was demonstrated to be associated with an increased risk of CVD events (129), distal symmetric polyneuropathy (130) and incidence of chronic kidney diseases (131) in type 1 diabetic adults. New terminology updated from NAFLD- metabolic dysfunction-associated fatty liver disease (MAFLD) was established recently. The global prevalence of MAFLD estimated by repurposing existing data on fatty liver disease was 33.78% in the general population and 44.94% in obesity clinics (132).

The metabolic comorbidities such as insulin resistance, hyperinsulinemia, dyslipidemia or impaired glucose tolerance as well as the psychological comorbidities including anxiety, depression, eating disorders, low self-esteem, psychosexual dysfunction or poor quality of life occurring in both obesity and diabetes can lead to development of polycystic ovary syndrome (PCOS), characterized by ovarian dysfunction and hormonal imbalance, often starts during adolescence (133). PCOS appears frequently in adolescent girls with T1D because of exposing the ovaries and the adrenals to excessive insulin concentrations (134). It is an important consequence since women with PCOS exhibit problems with fertility or even higher risk for the development of endometrial cancer (135).

Additionally, a study in a large cohort of T1D patients has provided genetic evidence for a causal association between obesity and kidney diseases in T1D. Mendelian randomization analysis with a genetic risk score comprised of 32 validated BMI loci showed a U-shaped relationship between BMI over lifespan conferring an increased risk for the development of microalbuminuria, diabetic kidney disease and end-stage renal disease (136).

TREATMENT OF OBESITY IN T1D, A PEDIATRIC PERSPECTIVE

Because type 2 diabetes is more common than type 1, and the prevalence of overweight or obesity is higher in patients with T2D than in those with T1D, most weight control strategies have been described for people living with type 2 diabetes. However, it is not known if the same interventions are safe and effective for patients with T1D. These individuals face many difficulties when attempting to control their weight, such as experiencing hypoglycaemic episodes during fasting, dietary carbohydrate restriction or exercise (71) For instance, in order to maintain normal blood glucose levels, people with T1D often need a supply of simple carbohydrates, which do not have ideal nutritional value but instead are a source of extra calories. Therefore, the management of type 1 diabetes may be challenging and not compatible with the principles of healthy eating and active lifestyle that are the basis of optimal weight management (137). Many both pharmacological and non-pharmacological approaches to the treatment of obesity in type 1 diabetes are described below.

TABLE 3 | Consequences of overlapping T1D and obesity complications.

T1D complications	Obesity complications	Consequences of overlapping
Macrovascular: atherosclerosis, thrombosis atherogenic lipid profile	Dyslipidemia Increased coronary artery calcium index Increased thickness of intima-media	Hypertension Increased risk of premature cardiovascular diseases Strokes
Hyperglycaemia	Insulin resistance Abdominal obesity Hyperlipidemia Hypertension	Metabolic syndrome
Hyperglycaemia	Adipose tissue dysfunction Elevated alanine aminotransferase	NAFLD-> CVD events, polyneuropathy, incidence of chronic kidney diseases
Impaired glucose tolerance Hyperinsulinemia	Dyslipidemia Insulin resistance Psychological comorbidities	PCOS: problems with fertility or higher risk for the development of endometrial cancer
Increased risk of nephropathy and albuminuria	High blood cholesterol Albuminuria	Kidney diseases, including end-stage renal disease

Weight Management

In its latest guidelines, the American Diabetes Association (ADA) recommends weight control and weight loss in people with both types of diabetes or pre-diabetes and coexisting overweight or obesity (138). Many dietary interventions used for weight reduction have been studied in people with or without diabetes, but very few studies have focused strictly on patients with type 1 diabetes (99). It is known that reduction in total calorie intake plays a key role in weight loss in obese individuals, regardless of the occurrence of diabetes (83). Nevertheless, the recommended management of weight reduction in T1D is a low-calorie diet, which is based on the Mediterranean diet pattern, where 40-50% of energy comes from carbohydrates with a low glycaemic index and high content of fiber, 15-25% of energy comes from protein and 30-35% from fat (with high content of monounsaturated fats and low content of trans and saturated fats) (83). At this point it is worth mentioning that recently trending low carbohydrate or ketogenic diets should be used with caution in people with type 1 diabetes. Despite their potential to improve metabolic control, they may cause potentially dangerous side effects such as increased risk of diabetic ketoacidosis and deterioration of the lipid profile (139). Children and adolescents with type 1 diabetes should not significantly restrict carbohydrate intake, as this may adversely affect their growth, worsen the metabolic profile, increase the risk of developing eating disorders and hypoglycemia and impair the effect of glucagon in its treatment (140). Therefore, the ADA recommends an individualized approach to nutrition therapy for children and adolescents with T1D under the guidance of an experienced dietitian as an important part of their treatment plan (100).

Physical Activity

Physical activity carries a number of physical and mental health benefits for youth with type 1 diabetes. Studies have shown that a combination of aerobics and strength training is beneficial in reducing not only BMI, but also HbA1c, triglycerides and total cholesterol in children with T1D. In addition, exercise significantly reduces depression, anxiety, and emotional disturbance, which may be important for youth with T1D who are at increased risk for depression (141). It has also been demonstrated that exercise increases lean body mass and energy expenditure at rest, reduces total and visceral adiposity and increases insulin sensitivity in children and adolescents (142). According to the recent American Diabetes Association (ADA) guidelines, all youth with type 1 diabetes are recommended to do 60 minutes of daily aerobic exercise of moderate- to vigorous-intensity and to perform vigorous muscle- and bone-strengthening exercises at least 3 times a week. To manage exercise-related hypo- and hyperglycemia, frequent measurement of blood glucose level before, during and after exercise is also recommended (100). Unfortunately, about two thirds of adolescents do not achieve the recommended time of physical activity. It is also a fact that children with type 1 diabetes are less physically active than their healthy peers (143). The main barrier for undertaking physical activity by these people appears to be the fear of hypoglycemia. Because of this, youth with T1D avoid or prematurely discontinue exercise or consume extra amounts of carbohydrates and calories, which eliminates the negative energy balance achieved through exercise (137). In parallel to carbohydrate consumption, insulin dose adjustment is a key factor in controlling blood glucose levels during and after exercise. Reducing insulin doses to protect against hypoglycemia induced by exercise is usually necessary for prolonged (more than 30 minutes) moderate-intensity activity. Fortunately, rapidly progressing diabetes technology helps patients with T1D to manage their blood glucose levels during exercise and gives them many tools, such as smartphone apps, insulin pumps, CGM and closed-loop technology (144). What is important, if proper measurements before exercise are carried out, patients with type 1 diabetes should be able to safely engage in both aerobic and weightlifting physical activities (99). Physical activity, which is an effective weight management intervention, presents many challenges for youth with type 1 diabetes and overweight or obesity. Thus, there is a need to develop safe strategies for undertaking physical activity in these patients.

Multifactorial Approach to Obesity Prevention in Type 1 Diabetes

As mentioned earlier, the co-occurrence of obesity and type 1 diabetes in children increases the risk of developing vascular complications of diabetes and may also cause additional diseases. Therefore, early interventions are needed to maintain a healthy body weight in such patients. Prevention and management of excessive weight gain are crucial in the care of youth with type 1 diabetes. Guidelines concerning correct nutritional habits and physical activity for the entire family are required, because parents and family members have a significant impact on a child's lifestyle. Thus, the family approach in the management of excessive body weight seems to be the most efficient, considering that parents are most often children's role models who can influence their dietary choices and encourage them to exercise. In order to promote patient's physical activity, advice needs to be given about their likely blood glucose responses. The counseling should include safety information and be suited to each person (140, 145). Hence, education of patients and their families on nutritional therapy and management of exercise-induced hypo- and hyperglycemia by qualified professionals is essential for self-management of the disease (146).

Pharmacotherapy

Interestingly, it has been shown that insulin adjunctive therapies may have positive effects on weight management in people with type 1 diabetes, even though they were designed to improve glycaemic control (71). Although they are gaining popularity among adults, they have not been sufficiently researched with regard to children (137). The increasing prevalence of obesity additionally exacerbates the already existing insulin resistance among patients with T1D, which creates a need for adjunctive therapies in type 1 diabetes to reduce the risk of cardiovascular disease (147).

Metformin

Metformin is a widely used oral antidiabetic drug that improves glycaemic in patients with type 2 diabetes in several ways, such as reducing glucose production in the liver and promoting glucose uptake in peripheral tissues, especially in the muscle (148). In adult patients with type 2 diabetes, this drug has been demonstrated to reduce the risk of cardiovascular disease and improve their body composition (147). Results from multiple studies show the promising potential of metformin as an insulin adjunctive therapy for poorly controlled overweight youth with type 1 diabetes to reduce cardiovascular disease risk (149). The findings of a randomized, double-blinded and controlled trial performed by Bjornstad et al. indicate that 3-month metformin therapy, which was additional to insulin administered to adolescents with type 1 diabetes mellitus, improved their insulin sensitivity and vascular health and also reduced their weight, body mass index and adiposity (147). These results are confirmed by the study by Libman et al. which also shows that adding metformin to insulin does not improve glycaemic control in overweight adolescents with type 1 diabetes after 6 months of such therapy (148). Consistent with these results are those by Nadeau et al. which also show a beneficial effect of metformin on insulin sensitivity, BMI and body composition in young people with type 1 diabetes (150). Metformin is found to be safe for adolescent children, with only a risk of minor gastrointestinal side effects, while acute side effects were not present in the conducted studies (149).

GLP-1 Receptor Agonists and DPP-4 Inhibitors

GLP-1 is a hormone secreted from intestinal enteroendocrine cells. In a glucose-dependent manner it increases the secretion of insulin and inhibits the release of glucagon. It also delays gastric emptying and reduces appetite, leading to weight loss. It is inactivated by the enzyme dipeptidyl peptidase-4 (DPP-4) (72). GLP-1 receptor agonists such as exenatide or liraglutide are now more commonly used in the treatment of type 2 diabetes. These are not yet authorized for the treatment of patients with type 1 diabetes, but there is growing interest in their safety and efficacy as adjunctive therapy to insulin in patients with T1D (137). The primary mechanism of action of these drugs is to increase postprandial insulin secretion, which is not possible in patients with type 1 diabetes, but these patients may be able to take advantages from other effects provided by the drug (97). It has been demonstrated that both exenatide and liraglutide reduce A1C, body weight, fasting and postprandial blood glucose levels and insulin doses in adults with type 1 diabetes. At the same time, the risk of hypoglycemia in these patients is not increased, but there is an enhanced prevalence of gastrointestinal side effects (151, 152).. The results of the Traina et al. study, which investigated the use of once-weekly treatment with exenatide as add-on therapy to insulin for adult patients with uncontrolled type 1 diabetes, are consistent with this observation (153). Furthermore, Ghanim et al. in their study revealed that the use of liraglutide in adults with coexisting overweight or obesity and type 1 diabetes significantly reduces body weight (primarily through fat mass loss), improves the glycemic profile and lowers systolic blood pressure. Therefore, GLP-1 receptor agonists may be considered as adjunctive therapy to insulin in adult patients with type 1 diabetes, whereas there are no data regarding the use of these drugs in children (151, 154).

The review by Wang et al. indicates that DPP-4 inhibitors do not significantly reduce HbA1c levels or insulin dose and do not affect body weight and BMI in patients with T1DM (155). However, as GLP-1 receptor agonists show potential for the treatment of overweight and obesity in type 1 diabetes, more emphasis should be placed on research not only in adults but also in children and adolescents with this disease. Unfortunately, there is lack of data regarding the use of these drugs in this group.

Pramlintide

As a synthetic analogue of amylin, a hormone that is secreted together with insulin, pramlintide supplements the action of insulin by decreasing postprandial glucagon output, delaying gastric emptying and promoting satiety (156). The Food and Drug Administration (FDA) has officially approved pramlintide as the only drug that can be used as a additional therapy to insulin to treat patients with type 1 diabetes in United States, but it should be noted that it is not approved for use in Europe (157, 158). Studies show that pramlintide as an add-on therapy to insulin for patients with type 1 diabetes brings beneficial effects such as improving glycaemic control, reducing insulin dose and body weight, while causing transient hypoglycemia and gastrointestinal side effects such as nausea, vomiting and anorexia at the beginning of treatment (159). There are limited data regarding the use of this drug in children with type 1 diabetes, but few studies show the beneficial effect of using such therapy in the pediatric population (160, 161).

SGLT-2 Inhibitors

The sodium-glucose transporter SGLT-2, which is present in the proximal tubule and overexpressed in diabetic patients, promotes glucose reabsorption in this part of the nephron. Therefore, drugs that inhibit this transporter affect this process (83). SGLT2 inhibitors are available for the treatment of type 2 diabetes and the results of many studies have proven that they are safe and effective in treating this disease (157). In some countries, a few SGLT-inhibitors can be used as add-on therapy for patients with type 1 diabetes. Dapagliflozin has been approved for the treatment of adults with this disease in the United Kingdom, while the European Commission approved both dapagliflozin and sotagliflozin in 2019 for adults with T1D with BMI ≥ 27 kg/m2. In Japan, ipragliflozin can be used to treat such patients with T1D from 2018, while the United States FDA has not authorized any SGLT-2 inhibitor for type 1 diabetes patients (162).

The results of the meta-analysis conducted by El Masri et al. clearly indicate that SGLT2 inhibitor therapy in comparison to placebo resulted in a reduction in HbA1c, body weight, and total daily insulin dose in patients with type 1 diabetes. Since the combination of these drugs with insulin results in a reduction in total daily insulin dose, it may have the positive effect of reducing the frequency of dose-related insulin side effects such as

hypoglycemia and weight gain (163). Importantly, the use of SGLT-2 inhibitors may be associated with the occurrence of euglycaemia diabetic ketoacidosis in both T1D and T2D patients. It is worth noting that type 1 diabetic patients treated with insulin pumps appear to be the highest risk group due to not taking long-acting insulin and sometimes experiencing infusion difficulties (164). In addition, other studies have shown an increased incidence of side effects, such as urinary tract and genital infections, which were associated with the use of these drugs (165). Unfortunately, published studies regarding the use of SGLT-2 inhibitors as an add-on therapy to insulin for the treatment of type 1 diabetes have focused mainly on the adult population (137), although in one study by Biester et al. indicated a potential benefit of using dapagliflozin in children over 12 years of age in reducing the average dose of insulin (166).

All of this points to the fact that the use of non-insulin drugs in the treatment of children with coexisting obesity and type 1 diabetes may have great potential for improving their glycaemic profile and reducing body weight. However, most of the studies are focused on the use of these drugs in adults, so further research is needed to provide evidence of the safety and efficacy of using insulin adjunctive therapies in the paediatric population.

Bariatric Surgery

Bariatric surgery may be an option for people with type 1 diabetes who find it impossible to overcome obesity with the aforementioned methods (71). It has many advantages for individuals with T1D, such as weight reduction, lower total daily insulin dose, and less presence of with obesity comorbidities. However, it may have minimal impact on glycaemic control of these patients in the long term (167). Landau et al. conducted a study to examine the short- and long-term implications of bariatric surgery in patients with coexisting obesity and type 1 diabetes. They found that patients who underwent bariatric surgery experienced significant weight loss and improvements in both blood pressure and lipid profile. In contrast, the surgery did not result in improved glycaemic control in these patients. It should be noted that after surgery, 15% of subjects experienced diabetic ketoacidosis while 23% developed acute hypoglycemic episodes (168). Multiple studies confirm the occurrence of these side effects of bariatric surgery in patients with T1D (169). There are limited data on the outcomes of these surgeries in youth with type 1 diabetes. Nevertheless, there are two case reports describing the results of bariatric surgeries due to severe obesity in two adolescents with type 1 diabetes. The first obese male underwent a vertical sleeve gastrectomy. One year after surgery, he weighed 91 kg compared with 125 kg on admission. His total insulin requirement was lower as well, and one year after surgery there was a reduction in LDL levels from 180 mg/dl to 81 mg/dl and an increase in HDL levels from 32 mg/dl to 45 mg/dl. HbA1c levels remained unchanged. The second female patient with a primary diagnosis of type 2 diabetes treated only with metformin underwent Roux-en-Y gastric bypass surgery, and one month after surgery she developed diabetic ketoacidosis with the detection of positive islet antibodies, which turned out to be present also before surgery. As a result, the diagnosis was changed to T1D and the administration of insulin was started. 28 months after surgery, her BMI decreased by 42% and similar to the first patient, her lipid profile improved and her insulin requirement decreased. In contrast, her HbA1c level increased from 6.3% to 10% (170). The use of this method for the management of obesity in patients with type 1 diabetes requires further investigation to determine if the benefits of bariatric surgery outweigh its risks, such as occurrence of diabetic ketoacidosis and hypoglycemia, also in relation to paediatric population. A review of potential adjuvant pharmacological therapies to insulin in obese patients with type 1 diabetes is presented in **Table 4**.

CONCLUSIONS

More and more children with T1D exhibit excessive body weight which previously was the domain of T2D patients. The prevalence of obesity in children with T1D creates many difficulties in the diagnostic process, mainly due to the overlapping phenotypes of both type 1 and type 2 diabetes, which has led to the distinction of the term double diabetes. It is worth adding that the coexistence of obesity and type 1 diabetes can alter or even disrupt the classic course of type 1 diabetes. Obesity can both contribute to the development of T1D and also be its result. Even though insulin is the treatment of choice in T1D, in addition to lack of physical activity, improper diet and

TABLE 4 | A review of potential adjuvant pharmacological therapies to insulin in obese patients with type 1 diabetes.

Name of the drug	Mechanism of action	The benefits for obese patients with T1DM	Side effects
Metformin	• Reducing gluconeogenesis in the liver, promoting glucose uptake in peripheral tissues	• Reducing cardiovascular risk, improving insulin sensitivity, reducing weight, BMI and adiposity	• Increased prevalence of minor gastrointestinal side effects
GLP-1 receptor agonists	• Increasing the secretion of insulin and decreasing the release of glucagon in a glucose-dependent manner	• Reducing HbA1c, BMI, fasting and postprandial blood glucose levels and insulin dose	• Increased prevalence of gastrointestinal side effects
DPP-4 inhibitors	• Inhibition of GLP-1 degradation	• No significant effect on the reduction of HbA1c, body weight, BMI or insulin dose	• Unknown
Pramlintide	• Decreasing postprandial glucagon output, delaying gastric emptying, promoting satiety	• Improving glycaemic control, reducing insulin dose and body weight	• Transient hypoglycemia, gastrointestinal side effects
SGLT-2 inhibitors	• Inhibiting glucose reabsorption in proximal tubule of nephron	• Reduction in HbA1c level, body weight and total daily insulin dose	• Euglycemic diabetic ketoacidosis, urinary tract and genital infections

fear of hypoglycaemia, it is also one of the factors contributing to weight gain in children with T1D.

Studies clearly show that, in comparison to their normal weight peers, overweight/obese children have a higher incidence of diabetes complications and further diseases resulting from them. Obesity in children with T1D should definitely be treated as early as possible to prevent especially cardiovascular risk factors, since cardiovascular diseases persist the leading causes of premature death among T1D patients. Although insulin therapy remains the most effective drug in improving glycaemic control in people with T1D, its use carries a risk of unexpected weight gain. Therefore, there is a need for new adjunctive drugs to insulin treatment that would help manage excessive body weight in patients with type 1 diabetes. Although such potential is demonstrated by drugs widely used in the treatment of T2D, there are still few reports describing the safety and efficacy of their use in people with T1D, especially in paediatric population.

It must not be forgotten that maintaining a healthy lifestyle at every stage of treatment remains a very important factor in the weight loss process. The problem of obesity in youth diagnosed with type 1 diabetes is a huge challenge for all patients, parents and diabetic medical teams, and everything points to the fact that this problem will continue to grow. Thus, most studies suggest that excessive weight gain reduces or nullifies the benefits of metabolic control. Consequently, further research is required to solve the issue of co-occurrence of obesity and type 1 diabetes in the paediatric population.

AUTHOR CONTRIBUTIONS

SC, EK - screened the literature search results and assessed for the eligibility for inclusion criteria, made substantial equal contribution to study design and conception, acquisition, analysis and interpretation of data, and wrote the original version of the paper. BG-O supervised the project, made substantial contribution to study design and conception, acquisition, analysis and interpretation of data, and revised the paper. AB was involved in the design, conception, analysis, and revised the paper. All authors contributed to discussion, read, and approved the final version of the manuscript.

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