



Vitamin B₁₂ Supplementation and NT-proBNP Levels in COPD Patients: A Secondary Analysis of a Randomized and Controlled Study in Rehabilitation

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Purpose: There is evidence of complex interaction between vitamin B₁₂ (vB₁₂) level, hyperhomocysteinemia (HyCy), and natriuretic peptide secretion. Exercise training could also modulate such interaction. In this secondary analysis of a Randomized Clinical Trial performed in a chronic obstructive pulmonary disease (COPD) rehabilitation setting, our primary objective was to investigate the interaction between vB₁₂ supplementation, exercise training, and changes in NT-proBNP levels after 8 weeks of intervention. Secondary objectives were to explore the correlations between acute changes in NT-proBNP levels with (i) acute exercise and (ii) oxygen uptake ($V'O_2$) kinetics during rest-to-exercise transition.

Methods: Thirty-two subjects with COPD were randomized into four groups: Rehabilitation+vB₁₂ ($n = 8$), Rehabilitation ($n = 8$), vB₁₂ ($n = 8$), or Maltodextrin ($n = 8$). They were evaluated at baseline and after 8 weeks, during resting and immediately after maximal exercise constant work-rate tests (CWTs, T_{lim}), for NT-proBNP plasmatic levels.

Results: After interaction analysis, the supplementation with vB₁₂ significantly changed the time course of NT-proBNP responses during treatment ($p = 0.048$). However, the final analysis could not support a significant change in NT-proBNP levels owing to high-intensity constant work-rate exercise (p -value > 0.05). There was a statistically significant correlation between $V'O_2$ time constant and Δ NT-proBNP values ($T_{lim} - rest$) at baseline ($p = 0.049$) and 2 months later ($p = 0.015$), considering all subjects ($n = 32$).

Conclusion: We conclude that vB₁₂ supplementation could modulate NT-proBNP secretion. Moreover, possibly, the slower the initial $V'O_2$ adjustments toward a steady-state during rest-to-exercise transitions, the more severe the ventricular chamber volume/pressure stress recruitment, expressed through higher NT-proBNP secretion in subjects with larger $V'O_2$ time constants, despite unchanged final acute exercise-induced neurohormone secretion.

Keywords: COPD, exercise training, hyperhomocysteinemia, natriuretic peptides, vitamin B₁₂

INTRODUCTION

In a recent Randomized Controlled Trial (RCT), we showed a slight but significant increase in maximal exercise tolerance (T_{lim}) in patients with chronic obstructive pulmonary disease (COPD) supplemented with vitamin B₁₂ during physical training, but without effects on oxygen uptake ($V\dot{O}_2$) kinetics beyond training alone (Paulin et al., 2017). Individuals with COPD are at risk of vitamin B₁₂ deficiency (Solomon, 2016) and hyperhomocysteinemia (HyCy) (Seemungal et al., 2007; Fimognari et al., 2009). Accordingly, HyCy is linked to numerous cardiovascular alteration (Ganguly and Alam, 2015) including histological changes in the heart (Piquereau et al., 2017), impaired global and segmental cardiac contractility (Kaya et al., 2014), or increased N-terminal-pro-B-type natriuretic peptide (NT-proBNP) secretion (Herrmann et al., 2007; Guéant Rodriguez et al., 2013). This prepeptide is synthesized and stored as a high molecular weight mass propeptide from both the atria and ventricles, and released mainly under pressure/volume overload of the cardiac chambers, after cleavage of the active form of BNP, inducing natriuresis and vasodilatation (Calzetta et al., 2016). However, NT-proBNP has a longer plasma half-life and attains larger concentrations, besides being described as a significant marker of prognosis in heart failure (HF) (Cipriano et al., 2014). Moreover, vitamin B₁₂ or folic acid supplementation reduced NT-proBNP levels after 2 months of supplementation in subjects with NT-proBNP > 40 pg/mL (Herrmann et al., 2007), mitigated HyCy-induced cardiac dysfunction (Jeremic et al., 2018), and proved to be protective for mitochondrial function and cardiac contractile properties in a murine model, with lessening of upregulated atrial brain natriuretic peptide (ANP) (Piquereau et al., 2017). In contrast, an experimental study was negative for cardiac morphological alterations during HyCy induction (Taban-Shomal et al., 2009).

It is recognized that physical training causes a reduction in cardiac natriuretic peptides in HF (Cipriano et al., 2014) as well as which, a beneficial interaction between physical training and vitamin B₁₂ or folate supplementation in reducing HyCy has been suggested (König et al., 2003; Tyagi and Joshua, 2014). Of note, there is evidence of increased secretion of BNP associated with increased pulmonary vascular resistance during acute exercise in COPD (Fujii et al., 1999); a mechanism which demonstrates potential for attenuation through physical training. Thus, this secondary analysis aims primarily to explore the interaction between vitamin B₁₂ supplementation, physical training, and resting/exercise levels for NT-proBNP in a stable population of COPD patients. In addition, we sought to analyze possible associations between acute changes in NT-proBNP levels during exercise with T_{lim} , delivered power (watts, w), and oxygen uptake kinetics ($V\dot{O}_2$ time constant) on an ergometer, in order to explore the determinants of these possible changes. The central hypothesis was that there would be attenuation of neurohormone alterations with supplementation alone or combined with physical training.

MATERIALS AND METHODS

As this is a secondary study of an already published RCT, the entire methodology has been previously described in detail (Paulin et al., 2017). Similarly, ethical considerations and consent details are published and recorded in the Brazilian Clinical Trials Registry (ReBEC number RBR-55f97c/2014). Additional unpublished methods will be considered in this exploratory study.

Participants and Study Design

In the final analysis, 32 stable COPD patients were consecutively randomized to four groups: (1) 8-weeks physical rehabilitation (REHA) group, (2) 8-weeks physical rehabilitation group with daily vitamin B₁₂ supplementation of 500 mg (REHA+B₁₂), (3) supplementation group as stand-alone with daily vitamin B₁₂ supplementation of 500 mg (B₁₂), and (4) placebo group (maltodextrin 500 mg) (P). All groups continued with their usual optimized pharmacological treatment for COPD. Among the subjects who completed the study, 28/32 were already being followed up at the specialized COPD clinic and had an echocardiogram performed within the previous 6 months. Mild mitral or aortic reflux and ventricular hypertrophy were accepted in the inclusion criteria. Patient history and further detailed physical examinations did not show signs of associated heart disease in the remaining subjects recruited without echocardiography.

Standard Doppler Echocardiography

All transthoracic echocardiography followed standard guidelines (Lang et al., 2005). Measurements of the cardiac cavities, interventricular septum, and left ventricular posterior wall thickness were collected by M-mode and two-dimensional analysis. Left atrial volume used the biplane Simpson method and was indexed by body surface. The ejection fraction was measured by the Teichholz method. The tricuspid reflux velocity was obtained by continuous Doppler in the right ventricle inlet, and the sPAP value was calculated by adding 10 mmHg of pressure in the right atrium.

Cardiopulmonary Exercise Testing (CPET)

All subjects were invited to perform an incremental cardiopulmonary exercise testing (CPET) and two equal constant work-rate tests (CWTs, 75% of the maximum incremental CPET load) to calculate time constants (τ , τ) for $V\dot{O}_2$ during the rest-to-exercise transition. Detailed CWT methods and oxygen uptake kinetics analysis were previously published (Paulin et al., 2017; Müller et al., 2019). Blood samples were collected at rest and T_{lim} during the first CWT, before and after 8 weeks, for NT-proBNP plasmatic level analysis (Figure 1).

NT-proBNP Analysis

After a suitable rest period, before the CWT, a polyurethane 22G catheter (Injex-Cath, Ourinhos, Brazil) was inserted on the back of the patient's right hand for venous blood sample

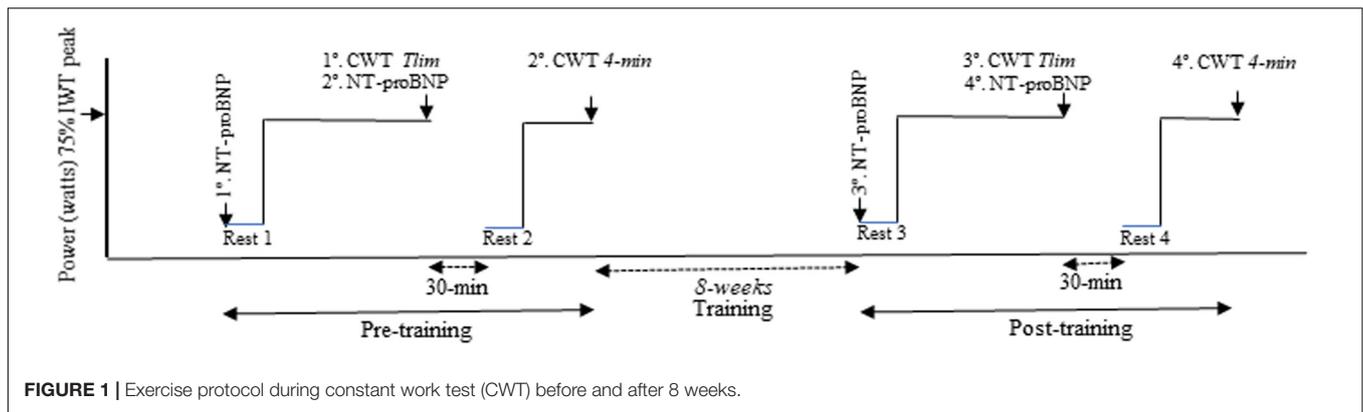


TABLE 1 | Selected baseline clinical and physiological characteristic of the four groups of COPD patients.

	REHA+B ₁₂	REHA+P	B ₁₂	P	p-value
Subjects, n	8	8	8	8	1.000
Anthropometry					
Age (years)	56.5 ± 5.0	65.2 ± 6.0	63.4 ± 5.2	58.1 ± 10.3	0.156
Gender (M/F)	3/5	3/5	5/3	5/3	0.289
BMI (kg/m ²)	24.5 ± 2.9	25.1 ± 6.0	24.5 ± 4.0	28.3 ± 5.5	0.332
Lung function					
FVC, % pred	65.2 ± 16.0	72.2 ± 10.0	66.0 ± 13.0	73.0 ± 13.1	0.534
FEV ₁ , % pred	34.0 ± 11.0	39.2 ± 6.8	32.8 ± 7.6	41.7 ± 12.3	0.235
FEV ₁ /FVC, %	40.7 ± 10.4	43.5 ± 9.2	41.2 ± 11.4	44.9 ± 6.8	0.695
PaO ₂ , mmHg, rest	66.2 ± 9.3	73.8 ± 9.1	76.4 ± 16.0	75.5 ± 7.6	0.348
CPET data					
V'O _{2peak} , % pred	50.5 ± 16.1	65.1 ± 17.7	68.8 ± 18.2	64.7 ± 18.2	0.191
W _{peak} , % pred	43.6 ± 12.4	46.8 ± 13.1	34.7 ± 12.0	43.1 ± 15.2	0.324
T _{lim} , s	410 ± 311	314 ± 230	259 ± 110*	436 ± 143	0.041
τ, s	65 ± 37	84 ± 39	69 ± 22	69 ± 25	0.617
k, 10 ⁻³ /s	17.7 ± 7.1	13.1 ± 6.2	14.3 ± 4.5	15.7 ± 4.4	0.443
Blood analysis					
Vitamin B12 (pre), pg/mL	385 ± 207	454 ± 309	451 ± 215	467 ± 114	0.877
Vitamin B12 (post), pg/mL	567 ± 227 [†]	358 ± 177	544 ± 145	442 ± 204	0.148
ΔVitamin B12, pg/mL	182 ± 206	-71 ± 175	93 ± 262	-16 ± 103	0.060
Vitamin B12 < 300 pg/mL, n	3	4	3	1	0.340
Creatinine, mg%	0.8 ± 0.2	0.9 ± 0.2	0.8 ± 0.1	0.8 ± 0.1	0.314
Hematocrit, %	47 ± 3	46 ± 4	43 ± 5	43 ± 3	0.118
Hemoglobin, g %	15 ± 1	15 ± 1	14 ± 2	14 ± 1	0.102
Echocardiography					
LVM, g/m ²	156 ± 22	132 ± 21	108 ± 17	122 ± 35	0.332
EF, %	70 ± 3.8	66 ± 2.1	69 ± 4.8	65 ± 4.2	0.126
LA, mm	36 ± 1.4	37 ± 4.5	30 ± 1.2	31 ± 2.4	0.201
LVDD, mm	46 ± 2.1	49 ± 1.9	45 ± 2.1	44 ± 2.9	0.133
PAP, mmHg	32 ± 2.1	29 ± 1.7	32 ± 1.9	29 ± 2.3	0.070

Abbreviations: BMI, body mass index; FVC, forced vital capacity; FEV₁, forced expiratory volume in 1 s; DL_{co}, carbon monoxide diffusion capacity; PaO₂, partial pressure of oxygen in arterial blood; V'O_{2peak}, peak oxygen consumption; T_{lim}, maximal tolerance exercise time; τ, time constant (tau); TD, time delay; k, rate of V'O₂ increase toward steady-state; LVM, left ventricular mass; EF, ejection fraction; LA, left atrium; LVDD, left ventricular diastolic diameter; PAP, pulmonary arterial pressure. *p < 0.05 B₁₂ vs P; [†]p < 0.05 B₁₂ pre vs B₁₂ post (Group REHA+B₁₂). The Bold is only to highlight significant values, i.e. p-value < 0.05.

collections at rest and during exercise. The material was collected in tubes containing a mixture of plasma-lithium, as recommended by the manufacturer, and incubated at -20°C for 30 min. The electrochemiluminescence sandwich immunoassay by COBAS e602® system (Roche Diagnostics, Germany) measurement method was used, with a measurement range of 5–35,000 pg/mL and predicted coefficient of variation < 3.1%.

Data Analysis and Statistics

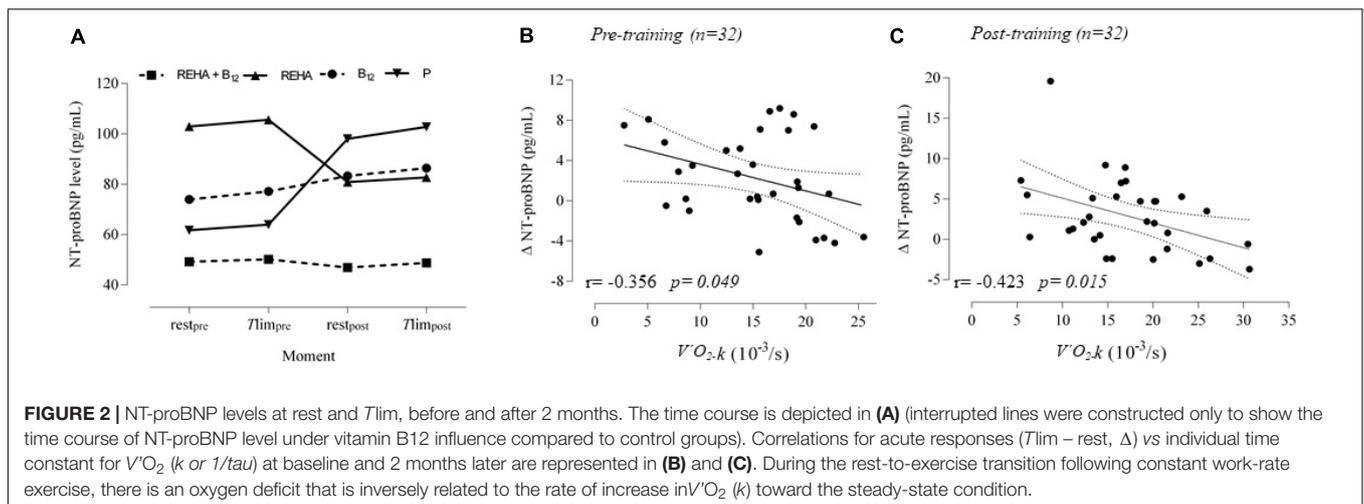
Data are expressed as mean ± standard deviation (SD) or median (IQR), mirroring, respectively, Gaussian or non-Gaussian distribution after the Shapiro–Wilk test. As τ (time constant, *tau*)—representing the time to attain 63% of the steady-state $\dot{V}O_2$ during a CWT—demonstrates marked skewness, we performed a reciprocal transformation and used k ($k = 1/\tau$) instead of τ . In exponential growth, k is equal to the reciprocal of the time constant *tau*, i.e., $k = 1/\tau$ and represents the rate of increase in $\dot{V}O_2$ toward a steady-state plateau. Hence, the faster the rate of adjustment (k) of $\dot{V}O_2$ toward the steady-state during rest-to-exercise constant work-rate exercise transitions, the lower the time constant *tau*. Thus, we chose to perform Pearson product-moment

coefficients for correlations using k , as k generates a more robust Gaussian distribution (Bland and Altman, 1996) and would imply similarly to a plausible physiological significance, as we recently suggested (Müller et al., 2019). The slopes of linear regression for pre- and post-training moments were compared with Univariate ANCOVA. In the temporal analysis of the effects of training and supplementation, we performed three-way repeated measures (RM) ANOVA with two between-subjects factors (training and supplementation) and four within-subjects RM (NT-proBNP level at rest/*Tlim* at baseline and rest/*Tlim* after 2 months), taking into account the sphericity standard and the Greenhouse-Geisser correction. In addition, one-way ANOVA for groups was performed for comparison of baseline characteristics and one-way RM ANOVA for time-dependent within-group NT-proBNP level changes. The calculated study power > 0.8, with an alpha risk of 0.05 (two-tailed), was calculated from the placebo Group with their respective SD differences. Thus, we considered the average within-subjects SD difference of 1 pg/mL for NT-proBNP and, two between-subjects factors, B₁₂ and exercise, with measured SD of the difference of 103 pg/mL and 100 s, respectively, in a three-way RM ANOVA design. We defined a p -value < 0.05 as statistically significant. The statistical program

TABLE 2 | NT-proBNP levels during acute exercise (rest and *Tlim*) evaluated at baseline and after 2 months of intervention.

Group	Test time				One-way RM ANOVA ¹ p -value	Three-way RM ANOVA p -value
	Baseline		2 Months			
	Rest	<i>Tlim</i>	Rest	<i>Tlim</i>		
REHA+B ₁₂	41.1 (25.8–77.1)	42.7 (23.7–82.3)	50.1 (30.1–64.1)	51.0 (30.2–69.4)	0.958	0.075
REHA+P	95.0 (43.9–140.4)	100.3 (45.2–146.8)	49.5 (21.8–157.1)	50.5 (25.9–157.0)	0.204	0.917
B ₁₂	55.9 (41.1–121.8)	60.9 (43.7–126.8)	72.9 (40.8–134.7)	75.7 (47.6–133.2)	0.639	0.048
P	20.7 (18.8–111.6)	22.1 (20.1–118.2)	74.4 (21.9–175.1)	75.3 (25.2–177.6)	0.120	0.525
Average (min-max)	49.5 (27.9–123.3)	51.0 (24.2–130.4)	64.0 (27.8–99.4)	68.9 (29.7–105.0)	–	–
One-wayANOVA ² p -value	0.090	0.127	0.400	0.372		

Abbreviations: REHA, rehabilitation; B₁₂, vitamin B₁₂; P, placebo; *Tlim*, time for maximal exercise tolerance. ¹within-subjects one-way RM ANOVA; ²between-subjects one-way RM ANOVA. The Bold is only to highlight significant values, i.e. p -value < 0.05.



SPSS 20.0 was used for all statistical analysis (SPSS, IBM Corp, United States, 2011).

RESULTS

Selected baseline characteristics are described in **Table 1** and detailed data have been previously published (Paulin et al., 2017). The groups were relatively balanced, with a significant difference only for T_{lim} at baseline ($p = 0.041$, **Table 1**). Despite a similar baseline PaO₂, the groups as a whole ($n = 32$) presented significantly reduced hemoglobin saturation through peripheral oximetry post-exercise ($p < 0.0001$). After interaction analysis, the supplementation with vitamin B₁₂ significantly changed the time course of NT-proBNP level during treatment ($p = 0.048$, **Table 2** and **Figure 2A**). In addition, the final analysis did not support a significant change in NT-proBNP levels owing to high-intensity constant work-rate exercise (three-way RM ANOVA analysis and one-way RM ANOVA analysis with p -value > 0.05 for both, **Table 2**). A statistically significant correlation was observed only between $\dot{V}O_2$ time constant and Δ NT-proBNP level ($T_{lim} - rest$) at baseline ($p = 0.049$, **Figure 2B**) and 2 months later ($p = 0.015$, **Figure 2C**), considering all subjects ($n = 32$), with an absence of significant correlations between Δ NT-proBNP level and T_{lim} or delivered Power on the cycle ergometer ($p > 0.05$ for both). The pre- and post-training slopes representing the correlation between the $\dot{V}O_2$ time constant and Δ NT-proBNP (**Figures 2B,C**) were not significantly different for the groups as a whole ($p = 0.259$).

DISCUSSION

This exploratory study suggests that B₁₂ measurements and/or supplementation should be considered in future studies on cardiovascular morbidity in COPD subjects. This is in line with the overall pathophysiology of cardiovascular morbidity in COPD which has not yet been totally unraveled. In addition, we did not detect acute changes in plasma levels for NT-proBNP during high-intensity constant work-rate exercise. However, during the rest-to-exercise transition, patients with slower $\dot{V}O_2$ adjustments toward a steady-state appeared to secrete higher NT-proBNP levels.

Normal levels of vitamin B₁₂ do not rule out the possibility of cobalamin deficiency, and *low-normal* levels (200–300 pg/mL), when associated with risk factors for increased oxidative stress, demonstrate metabolic evidence of deficiency, with high levels of HyCy or methylmalonic acid (MMA), since this vitamin is inactivated by oxidation (Solomon, 2016). Our study population contained at least three subjects in each group with levels < 300 pg/mL of vitamin B₁₂ and supplementation significantly increased their levels after 8 weeks for the REHA+B₁₂ group and not significantly for the B₁₂ group (Paulin et al., 2017).

Several previously cited animal and human studies have described a relationship between vitamin B₁₂ or folic acid level, HyCy, and NT-proBNP levels. Although preliminary,

our study points to an attenuating effect on NT-proBNP secretion with vitamin B₁₂ supplementation, in agreement with previous studies (Herrmann et al., 2007; Guéant Rodriguez et al., 2013), despite weak evidence. In monitoring the evolution of HF, attenuated or stable levels of NT-proBNP secretion were associated with fewer total cardiovascular events (Franke et al., 2011). Although the mechanisms underpinning this small but significant effect are largely unexplored, downregulation of natriuretic peptide production by reducing homocysteine and MMA accumulation under certain conditions is possible (Piquereau et al., 2017).

Two important secondary findings were described. Although we did not find an acute change in NT-proBNP owing to acute exercise during high-intensity CWT, there was a small but significant relationship between an acute change in this neurohormone (Δ) and oxygen uptake kinetics. Our study differs from a previous study, which showed increased secretion of BNP after exercise in a population similar to ours during CWT (Fujii et al., 1999). These contradictory results occurred despite both presenting significant hemoglobin desaturation during exercise. Arterial hypoxemia is a known trigger for natriuretic peptide secretion (Fujii et al., 1999). Surprisingly, our study agrees with another study for NT-proBNP also under CWT in mild-to-moderate COPD (Wang et al., 2011). Differences may reflect other factors, such as the secretion-to-metabolization ratio of natriuretic peptides and differences in exercise protocol. Our data suggest that much of the secretion of natriuretic peptides during CWT occurs at the time of initial adjustment during rest-to-exercise transitions, where the sudden change in the cardiac chamber pressure/volume condition is additionally stressed by the direct effect of transmural pressure, due to the abrupt increase in intrathoracic respiratory pressure swings, following the kinetics of the increase in pulmonary arterial pressure during the first minute and posterior decay during exercise (Lonsdorfer-Wolf et al., 2004). In this sense, our study is in agreement with a previous study that showed a direct relationship between BNP secretion and oxygen deficit during constant work-rate exercise in HF (Brunner-La Rocca et al., 1999).

As a limitation of this study, we cite the small number of individuals, notwithstanding this being partially tempered by the strong study design. In addition, we did not measure homocysteine and MMA levels. Four subjects were not evaluated by echocardiography; however, clinical data, NT-proBNP levels, and CPET analysis were not compatible with major heart disease.

CONCLUSION

We suggest that vitamin B₁₂ supplementation could modulate NT-proBNP secretion, but the effects are small and further studies are needed. Moreover, we did not find an increase in neurohormone level caused by acute exercise *per se*; however, there was an association with slower $\dot{V}O_2$ adjustment during the rest-to-exercise transition. The significance of this neurohormone dynamic warrants more detailed studies, considering different modalities of exercise training,

e.g., high-intensity 1-min bouts, with unexplored cardiac neuro-hormone signaling and unknown cardiovascular consequences.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Human Research Ethics Committee, affiliated to Brazilian Clinical Trial Registry and Federal University of Mato Grosso Do Sul. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

PM contributed to study design, literature search, data collection, analysis of data, and manuscript preparation

REFERENCES

- Bland, J. M., and Altman, D. G. (1996). Transforming data. *B.M.J.* 312:770.
- Brunner-La Rocca, H. P., Weilenmann, D., Follath, F., Schlumpf, M., Rickli, H., Schalcher, C., et al. (1999). Oxygen uptake kinetics during low level exercise in patients with heart failure: relation to neurohormones, peak oxygen consumption, and clinical findings. *Heart* 81, 121–127. doi: 10.1136/hrt.81.2.121
- Calzetta, L., Orlandi, A., Page, C., Paola, R., Barbara, R., Giuseppe, R., et al. (2016). Brain natriuretic peptide: much more than a biomarker. *Int. J. Cardiol.* 221, 1031–1038. doi: 10.1016/j.ijcard.2016.07.109
- Cipriano, G., Cipriano, V. T., da Silva, V. Z., Cipriano, G. F., Chiappa, G. R., de Lima, A. C., et al. (2014). Aerobic exercise effect on prognostic markers for systolic heart failure patients: a systematic review and meta-analysis. *Heart Fail. Rev.* 19, 655–667. doi: 10.1007/s10741-013-9407-6
- Fimognari, F. L., Loffredo, L., Di Simone, S., Sampietro, F., Pastorelli, R., Monaldo, M., et al. (2009). Hyperhomocysteinaemia and poor vitamin B status in chronic obstructive pulmonary disease. *Nutr. Metab. Cardiovasc. Dis.* 19, 654–659. doi: 10.1016/j.numecd.2008.12.006
- Franke, J., Frankenstein, L., Schellberg, D., Bajrovic, A., Wolter, J. S., Ehlermann, P., et al. (2011). Is there an additional benefit of serial NT-proBNP measurements in patients with stable chronic heart failure receiving individually optimized therapy? *Clin. Res. Cardiol.* 100, 1059–1067. doi: 10.1007/s00392-011-0340-1
- Fujii, T., Otsuka, T., Tanaka, S., Kanazawa, H., Hirata, K., Kohno, M., et al. (1999). Plasma endothelin-1 level in chronic obstructive pulmonary disease: relationship with natriuretic peptide. *Respiration* 66, 212–219. doi: 10.1159/000029380
- Ganguly, P., and Alam, S. F. (2015). Role of homocysteine in the development of cardiovascular disease. *Nutr. J.* 14:6. doi: 10.1186/1475-2891-14-6
- Guéant Rodriguez, R. M., Spada, R., Pooya, S., Jeannesson, E., Moreno Garcia, M. A., Anello, G., et al. (2013). Homocysteine predicts increased NT-proBNP through impaired fatty acid oxidation. *Int. J. Cardiol.* 167, 768–775. doi: 10.1016/j.ijcard.2012.03.047
- Herrmann, M., Stanger, O., Paulweber, B., Hufnagl, C., and Herrmann, W. (2007). Effect of folate supplementation on N-terminal pro-brain natriuretic peptide. *Int. J. Cardiol.* 118, 267–269. doi: 10.1016/j.ijcard.2006.07.034
- Jeremic, J., Nikolic Turnic, T., Zivkovic, V., Jeremic, N., Milosavljevic, I., Srejskovic, I., et al. (2018). Vitamin B complex mitigates cardiac dysfunction in

and review. FP contributed to literature search, data collection, analysis of data, and manuscript preparation and review. LG contributed to study design, literature search, data collection, analysis of data, and manuscript review. All authors contributed to the article and approved the submitted version.

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- high-methionine diet-induced hyperhomocysteinemia. *Clin. Exp. Pharmacol. Physiol.* 45, 683–693. doi: 10.1111/1440-1681.12930
- Kaya, Z., Bicer, A., Yalcin, F., Er, A., Camzucuoglu, A., Korkmaz, N., et al. (2014). Impaired global and segmental myocardial deformation assessed by two-dimensional speckle tracking echocardiography in patients with vitamin B12 deficiency. *Cardiol. J.* 21, 60–66. doi: 10.5603/CJ.a2013.0059
- König, D., Bissé, E., Deibert, P., Müller, H. M., Wieland, H., and Berg, A. (2003). Influence of training volume and acute physical exercise on the homocysteine levels in endurance-trained men: interactions with plasma folate and vitamin B12. *Ann. Nutr. Metab.* 47, 114–118. doi: 10.1159/000070032
- Lang, R. M., Bierig, M., Devereux, R. B., Flachskampf, F. A., Foster, E., Pellikka, P. A., et al. (2005). Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. *J. Am. Soc. Echocardiogr.* 18, 1440–1463. doi: 10.1016/j.echo.2005.10.005
- Lonsdorfer-Wolf, E., Bougault, V., Doutreleau, S., Charloux, A., Lonsdorfer, J., and Oswald-Mammosser, M. (2004). Intermittent exercise test in chronic obstructive pulmonary disease patients: how do the pulmonary hemodynamics adapt? *Med. Sci. Sports Exerc.* 36, 2032–2039. doi: 10.1249/01.mss.0000147631.59070.7d
- Müller, P. T., Nogueira, J. Z., Augusto, T. R., and Chiappa, G. R. (2019). Faster oxygen uptake, heart rate and ventilatory kinetics in Stepping compared to cycle ergometry in COPD patients during moderate intensity exercise. *Appl. Physiol. Nutr. Metab.* 44, 879–885. doi: 10.1139/apnm-2018-0662
- Paulin, F. V., Zagatto, A. M., Chiappa, G. R., and Müller, P. T. (2017). Addition of vitamin B12 to exercise training improves cycle ergometer endurance in advanced COPD patients: a randomized and controlled study. *Respir. Med.* 122, 23–29. doi: 10.1016/j.rmed.2016.11.015
- Piquereau, J., Moulin, M., Zurlo, G., Mateo, P., Gressette, M., Paul, J. L., et al. (2017). Cobalamin and folate protect mitochondrial and contractile functions in a murine model of cardiac pressure overload. *J. Mol. Cell Cardiol.* 102, 34–44. doi: 10.1016/j.yjmcc.2016.11.010
- Seemungal, T. A., Lun, J. C., Davis, G., Neblett, C., Chinyepi, N., Dookhan, C., et al. (2007). Plasma homocysteine is elevated in COPD patients and is related to COPD severity. *Int. J. Chron. Obstruct. Pulmon. Dis.* 2, 313–321. doi: 10.2147/copd.s2147

- Solomon, L. R. (2016). Low cobalamin levels as predictors of cobalamin deficiency: importance of comorbidities associated with increased oxidative stress. *Am. J. Med.* 129, 115.e9–115.e16. doi: 10.1016/j.amjmed.2015.07.017
- Taban-Shomal, O., Kilter, H., Wagner, A., Schorr, H., Umanskaya, N., Hübner, U., et al. (2009). The cardiac effects of prolonged vitamin B12 and folate deficiency in rats. *Cardiovasc. Toxicol.* 9, 95–102. doi: 10.1007/s12012-009-9038-2
- Tyagi, S. C., and Joshua, I. G. (2014). Exercise and nutrition in myocardial matrix metabolism, remodeling, regeneration, epigenetics, microcirculation, and muscle. *Can. J. Physiol. Pharmacol.* 92, 521–523. doi: 10.1139/cjpp-2014-0197
- Wang, H. Y., Xu, Q. F., Xiao, Y., Zhang, J., and Sperry, A. (2011). Cardiac response and N-terminal-pro-brain natriuretic peptide kinetics during exercise in patients with COPD. *Respir. Care* 56, 796–799. doi: 10.4187/respcare.00935a

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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