

Research Article

The Effect of Upright Lower Body Negative Pressure on Muscle Activity and Hemodynamics during Exercise

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Abstract

Purpose: The aim of the study was to evaluate the changes that occur in parameters relating to muscle work and muscle hemodynamics under the influence of upright Lower Body Negative Pressure (LBNP) application during walking.

Methods: 18 young females were included in this study. All 18 subjects participated in 2 trials of a 12-minute walking exercise on a treadmill equipped with a LBNP chamber, on a constant speed of 5 km/h. The first trial was executed under the application of a LBNP program (3 stages of -15, -25 and -30 mbar), while the second trial was similar, but without the activation of the negative pressure chamber. During both trials, the Vastus Lateralis (VL) muscle hemodynamic conditions were monitored continuously with a Functional Near-Infrared Spectroscopy (fNIRS) device and parallelly, VL activation was monitored via a Surface Electromyography (sEMG). Heart Rate (HR) values were recorded in the beginning and during the 10th min of each trial, after which, the difference between the 2 values was calculated. Immediately after the conclusion of each trial, participants were asked to provide a score for perceived exertion using Borg CR-10 scale (10 for maximum exertion).

Results: During the LBNP trial, Total Hemoglobin (tHb) and Oxy-Hemoglobin (O₂Hb) concentrations in the Vastus Lateralis (VL), was significantly lower compared to the control (p=0.007, p=0.001), but with a significant increase rate in deoxy-hemoglobin (HHb) (p=0.001). Tissue Saturation Index (TSI%) showed no significant alteration between trials (p= 0.668). All calculated parameters relating to work output showed a significant difference in the LBNP trial compared to control. HR was increased (p= 0.001), there were an increase in MPF and RMS amplitude in the VL (p=0.006, p < 0.001, respectively) and subjects reported higher rate of perceived exertion (p=0.001).

Conclusions: The application of LBNP showed elevated work characteristics, mainly on the work output and less local muscle hemodynamics. This could be a time efficient training tool for stressing the musculoskeletal system, faster improve body composition and potentially enhancing cardio respiratory fitness.

Keywords: Negative air pressure and exercise; Muscle activation and hemodynamics

Introduction

It is well documented that performing regular Physical Activity (PA) promotes good health and plays a vital role in the prevention of non-communicable diseases, such as type 2 diabetes [1,2], coronary heart disease [2,3], breast cancer [2,4]

and obesity [5,6]. Among the adult population, walking has been reported to be the most common type of PA [7–9], and it is considered to be feasible and safe form of PA to undertake regardless of age, health status, and ability [10,11]. While there

is robust evidence that regular walking at low intensities has potent health effects in terms of producing favorable changes in the lipoprotein profile [12] and improving glycemic control [13,14], accumulating data suggest that there may be additional physiological benefits by performing PA at higher intensities, especially in the context of obtaining pronounced improvements in cardio respiratory fitness (Duncan et al. 1991 and promoting skeletal muscle hypertrophy [15,16], which accumulating evidence suggest attenuates age-related loss in muscle function [17,18].

However, it has been reported that performing more intense PA may be difficult for certain populations (e.g., adults with mobility disability and seniors) [7,8]. Several training equipment and methods have been used to increase the intensity of gait-based activities for these populations, including walking with weighted vests [19,20], uphill walking [21], downhill walking [22] and walking while pushing a sled [23]. Still, there are environmental conditions where performing any kind of PA may be challenging or impractical, such as during spaceflights and bed rest, which predisposes these populations to microgravity-induced physiological deconditioning [24], and consequently increases the risk for muscle atrophy [25], immobility [25], orthostatic intolerance [26,27], cardiovascular morbidity [27] and all-cause mortality [27]. Hence, there has been a need for methods that simulate the Earth's gravity, 1g environment, during microgravity conditions to protect against these detrimental health effects. One such alternative is a utilizing a treadmill within a Lower Body Negative Pressure (LBNP) chamber, allowing participants to walk and run in a simulated hypergravity environment [24]. This device and tool combined with exercise, either in a supine or upright position, has been proposed to be very effective for simultaneously stress the cardiovascular and musculoskeletal system in clinical trials, with simulated microgravity conditions [24,28].

The method was originally invented to produce dry operating fields during surgery and to remove blood from diseased organs, and has later mainly been applied in clinical research to study the hemodynamic and neurohormonal responses following central hypovolemia [24]. It is based on transiently inducing orthostatic stress that simulates a state of central hypovolemia, which consequently activates several compensatory mechanisms that have been proposed to induce mechanical and cardiovascular responses that may be comparable to exercising in Earth's 1g environment [24,29]. This is accomplished by progressively reducing the pressure surrounding the lower body extremities inside the chamber, which redistribute blood volume from upper (above the iliac crest) to lower body compartments, diminishing central venous pressure and stroke volume, and sequentially reduces cardiac output and mean arterial pressure [24,30].

This initiates integrated compensatory responses that quickly enhance sympathetic tone to stabilize the mean arterial pressure, which includes increasing the Heart Rate (HR) and systemic vascular resistance [24,30]. During exercise, this been shown to provide a cardiovascular load that may be comparable to more intense forms of PA in 1g environments [26,28]. Additionally, there is also evidence that the LBNP chamber generates footward forces, or Ground Reaction Forces (GRFs), that is proportional to the exposed negative pressure [29,31], which has been suggested to have applications during orthopedic rehabilitation [24,32] and in space medicine to prevent lower body muscle atrophy for space travelers [26,28]. This is briefly accomplished by

uses a neoprene skirt sealed at the superior iliac crest inside the LBNP treadmill chamber, was the cross-sectional area of body at seal, in conjunction with the pressure differences between the external ambient and internal chamber environment, actively pulls the participant downwards and generates GRFs [31].

Furthermore, LBNP in combination with exercise has been proven to provide a load bearing that is adequate to maintain exercise capacity [28] and leg lean body mass [33] during simulated microgravity environments (bed rest). For Instance, Watenpaugh et al., and colleagues (2000) [28] demonstrated that supine lower body negative pressure exercise (walking and running) in the LBNP chamber for 40 min per day maintained aerobic fitness (VO_2 max) and sprint speed during bed rest, for a period of 15 days. Although, there is evidence that LBNP exercises (walking and running) may countermeasure physiological deconditioning during long duration spaceflight and bed rest [24,28,33], most studies was conducted in a supine position, leaving an uncertainty of the effects during upright LBNP exercise. Moreover, while there is data suggesting that LBNP during intense restive exercise may increase the muscle activity, Surface Electromyography (sEMG) amplitude, of the Vastus Lateralis (VL) and simultaneously elevate Total Hemoglobin (tHb) and Oxy-Hemoglobin (O_2 Hb) concentrations in the VL [30], it remains uncertain if continuous treadmill walking with LBNP induces similar electromyographical and hemodynamic response patterns, and if they are different from normal treadmill walking without LBNP. Additionally, it is also unclear whether the effects of LBNP occur due to the pressure change affecting the skeletal muscle oxidative metabolism or the increased mechanical load that is provoked by the simulated hypergravity condition in the LBNP chamber. Therefore, the aim of this study was to evaluate the impact of upright LBNP exercise on parameters relating to muscle work and hemodynamics during walking.

Material & Methods

Subjects

Eighteen female subjects aged 26.0 (± 5.0) volunteered for this study. Subjects were given oral and written explanation of the testing procedures. Subjects signed a written consent prior to the study. This study was approved by the Bioethics committee, Department of Physical Education and Sports Science, University of Thessaly.

Table 1: Characteristics of the subjects (n = 18).

Characteristics	(Mean \pm SD)
Age (years)	26.0 + 5.0
Height (cm)	167.8 + 4.8
Weight (kg)	63.5 + 14.5

Note: SD = Standard Deviation

Protocol

The study's crossover design consisted of two trials where subjects were placed in a specialized LBNP treadmill chamber (VACUPOWER, TMThessaloniki, Greece). Subjects wore costume-made neoprene skirts for ensuring airtight sealing at the iliac crest, and performed a 12 minute walking exercise inside the chamber, on a constant speed of 5 km/h (Figure 1). During trial 1, they walked for 2 minutes without any existing pressure change and at the beginning of the third minute, the LBNP program was activated. The program started with a pressure of -15 mbar inside the chamber for 2 minutes, continued with a pressure of -25 mbar for the next 2 minutes, and finally pressure was

maintained at -30 mbar for 4 minutes. For the remaining 2 minutes, the LBNP was terminated, and subjects continued walking unhindered as a cool down period. After a 30 minutes rest, the trial 2 was performed. It was identical to the LBNP trial, but without the engagement of the LBNP program. Upon their arrival at the laboratory, subjects participated in a small interview where they provided us with their history of physical activity and then were given the necessary instructions for the procedure, in order to familiarize themselves with the upcoming task. During both trials, we collected data related to cardiovascular and muscular work (i.e., HR, Borg Scale and sEMG data) and muscle hemodynamics (i.e., fNIRS data) from all participants. Prior to the commencement of the experiment, resting heart rate was obtained with the use of a standard handheld oximeter and their bodyweight was calculated by standing on top of a stationary force plate. Further, to obtain a reliable fNIRS and sEMG signal, the skin of subjects on the VL area was shaved, cleaned with alcohol, and lightly abraded using fine sandpaper at the fixation sites.

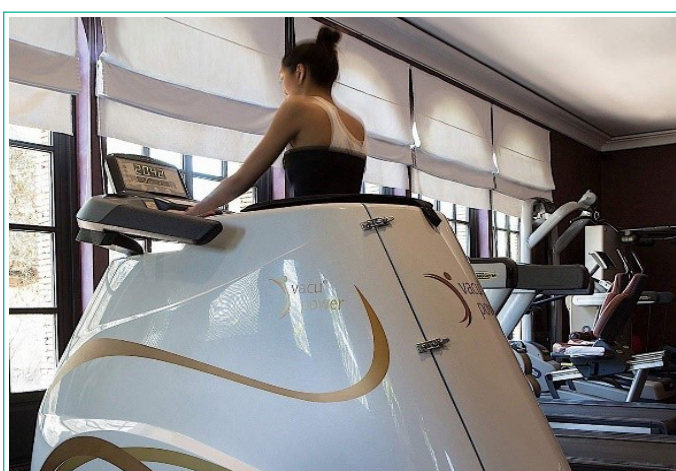


Figure 1: The LBNP treadmill chamber.

Except the program trials, we conducted in another day a validation procedure with one subject using a portable force plate (PASCO CAPSTONE TM), which was positioned inside the LBNP chamber. The subject was standing still on top of the force plate throughout this trial, wearing a neoprene skirt, which sealed the chamber opening and emulated the same hindering forces that the chamber provided. The program took 6 minutes and began with a pressure level of -15 mbar inside the chamber for 2 minutes, continued with an increase to -25 mbar for the next 2 minutes, and continued under -30 mbar for the last two 2 minutes. The force plate was used to track the GRFs of standing still during the different pressure levels, (Newtons). The purpose of this trial was based on the idea that negative pressure might increase the GRF due to mechanical/gravity effect that may appear. The motive for only assessing a single subject for this validation was because it is well-established that gravity acts the same on all masses and pulling all the objects to the Centre of the Earth [34]. Thus, there was no need to add extra participants.

Surface Electromyography

A wireless EMG system (Myon AG, Switzerland) were used to examine changes in muscle activation in the VL muscle. The sEMG bipolar electrodes were placed within two thirds of a straight line from the spina iliaca to the lateral condyle on the VT on the right leg in accordance with SENIAM guidelines [35]. The EMG signal was then processed and smoothened by band-pass filtering at 10-400 Hz (4th order Butterworth filter). A Fast-

Fourier Transform (FFT) window analysis was applied to determine the cut off frequencies, Further, the FFT was also used to obtain the Mean Power Frequency (MPF). The signal amplitude of sEMG was rectified and calculated as Root Mean Square (RMS) values. Subsequently, the MPF and RMS amplitude was used for statistical analysis.

Near-Infrared Spectroscopy

A portable NIRS system (Portalite, Artinis Medical Solutions, Netherlands) were used to monitor changes in total hemoglobin and muscle perfusion in the VL muscle. A NIRS probe were placed ~ 15 cm above the proximal border of the patella and was fixed to the VL on the right leg, using a dark 7.5 cm dynamic tape to avoid external light and artifacts.

During each trial, muscle perfusion and activation were continuously monitored using both the NIRS and an sEMG respectively. The three time-frames that were separated during our data analysis and the mean tHb was calculated for each LBNP phase.

Statistical Analysis

The data are presented as means \pm Standard Deviations (SD) unless otherwise stated. The differences between the LBNP trial and the control was normally distributed and was checked using the Shapiro–Wilk test. A paired t-tests were carried out to compare the means of the hemodynamical and work-related parameters in each condition.

For intra-trial analysis of the tHb concentration between each LBNP phase, statistical analysis was performed through generalized linear models' analysis. All statistical analyses were executed using SPSS ver. 28.0 statistical program for Windows (SPSS Software, IBM Inc., Chicago, IL, USA). The level of significance was set at $p < 0.05$.

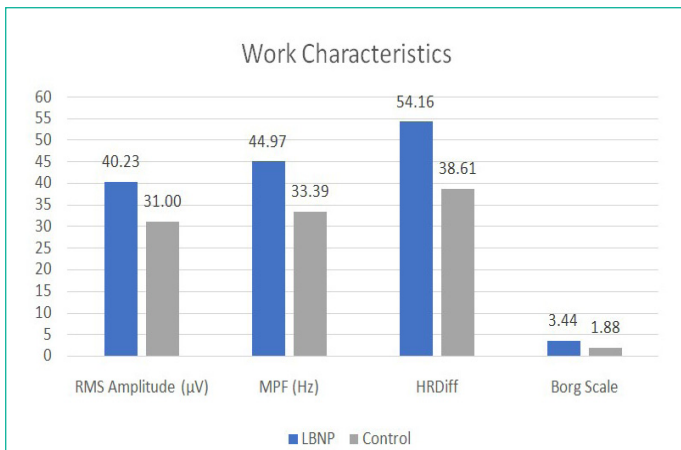
Results

Work Characteristics

There was a significant difference in all parameters relating to work output between the LBNP trials compared to the control. The mean MPF and RMS amplitude was significantly higher in the LBNP condition compared to the control ($p < 0.001$ and $p = 0.036$, respectively). In addition, the mean difference between resting HR and the HR after 10 minutes of exercise was 54.16 ± 12.70 for the LBNP and 38.61 ± 11.42 for the control ($p < 0.001$). Subjects also reported a higher value for the rate of perceived exertion, evaluating their exhaustion with a mean value of 3.44 ± 1.29 on the 10-point Borg scale for the LBNP trial and 1.88 ± 0.90 for the control ($p < 0.001$) (Table 2, Graf. 1).

Table 2: Work Characteristics; Values are means \pm SD. LBNP = Lower body negative pressure, RMS = Root mean square (μ V), MPF = Mean power frequency (Hz), HRDiff = Heart rate difference is the difference between Heart Rate during rest and Heart Rate during the 10th minute of each trial.

Parameter	Exercise session		p-value
	LBNP (n=18)	Control (n=18)	
RMS Amplitude (μ V)	40.23 \pm 30.05	31.00 \pm 25.23	0.036
MPF (Hz)	43.97 \pm 27.33	33.39 \pm 20.29	0.006
HRDiff	54.16 \pm 12.70	38.61 \pm 11.42	<0.001
Borg Scale	3.44 \pm 1.29	1.88 \pm 0.90	<0.001



Graf 1: Work Characteristics. Values are means. HRDiff is the difference between Heart Rate during rest and Heart Rate during the 10th minute of each trial.

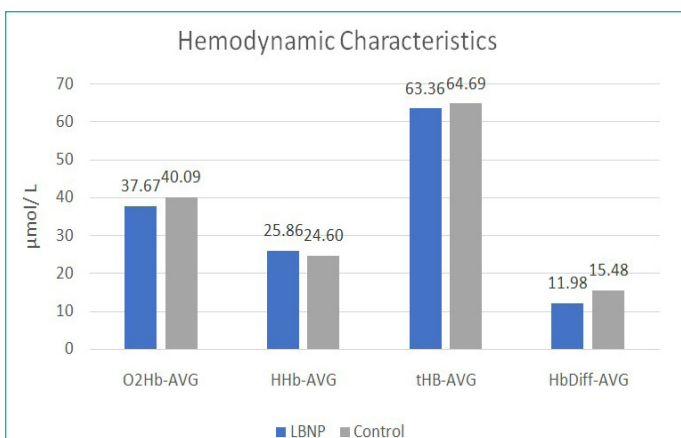
Hemodynamic Characteristics

During exercise under the application of LBNP, mean concentration of both tHb and O₂Hb were smaller, compared to the control trial (p=0.007, p <0.001, respectively). However, the opposite effect was observed for the mean concentration of HHb (µmol/L) since exercise under the application of LBNP showed a statistically significant increase (p <0.001) in HHb concentration within the muscle. The difference between O₂Hb and HHb was smaller during the LBNP trial. However, TSI% showed small and insignificant alteration between trials (Table 3, Graf. 2). No sliding of the NIRS probe was documented between the trials.

Table 3: Hemodynamic Characteristics

Parameter	Exercise Session		p-value
	LBNP (n=18)	Control (n=18)	
O ₂ Hb (µmol/L)	37.67 ± 6.32	40.08 ± 7.10	<0.001
HHb (µmol/L)	25.85 ± 3.99	24.60 ± 4.13	<0.001
tHb (µmol/L)	63.36 ± 10.12	64.68 ± 10.95	0.007
HbDiff (µmol/L)	11.98 ± 3.45	15.48 ± 3.89	<0.001
TSI%	99.94 ± 0.07	99.93 ± 0.05	0.668

Values are means ± SD. O₂Hb= Oxyhemoglobin concentration, HHb= Deoxyhemoglobin concentration, tHb= total hemoglobin concentration, HbDiff= Difference between O₂Hb and HHb, TSI %= Tissue Saturation Index %.



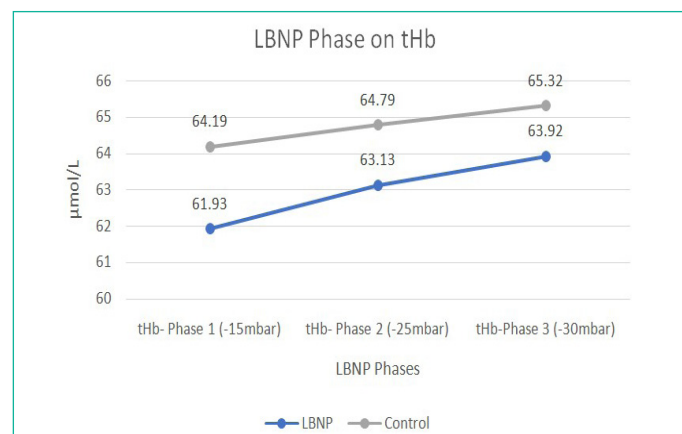
Graf 2: Hemodynamic Characteristics. Values are means. O₂Hb= Oxyhemoglobin concentration, HHb= Deoxyhemoglobin concentration, tHb= total hemoglobin concentration, HbDiff= Difference between O₂Hb and HHb.

The three separate LBNP phase time-frames were separated and mean tHb was calculated for each phase for both trials. In the LBNP trial, tHb showed lower values for all three stages, but with a higher increase rate between phases than in control. In LBNP, mean tHb for phase 1 was 61.93 µmol/L, while for control was 64.19 µmol/L. For phase 2, mean tHb for LBNP was 63.13 µmol/L, while in control were 64.79. Finally, in phase 3, mean tHb for LBNP was 63.92 µmol/L, while in control was 65.32 µmol/L, (Table 4, Graf 3).

Table 4: LBNP Phase Characteristics.

Parameter	LBNP Phase			p-value
	tHb- Phase 1 (-15mbar)	tHb- Phase 2 (-25mbar)	tHb- Phase 3 (-30mbar)	
LBNP	61.93 ± 9.91	63.13 ± 10.05	63.92 ± 10.14	0.948
Control	64.19 ± 10.87	64.79 ± 11.11	65.32 ± 10.97	0.992

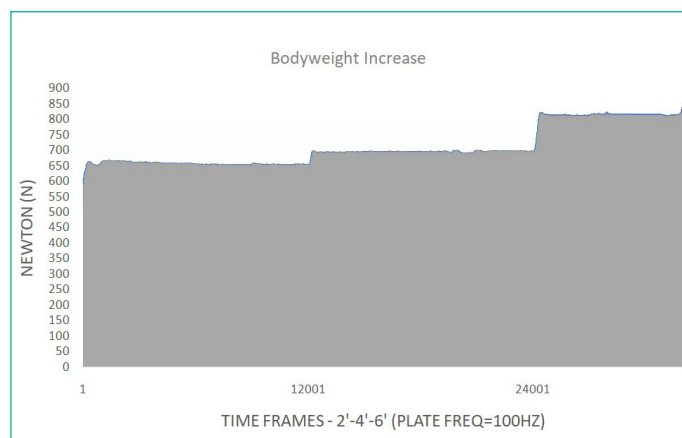
Values are means ± SD. tHb= total Hemoglobin.



Graf 3: Values are means. tHb= total Hemoglobin (µmol/L). Phase 1,2,3 are the separate time frames that were defined by LBNP change (-15 mbar, -25 mbar, -30 mbar)

Validation Procedure

There was a significant difference in GRFs of standing still with a pressure level of -15 mbar, -25 mbar & -30 mbar inside the chamber compared to standing still with no negative pressure (p=0.003, p <0.001, p < 0.001, respectively). The force plate analysis revealed that the GRFs of standing increases proportionally to the negative pressure exerted by the LBNP chamber, which consequently simulates a higher bodyweight (see Graf 4).



Graf 4: Bodyweight changes during LBNP changes (-15 mbar, -25 mbar, -30 mbar)

Discussion

The aim of this study was to evaluate the impact of upright LBNP exercise on parameters relating to muscle work and hemodynamics during walking. The main findings were that all parameters related to work output were significantly higher in LBNP trial compared to the control (Table 2), but not for the hemodynamic-related variables (Table 3). Of note, we observed in that the subjects during LBNP trial had significantly higher HHB values, and lower O_2Hb and $HbDiff$ values in trial 1 compared to the control in trial 2.

This suggest that upright LBNP walking may induce a stronger stress on micro vascular oxygenation than normal walking [36]. This is in line with previous research suggesting that LBNP may impact sympathetically mediated vasoconstriction (i.e., functional sympatholysis, a protective mechanism that facilitates blood flow), venous return and minimize blood pooling in the lower extremity muscles [36-38]. Interestingly, the average volume of tHB was also significantly lower in the LBNP trial compared to the control, indicating that the blood volume decreased to a greater extent in VL muscle during the LBNP condition [39]. These results contradicts some earlier research suggesting that the concentration of tHB tend increase during LBNP exercise [36,40] or remain constant [41].

The discrepancy in our findings may be explained by differences in LBNP exercises (e.g., leg press exercise vs walking) [42], the intensity (e.g., near-maximal vs. sub maximal [43] and differences in muscle used (e.g., calf vs vastus laterals) [36] compared to previous work. Intriguingly, TSI% (i.e., muscle oxygenation) were not statistically significantly between the LBNP and control conditions, which also inconsistent with other studies using LBNP [42,44]. For instance, a study by Parganlija et al., (2020) found that the TSI% decreased while the HHB simultaneously increased with graded LBNP exercise, which supports the notation that exercise under LBNP may rely more on energy production from oxidative metabolism compared to normal exercise [30].

However, based on our results, there may be minor differences in energy dependence from muscle oxidative metabolism during light intensity LBNP exercise in comparison to normal light intensity exercise, as light aerobic exercise (in normal conditions) already predominantly relies on the oxidative phosphorylation to meet increasing metabolic demands [45]. Still, we found that HRDiff was significantly higher in LBNP trial compared to the control (Table 2). This implies that LBNP treadmill walking may nevertheless more strongly stress the cardiovascular system compared normal treadmill walking, even at low intensities. These findings are coherent with previous research suggesting that HR increases by being exposed to greater negative pressures in a LBNP chamber, independent of performing any exercise [24,46], as a protective response to counterbalance the decreased stroke volume and maintain adequate mean arterial pressure [24]. Furthermore, we also found that the average MPF and RMS amplitude, and borg scale was significantly elevated in the LBNP condition compared to the control (Table 2), indicating that the subjects had higher muscle activation in the VL muscle during the LBNP exercise, but also exercised more vigorously and in total performed more mechanical work. Although, the average MPF commonly decreases during sub maximal sustained contraction as the muscle begins to fatigue [47], there is also data indicating that an increased MPF may be caused by higher proportion of type II fiber recruitment [48,49]

Moreover, there is also evidence that RMS tends to increase proportionally with increasing force [47]. Therefore, we can speculate that the LBNP trial demanded a higher contractile force from the VL muscle and consequently the increased MPF reflects a higher engagement of type II muscle fibers.

This supports the premise that LBNP combined with exercise may also be a time efficient and an applicable tool for stressing the musculoskeletal system [24,50]. Interestingly, we observed in the validation procedure that standing inside a LBNP chamber with ≤ 15 mbar can generate a higher mechanical load on the lower body, though increasing the GRFs, independent of performing any movement (Graf 4). This suggest that the increased energy expenditure, but also the higher RMS amplitude and MPF, during LBNP exercise may be more related to the higher mechanical load on the lower body though stimulating a higher bodyweight, as it is well documented that moving a higher body mass requires more muscular force [51,52] and expands more energy per unit of time at the same exercise intensity [53]. Moreover, this also provides evidence for the potential use of LBNP as training equipment for improving body composition (i.e., reduce subcutaneous adipose tissue), by helping to increase total daily energy expenditure and consequently lower the risk of obesity.

Noteworthy, we also found that the energy consumption was significantly elevated in the LBNP trial compared to the control, independently of changes in oxidative muscle metabolism. To the best of our knowledge, this is the first study that demonstrated that the higher metabolic cost during LBNP exercise may predominantly be related to the provoked mechanical load on the lower body via simulating a higher bodyweight and a hyper gravity condition. The present study may therefore provide new insight in sport and space medicine. However, there are some limitations with this study. For instance, the subjects were exclusively females, which limit the generalization of the results. Secondly, there is strong evidence that the NIRS signal is directly influenced by the thickness of the subcutaneous adipose tissue [54]. Thirdly, the subjects had no previous experience with this training method, which may potentially have influenced the findings in our study. However, since the LBNP exercise was at a light intensity and the task was non-complex, this probably had minimal effects on our results.

Conclusions

Although an LBNP treadmill exercise might be expensive for personal use, these findings suggest that its application may elevate training effects in a more time efficient manner compared to normal exercise. Exercise under the application of LBNP mainly seems to increase work characteristics and less local muscle hemodynamics. These findings suggest that exercising with a LBNP treadmill might be a time efficient training tool for stressing the musculoskeletal system, more rapidly improve body composition, and potentially more effectively enhance cardiorespiratory fitness in the general adult population.

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