SIX HIT TREADMILL SESSIONS IMPROVE LIPID OXIDATION AND VENTILATORY THRESHOLD INTENSITIES

SEIS SÉSSÕES DE HIT EM ESTEIRA AUMENTAM A OXIDAÇÃO DE LÍPIDOS E LIMIARES VENTILATÓRIOS

SEIS SESIONES DE HIT EN CINTA DE CORRER AUMENTAN LA OXIDACIÓN DE LÍPIDOS Y UMBALES VENTILATORIOS

ABSTRACT

Introduction: High-intensity interval training (HIT) has been used as an alternative to cardiorespiratory training performed continuously at submaximal intensity and over long periods. Objectives: Propose a treadmill HIT protocol and verify the influence of six HIT sessions with this protocol on ventilatory anaerobic thresholds (VATs) and substrate oxidation pattern during submaximal continuous exercise (SCE). Methods: Fifteen sporadically active subjects underwent maximal progressive testing before and after six HIT treadmill running sessions to determine peak oxygen uptake (VO2peak), peak velocity (Vpeak), and VATs followed by SCE to determine lipid (LIPox) and carbohydrate (CHOox) oxidation rates. The HIT sessions consisted of eight sets of 60s at 100%Vpeak interspersed with 75s of passive recovery between sets and a 48h interval between sessions. Results: Our results showed increases in VAT intensities of 4.4% for VAT1 and 8.8% for VAT2, a decrease of 12.8% in CHOox and an increase of 23.7% for LIPox; accordingly, the relative energy derived from LIPox was 20.3% higher after the training period. Conclusion: The proposed protocol produced adaptations and intensities which are similar to those described in the literature, but unlike others, it can be applied in sporadically active individuals. Level of Evidence II; Comparative prospective study.

Keywords: High-intensity interval training; Physical Endurance; Lipid Metabolism.

RESUMO

Introdução: O treinamento cardiorrespiratório intervalado de alta intensidade (HIT) tem sido utilizado como alternativa ao treinamento cardiorrespiratório contínuo de intensidade submáxima e duração prolongada. Objetivo: Propor um protocolo de HIT utilizando corrida em esteira e verificar a influência de seis sessões de HIT com esse protocolo sobre os limiares anaeróbicos ventilatórios (LAVs) e o padrão de oxidação de substratos durante o exercício contínuo de intensidade submáxima (ECIS). Métodos: Quinze indivíduos irregularmente ativos foram submetidos, antes e após seis sessões de HIT com corrida em esteira, a teste progressivo máximo para determinação do pico de consumo de oxigênio (VO2peak), velocidade de pico (Vpeak) e LAVs seguidos de SCE para determinação das taxas de oxidação de lípidos (LIPox) e carboidratos (CHOox). As sessões de HIT foram compostas por oito séries de 60 segundos a 100%Vpeak com 75 segundos de recuperação passiva entre as séries e com 48 horas de intervalo entre as sessões. Resultados: Observaram-se aumentos das intensidades de ocorrência das LAVs (4,4% para LAV1 e 8,8% para LAV2), redução de 12,8% em CHOox e aumento de 23,7% para LIPox; de acordo, a energia relativa derivada de LIPox apresentou-se 20,3% maior após o período de treinamento. Conclusão: O protocolo proposto promoveu adaptações e intensidades similares às descritas na literatura, mas contrariando outros, é aplicável em indivíduos irregularmente ativos. Nível de Evidência II; Estudo prospectivo comparativo.

Descritores: Treinamento intervalado de alta intensidade; Resistência Física; Metabolismo dos Lipídeos.
INTRODUCTION

Cardiorespiratory endurance, which is expressed by oxygen consumption (VO₂peak), is one of the most important components of physical fitness. Training increases mitochondrial oxidative activity, pulmonary diffusion, and hemoglobin saturation, optimizing the oxidation of lipids (LIP), the reduction in muscle synthesis of lactic acid, and the accumulation of lactate and hydrogen ions in the blood.²,³

Different protocols for cardiorespiratory exercise (CRe) have been proposed to optimize the use of fatty acids (FA), since low rates of lipid oxidation may be involved with the development of excess weight, type II diabetes, and cardiovascular diseases.⁴

High-intensity interval training (HIT), characterized by short periods of vigorous activity interspersed with periods of rest or low intensity exercise,² has been used as an alternative to traditional cardiorespiratory training (TCT), which is performed continuously at submaximal intensity and for a prolonged duration. Recent studies have observed similar or greater adaptations promoted by HIT, regarding muscle oxidative activity,⁷⁻⁹ use of energy substrates during or after the exercise,⁴,⁶,⁷,⁹⁻¹¹ and cardiovascular function,⁶,¹²,¹³ among others, suggesting potential implications related to the health, including type II diabetes, overweight/obesity, and cardiovascular disease.⁶,¹⁴ in addition to the obvious increase in performance.¹⁵⁻¹⁶

However, most HIT studies use cycle ergometry protocols derived from the Wingate test.¹⁵ Training protocols with these characteristics require, in addition to a specialized ergometer, an extremely high motivation level of the subjects, and given the extreme nature of the exercise, it is questionable whether the general population can adopt this model in a safe and practical manner.⁵

Thus, the objectives of this study were: a) to propose an alternative HIT protocol using a treadmill, as the literature presents different training protocols using cycle ergometry as the predominant model, (b) assess the influence of six HIT sessions of treadmill running on the intensities in which the ventilatory anaerobic thresholds (LAVs) occur, and c) on the substrate oxidation pattern during continuous exercise of submaximal intensity (CESI), 45 min of treadmill running at the intensity of the first ventilatory anaerobic threshold, LAV1).

MATERIALS AND METHODS

All procedures were submitted to and approved by the Ethics Committee for Research Involving Human Subjects of the Cidade de São Paulo University (CAAE 31998914.8.0000.0064), and all subjects signed an Informed Consent Form. The experiment was conducted in the Laboratory of Physiology and Metabolism of the Cidade de São Paulo University.

The study involved 15 men who were irregularly active according to their IPAQ, were non-smokers, and were not using any medication or nutritional ergogenic substance during the study period. The mean values ± standard error for age, weight, height, body fat percentage and peak oxygen consumption (VO₂peak) relative to the total body mass of subjects were 25.8 ± 1.2 years, 72.3 ± 1.4 kg, 175.2 ± 2.1 cm, 16.3 ± 0.9 %, and 42.5 ± 1.3 mL/kg/min, respectively. The subjects were instructed to maintain their number of daily meals, type of food consumed, and preparation throughout the study.

After clinical evaluation, the subjects were tested to determine their VO₂peak, peak velocity (Vpeak), and LAVs. Two days after the completion of the initial evaluations, the subjects were submitted to CESI to determine rates of lipid (LIPox) and carbohydrate (CHOox) oxidation, after 4 hours of food restriction followed by ingestion of maltodextrin (1 g/kg, 12% solution), 30 min before the start of the training. Subsequently, the subjects underwent six sessions of HIT with 48-h intervals between the sessions. At the end of the training period, the subjects were again tested for determination of VO₂peak, Vpeak, and LAVs, and were submitted to new CESI to determine the rates of LIPox and CHOox under the same conditions cited above. (Figure 1)

Prior to the determination of VO₂peak, the subjects remained lying and rested for 10 min to determine ventilatory data and initial heart rate (HR). The test protocol consisted of running on a treadmill (ATL Model, Inbrasport Ltda., Brazil) with an initial velocity of 6 km/h, followed by increasing increments of 1 km/h every minute until voluntary exhaustion of the subjects.

The ventilatory parameters were collected at rest and continuously throughout the tests, at each respiratory cycle and analyzed at an average of 20 sec through a computerized gas analyzer (VO2000; Inbrasport Ltda, Brazil). The gas analyzer was calibrated to a standard volume and to the concentration of gases immediately before the first test of the day and re-calibrated after each test, according to the standardization of the manufacturer. The HR was recorded using a cardiac monitor (model Sport Test; Polar Electro Oy, Finland), continuously throughout the tests. After exhaustion, there were two recovery periods of two min each, with 50% and 25% of the highest speed reached. Only HR was monitored during the recovery periods.

<table>
<thead>
<tr>
<th>VO₂peak LAVs</th>
<th>ECIS Fasting (4 h) 1 g/kg Malto (30 min) 45 min LAV1</th>
<th>6 HIT sessions</th>
<th>VO₂peak LAVs</th>
<th>ECIS Fasting (4 h) 1 g/kg Malto (30 min) 45 min LAV1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Session 1 Day 1</td>
<td>Session 2 Day 3</td>
<td>Sessions 3 to 8 Days 5 to 15</td>
<td>Session 9 Day 17</td>
<td>Session 10 Day 19</td>
</tr>
</tbody>
</table>

Figure 1. Experimental study.
The criteria for the determination of VO$_{2\text{peak}}$ and exhaustion were as follows: occurrence of a plateau in VO$_2$ (characterized by increases of 2 mL/kg/min or less) and the inability to maintain the velocity, respectively. The V$_{peak}$ corresponded to the highest velocity reached during the test. 

The LAVs were determined from the ventilatory equivalents (VE/VO$_2$ and VE/VCO$_2$), end expired fractions (FEO$_2$ and FEO$_2$), and respiratory quotient (RQ) and expressed as a function of VO$_2$ (mL/kg/min). LAV1 corresponded to the lowest value of VE/VO$_2$ before its continued rise associated with the beginning of the abrupt change and the continued increase of the RQ. LAV2 corresponded to the point at which the non-linear increases of VE/VO$_2$, VE/VCO$_2$, and FEO$_2$ coincided with the fall of FEO$_2$. 

Before and after the training period, the subjects performed 45 min of CEIS at the intensity of LAV1 (pre and post-training CESI) after 4 hours of food restriction followed by ingestion of maltodextrin (1 g/kg, 12% solution), 30 min before the start of the activity. The purpose of food restriction and ingestion of CHO before the CESI sessions was to enable similar metabolic states among subjects during the experimental sessions. Ventilatory parameters were collected during rest and continuously over the CESI, at each respiratory cycle, and analyzed in averages of 20 sec by a computerized gas analyzer (model VO2000; Inbrasport Ltda, Brazil) to determine LIPox and CHOox rates. 

The rates of LIPox and CHOox were determined in blocks of 10 min throughout the CESI, from mean values of VO$_2$ and VCO$_2$ (L/min), corresponding to the last two min of each block. Oxidation rates (in g/min) were calculated using Frayn's stoichiometric equations, assuming an insignificant nitrogen excretion rate. The energy provided by LIPox and CHOox (LIPkg and CHOkc, respectively, in kcal/min) was calculated from their respective energy equivalents (9.75 and 3.87 kcal/g for LIP and CHO, respectively).

The subjects underwent six HIT sessions, with 48-h intervals between sessions. The HIT protocol of treadmill running consisted of two initial warm-up periods (2 min each, 25% and 50% VO$_{peak}$), followed by eight series of 60 sec at 100% VO$_{peak}$, 75 sec of passive recovery, two final slowing periods (2 min each, at 50% and 25% VO$_{peak}$), Ventilatory parameters and HR were measured in each training session to determine the relative effort intensity.

### Statistical analysis

Results are presented as mean ± standard error. The homogeneity of the variances was verified by the Levene test. Ventilatory parameters, HR, running velocity, and oxidation rates of the experimental sessions were compared using the Student’s t-test or Sign test for paired data. 

The relative intensities of effort between training sessions and running series were compared through single-factor analysis of variance, followed by the Tukey HSD post-hoc test. The level of significance was set at $p < 0.05$. Statistical analysis was performed using the Statistics for Windows software (version 8.0, 2007, Statsoft, Inc., United States).

### RESULTS

No significant differences were observed between the absolute pre and post-training values at the peak effort in VO$_2$, HR, and velocity (Table 1). 

The six training sessions led to an increase in the intensities of the occurrence of the LAVs, relative to the VO$_{2\text{peak}}$ of 4.4% for LAV1 ($p = 0.23$) and 8.8% for LAV2 ($p = 0.01$). Discrete increases were observed but were not significant for HR and velocity in the intensities of the LAVs, relative to VO$_{2\text{peak}}$ (Table 2).

The mean pre- and post-training RQ and substrate oxidation values are presented in Table 3. After the training sessions, there was a reduction of 12.8% of CHOox ($p = 0.01$) and an increase of 23.7% in LIPox ($p = 0.04$). As a result, the relative energy derived from the LIPox (Figure 2) was 20.3% higher after the training period (29.2 ± 2.5 to 35.1 ± 2.2 %kcal/min, pre and post-training, respectively; $p = 0.01$).

The V$_{peak}$ was 14.9 ± 0.4 km/h, leading to relative intensities of 83.8 ± 0.6% VO$_{2\text{peak}}$ and 91.4 ± 0.2% FC$_{peak}$ with training. No significant differences were observed among the six training sessions (s) for %V0$_{2\text{peak}}$ and %FC$_{peak}$ (Figures 3 and 4, respectively). 

No significant differences were observed between the eight running bouts (c) for %VO$_{2\text{peak}}$ (Figure 5), however, the %FC$_{peak}$ presented significant differences on comparison between the initial and final series (Figure 6).

### Table 1. Maximal test: absolute values for performance parameters.

<table>
<thead>
<tr>
<th></th>
<th>VO$_2$ (mL/kg/min)</th>
<th>HR (bpm)</th>
<th>VEL (km/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAV1</td>
<td>Pre</td>
<td>204 ± 0.5</td>
<td>130 ± 3.2</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>209 ± 1.8</td>
<td>127 ± 2.7</td>
</tr>
<tr>
<td>LAV2</td>
<td>Pre</td>
<td>309 ± 0.9</td>
<td>165 ± 3.8</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>328 ± 2.6</td>
<td>162 ± 3.0</td>
</tr>
<tr>
<td>PEAK</td>
<td>Pre</td>
<td>424 ± 1.2</td>
<td>191 ± 2.9</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>406 ± 3.1</td>
<td>187 ± 2.5</td>
</tr>
</tbody>
</table>

Mean values ± standard error; n = 15. P <0.05 for CHOox and LIPox between pre- and post-training. 

### Table 2. Maximal test: values relative to the effort peak for performance parameters.

<table>
<thead>
<tr>
<th></th>
<th>VO$_2$ (%)</th>
<th>HR (%)</th>
<th>VEL (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAV1</td>
<td>PRE</td>
<td>48.5 ± 1.5</td>
<td>67.9 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>POST</td>
<td>50.7 ± 1.8</td>
<td>68.7 ± 1.5</td>
</tr>
<tr>
<td>LAV2</td>
<td>PRE</td>
<td>73.0 ± 1.3</td>
<td>86.6 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>POST</td>
<td>79.4 ± 1.5</td>
<td>87.0 ± 1.3</td>
</tr>
<tr>
<td>Δ%</td>
<td>LAV1</td>
<td>4.4</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>LAV2</td>
<td>8.8</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Mean values ± standard error; n = 15. LAV1, first ventilatory anaerobic threshold; LAV2, second ventilatory anaerobic threshold; Δ%, relative variation; VO$_2$, oxygen consumption; HR, heart rate; VEL, velocity. * indicates $p <0.05$ vs. Pre. ** indicates $p <0.05$.

### Table 3. Oxidation of substrates between the training periods.

<table>
<thead>
<tr>
<th></th>
<th>CHOox RQ (g/min)</th>
<th>CHOox LiPox (g/min)</th>
<th>Δ%</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECIS</td>
<td>0.01 ± 0.01</td>
<td>-1.28 ± 0.23</td>
<td>23.7</td>
</tr>
<tr>
<td>PRE</td>
<td>0.08 ± 0.01</td>
<td>1.71 ± 0.06</td>
<td>0.28 ± 0.02</td>
</tr>
<tr>
<td>POST</td>
<td>0.14 ± 0.01</td>
<td>1.49 ± 0.05</td>
<td>0.34 ± 0.02</td>
</tr>
</tbody>
</table>

Mean values ± standard error; n = 15. ECIS, continuous exercise of submaximal intensity; RQ, respiratory quotient; CHOox, oxidation of carbohydrates; LiPox, oxidation of lipids; Δ%, relative variation. * indicates $p <0.05$ vs. PRE. ** indicates $p <0.05$.
that depend fundamentally on oxidative energy metabolism, due in large part to an increase in the capacity of skeletal muscles in transporting and using O₂ and LIP. For this reason, different CrE protocols have been proposed to optimize the use of FA, since low rates of LIPox may be involved with the development of those who are overweight or have type II diabetes and cardiovascular diseases.

Several studies have reported significant differences in responses during the recovery period after exercise depending on the mode of execution of the CrE associated with its effort intensity. Kaminsky et al., 20 Bahr & Sejersted 21, and Borsheim & Bahr 22 observed greater magnitude and duration of EPOC promoted by CrE conducted in periods of vigorous activity interspersed with periods of low intensity exercise, implying a higher caloric expenditure and LIPox. On the other hand, during physical activity, LIPox is directly related to the intensity of exercise performed. Published studies, for example, indicate intervals of intensity for maximum LIPox between 44-49% of VO₂max and 60-64% of HRmax 23,24 in other words, at intensities close to LAn1.

However, the majority of studies involving HIT cycle ergometry use protocols derived from the Wingate test, 15 with maximum repeated efforts. Little et al., 25 for example, used a cycle ergometry protocol comprising 4 sets of 30 sec each with a load of 0.075 kg/kg body mass at maximum speed and with 4 min of passive rest between sets. Training protocols with these characteristics require, in addition to a specialized ergometer, an extremely high level of motivation of the subject, and given the extreme nature of the exercise, it is questionable whether the population may adopt this model in a safe and practical manner.

Accordingly, different studies have proposed alternative HIT protocols involving maximum or above maximum effort levels. In a previous study Little et al., 8 observed that eight to twelve series of cycle ergometry for 60 sec with approximately 350 W, interspersed by 75 sec with 30 W protocol effort intensities compatible with adaptations promoted by cycle ergometry protocols derived from the Wingate test. When comparing calisthenics exercises with cycle ergometry similar to the Wingate test, Gist et al., 26 observed that four series of 30 sec with the highest number of possible repetitions of calisthenic exercise promoted similar intensities to the cycle ergometry protocol, around 80%VO₂peak and 85%FCpeak. Weston et al., 19 report mean intensities of 85-95%FCpeak for HIT training for the general population, suggesting a classification based on these intensities to characterize different interval exercise protocols such as HIT.

In accordance with the literature, our results suggest that the treadmill running protocol leads to a high-enough intensity to characterize it as
high intensity; however, unlike others, this protocol can be applied in untrained physically active individuals.

The change in performance capability and the pattern of oxidation of substrates promoted by the HIT can be explained by the increase of the muscular oxidative activity and use of energy substrates, during or after the exercise, among others. Talanian et al. observed a change in the pattern of oxidation of substrates in physically active women subjected to seven HIT sessions on a cycle ergometer (10 sets of 4 min at 90% VO\textsubscript{peak} with 2 min rest between sets) over 13 days. Perry et al. found the same adaptations when reproducing the protocol described above in untrained active men for six weeks. In a later study, Talanian et al. found that training with this protocol increased the levels of sarcoplasmic and mitochondrial FA transporters (FAT/CD36 and FABPpm) and increased oxidative enzyme activity, which are adaptations responsible for the increase of LiPox and changing the pattern of oxidation of substrates. Little et al. found that after six HIT sessions on a cycle ergometer (8 to 12 series of 60 sec at 100% of the maximum load with 75 sec to recover 10% of the maximum load between series), there was a stimulus of mitochondrial biogenesis due to the increased content of PGC-1α (peroxisome proliferator-activated receptor γ co-activator 1α); this adaptation was also observed after a single HIT session with 4 sets of 30 sec with 0.075 kg/kg at the maximum speed of 4 min of rest between series.

The adjustments indicated above, along with an increase of the sarcoplasmic and mitochondrial FA transporters together with mitochondrial biogenesis, potentiate LiPox in detriment of the use of CHO\textsuperscript{27} and may be the probable mechanism of interference of HIT in the oxidation of substrates.

CONCLUSION

Our results suggest, in agreement with the literature, that the proposed protocol leads to intensities sufficient to characterize it as high intensity; however, unlike others, this can be applied in untrained physically active individuals.

Our results also indicate that six HIT sessions with a treadmill running protocol promotes changes: (a) in the intensities of the occurrence of LAVs, expressed in function of the VO\textsubscript{2peak}, especially for LAV2, and (b) in the pattern of oxidation of substrates, while running at submaximal intensity with increased oxidation and contribution of LiP to maintain the energy demand.

All authors declare no potential conflict of interest related to this article.

AUTHORS’ CONTRIBUTIONS: Each author made significant individual contributions to this manuscript. MLM (0000-0002-6593-941X)*: preparation of the entire research project, data analysis, drafting of the article and review; CFWA (0000-0002-0363-2103)* and FRL (0000-0002-9710-0803)*: training sessions, data collection and review; JMLA (0000-0003-2859-9299X)*: experimental design, training sessions, data collection and review; MSC (0000-0001-7667-9492)*: training sessions and data collection. All authors approved the final version of the manuscript. *ORCID (Open Researcher and Contributor ID).

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