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Inclusion of sprints during moderate-intensity continuous exercise enhances post-exercise fat oxidation in young males

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Abstract

To assess the physiological demand of including high-intensity efforts during continuous exercise, we designed a randomized crossover study, where 12 physically active young males executed three different exercises in random order: *FATmax* - continuous exercise at the highest fat oxidation zone (*FATmax*); *2min-130%* - *FATmax* interspersed by a 2-min bout at 130% of the maximal oxygen uptake associated intensity (iVO_{2max}); and *20s:10s-170%* - *FATmax* interspersed by four 20-s bouts at 170% iVO_{2max} interpolated by 10s of passive recovery. We measured oxygen uptake (VO_2), blood lactate concentration ([LAC]), respiratory exchange rate (RER), fat and carbohydrate (CHO) oxidation. For statistical analyses, repeated measures ANOVA was applied. Although no differences were found for average VO_2 or carbohydrate oxidation rate, the post-exercise fat oxidation rate was 37.5% and 50% higher during *2min-130%* and *20s:10s-170%*, respectively, compared to *FATmax*, which also presented lower values of RER during exercise compared to *2min-130%* and *20s:10s-170%* ($p < 0.001$ in both), and higher values post-exercise ($p = 0.04$ and $p = 0.002$, respectively). The [LAC] was higher during exercise when high-intensity bouts were applied ($p < 0.001$ for both) and higher post-exercise on the intermittent one compared to *FATmax* ($p = 0.016$). The inclusion of high-intensity efforts during moderate-intensity continuous exercise promoted higher physiological demand and post-exercise fat oxidation.

Novelty bullets

- The inclusion of 2-min efforts modifies continuous exercise demands
- Maximal efforts can increase post-exercise fat oxidation
- 2-min maximal efforts, continuous or intermittent, presents similar demands

Keywords: HIIT; weight loss; indirect calorimetry; energy substrates.

1. Introduction

Several studies have found that fat oxidation rates increase from low- to moderate-intensity exercises and are reduced when the intensity increases (Romijn *et al.*, 2000; Skovgaard *et al.*, 2016). The maximal fat oxidation rate (FATmax) occurs near 40-55% of the maximal oxygen uptake ($\text{VO}_{2\text{max}}$) (Maunder, Plews and Kilding, 2018) and is commonly prescribed as the intensity for Moderate-Intensity Continuous Exercise (MICT), especially when the goal is fat loss (Achten, Gleeson and Jeukendrup, 2002). However, even though it presents lower fat oxidation rates during exercise, High-Intensity Interval Training (HIIT) seems to promote equal or superior fat loss than MICT in chronically (Türk *et al.*, 2017; Weweg *et al.*, 2017). While MICT provides a better environment for fat utilization during exercise (i.e., low glycolytic activity and lactate levels), HIIT seems to promote higher post-exercise fat oxidation, probably due to catecholamines release, the need for lactate and H^+ clearance, and ATP resynthesis (Borsheim *et al.*, 1998). In this sense, the combination of MICT and HIIT on the same session could be a successful approach when aiming at fat oxidation.

The combination of MICT and HIIT (hybrid training) during the same session is not a new procedure. A recent study observed that 60 min of continuous exercise interspersed with 30s sprints every 10 min elevated cellular autophagy markers in the muscle and AMPK phosphorylation after the training session (Brandt *et al.*, 2018), which are related to improvements in strength and mitochondrial biogenesis (Schwalm *et al.*, 2015). Another study demonstrated that the hybrid model generates a higher concentration of lactate and adrenaline, and a reduction in the phosphocreatine stock compared to the moderate continuous model and, from a chronic point of view, the addition of sprints could cause adaptations in the muscular oxidative system (Gunnarsson *et al.*, 2019). With these findings, questions arise about the physiological effects of manipulating the inserted high-intensity exercise variables, such as done intermittently or continuously.

To the best of our knowledge, no studies have assessed the effects of including high-intensity bouts in moderate-intensity exercises on substrate

oxidation during and after effort. Thus, the present study aimed to measure the heart rate, blood lactate, oxygen consumption, and substrate oxidation responses during and after moderate continuous exercise in the FATmax zone with the insertion of high-intensity efforts. We hypothesize that inserting supramaximal-intensity bouts will increase physiological demand and provide higher subsequent lipid oxidation.

2. Materials and Methods

2.1 Study design

We designed a randomized crossover study with repeated measures to assess physiological (heart rate, blood lactate, and oxygen consumption) and bioenergetic (substrate oxidation) demands of including high-intensity sprints, repeated or not, into continuous exercise protocols in the FATmax zone. The independent variables were the exercise mode (FATmax, repeated or high-intensity bout) and the times (pre, during, and post-exercise). The study design is presented in Figure 1.

2.2 Participants

Twelve physical education students composed the sample, recruited by posters spread across the university (May – June 2019). The inclusion criteria were as follows: 1) being male; 2) being between 18 and 35 years old; 3) declaring experience with high-intensity efforts (e.g. interval training, team sports matches, weight training). As exclusion criteria, we considered: 1) being a smoker; 2) being overweight or obese; 3) using β -blockers; 4) being an athlete. All subjects signed the informed consent, and the Ethical Committee of the Federal University of Pelotas approved the research project (protocol number 29945519.5.0000.5313).

2.3 Settings

Each subject went to the laboratory four times, with at least 30 hours between each visit. They were asked to maintain their food and sleep routine and

We measured their body weight (Soehnle®) and height (Sanny®) on the first visit. Afterward, we measured their heart rate (Polar, RS800CX) for 15 minutes in a supine position. Then, we conducted a maximal graded exercise test (GXT) on a mechanical cycle ergometer (Cefise, Biotec 2100) to determine their FATmax zone, their maximal oxygen uptake ($\text{VO}_{2\text{MAX}}$), and its related intensity ($\text{iVO}_{2\text{MAX}}$).

Maximal graded exercise test

After the same 5-minute warm-up, the participants completed the following training protocols: 1) *Continuous at FATmax zone (FATmax)*: 30 minutes at an intensity correspondent to the FATmax zone determined on GXT. 2) *Continuous at FATmax zone with a high-intensity bout (2min-130%)*: 12 minutes at the same FATmax zone + 2 minutes at 130% of the $\dot{V}O_{2max}$ + 16 minutes at FATmax. 3) *Continuous at FATmax zone with repeated high-intensity bouts (20s:10s-170%)*: 12 minutes at FATmax + 4 all-out bouts of 20 seconds at 170% of the $\dot{V}O_{2max}$ interspersed by 10 seconds of rest + 16 minutes at FATmax.

The cadence was fixed at 65 to 75 rpm during all protocols, except when specified differently. The subjects remained seated for 30 minutes after the protocols, and VO_2 , [LAC], and HR were collected.

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2.5 Data collection

2.5.1 Gas exchange, heart rate, and associated parameters

The VO_2 was estimated at every three breaths using open-circuit spirometry (VO2000™, Medical Graphics, St. Paul, MN), calibrated following the manufacturer's recommendations, and registered in proper software (BREEZESUITE™ Diagnostic Testing Software, MedGraphics, St. Paul, MN), where the mean values for during and post-exercise were extracted. The $\text{VO}_{2\text{MAX}}$ was achieved when a plateau of $\leq 150\text{ml}$ was observed, and its related load was called $\text{iVO}_{2\text{MAX}}$. The participants used a *Neoprene*™ mask, with a high-flow pneumotachograph during exercise and low-flow during rest, connected by an umbilical to VO000™. The mean and maximum heart rate were collected and registered using a Polar® monitor (V800, Polar Electro Oy, Kempele, Finland).

2.5.2 FATmax zone calculation

We determined the FATmax zone following previously reported methods (Achten, Gleeson and Jeukendrup, 2002). Briefly, we registered the intensity related to the peak of fat oxidation during the GXT, indicated by the gas analyzer and its respective software (BREEZE SUITE™ Diagnostic Testing, MedGraphics, St. Paul, MN), which occur on the lowest obtained RER value. We considered the highest value of VO_2 as the $\text{VO}_{2\text{max}}$ (Trapp *et al.*, 2008), and the load related to it, the $\text{iVO}_{2\text{MAX}}$. To register the HR, we used a specific monitor (Polar® V800, Kempele, Finland), and we considered the highest value of HR during the GXT as the HR_{max} .

2.5.2 Blood analysis

A trained researcher (BNMS) collected blood for [LAC] analysis post-warm-up and on minutes 5, 15, 30, 45, and 60 after the start of the training protocol. To do that, after cleansing the participant's fingertip with 70% alcohol, he used a proper lancet of 3.25mm length and 0.04mm diameter (Twist Lancets, Medlevensohn®), drained 15 μL of blood to a heparinized capillary, and transferred it to an *Eppendorf*™ tube with 30 μL of EDTA anticoagulant. The

analysis was then performed on specific equipment (YSI 2300, Ohio, United States).

2.5.3 Substrates oxidation and energy expenditure calculation

To calculate the fat and CHO oxidation rates, respectively, we used the following equations: Fat oxidation rate (g/min^{-1}) = $1.695 \times \text{mean oxygen uptake (L}/\text{min}^{-1}) - 1.701 \times \text{mean exhaled carbon dioxide (L}/\text{min}^{-1})$ (Jeukendrup and Wallis, 2005); Carbohydrate oxidation rate (g/min^{-1}) = $4.585 \times \text{mean exhaled carbon dioxide (L}/\text{min}^{-1}) - 3.226 \times \text{mean oxygen uptake (L}/\text{min}^{-1})$ (Péronnet and Massicotte, 1991); and for the energy expenditure calculation, we adopted the following: Energy expenditure ($\text{Kcal}/\text{min}^{-1}$) = $4.82 \times \text{mean oxygen uptake (L}/\text{min}^{-1})$ (McArdle et al., 2010).

2.5.4 Bias control

Before each experimental session, participants were asked about their last mealtime, sleep hours, and how they commuted to the laboratory and were told to keep the same routine. All procedures were conducted by the same researcher (BNMS), in the same period of the day, and in the same environment-controlled laboratory.

Considering that the CO_2 from the buffering system during high-intensity exercise can skew the equation premise that the CO_2 comes from the oxidation process (Jeukendrup and Wallis, 2005), to measure the oxidation of the *2min-130%* and *20s:10s-170%* protocols, we adopted the following procedures:

Two trained and independent researchers (BNMS and GVP) determined when each subject returned to a steady state after the sprints by analyzing the VO_2 and VCO_2 curves. Then, the moment when the CO_2 from the buffering system was higher was discarded from the fat oxidation analyses. Therefore, we considered the mean of the pre-sprint moment (until minute-12) and after-sprint moment (from the time determined by the researchers until the end of the session) for the oxidation analyses. For the energy expenditure analyses we considered the whole session.

2.5.5 Statistical analysis

Energy expenditure and CHO oxidation were higher during exercise than post-exercise. Fat oxidation presented no differences or interaction between times. Figure 3 shows the substrate oxidation curves generated by the mean of all subjects.

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>>>>>>> please, insert figure 3 near here <<<<<<<<<<

The [LAC] presented significant interaction ($p < 0.001$), and the Bonferroni *post-hoc* test indicated that FATmax presented lower values than *2min-130%* ($p < 0.001$) and *20s:10s-170%* ($p < 0.001$) on minutes 15 and 30; and lower than 170% on minute 45 ($p = 0.016$). The pre-training, 5, and 60-minutes values were not different between protocols. Minutes 15 and 30 at 130% and 170% were higher than the other times, but not different between them ($p = 0.016$). The [LAC] curve is presented in Figure 4.

The RER presented significant interaction between training protocols and times ($F=26.01$; $p<0.001$). Between protocols, on minutes 15 and 45, *FATmax* presented lower values than *2min-130%* ($p<0.001$; $p=0.04$, respectively) and *20s:10s-170%* ($p<0.001$; $p=0.002$). There were no differences between the others.

For the [LAC], 2min-130% and 20s:10s-170% presented the same behavior between the times. There were differences between minutes 5 and 15 ($p<0.001$; $p<0.001$, respectively), 30 ($p=0.014$; $p=0.014$), 45 ($p=0.035$; $p=0.007$), 60 ($p=0.030$; $p=0.002$), differences between 15 and 30 ($p<0.001$; $p<0.001$), 45 ($p<0.001$; $p<0.001$), 60 ($p<0.001$; $p<0.001$), and differences between 30 and 45 ($p=0.015$; $p=0.001$), 60 ($p=0.007$; $p=0.001$). For the *FATmax*, minute 5 was different from 15 ($p=0.006$), 30 ($p=0.001$) and 60 ($p=0.009$), minute 15 was different from 60 ($p=0.002$), and minute 30 was different from 45 ($p=0.040$) and 60 ($p=0.001$).

studies showed that similar high-intensity protocols could elicit a peak HR near 90% HR_{max} (Buchheit and Laursen, 2013; Protzen *et al.*, 2020). Still, re-establishing steady HR values after efforts takes considerable time, depending on the subject's physical fitness (Imai *et al.*, 1994). Considering that our sample was relatively low fit, it may explain the differences in average HR.

The VO_2 during exercise is appointed as a determinant to enhance the cardiovascular system function (Buchheit and Laursen, 2013). We observed a higher peak of VO_2 when high-intensity bouts were included. However, there were no significant difference in average VO_2 . Considering that previous investigation shown that the execution of four bouts at 170% $\text{VO}_{2\text{max}}$ requires just around 4.5 L of oxygen (Protzen *et al.*, 2020), we suppose that this exercise protocol is not able to increase statistically the average VO_2 of the whole session.

The anaerobic lactic system is importantly used to perform high-intensity efforts, and the [LAC] indicates its utilization (Di Prampero and Ferretti, 1999). We found higher [LAC] during exercises with high-intensity efforts, similar to the findings of Gunnarsson *et al.* (2019), who also tested MICT with and without the inclusion of high-intensity bouts and found the same pattern. However, they reported slightly lower values, probably due to the protocol characteristics since our [LAC] values were similar to Protzen *et al.* (2020) during the 170% intermittent exercise. The higher [LAC] was an expected result since higher intensities require higher anaerobic contributions to be sustained (Ferguson *et al.*, 2018).

In our study, the RER remained stable during the MICT and decreased after exercise cessation. This ratio between carbon dioxide production and oxygen consumption indicates the predominantly used substrate (Weir, 1948). While an RER higher than 1 indicate mainly CHO contribution, lower-than-1 values indicate higher lipidic oxidation (Jeukendrup and Wallis, 2005). The RER behavior during MICT was different from the exercises with sprints, in which the RER achieved very high values during efforts, due to the high carbon dioxide produced by the energetic pathways and acid-base balance (Jeukendrup and Wallis, 2005). On the other hand, after the high-intensity section, the RER reduced continuously and presented lower post-exercise values than MICT. During exercise, it has been evidenced that lower RER-values, and consequently

higher fat oxidation, are found: in long-duration, low-intensity exercises (Tsiloulis and Watt, 2015); while fasting (Maughan, Fallah and Coyle, 2010; Zouhal *et al.*, 2020); or while adopting CHO-restricted diets (Macedo *et al.*, 2020). Despite this, the higher the intensity during exercise, the lower the post-exercise RER (Phelain *et al.*, 1997; Tucker, Angadi and Gaesser, 2016; Nakagata, Yamada and Naito, 2018). This seems to be explained by many factors, such as the release of catecholamines after high intensity exercises (Bracken, Linnane and Brooks, 2009), which increases the blood fatty acids concentration (Borsheim *et al.*, 1998); the ATP resynthesis derived from phosphocreatine degradation and muscular triacylglycerol stores due to the possible inhibition of anaerobic glycogenolysis; and the need to remove lactate and H^+ (Gaitanos *et al.*, 1993). Thus, the inclusion of high-intensity efforts seems to favor lipid oxidation subsequently, and the effect is indicated by the lower RER-values after exercise.

During exercise, energy expenditure has a significant role in generating energy deficit, a relevant aspect for weight loss and obesity prevention (Donnelly *et al.*, 2009). The exercise models did not present differences between them, but only between the times, being higher during (~7Kcal/min) than post-exercise (~1.5Kcal/min). In opposition to Crisp *et al.* (2012), who demonstrated that the inclusion of 4-second sprints every two minutes could promote higher energy expenditure without impairing fat oxidation during MICT (Crisp *et al.*, 2012), our protocols with high-intensity efforts did not change the caloric expenditure. This difference allows the comparison between isocaloric protocols, including in the post-exercise period. It is essential to consider more than just the quantity of energy, but from where it comes. Although we could not observe differences in energy expenditure, the fat contribution was higher, partially explaining the favorable results found in interventions with high-intensity exercises (Türk *et al.*, 2017), which are also explained by chronic adaptations related to fat oxidation (Trapp *et al.*, 2008).

Increasing fat oxidation at rest is challenging since it is already predominant and regulated by many factors (Melzer, 2011; Jordy and Kiens, 2014; Pownall and Moore, 2014). The inclusion of high-intensity bouts can enhance fat oxidation in the post-exercise period (Børsheim and Bahr, 2003). Our

study provides a relevant insight for endurance exercise interventions aiming to enhance fat oxidation or reduce stored body fat.

The following should be mentioned as limitations of our study: our sample was composed only of males, and we suggest new studies with females to confirm the findings in this population; Although we used the gold standard to assess substrate metabolism (indirect calorimetry), the method has its limitations, especially in high-intensity exercises, when shifts in acid-base balance promote non-oxidative CO₂ (Hetlelid *et al.*, 2015). However, we adopted a strategy to overcome this problem, as cited in the Methods section (bias control); it is important to highlight that this strategy, to the best of our knowledge, is not validated yet. Furthermore, we did not consider the first seven minutes after exercise due to the need to change the pneumotachograph. Nevertheless, the equations were not created considering Excess Post-Exercise Oxygen Consumption (Jeukendrup and Wallis, 2005).

5. Conclusion

Our study found that the inclusion of supramaximal sprints, repeated or not, in moderate-intensity continuous exercise promotes higher cardiorespiratory and metabolic demand and improves post-exercise fat oxidation, even though it does not modify energy expenditure.

Author statements

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Competing interests

The authors declare there are no competing interests.

Contributors' statement

Conceptualization, methodology and formal analysis: BMS and FBV; data curation, investigation, and writing – original draft: BMS; writing – review and editing: BMS, GVP, and FBV.

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565

Figure captions

Figure 1 - Study design to analyze the effects of including high-intensity bouts on FATmax exercise.

From left to right are the steps of data collection and processing; HR = Heart rate; VO = Oxygen uptake; VCO₂ = Expelled carbon dioxide; ♂ = exercise period; ♂ = pneumotachograph change period (from standard-flow to low-flow); ♀ = post exercise period.

Figure 2 - Heart rate comparison during FATmax exercise with or without high-intensity bouts insertion (n = 12).

* = average heart rate lower on *FATmax* than *2min-130%* (p=0.001) and *20s:10s-170%* (p<0.001); # = peak heart rate lower on *FATmax* than *2min-130%* (p=0.001) and *20s:10s-170%* (p=0.001).

Figure 3 - Substrate oxidation during and post continuous-moderate exercise with or without the inclusion of high-intensity bouts (n=12).

^CO₂ = period excluded from the analysis due to the high CO₂ production during the high-intensity bouts; bias control strategy that is better explained in the methods section.

Figure 4 - Blood lactate concentration and Respiratory Exchange Ratio (RER) during continuous moderate with or without high-intensity sprints (n=12).

Warmed-up = blood lactate concentration just after the end of the warm-up; 5, 15, 30, 45, 60 = current time of the session; * = *FATmax* different from *2min-130%* and *20s:10s-170%*; # = *FATmax* different from *20s:10s-170%*.

Table 1. Participants’ anthropometrical and physiological characteristics (n=12).

Descriptive variables	Mean ± SD
Age (years)	24 ± 4.2
Height (m)	1.76 ± 0.1
Body mass (kg)	80 ± 11.8
Body mass index	26 ± 3.4
Rest heart rate (bpm)	61 ± 7
Maximum oxygen uptake (mL/kg/min ⁻¹)	37.38 ± 5.6
Maximum heart rate (bpm)	176 ± 8
FATmax zone (%VO2 _{max})	40.95 ± 14.6
RPE on FATmax zone (6-20)	8 ± 1.5

SD = standard deviation; RPE = rating of perceived exertion.

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Table 2. Comparison of physiological responses to a continuous moderate exercise with or without the inclusion of high-intensity bouts (n=12).

	<i>2min-130%</i>	<i>20s:10s-170%</i>	<i>FATmax</i>	Between groups	
	Mean±SD	Mean±SD	Mean±SD	F	<i>p</i>
During exercise					
VO ₂ peak (mL·kg ⁻¹ ·min ⁻¹)	36±7	34±6	21±3 ^{&}	32.36	< 0.001
VO ₂ average (mL·kg ⁻¹ ·min ⁻¹)	18±3	18±3	17±3	0.79	0.46
Fat oxidation (g·min ⁻¹)	0.12±0.19	0.03±0.17	0.05±0.15	0.55	0.58
Carbohydrate oxidation (g·min ⁻¹)	1.42±0.5	1.51±0.46	1.74±0.38	1.5	0.24
Energy expenditure (kcal·min ⁻¹)	7.18±1.37	6.85±1.06	6.62±1.35	0.86	0.44
Non-parametrical variable	Median±IQR	Median±IQR	Median±IQR	X ²	<i>P</i>
Eligible analysis time (min)	20±1.22	20±1.29	30±0.64 ^a	18.66	< 0.001
Post-exercise					
	Mean±SD	Mean±SD	Mean±SD	F	<i>P</i>
HR average (bpm)	88±6	89±6	86±13	0.87	0.43
VO ₂ average (mL·kg ⁻¹ ·min ⁻¹)	4.11±0.7	4.01±0.42	4.1±0.53	0.17	0.84
Fat oxidation (g·min ⁻¹)	0.11±0.03	0.12±0.04	0.08±0.04 [#]	9.31	0.005
Energy expenditure (kcal·min ⁻¹)	1.59±0.26	1.55±0.24	1.52±0.25	0.44	0.65
Non-parametrical variable	Median±IQR	Median±IQR	Median±IQR	X ²	<i>p</i>
Carbohydrate oxidation (g·min ⁻¹)	0.1±0.1	0.1±0.1	0.2±0.13	5.91	0.052
Eligible analysis time (min)	23±2.2	23±1.3	22±0.7	2.66	0.26

SD = standard deviation; IQR = interquartile range; FATmax = FATmax zone continuous exercise; 130% = FATmax zone continuous exercise with a bout of 2 minutes @ 130% iVO_{2max}; 170% = FATmax zone continuous exercise with four bouts of 20 seconds @ 170% iVO_{2max} interspersed by 10 seconds of rest; HR = heart rate; VO₂ = oxygen uptake; &= differences between FATmax and 130% (p<0.001) and 170% (p<0.001); # = differences between FATmax and 130% (P= 0.001) and 20s:10s-170% (p=0.019). ^a = differences between FATmax and 2min-130% (p=0.003) and 20s:10s-170% (p<0.001).

Figure 1

Figure 2

Figure 3

Figure 4