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Effect of exercise intensity on postprandial lipemia, markers of oxidative stress and endothelial function after a high-fat meal

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Abstract

Purpose: The aim of this study was to compare the effect of two different exercise intensities on postprandial lipemia, oxidative stress markers and endothelial function after a high-fat meal (HFM).

Material and methods: Eleven young men completed 2-day trials in three conditions: Rest, moderate-intensity exercise (MI-Exercise) and heavy-intensity exercise (HI-Exercise). Subjects performed an exercise bout or no exercise (Rest) on the evening of day 1. On the morning of day 2, a HFM was provided. Blood was sampled at fasting (0 h) and every hour from 1 to 5 h during the postprandial period for triacylglycerol (TAG), thiobarbituric acid reactive substance (TBARS) and nitrite/nitrate (NOx) concentrations. Flow-mediated dilatation (FMD) was also analyzed.

Results: TAG concentrations were reduced in exercise conditions compared to Rest during the postprandial period ($P < 0.004$). TAG incremental area under the curve (iAUC) was smaller after HI-Exercise compared with Rest ($P = 0.012$). TBARS concentrations were reduced in MI-Exercise compared to Rest ($P < 0.041$). FMD was higher in exercise conditions than Rest at 0 h ($P < 0.02$) and NOx concentrations were enhanced in MI-Exercise compared to Rest at 0 h ($P < 0.01$).

Conclusions: These results suggest that acute exercise can reduce lipemia after a HFM. However, HI-Exercise showed to be more effective in reducing iAUC TAG, which might suggest higher protection against postprandial TAG enhancement. Conversely, MI-Exercise can be beneficial to attenuate the susceptibility of oxidative damage induced by a HFM and to increase endothelial function in the fasted state compared to Rest.

Keywords: Postprandial responses, oxidative damage, lipid peroxidation, nitrites/nitrates, flow-mediated dilatation.

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Introduction

Regular consumption of a high-fat meal (HFM) can increase circulating triacylglycerol (TAG) concentration and contribute to the long-term development of cardiovascular diseases, even in healthy young men (Bae et al. 2001; Tushuizen et al. 2006). Moreover, the consumption of a HFM produces an imbalance in postprandial metabolism, increasing the susceptibility of oxidative damage and vascular endothelial dysfunction (Wallace et al. 2010). The increase in reactive oxygen species production enhances peroxynitrite (ONOO^-) formation and, by consequence, decreases nitric oxide (NO) bioavailability, causing endothelial dysfunction (Forstermann and Munzel 2006; Wallace et al. 2010). While direct assessment of NO is extremely difficult, the combination of nitrites/nitrates (NO_x), NO end-products, is widely used to indirectly estimate NO production (Casey et al. 2007). Another extensively-used technique to evaluate endothelial function is flow-mediated dilatation (FMD) of the brachial artery in response to hyperemia (Tyldum et al. 2009; Wallace et al. 2010; Sedgwick et al. 2013; Sedgwick et al. 2014).

In this sense, previous studies have shown that exercise performed a few hours before the consumption of a HFM can attenuate oxidative damage and endothelial dysfunction induced by the meal (Bae et al. 2001; Clegg et al. 2007; Mc Clean et al. 2007; Jenkins et al. 2011; Krüger et al. 2015). However, only few studies have investigated the effect of exercise intensity (Tyldum et al. 2009; Gabriel et al. 2012; Canale et al. 2014). For instance, Tyldum *et al.* (2009) tested the effect of two different aerobic exercise intensities with the same energy expenditure (at 85 - 90% and at 60 - 70% of maximal heart rate (HR)). Total antioxidant status (TAS) was increased after

both exercise sessions compared with rest, but the high-intensity exercise increased the antioxidant status more. In addition, FMD was increased in both exercise conditions compared to rest (Tyldum et al. 2009). Gabriel et al. (2012) also compared the effect of two different exercise intensities with different energy expenditures (high-intensity interval cycling vs. continuous walking for 30 min). The authors reported that thiobarbituric acid reactive substance (TBARS) concentrations were decreased after the HFM only in the interval cycling condition, which had lower energy expenditure compared to the walking condition. Conversely, Canale et al. (2014) analyzed the effect of three intensities of cycling exercise (60 min, 60 s and 15 s) and did not find any difference between conditions for lipid peroxidation.

It is evident that there is a gap in the literature that cannot explain the effect of exercise intensity on postprandial oxidative stress and endothelial function responses. Different exercise methodologies have been tested and the results are contradictory. It is known that to analyze the effect of the exercise intensity, the exercise bouts need to be matched in terms of energy expenditure, considering that the energy expenditure during an exercise session can affect the postprandial metabolism responses (Pettit and Cureton 2003). The only previous study that have matched the energy expenditure between both exercise intensities sessions (Tyldum et al. 2009) did not directly evaluated any prooxidant stress markers. In addition, the exercise intensities were prescribed based on the %HR, which it is a less reliable method to prescribe individualized exercise compared to the ventilatory threshold based method (Wolpern et al. 2015).

Answering this scientific question about the effect of exercise intensity on the postprandial metabolism is crucial because exercise intensity is an important component of training prescription. Therefore, to clarify this question, the purpose of this study was to compare the effect of two different isoenergetic aerobic exercise intensities determinate by the ventilatory threshold based method (moderate and heavy) on postprandial lipemia (PPL), oxidative stress markers and endothelial function after the consumption of a HFM. Based on the previous study done by Tyldum et al. (2009), we hypothesized that exercise performed at heavy-intensity would cause a greater attenuation of PPL and prooxidant stress markers and increase both antioxidant defense and FMD response than moderate–intensity exercise.

Materials and methods

Study population

Eleven healthy young men volunteered to participate in this study. To be included in this study, all participants were required to be physically active (exercise at least three times a week), eutrophic (body mass index: 18.5 - 25 kg/m²) and have a VO_{2max} greater than 35 mL/kg/min. Smokers, participants who were receiving medical treatment or had any history of severe disease related to lipid metabolism were excluded. All participants provided written informed consent. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and was approved by the ethics committee of the Federal University of Rio Grande do Sul, Brazil.

Preliminary visit

At the preliminary visit all participants performed an analysis of basal metabolic rate (BMR), body composition and $\text{VO}_{2\text{max}}$. The BMR analysis consisted in 30 min of exhaled gas collection in dorsal decubitus performed during the first morning hours (between 7.00 and 8.30 am) while exhaled gases were analyzed by a breath-by-breath collection system (Quark CPET, COSMED, Rome, Italy). The first 10 min of gas collection were excluded from the analysis; thus, VO_2 and VCO_2 (L/min) obtained during the final 20 min of each collection (mean value of the period) were used for the calculation of BMR. BMR was calculated according to the Weir equation (Weir 1949) and then converted to BMR for a 24-h period. Prior to this analysis, participants were asked to fast for 12 h and to have a good night sleep of at least 8 h. Furthermore, they were strongly encouraged to avoid moderate to high energy expenditure activities 24 h before the BMR analysis. The marking of the anatomical sites and measurement technique of skinfolds followed the standards of the International Society for the Advancement of Kinanthropometry (ISAK). Body composition was calculated using a five-component method (Marfell-Jones et al. 2006). An incremental exercise test was conducted to determine $\text{VO}_{2\text{max}}$ using the breath-by-breath method and an open-circuit spirometry system (Quark CPET, COSMED, Rome, Italy). The progressive exercise test was performed on a treadmill and lasted for 8 - 12 min according to the recommendations of the *American College of Sports Medicine (ACSM)* (ACSM 2009). The test ended if the participant reached volitional exhaustion, respiratory exchange ratio (RER) ≥ 1.15 or HR $\geq 95\%$ of age-predicted maximum. The first ventilatory threshold (VT_1) was identified as the minimum workload at which the ventilatory equivalent ratio for oxygen (VE/VO_2) systematically increased without an increase in the

ventilatory equivalent ratio for carbon dioxide (VE/VCO_2) and the second ventilatory threshold (VT_2) as the lowest workload where both VE/VO_2 and VE/VCO_2 increased. VO_{2max} was determined when a plateau in the VO_2 occurred when further increase of exercise intensity. The plateau was considered when the VO_2 varied by less than 1.5 mL/kg/min even with an increase in the workload (Cunha et al. 2011). VT_1 , VT_2 and VO_{2max} were obtained by visual inspection of graphs by 3 independent observers.

Intervention

After the preliminary visit, the participants were randomly assigned to perform 2-day trials in three different conditions (interspaced by at least one week):

- (i) Rest: the participant rested for 45 min;
- (ii) Moderate-intensity exercise (MI-Exercise): the participant ran on a treadmill at a speed corresponding to VT_1 ;
- (iii) Heavy-intensity exercise (HI-Exercise): the participant ran on a treadmill at a speed corresponding to 10% below VT_2 .

The exercise-intensities were defined based on the “intensity domain” model described by Hill et al. (2002), in which moderate intensity is associated with an increase in VO_2 to a steady state (i.e. close to VT_1), whereas heavy intensity corresponds to work rates close to the lactate threshold or VT_2 (Hill et al. 2002). Both exercise bouts were monitored online by an open-circuit spirometry system. The exercise duration was determined to correspond to 15% of the BMR in kcal. This value corresponded to an average of 306.2 ± 28.2 kcal/per session of MI-Exercise and 298.4 ± 27.8 kcal/per session of HI-Exercise and it met the ACSM recommendations

associated with lower rate of cardiovascular disease, which it is about 1000 kcal/week or 150 min/week performed on ≥ 3 days/week (Garber et al. 2011). Both exercise bouts were isoenergetic and were monitored online by an open-circuit spirometry system (Quark CPET, COSMED, Rome, Italy). The energy expenditure during exercise was calculated based on the metabolic equivalent method (Ainsworth et al. 2000). The energy expenditure was calculated at the end of every minute and exercise stopped once the target energy expenditure had been completed.

On the evening of day 1, participants performed one of the three different conditions. Ten minutes after the protocol a standard meal was provided. Participants then went home and were instructed to fast, have a good night sleep and to avoid physical activity for the following 12 h. In the early morning (day 2), participants arrived at the laboratory for a fasted blood collection and FMD analysis (0 h). A HFM was then provided and blood samples were taken every hour during the postprandial period (from 1 to 5 h after the meal consumption). Additional FMD analyses were performed at 1 and 3 h.

Diet Control

To eliminate the confounder effects of food consumption on analyzed parameters, a 24-h diet recall interview was performed by a nutritionist during day 2 of each trial. This interview consisted of recording the amount of food and beverage consumed during day 1. After the first condition, participants were asked to follow the same diet for the other conditions to avoid intraindividual variance between the conditions.

The standard meal consisted of a four-cheese pizza and a maltodextrin beverage. The percentage of each macronutrient was: 60% carbohydrate, 20% lipid and 20% protein. The HFM was white sandwich bread with table cream and mozzarella cheese and fat milk with chocolate powder. The percentage of each macronutrient was: 35% carbohydrate, 50% lipid and 15% protein. Previous studies have shown that the minimal absolute lipid load that leads to significant changes on TAG can vary between 30 and 50 g (Cohen and Schall 1988; Dubois et al. 1998). In this study, 50% of energy from fat provided an average of 52.4 g fat. The size of both meals was individually calculated to be equivalent in energy to 50% of daily BMR. The participants had between 10 - 20 min to ingest each meal.

Blood collection and biochemistry

Blood samples (0 – 5 h) were collected every hour from the arm antecubital region via a disposable sterile cannula. At 0, 1, 3 and 5 h, blood was collected to analyze TAG, total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), glucose, insulin, TBARS, NOx and total thiols. At 2 and 4 h, blood was taken to analyze TAG only. Blood samples were collected in tubes containing EDTA and then centrifuged at 1500 rpm for 15 min at 4 °C. The plasma was stored and frozen at –80 °C until analysis.

TAG, total cholesterol, HDL-C and glucose were analyzed using an automatic colorimetric method (Cobas C111 analyzer, Roche, Basel, Switzerland). LDL-C was calculated from the Friedewald equation (Friedewald et al. 1972). Insulin concentrations

were determined by ELISA with standard test kits (ref. EIA-2935, DRG International, Springfield, United States), according to the manufacturer's instructions.

The NO_x concentrations were analyzed according to a previously described methodology by Miranda et al. (2001). The samples were analyzed in a microplate reader (Multiskan Go, Thermo Scientific, Vantaa, Finland) with the specified wavelength of 540 nm and the results were expressed in $\mu\text{M/L}$. TBARS concentrations were analyzed following a previously described technique by Ohkawa et al. (1979). The samples were analyzed in a microplate reader at 532 nm and the results were expressed in μM of MDA/L. Total thiols analysis was performed according to a methodology described by Ellman (1959). The samples were analyzed in a microplate reader at 412 nm and the results were expressed in mM of GSH/L.

Arterial flow-mediated dilatation (FMD)

FMD analysis consisted of acquiring images of the brachial artery using a 7.5 MHz linear array transducer ultrasound system (Nemio XG, Toshiba, Japan). A rapid inflation/deflation pneumatic cuff was positioned distal to the olecranon to create an ischemic stimulus. Doppler flow velocity measurements were obtained simultaneously using the lowest possible angle to interrogate maximum laminar flow. Participants remained at rest for 15 min before 30 s of baseline analysis of arterial diameter and blood flow were performed. Subsequently, the cuff was inflated (> 240 mmHg) for 5 min. Arterial diameter and blood flow were recorded on video tape at least 30 s prior to cuff deflation and for 2 min immediately after. The videotape was later analyzed by two

independent blinded observers according to published instructions (Corretti et al. 2002).

The results are expressed in %FMD variation from baseline.

Statistical Analysis

The Shapiro-Wilk test was used to assess the normality of data for each variable. Levene's test was used to test the homogeneity of variance assumption. Mauchly's test was used to assess the sphericity assumption. When sphericity was violated, the Greenhouse-Geisser correction factor was applied. Parametric data are presented as mean $\pm$ standard deviation (SD) and the non-parametric data (NOx) are presented as median [quartile 1 (Q1), quartile 3 (Q3)]. Differences between exercise bouts variables were assessed by paired *t*-test. A one-way analysis of variances (ANOVA) was performed to analyze the effect of macronutrient intake from the 24-h diet recalls between the conditions. All blood outcomes (except NOx) and FMD data were analyzed using a two-way ANOVA (3 conditions and 6 times) with repeated-measures. If results showed a significant main effect for condition or time, a subsequent Bonferroni post-hoc analysis was used to identify differences between conditions or times. If there was an interaction between condition and time, a series of paired *t*-tests with Holm-Bonferroni corrections was used to compare different conditions and time points (Holm 1979). The area under the curve (AUC) for TAG was calculated using the trapezoidal method, whereas the incremental area under the curve (iAUC) was calculated by the same method, but subtracting fasting levels from each postprandial response. The difference between conditions for AUC and iAUC was analyzed using a one-way ANOVA. For NOx, the Kruskal-Wallis test was used to perform intergroup (3 conditions) comparisons

at each time point. Time-point comparisons were performed by Friedman test. A 95% level of confidence was predetermined as the minimum criterion to denote a statistical difference ($P < 0.05$). The data was analyzed using the Statistical Package for Social Sciences version 19.0 (SPSS Inc., Chicago, United States). Using Altman's nomogram (Altman 1982), the total sample size calculation for the outcome AUC TAG was 11 participants, assuming a standardized difference of 1.07 (Gabriel et al. 2012), a power of 0.8 and a significance level of 0.05.

Results

The characteristics of the participants are presented in Table 1. There were no differences between the conditions for reported energy and macronutrient intake from the 24-h diet recalls ($P > 0.05$) (Table 2). There were no differences in energy expenditure between MI-Exercise and HI-Exercise (306.2 ± 28.2 kcal vs. 298.4 ± 27.8 kcal; $P > 0.05$). The average and the percentage of VO_2 during exercise were lower in MI-Exercise than HI-Exercise (31 ± 4 mL/kg/min and $65 \pm 3\%$ vs. 37 ± 4 mL/kg/min and $77 \pm 2\%$; $P < 0.01$). Exercise duration was longer in MI-Exercise than HI-Exercise (37.5 ± 4.7 min vs. 32.5 ± 3.9 min; $P < 0.01$), whereas mean HR was higher in HI-Exercise than MI-Exercise (165.5 ± 8.1 beats per minute (bpm) vs. 157.6 ± 6.6 bpm; $P = 0.02$).

Biochemistry and endothelial responses

The two-way ANOVA test showed no significant interaction between conditions and time for any of the blood outcomes. There was a condition effect for TAG concentrations ($F_{2,20} = 22.082$; $P < 0.001$). The Bonferroni post-hoc test showed a

difference between MI-Exercise and Rest ($P < 0.004$) and between HI-Exercise and Rest ($P < 0.001$) (Fig. 1A). There was also a significant time effect ($F_{5,50} = 15.988$; $P < 0.001$). TAG concentrations showed significantly higher value from 2 to 5 h compared to 0 h ($P < 0.032$). There was also an increase in TAG concentrations from 1 to 2 h ($P = 0.041$) and a decrease from 2 and 3 h compared to 5 h ($P < 0.044$).

The one-way ANOVA revealed a difference between the conditions for TAG AUC ($F_{2,32} = 8.488$; $P < 0.001$). TAG AUC was 29% smaller after HI-Exercise compared with Rest ($P < 0.01$) and 21% smaller after MI-Exercise compared with Rest ($P = 0.017$) (Fig. 1B). The one-way ANOVA revealed also a difference between the conditions for iAUC TAG ($F_{2,32} = 5.038$; $P = 0.013$). The iAUC TAG was 49% smaller after HI-Exercise compared with Rest ($P = 0.012$) (Fig. 1C).

There was no main effect of condition and time for total cholesterol ($P > 0.05$). There was also no main effect of condition for LDL-C, HDL-C, glucose or insulin ($P > 0.05$) (Table 3). There was a main effect of time for LDL-C ($F_{5,50} = 8.410$; $P < 0.001$). LDL-C concentrations were lower at 1, 3 and 5 h compared to 0 h ($P < 0.016$). There was a main effect of time for HDL-C ($F_{5,50} = 5.546$; $P < 0.001$). There was a decrease in HDL-C concentrations from 0 to 5 h ($P < 0.001$). There was also a time effect for glucose ($F_{3,30} = 16.763$; $P < 0.001$) and Insulin ($F_{3,30} = 83.001$; $P < 0.001$). Glucose concentrations decreased from 0 to 1 h ($P = 0.002$) and from 1 to 3 h ($P = 0.012$), whereas Insulin concentrations increased from 0 to 1 h ($P < 0.001$) and decreased from 1 to 3 h ($P < 0.001$) (Table 3).

There was a significant main effect of condition for TBARS ($F_{2,20} = 5.880$; $P = 0.012$) (Fig. 2). The Bonferroni post-hoc test showed a significant difference between

MI-exercise and Rest ($P = 0.041$). There was also a significant time effect for TBARS ($F_{3,30} = 6.393$; $P = 0.002$) (Fig. 2). The post hoc analysis showed difference between 0 and 3 h for TBARS ($P = 0.007$). There was no main effect of condition and time for total thiols ($P > 0.05$). A Kruskal-Wallis test revealed a significant difference for fasting NOx concentrations between MI-Exercise and Rest ($P < 0.01$) (Fig. 3). A Friedman test revealed no significant difference between the repeated measures (time) NOx concentrations.

The two-way ANOVA showed an interaction between the conditions and time for FMD ($F_{4,40} = 3.850$; $P = 0.019$). The paired sample *t*-test analysis showed a different dilatation between MI-Exercise and Rest at 0 h ($P = 0.02$) and between HI-Exercise and Rest at 0 h ($P = 0.008$) (Fig. 4). There was also a difference from 0 to 3 h and from 1 to 3 h in the Rest condition ($P < 0.02$) and from 0 to 1 h in the HI-Exercise condition ($P = 0.015$). It is important to mention that there was no difference in mean basal diameter of the artery between the three conditions ($P > 0.05$).

Discussion

The purpose of this study was to compare the effect of two different intensities of isoenergetic exercise on PPL, oxidative stress markers and endothelial function after a HFM. The main findings of this study are: (i) both exercise intensities reduce TAG concentrations after a HFM; and (ii) MI-Exercise causes a lower oxidative stress marker response and a greater endothelial function in the fasted state compared to Rest.

Independent of exercise intensity, a prior single bout of exercise reduced postprandial TAG concentrations compared to Rest. This is in agreement with previous

findings showing that single exercise bouts with equal energy expenditure cause similar reductions in postprandial TAG concentrations (Burnett et al. 1993; Miyashita et al. 2006; Ferreira et al. 2011). Although both exercise intensities were effective in minimizing the AUC TAG concentrations in this study, iAUC TAG was 49% lower in the HI-Exercise than Rest. Recent studies have shown that iAUC is an accurate method to describe TAG responses after the consumption of a HFM, since this method subtracts the fasting concentrations from all postprandial concentrations in order to evaluate just the postprandial rise in TAG (Freese et al. 2014). Thus, the lower iAUC TAG in the HI-Exercise condition compared to Rest suggests that high-intensity exercise offers more protection against the postprandial TAG enhancement (Freese et al. 2014). This larger reduction of iAUC TAG in HI-Exercise after the HFM may be important for clinical applications such as exercise prescription. It is likely that HI-Exercise enabled TAG clearance over a greater amount of time than MI-Exercise and thus, may be more effective in reducing the long-term risk for cardiovascular diseases. A plausible explanation for the enhanced TAG clearance after high-intensity exercises is the greater activation of muscle lipoprotein lipase (Trombold et al. 2013). There are some evidences showing that the lipoprotein lipase plays an essential role in plasma TAG clearance (Kersten 2014). However, the reduction in the synthesis and secretion of liver-derived very-low-density lipoprotein cholesterol (VLDL-C) and the increase in chylomicron and VLDL-C clearance after exercise can also play an important role in this process (Malkova et al. 2000). Thus, these factors together might have contributed to increase TAG clearance in this condition.

For insulin, there was an increase in the concentrations from 0 to 1 and 3 h during the postprandial state, as expected after a HFM (Zhang et al. 2007; Miyashita et al. 2008). Conversely, concentrations of glucose decreased during the postprandial period. Because there was no effect of condition, this time effect appears to be a consequence of the increase in insulin concentrations at this same time. There were no differences between conditions for LDL-C, HDL-C, glucose and insulin levels. These results support previous studies that also did not find difference between exercise and rest conditions on these variables concentrations during the postprandial period (Clegg et al. 2007; Gabriel et al. 2012).

We hypothesized that HI-Exercise would promote greater benefits on PPL and on the response of oxidative stress markers after a HFM. This study demonstrated that a single bout of exercise can attenuate TAG concentrations during the postprandial period, independent of exercise intensity. However, just the MI-Exercise decreased TBARS concentrations compared to Rest condition. TBARS concentrations increased from 0 to 3 h after the consumption of a HMF. This result is in accordance with previous studies that show that TBARS concentrations are enhanced during the postprandial period (Gabriel et al. 2012; Canale et al. 2014). Despite this fact, TBARS concentrations were reduced only in MI-Exercise condition compared to Rest. Therefore, different exercise intensities may cause distinct effects on lipid peroxidation. The HI-Exercise was performed at VO_2 approximately 15% higher than MI-Exercise. It is known that exercise performed at higher intensities contributes to an increase in reactive oxygen species production due to increased depletion of energy substrates and/or a disturbed redox state (Lovlin et al. 1987; Volvaard et al. 2005). Conversely, the lower intensity of

the MI-Exercise condition might not be sufficient to increase reactive oxygen species production and to cause an increase in the oxidative stress marker evaluated in this study. Thus, acutely, MI-Exercise produces a lower oxidative stress damage.

NOx concentrations and FMD responses did not differ between conditions during the postprandial period. This is in agreement with a similar study done by Mc Clean et al. (2007), in which no difference in NOx concentrations between exercise and rest during the postprandial period was observed. According to Mc Clean et al. (2007), the acute benefits of the exercise might occur despite of changes in NOx concentrations in postprandial state. Regarding FMD responses, there are two main explanations that may explain our results. Firstly, there is an evidence showing that NOx concentrations are well-correlated with brachial FMD responses in young adults (Casey et al. 2007). So, despite some studies having shown exercise-induced increases in %FMD after a HFM (Tyldum et al. 2009; Sedgwick et al. 2013; Sedgwick et al. 2014), the absence of changes in FMD response in the present study may be explained by the unchanged NOx concentrations. Secondly, the studies cited above evaluated different populations (adolescents and middle-aged men) and it is known that people from different age and training status present groups have different FMD responses. For instance, physically active and inactive men can present different FMD responses following a HFM (Johnson et al. 2011). While inactive men have a lower postprandial FMD response, FMD remains unchanged after a HFM in active men (Johnson et al. 2011). Since in the present study the participants were physically active, habitual physical activity may have attenuated endothelial impairments induced by the meal.

MI-Exercise also induced an increase in fasting NOx concentrations. It is known that when exercise is performed at 50% of $\text{VO}_{2\text{max}}$ (i.e. approximately the intensity of MI-Exercise) NOx production is increased. However, at higher intensities (75% of $\text{VO}_{2\text{max}}$), exercise can reduce NOx production due to greater reactive oxygen species production (Goto et al. 2003). As a result, exercise performed at moderate intensity can have a beneficial effect maintaining higher NOx plasmatic concentrations that can be observed up to 12 h after the exercise session. There was also an increase in FMD at 0 h for both exercise intensities compared to Rest. For MI-Exercise the increase in FMD corresponded to a similar increase in NOx. Conversely, FMD responses in HI-Exercise cannot be explained by higher concentrations of NOx. A possible explanation is that higher reactive oxygen species production after the exercise bout might have contributed to increase ONOO^- formation and, by consequence, decrease NO production. As a result, stable NO end-products were unchanged. The increase on FMD in HI-Exercise might be explained by increases in other vasoactive mediators that were not measured in this study.

There are a couple of limitations that need to be addressed. First, the combination of NOx and FMD analyses have been widely used to indirectly estimate NO production and vasodilation of the brachial artery, respectively, in response to hyperemia. However, both techniques have limitations regarding sensitivity, variability and reproducibility. Second, one of the main limitations of this study was the non-measurement of other cardiovascular risk markers. For instance, we did not find a statistical difference for total thiols between the conditions. This group of organic components represents about half of the total antioxidant defense in humans,

nevertheless, other components also play an important role to defend our organism against prooxidant agents such as TAS and vitamins C and E (Erel 2004). Thus, a clear limitation of this study was to not evaluate any of these parameters.

In summary, in young health men, a single aerobic exercise bout performed 12 h before the consumption of a HFM can attenuate TAG concentrations, suggesting that aerobic exercise can reduce lipemia independent of exercise intensity (moderate or heavy). However, HI-Exercise showed to be more effective in reducing iAUC TAG, which might suggest that this exercise intensity offers more protection against the postprandial TAG enhancement. It is known that regular exercise is an important strategy to decrease oxidative damage risk induced by a high-fat diet and also to reduce the long-term development of cardiovascular diseases. This study has shown that, acutely, MI-Exercise can be beneficial to attenuate the susceptibility of oxidative damage induced by a HFM compared to Rest. Other potential benefits of MI-Exercise are the increase in fasting NOx concentrations and the vasodilation response which likely indicate lower oxidative stress damage and greater endothelial function in the fasted state due to the exercise bout. However, more studies must be done to confirm this statement.

Conflict of interest

The authors declare that there are no conflicts of interest.

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Table 1. Participants Characteristics

	Participants (n = 11)
Age (years)	23 ± 3
Body mass (kg)	73.7 ± 9.8
Height (cm)	177.0 ± 1.0
BMI (kg/m ²)	23.3 ± 2.4
BMR (kcal/day)	1882.2 ± 157.6
VO _{2max} (mL/kg/min)	47.7 ± 6.1
Muscle mass (kg)	34.5 ± 6.1
Fat mass (kg)	18.7 ± 3.3
Sum of skinfolds (mm)	70.1 ± 18.5

Values are arithmetic mean ± SD. BMI: body metabolic index, BMR: basal metabolic rate, VO_{2max}: maximal oxygen uptake. Sum of skinfolds (triceps, subscapular, supraspinal, abdominal, thigh and medial calf).

Table 2. Reported energy and macronutrient intake in the 24-h diet recall between experimental trials

	Rest	MI-Exercise	HI-Exercise
Energy Intake (kcal)	2442 ± 889	2545 ± 1341	2167 ± 481
Carbohydrate (%)	55 ± 7	53 ± 15	56 ± 6
Carbohydrate (g)	321 ± 95	337 ± 85	299 ± 44
Protein (%)	19 ± 4	20 ± 6	20 ± 3
Protein (g)	110 ± 31	125 ± 73	111 ± 36
Fat (%)	26 ± 9	27 ± 11	24 ± 6
Fat (g)	64 ± 34	71 ± 29	58 ± 14

No significant difference for reported energy and macronutrient intake ($P > 0.05$).

Values are arithmetic mean ± SD. MI-Exercise: moderate-intensity exercise, HI-Exercise: heavy-intensity exercise.

Table 3. Biochemical markers in the fasted state and following a high-fat meal

	Time (h)			
	0	1	3	5
<i>Rest</i>				
LDL-C (mg/dL) *	115.7 ± 21.7	108.6 ± 21.4	100.7 ± 20.9	111.4 ± 21.3
HDL-C (mg/dL) †	41.5 ± 6.1	40.9 ± 6.4	39.2 ± 5.3	38.1 ± 6.1
Insulin (μIU/mL) ‡	7.6 ± 1.7	35.9 ± 23.2	15.6 ± 6.9	7.2 ± 2.0
Glucose (mg/dL)	93.6 ± 6.1	83.0 ± 19.0	80.0 ± 11.2	89.6 ± 5.2
<i>MI-Exercise</i>				
LDL-C (mg/dL) *	116.4 ± 21.1	109.8 ± 22.8	103.2 ± 24.1	109.2 ± 22.1
HDL-C (mg/dL) †	41.8 ± 5.0	41.0 ± 7.2	39.0 ± 5.8	39.8 ± 5.5
Insulin (μIU/mL) ‡	7.9 ± 1.7	33.7 ± 11.9	13.8 ± 7.0	6.8 ± 2.0
Glucose (mg/dL)	92.7 ± 4.3	78.3 ± 14.1	84.3 ± 9.0	89.7 ± 3.2
<i>HI-Exercise</i>				
LDL-C (mg/dL) *	120.2 ± 27.9	110.6 ± 29.4	107.1 ± 26.9	105.0 ± 20.5
HDL-C (mg/dL) †	43.1 ± 6.9	41.9 ± 5.3	39.1 ± 5.3	39.9 ± 5.1
Insulin (μIU/mL) ‡	7.4 ± 1.7	29.9 ± 12.9	11.2 ± 4.0	6.3 ± 1.5
Glucose (mg/dL)	92.0 ± 3.7	81.1 ± 20.2	87.1 ± 8.5	88.7 ± 6.8

Values are arithmetic mean ± SD. LDL-C: low-density lipoprotein cholesterol, HDL-C: high-density lipoprotein cholesterol, MI-Exercise: moderate-intensity exercise, HI-Exercise: heavy-intensity exercise.

* Time effect at $P < 0.001$: significant difference from 0 to 1, 3, and 5 h at $P < 0.016$.

† Time effect at $P < 0.001$: significant difference from 1 to 5 h at $P < 0.001$.

‡ Time effect at $P < 0.001$: significant difference from 0 to 1 h at $P < 0.001$; significant difference from 1 to 3 h at $P < 0.001$.

|| Time effect at $P < 0.001$: significant difference from 0 to 1 h at $P = 0.002$; significant difference from 1 to 3 h at $P < 0.001$.

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Figures

Fig. 1A. Plasma triacylglycerol (TAG) concentration during the experiment. Values are arithmetic mean $\pm$ SD. MI-Exercise: moderate-intensity exercise, HI-Exercise: heavy-intensity exercise.

* Time effect at $P < 0.001$: significant difference from 0 to 2, 3, 4 and 5 h at $P < 0.032$; significant difference from 1 to 2 h at $P = 0.041$; significant difference from 2 and 3 to 5 h at $P < 0.044$.

† Condition effect at $P < 0.001$: significant difference from MI-Exercise to Rest at $P = 0.004$; significant difference from HI-Exercise to Rest at $P < 0.001$.

Fig. 1B. The AUC TAG concentration for each condition. Values are arithmetic mean $\pm$ SD. MI-Exercise: moderate-intensity exercise, HI-Exercise: heavy-intensity exercise.

* Condition effect at $P < 0.001$: difference from MI-Exercise to Rest at $P = 0.017$; difference from HI-Exercise and Rest at $P < 0.01$.

Fig. 1C. The iAUC TAG concentration for each condition. Values are arithmetic mean $\pm$ SD. MI-Exercise: moderate-intensity exercise, HI-Exercise: heavy-intensity exercise.

* Condition effect at $P = 0.013$: difference from HI-Exercise and Rest at $P = 0.012$.

Fig. 2. Plasma thiobarbituric acid reactive substance (TBARS) concentration during the experiment. Values are arithmetic mean $\pm$ SD. MI-Exercise: moderate-intensity exercise, HI-Exercise: heavy-intensity exercise.

* Time effect at $P = 0.002$: significant difference from 0 to 3 h at $P = 0.007$.

† Condition effect at $P = 0.012$: significant difference from MI-Exercise to Rest at $P = 0.041$.

Fig. 3. Plasma nitrite/nitrate (NO_x) concentration during the experiment. Values are presented as median [Q1, Q3]. MI-Exercise: moderate-intensity exercise, HI-Exercise: heavy-intensity exercise.

† Kruskal-Wallis test: difference from MI-Exercise to Rest at $P < 0.01$.

Fig. 4. Flow-mediated dilatation (FMD) response during the experiment. Values are arithmetic mean $\pm$ SD. MI-Exercise: moderate-intensity exercise, HI-Exercise: heavy-intensity exercise.

Interaction between condition and time at $P = 0.019$.

* Significant difference from 0 to 3 h and from 1 to 3 h in the Rest condition at $P < 0.02$; significant difference from 0 to 1 h in the HI-Exercise condition at $P = 0.015$.

† Significant difference from MI-Exercise and Rest at $P = 0.02$; significant difference from HI-Exercise and Rest at $P = 0.008$.

A.

B.

C.

60x108mm (300 x 300 DPI)

160x96mm (300 x 300 DPI)

62x41mm (300 x 300 DPI)

160x96mm (300 x 300 DPI)