The effect of aerobic exercise intensity on attenuation of postprandial lipemia is dependent on apolipoprotein E genotype

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Objective: To investigate the effect of aerobic exercise intensity on postprandial lipemia according to allelic variants of the apolipoprotein E gene.

Methods: Three groups of 10 healthy men each were formed based genotyping of the APOE gene, rested or performed 500 Kcal tests in a random sequence separated by a minimum 48 h interval, as follows: (a) no exercise (control), (b) intense intermittent exercise, (c) moderate continuous exercise. Each test series was completed 30-min before ingestion of a high-fat meal (1 g fat/kg). Venous blood was collected before and at 1, 2, 3 and 4 h after the high-fat meal. Postprandial lipemia was assessed using the area under the curve approach as well as the kinetic profile of mean lipid variables. Statistical significance was adopted at \( P < 0.05 \) level.

Results: The main results show that, in the moderate continuous exercise, total postprandial cholesterolemia was higher in 34 than in 32 carriers, whereas under intense intermittent exercise, total and LDL cholesterolemia were higher in 34 than in 32 and 33 carriers. There was no difference in the lipemic profile of the subjects across APOE genotypes at baseline.

Conclusion: Moderate and intense exercise were effective in attenuating PPL in both 32 and 33 subjects, with 32 subjects being more susceptible to the lipid lowering effect of moderate training than 33 subjects. Carriers of the 34 allele, however, showed no attenuation of postprandial lipemia.

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1. Introduction

Postprandial lipemia (PPL) refers to a sequence of metabolic events related to high serum concentrations of lipoprotein particles rich in triglycerides (TG) and cholesterol, such as chylomicrons and very low-density lipoproteins (VLDL cholesterol) and their remnants, after fat intake [1].

Regular exposure to PPL is considered an indirect atherogenic factor, because it favors atherosclerotic plaque formation. Furthermore, PPL is associated with increased blood clotting, endothelial dysfunction, and increased systemic inflammation [2]. Despite the transient nature of PPL, the habit of eating three or more high-fat meals per day may translate into a perpetually unsatisfactory metabolic state, as people can spend up to two thirds of the day in the postprandial state [3].

Apolipoprotein E (APOE) has an important role in lipid metabolism, such as lipoprotein transport and reverse cholesterol transport [4,5]. Higher cholesterol levels and increased mortality due to coronary heart disease occur in subjects carrying the 4 allele [6,7]. A meta-analysis involving patients and controls showed that carriers of the 4 allele had a 42% higher risk for coronary heart disease compared to the 3/3 genotype, whereas the 2 allele showed no significant association [8].

Physical activity can be an effective tool to attenuate PPL, as already evaluated by chronic [9,10] and acute [11–13] exercise studies. Katso et al. [14] found that exercise of moderate intensity, but not an equal energy expenditure exercise of low intensity, attenuates PPL. Altena et al. [11] compared intermittent and continuous exercise of equal energy expenditure and found that only intermittent exercise caused reduction in postprandial TG.
Miyashita et al. [15] analyzed PPL in accumulated exercise, continuous exercise and control trials and found that both exercise sessions attenuated PPL.

Although studies demonstrating attenuation of PPL at acute exercise suggest similar mechanisms to explain the phenomenon [16–18], the data appear conflicting on the intensity and type of exercise to attenuate PPL. The hypothesis of this study involves the interaction of the APOE gene and exercise, suggesting that differences arising from APOE isoforms may in part modulate PPL response under different exercise intensities. Thus, the objective of this study was to investigate the effect of aerobic exercise intensity on postprandial lipemia according to allelic variants of the apolipoprotein E gene.

2. Methods

2.1. Subjects

Initially, 350 healthy adults aged between 20 and 40 years-old were screened using the International physical activity questionnaire — IPAQ and by genotyping of the APOE gene. Men who volunteered and met the criteria of being at least moderately active and carried either the ε2ε3, ε3ε3 or ε3ε4 genotypes were eligible. On day 1, each subject was assessed by an experienced cardiologist for additional exclusion criteria, as follows: current or prior smoking habit; signs or symptoms of active infection, inflammation or malignancy; neuropathies and/or orthopedic problems; use of lipid or glucose lowering medication. This study was approved by the institutional Research Ethics Committee and all men signed a written consent form.

2.2. Detection of APOE genotypes

Total DNA was isolated on day 1 from peripheral blood according to standard procedures. APOE genotypes were analyzed using a modified version of a refractory mutation system (ARMS) for multiplex polymerase chain reaction (PCR) to identify the classical ε2, ε3, and ε4 alleles [19]. In our conditions, PCR products were electrophoresed in 1.6% agarose gels. Each sample was run at least twice, and further checked only if the genotypes yielded by the first two productive analyses were in conflict.

2.3. Preliminary tests

On day 2, subjects underwent a physical examination and had their weight (kg), height (m), BMI (kg/m²), body composition (% body fat), maximal oxygen uptake (ml/kg/min⁻¹) and velocity corresponding to the anaerobic threshold (km/h) assessed. These tests were performed at temperature maintained between 21 and 23 °C and relative humidity between 40 and 60%.

Maximal oxygen uptake (VO₂max) was determined using a breath by breath method (Cortex — Metrolyzer 3B from Cortex Bio-physik — GER) during an incremental exercise test with 3-min stages and increments of 0.5 km/h. The initial velocity was individualized between 8 and 9 km/h on the treadmill (Brudden Equipamentos LTDA — Line Movement, model RT 400, São Paulo — BRA). The test was conducted until voluntary exhaustion or other criteria such as blood pressure >260/115 mmHg, rating of perceived exertion (RPE) > 19 on the Borg 6–20 scale [20] or S-T segment electrocardiogram with depression or elevation greater than 2 mm.

During each test, heart rate (HR) was measured continuously as well as blood pressure (BP), RPE, ventilation (VE), oxygen uptake (VO₂), carbon dioxide release (VCO₂), oxygen ventilation equivalent (VE/VO₂), carbon dioxide equivalent VE/VCO₂ and respiratory exchange ratio (RER). VO₂max was valid when at least two of the following criteria were met: RER > 1.15, VO₂ plateau ΔVO₂ ≥ 150 ml at the last minute, HR < 10 bpm of 220 — age and/or RPE ≥ 19.

Anaerobic threshold (AT) was determined based on VE/VO₂ and VE/VCO₂, measured at the point where VE/VCO₂ increased disproportionately compared to the increase in VE/VO₂ [21].

Each subject was instructed to avoid caffeine, alcohol and strenuous exercise 48 h before the experiments, as well as to eat normally and avoid foods not usual in their daily intake.

2.4. Study trials

After the maximal test, all subjects participated in three randomly selected trials, on three different days with at least 48 h between sessions: control (no exercise), MOD-EX (moderate exercise) and INT-EX (intense exercise). In the first experiment, subjects recorded their past 24 h food intake and were asked to reproduce the same food intake pattern (frequency and amounts) on the days preceding test days.

The MOD-EX trial was performed jogging continuously on a treadmill at a speed set at 85% of each individual AT, whereas the INT-EX trial was with a velocity of 115% of the AT in 3-min bouts with 1.5-min of passive recovery, with an equal energy expenditure of 500 kcal in both cases. Both trials began with a 3-min warm-up that started at the velocity of 70% of the AT and lasted until the 500 kcal expenditure was almost reached. A 3 min recovery activity at 70% AT was performed by lowering the treadmill speed to 5 km/h for 1-min and to 3 km/h for another 2-min. The energy expenditure was measured continuously and the energy generated in warm-up and recovery was computed as part of the 500 kcal. RPE was reported at the end of the warm-up and when 500 kcal was reached, and also at every bout on the INT-EX test and when 150, 300 and 450 kcal was reached on the MOD-EX trial.

After the 3-min of cool down, subjects sat for additional 27-min. Gas analysis with caloric expenditure and average RER were measured during the exercise, cool down and post-exercise rest period. After a 30-min rest, subjects were given a high-fat meal. After the meal, gas analyses were repeated every hour for 10-min. On control trials, subjects rested for 30-min and submitted to the same high-fat meal and subsequent measurements.

2.5. High-fat meal (HFM)

In the three HFM trials, subjects were instructed to consume the meal in approximated 15-min. Caloric composition was based on body weight and contained 1.0 g of fat, 0.3 g of protein and 0.62 g of carbohydrate per kg of body weight, and included foods found ordinarily in the typical Brazilian diet such as chocolate, Brazilian nuts, eggs, olive oil and milk powder, among others. The total fat content corresponded to 71% of the whole beverage calories. No other food was provided for 4 h after the HFM was ingested. Postprandial period water was available ad libitum in the first trial, and the ingested amount was replicated in the following trials.

2.6. Blood analysis

Blood samples were obtained through a venous puncture at baseline, immediately before the trials, and at 1, 2, 3 and 4 h postprandial. Blood samples were collected into vacutainer tubes and were centrifuged for 12-min at 4200 rpm after resting for 10-min. Serum TG, HDL cholesterol, total cholesterol and glucose were determined by enzymatic colorimetric methods using kits available commercially (Advia 2400, SIEMENS Healthcare Diagnostics Inc. Tarrytown, NY — USA, 2008). LDL cholesterol and VLDL cholesterol were estimated from formula [22]. Serum insulin was determined by fluoroimmunoassay using a commercially available

2.7. Calculations and statistical analyses

For each variable, the area under the curve (AUC) was calculated using trapezoidal methods [13]. AUC values for TG, HDL cholesterol, LDL cholesterol, VLDL cholesterol, total cholesterol and glucose variables are presented in mg.dL \(^{-1}\).h \(^{-1}\). For insulin, AUC is expressed as \(\mu\)U ml \(^{-1}\).h \(^{-1}\). After the development of a pilot study, we estimated an effect size of 0.96 and 1.00 for triglycerides and total cholesterol, respectively. Therefore, with an alpha of 0.05 and power of 0.80 for a two-tailed test, a sample of at least 10 participants per group would be required to detect a significant difference between treatment groups.

The Kolmogorov test confirmed that all dependent variables of interest were normally distributed. Data are presented as mean ± SD. The differences between the MOD-EX and INT-EX trials were analyzed using a paired t-test. Repeated-measures were analyzed using a variance analysis to examine the differences between each measurement in each trial (control, MOD-EX and INT-EX). One-way ANOVA followed by Scheffé post-hoc test was used to analyze groups according to allele frequency. The average of the three trials baseline was adopted as covariant. A P-value of less than 0.05 was considered significant. Data was further analyzed with the LSD post-hoc test. Statistical analyses were performed with SPSS for Windows version 11.5 (SPSS, Chicago, IL).

3. Results

Three groups of 10 healthy men each were formed based on the inclusion/exclusion criteria and statistical design. Table 1 shows mean values for biometric, performance and biochemical variables according to APOE alleles, and no differences across genotypic groups were found.

Table 2 presents data on the characterization of volume and intensity of MOD-EX and INT-EX trials according to APOE genotypes. Only energy expenditure was similar across trials, while all other variables differed between MOD-EX and INT-EX for all three allelic groups, demonstrating that, although both trials resulted in equal energy expenditure, physical activity in MOD-EX was significantly less vigorous than in INT-EX, characterizing exercise intensity as moderate and intense, respectively.

Table 3 shows AUC values for lipemic variables calculated from baseline to 4 h postprandial, with individuals gathered according to exercise intensity and APOE genotype. Both in MOD-EX and INT-EX, between-group analysis revealed a 27% higher AUC for total cholesterol in group c3c4, compared to c2c3 (p < 0.05). AUC for LDL cholesterol in group c3c4 was 41% and 29% higher than in c2c3 and c3c3 groups under INT-EX, respectively (p < 0.05).

In the intra-group analysis, two carriers showed an AUC decrease of 31% for TG, 11% for total cholesterol and 23% for VLDL cholesterol in MOD-EX, compared to control (p < 0.05). In INT-EX, a similar comparison revealed an AUC decrease of 25% for TG, 6% for HDL cholesterol and 7% for total cholesterol (p < 0.05). In group c3, intra-group analysis revealed a 15% lower AUC for TG in MOD-EX compared to control, and AUCs for TG and VLDL cholesterol were 15% and 13% lower in INT-EX, respectively, compared to control (p < 0.05). In the c4 group, intra-group analysis showed no differences between control, MOD-EX, and INT-EX trials.

Fig. 1 shows a comparison of TG levels in control, MOD-EX, and INT-EX trials according to APOE alleles at each time point, from baseline to 4 h after ingestion of HFM. In group c2, TG levels were attenuated between 1 and 4 h postprandial (at 4 time points) in MOD-EX and between 2 and 4 h postprandial (at 3 time points) in INT-EX, compared to control. There were no other differences between MOD-EX and INT-EX. In group c3, TG levels were attenuated between 3 and 4 h postprandial (at 2 time points) in MOD-EX and between 2 and 4 h postprandial (at 3 time points) in INT-EX, compared to control. There were no other differences between MOD-EX and INT-EX. In group c4, there were no differences in TG levels between trials.

A similar analysis was performed for total cholesterol, HDL cholesterol, LDL cholesterol, and VLDL cholesterol by comparing each time point from baseline to 4 h postprandial between APOE allele groups (Fig. 2). The sum of statistically significant changes at all time points for TG, total cholesterol, HDL cholesterol, LDL cholesterol, and VLDL cholesterol revealed that, in group c2, MOD-EX and INT-EX showed attenuation at 11 and 6 time points, respectively, compared to control. In group c3, MOD-EX and INT-EX showed attenuation at 2 and 4 time points, respectively, compared to control. In group c4, no difference was observed between control, MOD-EX, and INT-EX trials at any time point.

4. Discussion

Our initial analyses demonstrate no differences in clinical, biochemical and anthropometric variables, as well as in volume and intensity of trials between APOE allele groups. This homogeneity across groups, particularly on variables that were used to control the volume and intensity of trials, is an important factor since total energy expenditure is well established as a determining factor in the magnitude of PPL [14,15]. In line, there was no difference in AUCs for any variable tested across APOE allele groups in the pretest, control session. The set of variables representing performance (kcal, AT, HR, VO\(_2\) and RER) yielded similar ratings after both trials (MOD-EX and INT-EX), indicating that volume and intensity of the exercise were comparable, ruling out a possible influence of these variables in the PPL response in our assay given that exercise intensity has been a subject of controversy in studies conducted elsewhere on PPL [23,24].

In this sense, the main results of this study show that in the MOD-EX trial, total cholesterol AUC was higher for c4 than c2 carriers, and in the INT-EX trial, total cholesterol AUC and LDL cholesterol were higher in c4 carriers than in the c2 and c3 counterparts. Clinically, we can observe that, at moderate-intensity exercise conditions, carriers of the c2 allele show an overall improved lipemic response than c4 subjects, while at vigorous physical
activity, both ε2 and ε3 groups had better response than ε4. In regard to countering PPL, our finding suggests that subjects carrying the ε4 allele are less responsive to moderate exercise than those with ε2, and less responsive to intense exercise than ε2 and ε3 carriers, indicating a commitment of the ε4 allele with a proatherosclerotic hyperlipemic profile [25,26]. All analyses described above were performed with variables expressed based on the AUC approach. Taking into account that nutritional and life style aspects could impact results presented herein, similar analyses were conducted using the incremental AUC approach in order to rule out the effect of important inter-individual variations in baseline values, following method described elsewhere [13]. This subsequent round of analyses reassured the results obtained.

Only a few studies have been performed aiming to investigate the interplay between PPL, APOE allelic variation and acute exercise. Bernstein et al. [27], in a cross-sectional study with 1,708 subjects aiming to investigate whether the effect of APOE genotype on lipid profile is modulated by the level of physical activity found data similar to those reported herein. On what concerns chronic exercise, one study analyzed subjects with different APOE genotypes (35 ε2/3, 40 ε3/3, and 31 ε3/4) exercising aerobically at 75% of maximal HR for 40 min/day, four times weekly for 6 months, and found that ε4 carriers had higher plasma LDL cholesterol levels than ε3/3 subjects [28]. Another 24-week longitudinal study investigating the influence of aerobic training on HDL subfraction levels and particle size found that ε4 subjects had worse outcomes than subjects carrying other alleles [29]. One more study analyzed the interaction of APOE genotype and chronic aerobic exercise in lipemia and reported that ε4 subjects had worse plasma LDL cholesterol and HDL/LDL ratio than ε2 and ε3 carriers [30].

There are plausible explanations for this unfavorable attribute of ε4 subjects in exhibiting lower responsiveness to chronic or acute training. Seip et al. [28] believe that differences related to specific amino acids present in each isoform and their binding capacity to receptors would be the main contributors. Thompson et al. [30] state that ε2 carriers usually shows lower levels of total and LDL cholesterol due to a decreased binding affinity of ε2 for hepatic receptors, endogenously counteracted by reduced levels of total cholesterol released by the liver and by an upregulation of hepatic receptors for LDL cholesterol in ε2 subjects. In contrast, higher LDL cholesterol levels found in ε4/ε4/ε3/4 and in ε3ε3 subjects are likely to result from the lower content of the Apo ε4 isoform in HDL particles. Because approximately 30% of the plasma apolipoprotein E (apoE) concentration is associated with HDL cholesterol, the interplay between PPL, subsequent round of analyses reassured the results obtained. Bernstein et al.[27], in a cross-sectional study with 1,708 subjects baseline values, following method described elsewhere[13]. This analyses were conducted using the incremental AUC approach in order on the AUC approach. Taking into account that nutritional and life des described above were performed with variables expressed based on the AUC approach. Taking into account that nutritional and life style aspects could impact results presented herein, similar analyses were conducted using the incremental AUC approach in order to rule out the effect of important inter-individual variations in baseline values, following method described elsewhere [13]. This subsequent round of analyses reassured the results obtained. Only a few studies have been performed aiming to investigate the interplay between PPL, APOE allelic variation and acute exercise. Bernstein et al. [27], in a cross-sectional study with 1,708 subjects aiming to investigate whether the effect of APOE genotype on lipid profile is modulated by the level of physical activity found data similar to those reported herein. On what concerns chronic exercise, one study analyzed subjects with different APOE genotypes (35 ε2/3, 40 ε3/3, and 31 ε3/4) exercising aerobically at 75% of maximal HR for 40 min/day, four times weekly for 6 months, and found that ε4 carriers had higher plasma LDL cholesterol levels than ε3/3 subjects [28]. Another 24-week longitudinal study investigating the influence of aerobic training on HDL subfraction levels and particle size found that ε4 subjects had worse outcomes than subjects carrying other alleles [29]. One more study analyzed the interaction of APOE genotype and chronic aerobic exercise in lipemia and reported that ε4 subjects had worse plasma LDL cholesterol and HDL/LDL ratio than ε2 and ε3 carriers [30]. There are plausible explanations for this unfavorable attribute of ε4 subjects in exhibiting lower responsiveness to chronic or acute training. Seip et al. [28] believe that differences related to specific amino acids present in each isoform and their binding capacity to receptors would be the main contributors. Thompson et al. [30] state that ε2 carriers usually shows lower levels of total and LDL cholesterol due to a decreased binding affinity of ε2 for hepatic receptors, endogenously counteracted by reduced levels of total cholesterol released by the liver and by an upregulation of hepatic receptors for LDL cholesterol in ε2 subjects. In contrast, higher LDL cholesterol levels found in ε4/ε4/ε3/4 and in ε3ε3 subjects are likely to result from the lower content of the Apo ε4 isoform in HDL particles. Because approximately 30% of the plasma apolipoprotein E (apoE) concentration is associated with HDL cholesterol,
and ε4 has less binding capacity to HDL cholesterol, TG-rich particles (e.g., LDL cholesterol) are assumed to be formed more rapidly.

Additionally, the present hypothesis is further supported by intra-group comparisons performed in this study. In the ε2 group, AUC results for TG and total cholesterol in the control trial were higher than those in the MOD-EX and INT-EX trials, whereas HDL cholesterol and VLDL cholesterol became higher after INT-EX and MOD-EX, respectively. Also among the carriers of the ε2 allele, we found that MOD-EX and INT-EX showed significant improvements at 11 and 6 time points compared to control, respectively, indicating an advantage for moderate-intensity exercise in attenuation of PPL. Intra-group comparisons among ε3 homozygotes showed that AUC results for TG in the control trial were higher than those in MOD-EX and INT-EX, and that VLDL cholesterol AUC was higher when compared to INT-EX. After MOD-EX and INT-EX, these ε3/ε3 subjects showed significant differences compared to control at 2 and 4 time points, respectively, indicating that ε3 subjects can also be responsive to physical activity. Finally, intra-group comparisons among ε4 subjects showed that pre-test results were not different from those obtained after MOD-EX or INT-EX regardless of the time point. This demonstrates that carriers of the ε4 allele are not responsive to either forms of exercise tested herein, prompting for caution among these individuals in aspects as food choice since a diet high in fat should maintain them at an elevated PPL response even after moderate or intense exercise with an energy expenditure of 500 kcal.

As stated, the actual mechanisms by which APOE allelic variation can attenuate PPL regardless of the exercise intensity applied remains unclear, but attenuation is likely to result from differences related to lipid clearance and/or synthesis [30]. Also, it is known that APOE isoforms differently affect the hepatic clearance of chylomicrons, VLDL cholesterol, and LDL cholesterol through their interaction with LDL receptors [28]. Although these well-known differences have been documented at fasting rest, one cannot state that during exercise or in the postprandial period their mechanism of action will be similar. Moreover, physical activity reduces TG levels in part and accelerates VLDL cholesterol lipolysis [30]. In the present study, exercise significantly reduced TG and VLDL cholesterol levels, but only in the ε2 and ε3 groups. Therefore, we suggest that the group composed of carriers of the ε4 allele is less responsive than the other groups to continuous and intermittent exercise. One may consider the hypothesis that the non-attenuation of PPL in ε4 subjects is based on the discrete though non-significant higher baseline values observed for this group. Nonetheless, all trials analyzed here considered baseline data as covariant, with no influence on the main results.

Fig. 2. Mean and standard deviation of LDL-c (A), VLDL-c (C), total cholesterol (C), and HDL-c (D) at baseline and at 1, 2, 3 and 4 h after ingestion of a high-fat meal in control, moderate exercise (MOD-EX), and intense exercise (INT-EX) trials, according to apolipoprotein E (apoE) alleles. Legend: Significant difference between control and MOD-EX (p < 0.05), Significant difference between control and INT-EX (p < 0.05).
A limitation of this study is that the attenuation of PPL observed after exercise cannot be extrapolated to a time frame beyond 4 h or if a second meal was ingested, although there is evidence that an abbreviated 4-hour PPL test is a valid surrogate for an 8-hour test [31]. A possible mechanism to explain attenuation of PPL is the immune reaction of lipoprotein lipase (LPL); however, the peak of this action appears to occur at about 8 h after exercise stimulus [32]. In addition, hepatic uptake and recycling of circulating lipoproteins in the postprandial period may be related to exercise and the associated attenuation of PPL, as this was not observed in the control trial, indicating that fatty acid oxidation in the liver was elevated, which, in turn, would reduce the availability of TG for incorporation into VLDL cholesterol [15]. Katsanos [33] suggests that when exercise is completed immediately before the ingestion of a HFM, the mechanism involving the reduction of hepatic secretion of triglyceride-rich VLDL cholesterol may play a more important role in attenuating PPL than the increase in LPL activity. However, we cannot overlook the fact that hepatic uptake and recycling of circulating lipoproteins from a previous meal may affect the response to a subsequent meal, as well as the macronutrient content and timing of subsequent meals would affect day-long response to the first meal.

It is worth noting that the results of the present study might be different if the trial is to be carried out in women, since sex-related hormonal aspects may be associated with the physiology of attenuation of PPL. In addition, we also point out that the data refer to an acute exercise condition. Thus, further studies should be conducted to investigate PPL response in different genes and also in a chronic exercise condition. In conclusion, this study showed that moderate and intense exercise were effective in attenuating PPL in both c2 and c3 subjects, with c2 subjects being more susceptible to moderate training and c3 subjects to intense exercise. Carriers of the 4 allele, however, showed no attenuation of PPL.

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References
