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Increased blood cholesterol after a high saturated fat diet is prevented by aerobic exercise training

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ABSTRACT

A high saturated fatty acids diet (HSFAD) deteriorates metabolic and cardiovascular health while aerobic training improves them. The aim of this study was to investigate in physically inactive, overweight people if two weeks of HSFAD leads to hyperlipemia or insulin resistance and if concurrent aerobic exercise training counteracts those effects. Fourteen overweight (BMI: 27.5±0.6 $kg \cdot m^{-2}$) healthy-young individuals (24.8±1.8 yr old) were randomly assigned to a diet (D) or a diet plus exercise (D+E) group. During 14 consecutive days both groups increased dietary saturated fatty acids from 31±10 to 52±14 $g \cdot day^{-1}$ ($P<0.001$) while maintaining total fat intake. Concurrent to the diet, the D+E group underwent 11 cycle-ergometer sessions of 55 min at 60% $\dot{V}O_{2peak}$. Before and after intervention insulin sensitivity was estimated and body composition, plasma lipid profile, free fatty acids (FFA) composition, resting blood pressure (BP) and $\dot{V}O_{2peak}$ were measured. Body weight and composition, plasma FFA composition and insulin sensitivity remained unchanged in both groups. However, total cholesterol (T_C) and low-density lipoprotein cholesterol (LDL-$_C$) increased above pre-intervention values in the D group (147±8 to 161±9 $mg \cdot dL^{-1}$, $P=0.018$ and 71±10 to 82±10 $mg \cdot dL^{-1}$, $P=0.034$, respectively). In contrast, in the D+E group, T_C and LDL-$_C$ remained unchanged (153±20 to 157±24 $mg \cdot dL^{-1}$ and 71±21 to 70±25 $mg \cdot dL^{-1}$). Additionally the D+E group lowered systolic BP (6±2 mmHg, $P=0.029$) and increased $\dot{V}O_{2peak}$ (6±2 $ml \cdot kg^{-1} \cdot min^{-1}$, $P=0.020$). Increases in T_C and LDL-$_C$ induced by 14 days of HSFAD can be prevented by concurrent aerobic exercise training that in addition improves cardio-respiratory fitness.

Key words: Exercise training, LDL, Atherogenic diet, Insulin sensitivity, OGTT.

INTRODUCTION

A high-fat diet is associated with the development of obesity and an atherogenic blood lipid profile which increase the risk of developing cardiovascular and metabolic diseases (Nishida et al. 2004). An atherogenic blood lipid profile is characterized by increased total cholesterol (T_C), low-density lipoprotein cholesterol (LDL-$_C$), and triglycerides (TG) together with reduced high density lipoprotein cholesterol (HDL-$_C$) (Grundy et al. 2005). Diets high in saturated fatty acids (SFA) could promote an atherogenic blood lipid profile, even when total caloric intake is not increased (i.e., eucaloric high-SFA diets). For instance it has been recently reported that the substitution of dietary unsaturated for saturated fatty acids results in marked increases in T_C and LDL-$_C$ after 3 months of intervention (Vessby et al. 2001, Jebb et al. 2010). Even after just 5 weeks of a high saturated fatty acids diet (HSFAD), LDL-$_C$ increases have been reported (Allman-Farinelli et al. 2005). While the metabolic effects of sustained increases in total fat and dietary SFA intake are well known, scarce information is available about the effect of short-term increments in SFA intake. This is of potential importance as, for example, a short-term increase in dietary fat intake is common during weekends and holidays (Haines et al. 2003). If a short-term diet is able to deteriorate the blood lipid profile and counter-measures are not established, it is possible that one or repeated bouts of short-term high saturated diet could provoke sustained metabolic disturbances on blood lipids increasing the risk for cardiometabolic diseases development. Additionally, post-prandial lipemia and insulinemia are higher after a high saturated fat meal (Lopez et al.), and post-prandial lipid excursions are linked with cardiovascular disease pathogenesis (i.e., foam cells generation, thrombophylia, endothelial disfunction and oxidative stress; (Ceriello 2000)). To our knowledge, the blood lipid response to a short-term increase in dietary SFA (e.g. two weeks) has not been reported in the literature.

An increased flux of blood lipids is associated with the development of insulin resistance (Taskinen 2003) in a cause-effect fashion since acute elevations in plasma fatty acids concentrations by intralipid infusion causes increases in insulin resistance (Schenk and Horowitz 2007). Not only the amount but also the saturation of circulating fatty acids may be related to insulin resistance. In fact, in diabetes type 2 patients, 6-week of HSFAD worsens their insulin resistance (Christiansen et al. 1997). However, in non-diabetic but overweight subjects the effects of dietary SFA on insulin sensitivity are not that clear. Some authors have reported decreased insulin sensitivity associated with increases in dietary SFA intake (Vessby et al. 2001), while others did not find differences when monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) are substituted by SFA (Jebb et al. 2010, Haghighatdoost et al. 2012). To our knowledge, it is unclear if a short-term HSFAD would increase insulin resistance in overweight people that habitually consume a low SFA diet (i.e., Mediterranean population). We think that this question is especially relevant in overweight individuals on account of their recognized increased risk for the development of obesity, dyslipidemia and related cardiometabolic diseases.

Exercise training can reduce TG, T_C, and LDL-$_C$, and increase HDL-$_C$ particularly when caloric expenditure is at least 1200 kcal·week^{-1}(Durstine et al. 2001). Regular exercise improves the blood lipid profile and enhances insulin sensitivity in previously sedentary adults, even when fat intake is not reduced (Weintraub et al. 1989, Duncan et al. 2003). On the other hand, in overweight people with an atherogenic blood lipid profile, 4 months of supervised aerobic exercise training, enhanced insulin sensitivity (Yoshida et al. 2010) in presence of body weight loss. Improvements in insulin sensitivity have been described after short-term exercise training (seven exercise sessions) in healthy middle-aged men (Tanner et al. 2002). Metabolic short-term training benefits may be even more relevant in people with increased risk factors

(i.e., overweight), and more so when exercise is performed while individuals are consuming a HSFAD.

Exercise training in overweight individuals derives not only metabolic but also cardiovascular health benefits (Stensvold et al. 2010). Twelve weeks of aerobic interval training enhances peripheral endothelial function, increases $\dot{V}O_{2peak}$ and reduces systolic blood pressure in individuals with abdominal obesity and metabolic syndrome (Stensvold et al. 2010). Moreover, 12 weeks of endurance exercise training increased HDL-$_C$ and decreased LDL-$_C$ in overweight/obese subjects despite moderate weight loss (i.e., 2,7 kg) without dietary intervention (Greene et al. 2012). To our knowledge, there is no information about the cardiovascular adaptations of a short-term exercise training program performed concurrently to a HSFAD. We hypothesized that hyperlipidemia and reduced insulin sensitivity occurred even if the increase in dietary SFA lasted only 2 weeks. We also hypothesized that exercise counteracted the negative metabolic and cardiovascular consequences of a high-SFA diet.

METHODS

Participants. Fourteen young (24.8 ± 1.8 years old), overweight (BMI of 27.5 ± 0.6 $kg \cdot m^{-2}$), physically inactive (less than 120 min per week of moderate/vigorous self-reported physical activity) volunteers were recruited from the University's student and workers population. Participant's basal anthropometric and cardiovascular parameters are presented in Table 1. Women (two in each experimental group) were regularly menstruating and not using oral contraceptives. All subjects were weight stable for the 6 months previous to participation. The study was approved by the local Hospital's Ethics Committee and was conformed to the latest revision of the Declaration of Helsinki.

Preliminary testing. All volunteers underwent medical screening, oral glucose tolerance test (OGTT) and a complete blood lipid profile analysis. Medical screening excluded smokers and individuals with heart, metabolic (i.e., diabetes and hyperlipemia), intestinal and pulmonary diseases or those taking medications known to affect the outcome variables. A week before participation in the study subjects underwent a cycling graded exercise stress-test (increasing $25W \cdot min^{-1}$ until volitional fatigue) with ECG and indirect calorimetry monitoring (Quark B^2 plus Quark T12, 12-lead telemetry ECG, Cosmed, Italy). Results of this evaluation are depicted in the PRE columns in Table 1.

Design. Participants were randomly assigned to a diet alone (D group) or a diet plus exercise training group (D+E group). During 14-days all subjects received a diet that increased their SFA intake while maintaining their habitual total fat and daily calorie intake (Table 2). All ingested fat was individualized and provided to the subjects by the research team. In addition, the seven participants of the D+E group received the same diet but underwent 11 sessions of aerobic exercise concurrent to the diet. Before and 48 h after the last training session (D+E group) metabolic and cardiovascular responses were evaluated (Figure 1). Before and after treatments the tests took place after an overnight fast (> 8 h) that followed a

standardized low fat dinner composed of pasta and bread with tomato sauce (617 kcal; 18% kcal from fat, 69% kcal from CHO and 13% kcal from protein).

Diet. During the week prior to the start of the study (Pre-week, Figure 1) participants weighted (Delicia, Tefal, France) and recorded all foodstuff and beverages ingested and daily recorded their nude body weight (WildCat; Mettler-Toledo, USA) right after awakening and voiding. Computerized dietary analysis of their records (CESNID, Barcelona, Spain) allowed calculation of daily caloric intake, dietary fat content and composition (i.e., mono-unsaturated; MUFA, polyunsaturated; PUFA, and saturated fatty acids; SFA). During the 14 day intervention, subjects were requested to refrain from all dietary fat sources while were provided by the research team with SFA in the form of whole fat dairy products (i.e., milk, manchego cheese, butter, cream and cheesecake ice-cream). Only the source of fat was substituted and thus calories and total fat intake were maintained (Table 2) with some fluctuation in the D+E group due to the extra food provided after exercise to maintain the energy balance. The snack was composed of an 8% carbohydrates drink (provided during exercise) and a turkey breast sandwich plus bread with quince jelly, provided right after exercise (i.e., 554 ± 42 kcal). Participants were instructed to maintain their habitual ingestion of fruits, vegetables, cereals, bread, pasta and legumes (cooked with the provided butter). During the 14 days of intervention, subjects weighted and recorded the food consumed outside the dependencies of the lab. Diet logs were evaluated daily to check and adjust adherence to the diet intervention.

Exercise training. Subjects in the D+E group underwent 11 exercise sessions during 55-min on a cycle-ergometer (Ergoline 200, Cosmed, Italy). Exercise started with a 5-min of 50 W warm-up for women and 100 W for men. After the warm-up, it followed a workload that elicited the 65% of their individual $\dot{V}O_{2peak}$ during 5-min. This absolute workload was replicated during 5-min after the warm-up during the ten remaining exercise sessions with the purpose of evaluating training adaptations on heart rate, gross efficiency, blood lactate and

total fat oxidation rates for a given absolute workload. These 10 min of exercise were followed by 40-min of exercise at 65% of their individual $\dot{V}O_{2peak}$. As training adaptations developed, the workload during those 40 min was progressively increased to maintain target heart rate (Lucia et al. 2000). Sessions concluded with a 5-min cool-down at the same workload of warm-up. Thus, participants cycled for 55 min at an average intensity of 60% their $\dot{V}O_{2peak}$ when warm-up and cool-down cycling were included.

Body composition. Weight and height were measured using a calibrated scale and stadiometer (Seca, Vogel & Halke, Germany) and BMI was calculated. Percent body fat was estimated by measuring 7 sites skinfold thickness (Holtain Tanner/Whitehouse Skinfold Caliper, Crymich, UK). All measurements were performed by the same technician and percent body fat calculated using gender specific formulae (Jackson and Pollock 1978, Jackson et al. 1980).

Metabolic measurements. After an overnight fast and at least 48-h after refraining from exercise, participants lay on a stretcher for 15 minutes in a thermoneutral (21–24ºC), dimly lit quiet room. Resting metabolic rate (RMR) was determined by indirect calorimetry (Quark B^2, Cosmed, Italy) during 15 minutes after steady-state readings were obtained. Following the RMR measurements, an intravenous catheter (BD Insyte, Becton-Dickinson, Spain) was inserted in an antecubital vein. Five mL of blood were extracted and the catheter was frequently flushed with 0.9% saline solution, (Salina Fisiológica, Grifols, Spain) to ensure patency. Five minutes after blood extraction, an oral glucose tolerance test (OGTT) was started with the ingestion of 75 g of glucose 1-hydrate (Guinama, Spain) diluted into 250 mL of water as proposed by Matsuda and Defronzo (Matsuda and DeFronzo 1999). After 15, 30, 45, 60, 90 and 120-min of ingesting the glucose load, 5 mL blood samples were obtained. Samples were mixed in tubes with 3K-EDTA (Vacuette, Greiner Bio-one, Spain) and centrifuged at 4000 rpm during 10 min at 4°C to obtain plasma. Glucose and lactate were

measured immediately with an automated analyzer (EML-105, Radiometer, Denmark) while the remaining of the plasma was stored at -80°C for further analysis. Insulin concentration was analyzed using human insulin ELISA immunoassay technique (96T, Cusabio Biotech Co., USA). Insulin sensitivity was estimated using the fasting glucose and insulin concentration calculating the HOMA-IR index (Matthews et al. 1985, Haffner et al. 1996) and using the OGTT data, Matsuda insulin sensitivity index was calculated (Matsuda and DeFronzo 1999).

Plasma TG, T_C, and HDL-$_C$ were analyzed using enzymatic assays and a multichannel spectrometer plate reader (Versamax, Molecular Devices, USA). Specifically, TG was analyzed using a glycerol phosphate oxidase reaction (BioSystems, Spain), T_C using a cholesterol oxidase reaction (BioSystems, Spain) and HDL-$_C$, using an Mg-cholesterol oxidase reaction (BioSystems, Spain). LDL-$_C$ was calculated as proposed by Friedewald (Friedewald et al. 1972). Free fatty acids composition was measured using gas chromatography-mass spectrometry (GC/MS; Agilent 5973 Networks, Mass Selective Detector; Agilent Technologies, USA) after calibration with standards of known concentrations (i.e., WAKO Chemicals, Germany). During exercise, substrate utilization and energy expenditure were calculated from indirect calorimetry (Quark B^2, Cosmed, Italy). $\dot{V}O_2$ and $\dot{V}CO_2$ were measured between 3-5 min and 35-40 min during the first, sixth and eleventh exercise session. Fat oxidation rates were calculated using the equation proposed by Frayn (Frayn 1983).

Cardio-respiratory measurements. Resting heart rate (HR) and blood pressure were measured just after the RMR assessment. HR was measured using a heart rate monitor (Polar S810i, Finland). Blood pressure was measured in triplicate using an automated blood pressure monitor (Tango Medical Instrument Inc., USA). The day when habitual diet recording started and the day following the end of the experimental interventions (i.e., 48-h after the last exercise bout in D+E group) participants underwent the post OGTT. Then subjects had breakfast and rested for 2-h prior to the post $\dot{V}O_{2peak}$ assessment on the same cycle-ergometer. After a

standardized 10-min warm-up, the initial power output was set (75 W in men, 50 W in women) and increased thereafter (i.e., 25 W · min^{-1}) until volitional fatigue or when pedaling cadence dropped repeatedly below 60 rpm. Oxygen uptake (i.e., $\dot{V}O_2$) was measured using an automated breath-by-breath indirect calorimetry system (Quark B^2, Cosmed, Italy) and data was averaged every 15 sec. The $\dot{V}O_{2peak}$ was defined as the highest $\dot{V}O_2$ value reached.

Statistics. Shapiro-Wilk test was used to assess normal distribution of data. Since the main variable under examination (blood lipid profile) did not show a normal distribution and the sample size was limited, non-parametric statistics were chosen for the analysis (i.e., PRE vs. POST intervention for the D and D+E groups). Wilcoxon test was performed to analyze dependent samples and Mann-Whitney U for independent samples. The level of significance was set at $P < 0.05$. Results were reported as means ± SEM. All the tests were performed with SPSS software version 18.

RESULTS

Body composition. There were no differences between groups in body weight, BMI, and percent body fat before the interventions. BMI, body weight and percent body fat were not different between the beginning and the end of the interventions in any of the groups (Table 1).

Diet manipulation. SFA ingestion increased by approximately 65% in both groups, and the percent of daily energy intake from SFA in the diet increased (12.4 ± 2.7% to 20.2 ± 4.8 %; P <0.001, Table 2) while percent MUFA (13.0 ± 3.8% to 10.5 ± 2.2%; P = 0.001) and PUFA (7.0 ± 1.5% to 2.7 ± 0.4 % P <0.001) were reduced. In the D+E group, energy intake increased (Table 2) as a result of the supplemented food provided to compensate for the energy expenditure during the exercise sessions. The increased energy intake in D+E group was associated with an increased intake of macronutrients, but only fat intake reach a significant difference (P=0.028). In the D group, calorie intake decreased 5% (NS; P =0.093), fat intake remained at pre-intervention levels, and as a consequence, relative fat intake increased by 6% (P = 0.047, Table 2).

Exercise training adaptations. The metabolic and cardiovascular adaptations to eleven sessions of exercise are depicted in Table 3. $\dot{V}O_{2peak}$ increased in the D+E group by 18 ± 5 % (P=0.020) while it remained unchanged in the D group (Table 1). Average energy expenditure during exercise was 554 ± 42 kcal and 3048 ± 208 kcal per week. The work rate needed to elicit the target HR increased 17% (P = 0.017; Table 3). As a consequence the energy cost of each exercise session increased across time (527 ± 39 to 580 ± 41 kcal; P=0.012). At a given absolute intensity (i.e., 124 ± 9 W), HR and blood lactate were markedly reduced (10 beat · min^{-1} and 1.6 mmol · L^{-1}; P = 0.019 and P = 0.022, respectively; Table 3) while exercise fat oxidation increased 14 fold (P = 0.034; Table 3) after eleven days of exercise training. Finally, the HR after five minutes of cool-down was significantly lower after training (P <0.001; Table 3).

Metabolic measurements. RMR was not different between groups before the interventions (1622 ± 110 and 1476 ± 92 Kcal · day^{-1} for D and D+E groups, respectively; P = 0.194) and did not change after the interventions in any of the two group (1676 ± 128 Kcal · day^{-1}, P = 0.999 and 1548 ± 132 Kcal · day^{-1}, P = 0.515 for D and D+E group, respectively). The high SFA diet was associated with a significant increase in T_C and LDL-$_C$ in the D group (147 ± 8 to 161 ± 9 mg · dL^{-1}, P = 0.018 and 71 ± 10 to 82 ± 10 mg · dL^{-1}, P =0.034, respectively; Figure 2), which was prevented in the D + E group (153 ± 20 to 157 ± 24, P =0.735, and 71 ± 21 to 70 ± 25 mg · dL^{-1}; P =0.866, respectively; Figure 2). Plasma concentration of HDL-$_C$, TG and FFA remained unchanged in both groups (Table 4). Before the interventions subjects display similar plasma distribution of FFA (38.8 ± 1.1 % of SFA; 47.9 ± 1.3 % MUFA and 13.3 ± 1.1% PUFA) which remained unchanged after the intervention in both groups. Of note plasma SFA increased in both groups from 38.8 ± 1.1 % to 40.3 ± 1.4 % without reaching significance (P = 0.253).

Fasting glucose and insulin did not change as a result of the intervention in any group (Table 4). Therefore, HOMA-IR remained unchanged in the D group and decrease by 24% in D+E group (i.e., improvement in insulin sensitivity) without reaching significance (P = 0.945 and P = 0.119, respectively). The insulin sensitivity index calculated from the 120 min OGTT (Matsuda index), remained unchanged in both groups (Table 4). Between groups estimated insulin sensitivity was not different at any time.

Cardio-respiratory measurements. In D+E group, there was a significant reduction in resting systolic blood pressure (i.e., 6 ± 2 mmHg; P=0.029) and resting HR (i.e., 9 ± 3 beats · min^{-1}; P= 0.027; Table 1). Conversely, in the D group there was not a significant change in resting systolic blood pressure or resting HR (Table 1).

DISCUSSION

During 14 days and under our direct supervision 14 overweight subjects increased by 65% their dietary SFA while reducing PUFA and MUFA intake with the aim to maintain total fat intake. As planned, this short-term eucaloric diet did not affect body weight or percent body fat. However, the subjects that received the diet alone (i.e., D group) increased their blood T_C and LDL-$_C$ by 9 and 16% respectively, (Figure 2; $P < 0.05$). This increase was not induced by the last meal ingested (standardized low-fat dinner provided the day before pre and post-intervention testing) but likely to the 14 days exposure to the SFA diet. The most of calories expended during exercise (D+E group) were replaced to avoid the confounding effects of a negative caloric balance. In addition, testing took place 48 h after the last exercise session (D+E group) with the aim to test the chronic not the acute effects of exercise. On note, short term endurance training (i.e., 11 sessions of 55 min on cycle ergometer at 60% $\dot{V}O_{2peak}$) concurrent to a HSFAD prevented the increases in blood cholesterol, while increasing $\dot{V}O_{2peak}$ (i.e., 18%), fat oxidation and reducing resting systolic blood pressure (i.e., 5%). Thus, our data suggest that the atherogenic blood lipid profile induced by a short-term high saturated fat diet can be prevented by concurrent aerobic exercise training while improving cardio-respiratory fitness.

We are not the first to show increased T_C and LDL-$_C$ in association with partial substitution of dietary unsaturated by SFA in overweight people (Vessby et al. 2001, Jebb et al. 2010). With subjects similarly overweighed (i.e., BMI ~ 27 kg · m^{-2}) and with a similar dietary intervention (i.e., increases in SFA from 14 to 18% vs. 12 to 20% EI presently) Vessby and co-workers reported less increase in blood cholesterol (i.e., 2% and 4% for T_C and LDL-$_C$ for Vessby vs. 9% and 16% increases, presently). Of note, our subjects had lower pre-intervention T_C (150 mg · dL^{-1} vs. 210 mg · dL^{-1} in Vessby's data). Thus, it seems that the plasma hyperlipemic response to increased dietary SFA could be also determined by the initial plasma

lipid concentration. In any extent, Vessby's subjects underwent a 3 month experimental diet, while our intervention lasted merely 2 weeks. To our knowledge, we are the first to show that a two week dietary substitution of unsaturated by SFA, without increasing the absolute amount of fat in diet, could raise blood cholesterol in normolipemic and healthy overweight individuals. We believe that this is relevant since short-term increases in dietary SFA are common during weekends and holidays (e.g., Easter, Christmas, summer breaks) and without a specific intervention (i.e., diet, exercise, medication) the increases in T_C and LDL-$_C$ could reach pathological levels (i.e., hyperlipidemia). Additionally, a high saturated fatty acids meal not only results in post-prandial lipemia but also in events linked with cardiovascular disease pathogenesis (i.e., oxidative stress, chylomicron remnants uptake by macrophages, LDL oxidation, and thrombosis activation (Ceriello 2000)).

Post-intervention testing was performed 24 hours after the end of the dietary intervention and 48 hours after the last bout of exercise on the D+E group. The 48h period was chosen to avoid the confounding effects of the last bout of exercise on blood lipids (i.e., TG decrease and HDL-$_C$ increase (Kantor et al. 1984, Grandjean et al. 2000, Crouse et al. 1997)). Crouse et al., (Crouse et al. 1997) reported reductions in LDL-$_C$ right after training, while no differences could be observed when comparing before and 48 hours after an exercise session. Similar results were reported by Ferguson et al., (Ferguson et al. 1998) using trained men. They did not observe differences in T_C and LDL-$_C$ between before exercise and 48 hours after exercise of different duration (i.e., 60, 81, 94 and 111 min of running at 70% $\dot{V}O_{2peak}$.). Based on these reports we thought that blood lipid concentrations 48h after the last exercise bout would be more likely related to the two weeks of training than to the acute effect of the last exercise session. However, a possible influence of the acute exercise effects on blood lipids cannot be ruled out after 48h as it has been recently shown by others (Grandjean et al. 2000, Crouse et al. 1995, Greene et al. 2012).

Vessby et al., showed that 3 months of increased dietary SFA not only affected blood lipids but also reduced insulin sensitivity in comparison to a group ingesting MUFA when fat intake was above 37% of daily energy intake. However, in a subsequent study with a higher pool of subjects and a similar intervention, Jebb et al. (Jebb et al. 2010) could not demonstrate an effect of dietary SFA on insulin sensitivity in accordance with a recent report in overweight women (Haghighatdoost et al. 2012). Thus, the effects of increased dietary SFA on insulin sensitivity are not well defined. Our subjects' fat intake was below 37% of daily energy intake and in accordance to Jebb's data, our indexes that estimate insulin sensitivity remained unchanged in both groups. Noteworthy, our short-term dietary intervention did not raise the percent of SFA in plasma. Longer interventions (i.e. 3 months, (Andersson et al. 2002)) have shown to increase the contribution of SFA to the plasma lipid profile. It is possible that an increased presence of SFA in the circulation may be necessary to provoke insulin resistance when total fat intake is not increased like at present.

It is somewhat surprising that eleven session of exercise had such a large impact on cardiovascular health indexes (18% increase in $\dot{V}O_{2peak}$) and 6 mmHg reduction in resting systolic blood pressure), on account that two weeks (6 sessions) of spring interval training in sedentary overweight subjects raises $\dot{V}O_{2peak}$ 8.4% and lowers resting systolic blood pressure by 6 mm Hg (Whyte et al.). Habitual physical activity in our subjects was well below the minimal amount to obtain health benefits (i.e., 150 minutes/week) according to the 2008 Physical Activity Guidelines for Americans (U.S. Department of Health and Human Services 2008). In fact, our subjects started with a poor cardio-respiratory fitness level (i.e., $\dot{V}O_{2peak}$ below the 25th percentile matched by gender and age group (Balady 2000)) which may have permitted the large gain in $\dot{V}O_{2peak}$. Due to the high workout frequency (i.e., 5 sessions per week) , our D+E subjects expended 3048 ± 208 extra kcal per week which rated our program

as "very vigorous" according to the ACSM and AHA guidelines (Haskell et al. 2007). Our participants received a potentially atherogenic diet and thus we thrive to counteract that potential raising exercise frequency higher than what is usually reported in studies (i.e., 5 vs. 2-3 days a week). It is possible that a lesser volume and density of exercise could elicit the same cardio-respiratory adaptations observed in our participants, but the interaction of baseline fitness status, volume and intensity of exercise on the achievement of cardiometabolic adaptations are beyond the scope of the present research and further research is necessary to determine if more moderate and feasible exercise programs may have similar beneficial effects.

Exercise is a cornerstone intervention in the treatment and prevention of cardiometabolic diseases. The benefits of exercise counteracting risk factors as dyslipidemia and insulin resistance in overweight subjects have been evidenced (Stensvold et al. 2010). Nevertheless, most of the exercise related benefits have been associated with energy deficit and body weight loss. With the aim to assess if exercise was able to prevent a blood lipid profile deterioration or insulin resistance in absence of an energy deficit, we supplemented participants in D+E group with food to match the energy spent during the exercise sessions. Despite being in energy balance the D+E participants did not increase in T_C and LDL-$_C$ and nevertheless improved cardiometabolic fitness (i.e., resting systolic blood pressure, $\dot{V}O_{2peak}$ and fat oxidation rate during exercise). However the 11 exercise sessions neither improve blood lipid profile nor insulin sensitivity. It is possible that a negative carbohydrate balance is needed to increase insulin sensitivity when blood lipids remain unchanged as it has been suggested by other researchers (Harrison et al. 2009, Holtz et al. 2008, Newsom et al. 2010).

In summary, we presently show in physically inactive overweight adults that two weeks of substitution of dietary unsaturated for saturated fatty acids worsens their blood lipid profile (increase T_C and LDL-$_C$) without affecting their insulin sensitivity, body weight or body composition. Furthermore, 11 sessions of aerobic exercise concurrent to the HSFAD

prevented the increase in blood cholesterol and improved parameters associated with cardiovascular health ($\dot{V}O_{2peak}$ and resting systolic blood pressure).

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Table 1. Anthropometric and cardiovascular variables before and after the 2 weeks of treatment in the diet and diet plus exercise groups.

	DIET			DIET + EXERCISE		
	PRE	POST	*P*	PRE	POST	*P*
Body Weight (kg)	86 ± 4.1	85.7 ± 4.2	0.387	77.4 ± 2.7	77.1 ± 2.8	0.421
BMI ($kg \cdot m^{-2}$)	28.6 ± 1.0	28.5 ± 1.0	0.196	26.4 ± 0.4	26.3 ± 0.4	0.397
Body fat (%)	23.3 ± 7.5	23 ± 7.4	0.108	22.3 ± 5.1	21.9 ± 5.2	0.059
SBP (mmHg)	117 ± 3	119 ± 3	0.493	123 ± 3	117 ± 2	0.029 *
DBP (mmHg)	68 ± 2	68 ± 3	1.000	69 ± 2	66 ± 3	0.257
Resting HR ($beats \cdot min^{-1}$)	62 ± 6	60 ± 4	0.573	69 ± 5	60 ± 4	0.027 *
$\dot{V}O_{2peak}$ ($ml \cdot kg^{1} \cdot min^{-1}$)	35.2 ± 3.3	34 ± 2.9	0.144	36.2 ± 2.5	42.3 ± 2.8	0.020 *

Values are means ± SEM for 7 subjects in each group. * Significant difference between PRE and POST intervention ($P<0.05$). BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; HR: Heart rate; $\dot{V}O_{2peak}$: Peak oxygen consumption.

Table 2. Dietary intake before and after the 2 weeks of treatment in the diet and diet plus exercise groups.

	DIET			DIET + EXERCISE		
	PRE	POST	*P*	PRE	POST	*P*
Energy Intake (kcal · day^{-1})	2405 ± 234	2273 ± 244	0.093	2138 ± 99	2436 ± 143	0.027 *
Carbohydrates (g · day^{-1})	247 ± 25	236 ± 30	0.310	256 ± 10	307 ± 24	0.063
Proteins (g · day^{-1})	108 ± 17	107 ± 12	0.921	93 ± 6	96 ± 5	0.699
Fat intake (g · day^{-1})	90 ± 9	89 ± 10	0.738	74 ± 6	83 ± 7	0.028 *
Fat intake (% of EI)	34.3 ± 2.9	36.2 ± 36.2	0.047 *	31.1 ± 1.5	31.0 ± 2	0.690
SFA intake (g · day^{-1})	32 ± 4	53 ± 6	0.001 *	31 ± 4	51 ± 4	<0.001*
SFA intake (% of EI)	11.9 ± 1.1	21.7 ± 2.1	<0.001 *	12.9 ± 1.0	18.8 ± 1.4	0.003 *

Values are means ± SEM for 7 subjects in each group. * Significant difference between PRE and POST intervention (P<0.05). SFA: Saturated fatty acids.

Table 3. Training-related adaptations in the diet plus exercise group.

	DIET + EXERCISE		
	1^{ST} session	11^{TH} session	*P*
Heart Rate (beat · min^{-1}) †	149 ± 3	139 ± 4	0.019 *
Blood lactate (mmol · L^{-1})	5.4 ± 0.5	3.8 ± 0.3	0.022 *
WR at target HR (Watts) ‡	124 ± 9	145 ± 11	0.017 *
Fat oxidation rates (g · min^{-1})	0.01 ± 0.01	0.14 ± 0.04	0.043 *
Recovery HR (beats · min^{-1})	142 ± 3	130 ± 4	<0.001 *

Values are means ± SEM for 7 subjects in the diet + exercise group. WR: Work rate. * Significant difference from the first exercise session ($P<0.05$).) † HR at a fixed work rate (124± 9 W). ‡ Target HR: HR at 65% $\dot{V}O_{2peak}$).

Table 4. Carbohydrate and fat blood variables prior and after the 2 weeks of treatment in the diet and diet and exercise groups.

	DIET			DIET + EXERCISE		
	PRE	POST	*P*	PRE	POST	*P*
Fasting glucose ($mg \cdot dL^{-1}$)	92 ± 2	94 ± 2	0.299	92 ± 2	91 ± 2	0.397
Fasting Insulin ($\mu U \cdot dL^{-1}$)	23 ± 7	23 ± 7	0.959	23 ± 5	19 ± 4	0.184
HOMA-IR	5.3 ± 1.7	5.4 ± 1.7	0.945	5.1 ± 1.2	3.9 ± 0.8	0.119
Matsuda Index	3.0 ± 0.7	3.2 ± 0.7	0.673	2.7 ± 0.4	3.0 ± 0.4	0.111
HDL-$_C$ ($mg \cdot dL^{-1}$)	59 ± 3	61 ± 3	0.325	65 ± 4	68 ± 4	0.118
Triglycerides ($mg \cdot dL^{-1}$)	85 ± 6	87 ± 7	0.907	87 ± 19	96 ± 16	0.343
FFA ($nmol \cdot mL^{-1}$)	455 ± 46	417 ± 49	0.866	428 ± 85	422 ± 52	0.612

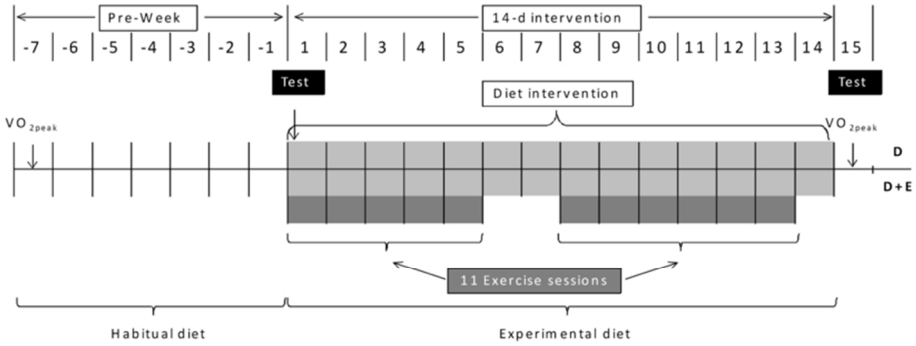
Values are means ± SEM for 7 subjects in each group. * Significant difference ($P<0.05$).

HDL-$_C$: High density lipoprotein cholesterol; TG: Triglycerides; FFA: Free fatty acids.

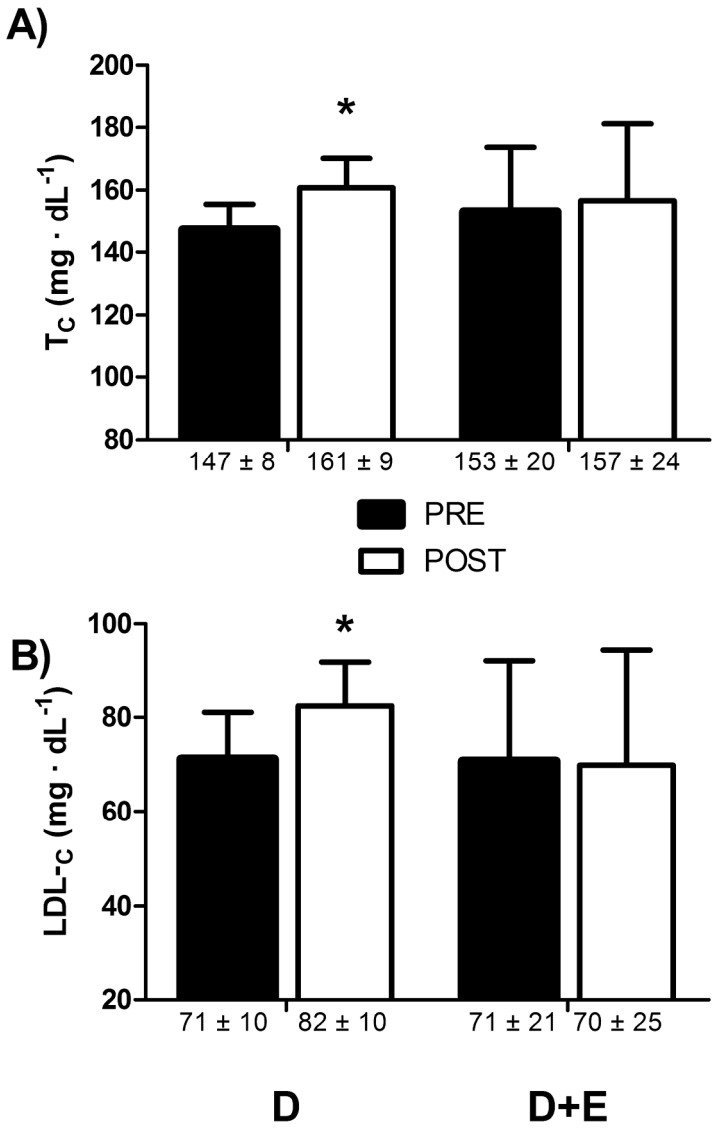
FIGURES CAPTIONS

Figure 1. Experimental design. Habitual diet was recorded during the Pre-week. ☐ Days of experimental diet. ■ Supervised cycle-ergometer training. Test Experimental testing

Figure 2. A) Total cholesterol (i.e., T_C) and B) Low density lipoprotein (i.e., LDL-$_C$) after 14 days of high SFA diet (D) and diet plus concurrent aerobic exercise training (D+E). Data are means ± SEM for 7 subjects in each group. * Denotes difference between PRE and POST intervention (P<0.05).



Experimental design. Habitual diet was recorded during the Pre-week. □ Days of experimental diet. □(Gray) Supervised cycle-ergometer training. ■ Experimental testing
76x29mm (300 x 300 DPI)



A) Total cholesterol (i.e., TC) and B) Low density lipoprotein (i.e., LDL-C) after 14 days of high SFA diet (D) and diet plus concurrent aerobic exercise training (D+E). Data are means ± SEM for 7 subjects in each group. * Denotes difference between PRE and POST intervention ($P<0.05$).
199x275mm (300 x 300 DPI)