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**ORIGINAL ARTICLE**

# Exercise training improves fat metabolism independent of total energy expenditure in sedentary overweight men, but does not restore lean metabolic phenotype

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**BACKGROUND:** Obesity is a dietary fat storage disease. Although exercise prevents weight gain, effects of chronic training on dietary fat oxidation remains understudied in overweight adults.
**OBJECTIVE:** We tested whether 2 months of training at current guidelines increase dietary fat oxidation in sedentary overweight adults like in sedentary lean adults.
**DESIGN:** Sedentary lean ($n = 10$) and overweight ($n = 9$) men trained on a cycle ergometer at 50% $VO_{2peak}$, 1 h $day^{-1}$, four times per week, for 2 months while energy balance was clamped. Metabolic fate of [$d_{31}$]palmitate and [1-$^{13}$C]oleate mixed in standard meals, total substrate use, total energy expenditure (TEE), activity energy expenditure (AEE) and key muscle proteins/enzymes were measured before and at the end of the intervention.
**RESULTS:** Conversely to lean subjects, TEE and AEE did not increase in overweight participants due to a spontaneous decrease in non-training AEE. Despite this compensatory behavior, aerobic fitness, insulin sensitivity and fat oxidation were improved by exercise training. The latter was not explained by changes in dietary fat trafficking but more likely by a coordinated response at the muscle level enhancing fat uptake, acylation and oxidation (FABPpm, CD36, FATP1, ACSL1, CPT1, mtGPAT). ACSL1 fold change positively correlated with total fasting ($R^2 = 0.59$, $P < 0.0001$) and post-prandial ($R^2 = 0.49$, $P = 0.0006$) fat oxidation whereas mtGPAT fold change negatively correlated with dietary palmitate oxidation ($R^2 = 0.40$, $P = 0.009$), suggesting modified fat trafficking between oxidation and storage within the muscle. However, for most of the measured parameters the post-training values observed in overweight adults remained lower than the pre-training values observed in the lean subjects.
**CONCLUSION:** Independent of energy balance and TEE, exercise training at current recommendations improved fitness and fat oxidation in overweight adults. However the improved metabolic phenotype of overweight adults was not as healthy as the one of their lean counterparts before the 2-month training, likely due to the spontaneous reduction in non-training AEE.

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## INTRODUCTION

While a clear line of evidence supports a key role of exercise in preventing weight gain and regain,[1,2] a number of specific questions need to be addressed to develop efficient guidelines. One key point is related to the exercise intensity and amount thresholds able to prevent excessive weight gain, as it has been shown that the dose–response relationship between exercise and health may be health outcome dependent.[3] Yet it has been suggested that the current physical activity (PA) guidelines for the general population[4] may be efficient to favorably affect fasting lipemia and insulin sensitivity, but fail to prevent additional weight gain or even more produce weight loss in overweight individuals.[5,6] Another interrogation concerns the respective role of exercise and spontaneous or daily PA in body weight regulation; daily PA, that accounts for a large percentage of total daily energy expenditure, has been associated with resistance to fat gain in response to overfeeding.[7] Furthermore, it has been suggested that an approach promoting small changes in lifestyle behaviors may be the adequate strategy to improve metabolic health in overweight subjects.[8]

One way to address these questions is to investigate the effect of PA on dietary fat balance. Obesity is primarily a fat storage disease that essentially represents an imbalance in the partitioning of fat between storage and oxidation[7] and a blunted capacity to oxidize fat was suggested a primary etiologic impairment of obesity, because weight reduction do not fully improve fat utilization.[9,10] Consequently, any effects of PA on body mass regulation must involve changes in total fat oxidation and more specifically in dietary fat oxidation.

A few studies reported that acute exercise have beneficial effects on dietary fat oxidation in both lean men[11] and animals.[12] We found that 2 and 3 months of bed rest, a model of severe physical inactivity, affect partitioning of dietary saturated fats toward storage in both normal-weight men and women.[13,14] Moreover, two studies indicate that exercise accelerates the capacity to increase total fat oxidation after a 1-week shift to a

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eucaloric high-fat diet in lean and overweight subjects,[15,16] suggesting that PA participates in the regulation of fat balance, independent of energy balance. However the effects of chronic exercise training on dietary fat trafficking and oxidation, beyond the effect of acute exercise, appear to be understudied. Using a 2-month training/detraining program at current recommendations in lean subjects, we showed that activity energy expenditure (AEE) was a strong predictor of dietary fat oxidation, which was mediated by a coordinated muscular response in genes and proteins effectors.[17]

Whether or not overweight affects this relationship remains questionable. We therefore submitted overweight men to a 2-month training protocol at current recommendations level similar to the one we used in a study comparing the effect of training and detraining on dietary fat metabolism in lean men.[17]

## MATERIALS AND METHODS

### Experimental protocol

The study was conducted from April 2008 to December 2010. Twelve sedentary lean ($20 \leqslant$ body mass index $< 25$ kg m$^{-2}$) and 12 overweight ($25 \leqslant$ body mass index $\leqslant 35$ kg m$^{-2}$) men, disease free and weight stable for at least 3 months before enrolment, were included in a 2-month outpatient training study (Supplementary Table S1). Sedentary status was defined using the MOSPA questionnaire.[18] Additional inclusion criteria included no family history of obesity or type 2 diabetes for the lean group, or at least one overweight or diabetic first-degree relative for the overweight group. Training was performed for 2 months in free-living conditions with three 60- min sessions per week at 50% $VO_{2peak}$ on a cycle ergometer performed in the laboratory plus an additional equivalent 60-min weekend session checked by trixial accelerometry. Maximal aerobic capacity ($VO_{2peak}$) was determined on a cycle ergometer. The diet was monitored and regularly adjusted in an effort to maintain subjects in stable energy balance. The study was approved by the Alsace IRB. All subjects signed informed consents. Two sets of identical tests were performed before and after training to assess fat metabolism, as previously reported for lean sedentary subjects.[17] Three overweight subjects did not respect the training protocol, one lean did not have tracers properly homogenized in the liquid meal and samples from another one lean were lost during the mass spectrometry analyses. Results are therefore presented for 19 subjects, 10 lean and 9 overweight. Supplementary Figures S1 and S2, respectively, present a flow chart and a protocol design summary.

### Energy expenditures and body composition

Resting metabolic rate (RMR) was measured by indirect calorimetry (Deltatrac II; General Electric, Indianapolis, IN, USA) for 1 h after an overnight fast.

Total energy expenditure (TEE) was determined by the doubly labeled water method over a 10-day period.[19] Subjects ingested a premixed 2 g kg$^{-1}$ estimated total body water (TBW) dose of doubly labeled water composed of 0.2 and 0.15 g kg$^{-1}$ estimated TBW of $H_2^{18}O$ and $^2H_2O$, respectively (CIL, Andover, MA, USA). Equilibration and end point urines were cleaned as previously described.[20] Deuterium and 18-oxygen isotopic abundances were analyzed by pyrolysis on a Flash HT (ThermoFisher Scientific, Waltham, MA, USA) connected to a continuous-flow IRMS (Delta V; ThermoFisher Scientific). Results were scaled using two laboratory standards. Analyses were performed in quadruplicate and repeated if the s. d. exceeded 2‰ for deuterium and 0.5‰ for 18-oxygen. TBW and TEE were calculated as previously described[21,22] using a food quotient of 0.86. Diet-induced thermogenesis was assumed to be 10% of TEE. AEE was calculated as 0.9 TEE minus RMR. Non-training AEE was calculated as AEE minus energy expenditures spent during each training sessions on the ergometers. Calculations were checked by proper adherence to the protocol with the help of triaxial accelerometers, notably during the out-of-laboratory activity sessions.

Fat-free mass was calculated from TBW using a hydration factor of 0.73[23] and fat mass was calculated from the difference with body mass.

### Dietary fat oxidation and trafficking

Standard meals (55% carbohydrate, 15% protein, 30% lipid of total energy intake) were provided 36 h prior each test. After an overnight fast, an intravenous catheter was inserted retrogradely in a forearm vein. After collecting baseline fasting breath, urine and arterialized blood samples, a breakfast was served (50% RMR as energy, 55% carbohydrates, 15% protein and 30% fat) composed of a liquid replacement meal (Renutril) in which 15 mg kg$^{-1}$ of $d_{31}$-palmitic acid and 10 mg kg$^{-1}$ of [1-$^{13}$C]-oleic acid (both $>$98% enriched; CIL) were homogenized at 65 °C. A lunch (73% carbohydrate, 10% fat and 18% protein) was served after 4 h after breakfast. Over 8 h, total substrate use was assessed by hourly indirect calorimetry. Hourly blood, urine and breath samples were collected to assess dietary fat oxidation and trafficking in triglycerides (TG)-rich lipoproteins and non-esterified fatty acids (NEFA). Urine and breath samples were collected by the subjects at home to calculate dietary fat oxidation over 24 h.

Chylomicrons and very low density lipoprotein (VLDL) were separated by ultracentrifugation as previously described.[17] Total lipids from chylomicrons and VLDL were extracted by a Folch procedure. TG and NEFA were further separated from plasma by solid phase extraction prior to derivatization.[13] Gas chromatography was used to analyze the fatty acid composition of plasma NEFA and TG.[24–26] Concentrations of individual fatty acids were calculated by reference to internal standards added to the plasma during lipid extraction (heneicosanoic acid, tripentadecanoyl glycerol). Isotopic enrichment of $d_{31}$-palmitic and [1-$^{13}$C]-oleic acids in TG and NEFA was analyzed by gas chromatography mass spectrometry (Agilent 5975).[13] To assess both the isotopic enrichment and the individual fatty acid concentrations, we designed a dual acquisition program in single-ion monitoring mode. The *m/z* ratios of 296 and 297 were acquired for oleate and 270 and 301 for palmitate. The concentration of each labeled fatty acid was calculated by multiplying its molar percent enrichment by the concentration of its corresponding unlabeled compound. Plasma NEFA was measured by NEFA C WAKO kit (SOBIODA S.A.S), glucose was measured using GLUCm glucose kit and TG using triglyceride GPO kit both from Clinical System SYNCHRON (Beckman Coulter, Villepinte, France). Plasma insulin was determined by immunoassay (ADVIA Centaur Insulin IRI Siemens).

[1-$^{13}$C]oleate oxidation was calculated as the instantaneous percentage recovery of $^{13}$C in expired $CO_2$ per hour over 8 h and measured in triplicate on a continuous-flow inlet system connected to an IRMS (GV Instruments, Manchester, UK) and corrected for isotope sequestration by assuming an acetate correction factor.[27,28] To measure d31-palmitate oxidation, $^2H/^1H$ ratios from urine samples were analyzed, as above described for the doubly labeled water method. The oxidation rate of palmitate was calculated from the cumulative recovery of $^2H$ in TBW. The detailed calculations of the percentage recoveries are detailed elsewhere.[29]

### Muscle analyses

Vastus lateralis muscle was biopsied at the end of the test day and froze in liquid nitrogen. Muscle were grounded in liquid nitrogen and total RNA was extracted using mirVana miRNA Isolation Kit (Applied Biosystems, Villebon sur Yvette, France).

First-strand cDNAs were synthesized from 500 ng of total RNA in the presence of 100 units of Superscript II (Invitrogen, Cergy Pontoise, France) using a mixture of random hexamers and oligo (dT) primers (Promega, Charbonnières les Bains, France). Real-time PCR assays were performed using a Rotor-GeneTM 6000 (Qiagen, Courtaboeuf, France). For quantification, a standard curve was systematically generated with six different amounts of cDNA. Each assay was performed in duplicate, and validation of the reverse transcriptase-PCR runs was assessed by evaluation of the melting temperature of the products and by the slope and error obtained with the standard curve. TBP mRNA level was determined in each sample and was used as an internal standard for normalization of target mRNA expression. The list of the PCR primers and the quantitative PCR assay conditions are available upon request. The quantitative reverse transcriptase PCR was applied to detect changes in the expression of LPL, FAT/CD36, FABPpm/GOT2, FATP1/SLC27A1, ACSL1, CPT1, mtGPAT/GPAM and PGC1α.

Proteins were extracted for western blot experiments only in a subgroup of subjects for which we had enough muscle tissue, four lean and four overweight participants. Anti-CD36, anti-FATP1, anti-GAPDH (Santa Cruz Biotechnology, Palo Alto, CA, USA) and anti-OXPHOS (#MS604; MitoSciences Inc., Eugene, OR, USA) antibodies were used. The signal was quantified by using the Image J program (version 1.45 s Java 1.6.0_45; Wayne Rasband, NIH) and normalized to GAPDH. Coomassie blue staining was performed to check for equivalent loading. Supplementary Figure S3 presents western blots. For 3-HAD enzyme activity, 8 µg proteins were added to an assay buffer containing 50 mmol l$^{-1}$ Tris (pH: 7.6), 2 mmol l$^{-1}$ EDTA, 50 µmol l$^{-1}$

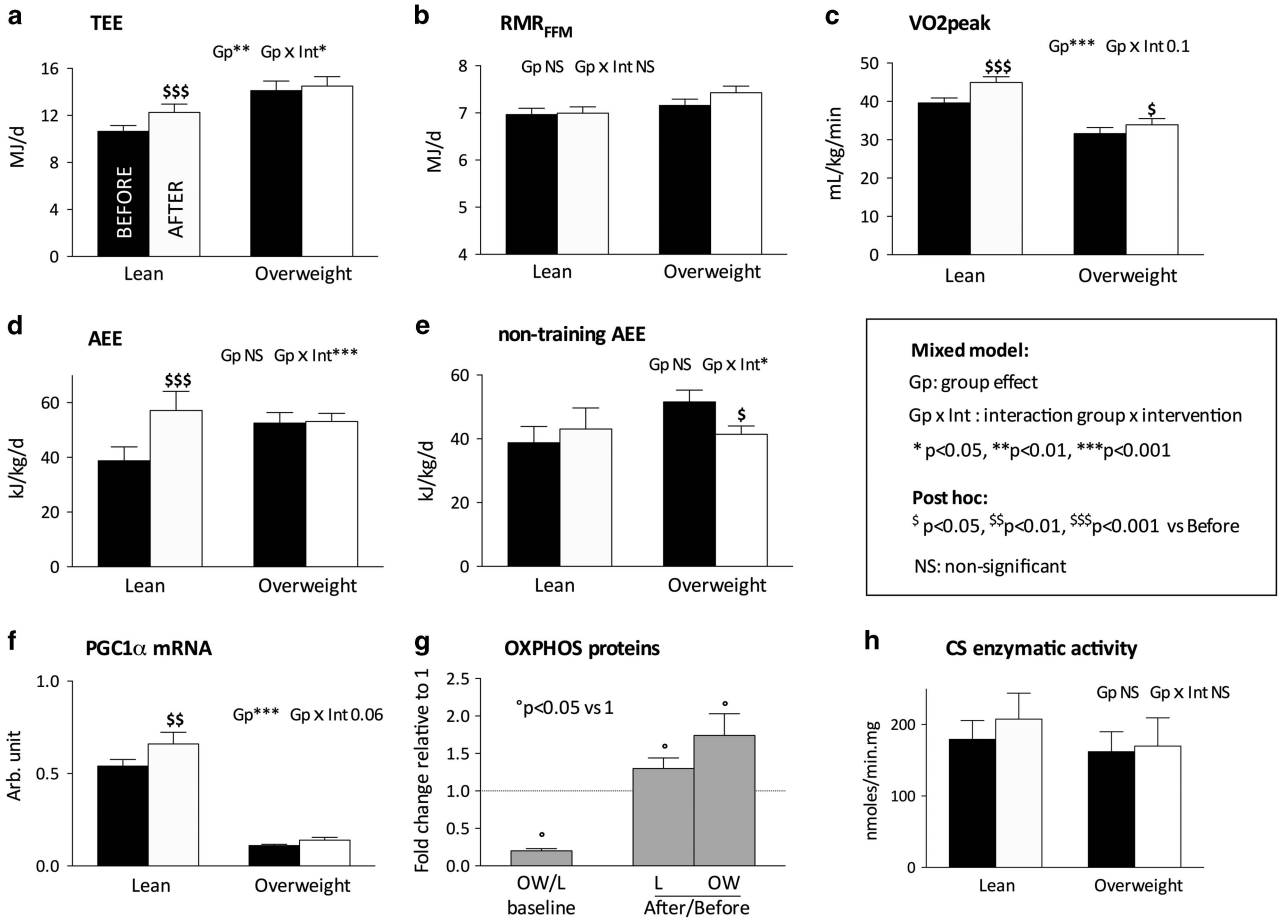


**Figure 1.** Energy metabolism. Whole-body energy metabolism: TEE (**a**, total energy expenditure), AEE (**d**, activity energy expenditure), non-training AEE (**e**, energy expenditure associated with activities of daily life excluding energy expended during training), RMR (**b**, resting energy expenditure), $VO_2$peak (**c**, maximal oxygen uptake) before and after a 2-month training at current recommendations in lean ($n=10$) and overweight ($n=9$) subjects. Muscle energy metabolism assessed through three parameters measured before and after the same training protocol: PGC1α mRNA (**f**, lean $n=10$, overweight $n=9$) and citrate synthase activity (**h**, CS, lean $n=4$, overweight $n=4$). The amount of OXPHOS proteins (**g**) is presented as the ratio between the values obtained in the overweight (OW, $n=4$) and lean (L, $n=4$) groups in baseline and as the change from pre to post intervention in both groups. In the calculations lean (for before/after comparision) and baseline (for lean/overweight comparison) are mathematically set to 1 for readability. Values are means ± s.e.m.

NADH, H+ and 0.01% Triton X-100 (vol:vol). NADH, H+ oxidation was fluorometrically recorded after the addition of 75 μmol $l^{-1}$ acetoacetyl CoA.[17] Measurements of citrate synthase (CS) activity were performed.[17]

### Statistical analysis

A sample size of 10 subjects per group was calculated to detect an 7% increase of the palmitate oxidation in each group of subjects with a 90% power and an alpha error of 0.05. To anticipate dropouts and missing data, 12 subjects were included in each group.

Data are presented as mean ± s.e.m. Area under the curve (AUC) of the concentration–time curve was calculated. After checking for basic statistical rules (normal distribution and homogeneity of variance), mixed linear models taking into account the repeated individual data over time, group, intervention, group-by-intervention interaction as fixed effects and individuals as random effects were used. *Post hoc* Tukey tests were used to assess the effect of intervention within groups. Protein fold changes only were tested using paired *t*-tests. Significance was set at 0.05 for main effects and 0.1 for interaction effects. Statistics were performed using SAS version 0.3 (SAS, Cary, NC, USA).

## RESULTS

### Baseline differences

At baseline, TEE was higher in overweight participants due to higher RMR and AEE. However, the differences in RMR and AEE disappeared after adjustment on fat-free mass and body mass, respectively (Supplementary Table S1 and Figures 1a, b and d). $VO_{2peak}$ was lower in overweight subjects, as well as muscle gene expression of the master regulator of muscle energy metabolism PGC1α (Figures 1c and f). OXPHOS proteins involved in mitochondrial energy production were significantly lower in overweight subjects while CS activity, a marker of mitrochondria density, was not different between the two groups (Figures 1g and h).

Overweight subjects had greater plasma glucose and insulin over the 8-h post-prandial periods (Figures 2a and b). No difference in glucose oxidation per kilogram of body mass was noted (data not shown). Despite the higher insulin response, the post-breakfast shift in substrate use towards glucose was blunted in overweight subjects, indicating a metabolically inflexible status. The 8 h kinetics showed that non-prontein respiratory quotient remained high and flat during the test day and did not reflect the expected variations induced by the two meal ingestions. Indeed, the non-prontein respiratory quotient at 4-h post breakfast remained significantly higher in the overweight group compared with their lean counterparts (Figure 2c). Lastly, the maximal suppression of lipolysis following breakfast as assessed by the drop in NEFA plasma concentration was lower in the overweight group (Figure 2e).

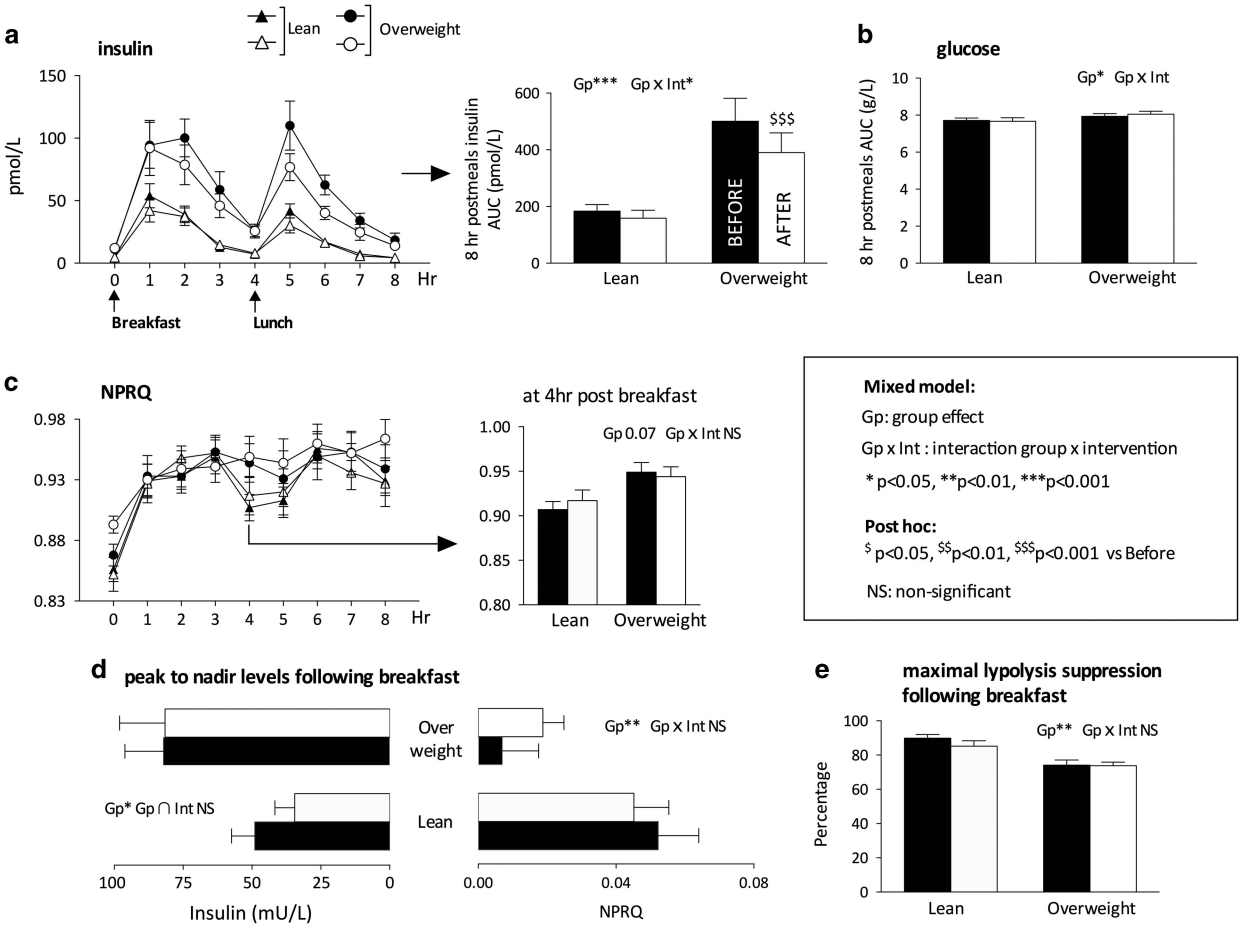


**Figure 2.** Metabolic flexibility. Eight-hour plasma insulin concentrations following standardized breakafast and lunch and the associated area under the curve (**a**) of post-prandial insulin and glucose concentration (**b**) in lean ($n=10$) and overweight ($n=9$) before (black) and after (white) exercise training. *Lower inset:* Indexes of metabolic flexibility selected as non-protein respiratory quotient (**c**, NPRQ), insulin kinetics further represented as their peak to nadir levels following the breakfast (**d**) and maximal lipolysis suppression following breakfast assessed as the decrease percentage in plasma NEFA concentration (**e**). $n=10$ in lean and $n=9$ in overweight. Values are means ± s.e.m.

Eight-hour post-prandial TG AUCs were higher in the overweight group compared with the lean group. This was mainly explained by differences in the chylomicrons TG, as VLDL were conversely lower. No between-group difference in post-meal NEFA was noted (Figures 3a–c). Whole-body fasting and post-prandial total fat oxidation, but not dietary (palmitate and oleate) fat oxidation, were lower in the overweight participants compared with the normal-weight ones (Figures 4a–c). Gene expression of muscle LPL and FABPpm, gene expression and amount of total proteins of fatty acid transporters (CD36 and FATP1; Figures 5a–d), which mechanistically contribute to the trafficking of lipids, as well as gene expression of proteins involved in their acetylation (ACSL1) and their transportation at the mitochondria level (CPT1) were significantly reduced in overweight subjects compared with the lean subjects (Figures 6a and b).

## Effects of training

As imposed by the protocol, body mass and fat mass were not affected by the exercise training (Supplementary Table S1). The increase in TEE observed in the lean subjects was only explained by the rise in AEE. By contrast, training had no significant impact on neither TEE nor AEE per kg body mass in overweight subjects (Figures 1a and d). In this later group, the training program was fully compensated by a reduction in non-training AEE during the rest of the day (Figure 1e). Post-training RMR was higher in the overweight group and this was not explained by the nonsignificant rise in fat-free mass (Figure 1b). Despite this differential effect on TEE, the training protocol increased $VO_{2peak}$ in both groups (Figure 1c). It is however important to note that although the $VO_{2peak}$ of the overweight group significantly rose after training, the post-training value remained well below the values of their lean counterparts before the intervention. At the cellular level, in the lean group only we observed that training raised muscle gene expression of the central energy regulator PGC1α. Conversely, OXPHOS proteins significantly rose after training in both groups while CS enzyme activity did not change following the intervention in both groups (Figures 1f–h).

Although exercise training decreased the 8 h post-prandial insulin responses in the overweight group, insulin remained twice higher than in the lean group (Figure 2a). Post meals plasma TG and NEFA did not change (Figures 3a–c). Metabolic flexibility, measured as the peak to nadir differences in insulin levels and non-prontein respiratory quotient following breakfast, was not affected by training in either group (Figure 2d). Lastly, the adipose tissue sensitivity to insulin, as assessed by the maximal lipolysis suppression of plasma NEFA following breakfast, was not affected by the intervention (Figure 2e).

At the whole-body level fasting and post-prandial total lipid oxidation increased after training in the overweight group only (Figure 4a). Dietary fat oxidation (oleate and palmitate) increased in both lean and overweight subjects; however, significance was not reached for dietary oleate in the overweight group (Figures 4b and c). These changes were not associated with modifications in

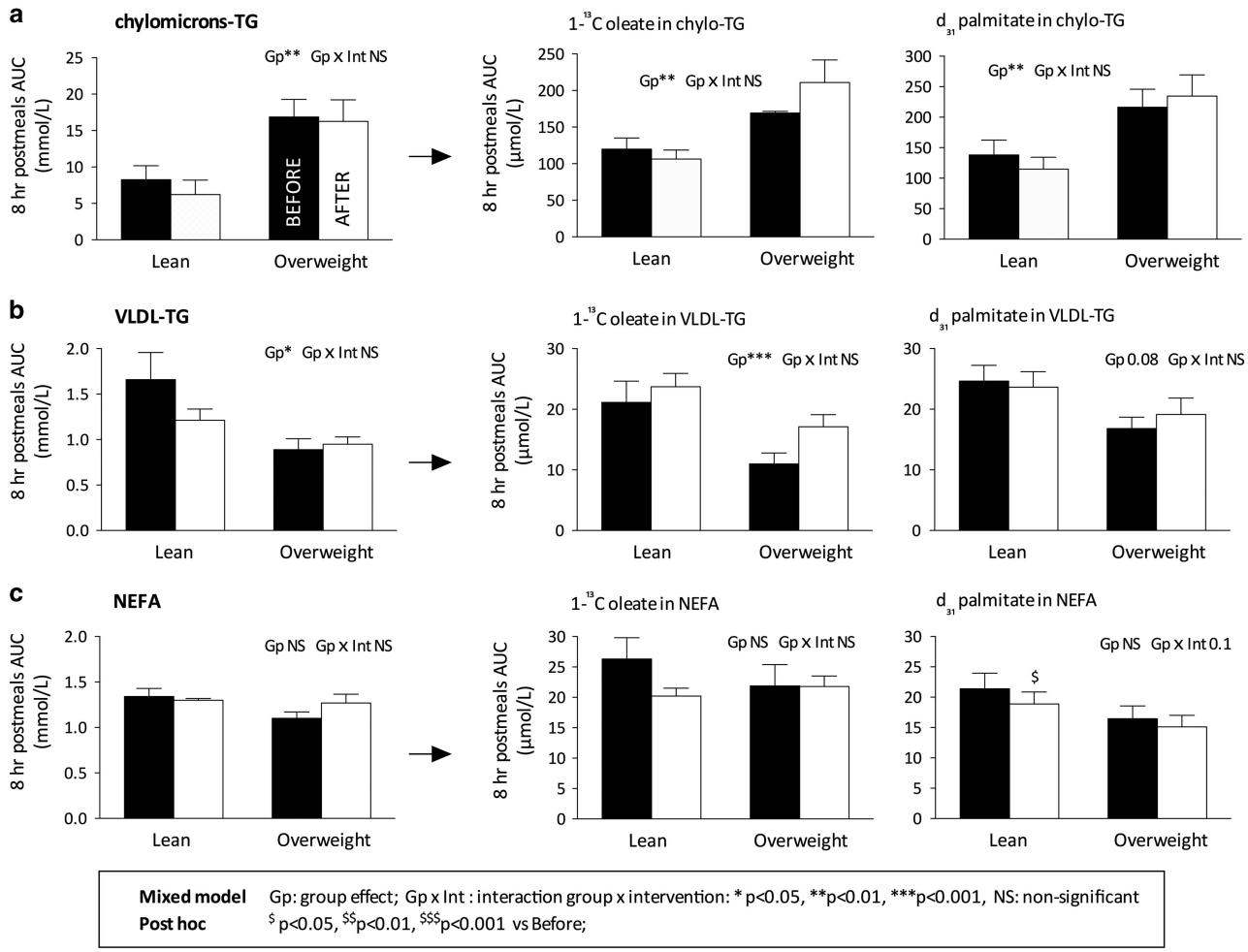


**Figure 3.** Dietary fat trafficking. Concentrations in TG-chylomicrons along with the concentrations of dietary $D_{31}$-palmitate and $^{13}C$-oleate in this triglycerides-rich lipoprotein fraction (**a**). Concentrations in TG-VLDL along with the concentrations of dietary $D_{31}$-palmitate and $^{13}C$-oleate in this triglycerides-rich lipoprotein fraction (**b**). Concentrations in NEFA along with the concentrations of dietary $D_{31}$-palmitate and $^{13}C$-oleate in NEFA (**c**). $n = 10$ in lean and $n = 9$ in overweight. Values are means ± s.e.m.

dietary fat trafficking in chylomicrons, VLDL and NEFA cumulated over the 8 h post meals' period (Figures 3a–c). The changes in gene expression and total amount of the proteins involved in hydrolysis of lipoproteins and transportations of fatty acids at the muscle level support an increased update of fatty acids at the muscle level. Indeed, whereas training increased muscle mRNA levels of CD36 in both groups, we observed an increase in CD36 protein levels in the lean group only (Figure 5c). FATP1 and FABPpm transporters mRNA tended to increase in both groups (Figures 5b and d). A trend was also noted for an increase in LPL following training in the lean group only (Figure 5a). Here again, for most molecular parameters—LPL mRNA, FABPpm mRNA, FATP1 mRNA, CD36 protein—the differences observed before intervention between the lean and overweight subjects were not modified by our training protocol, except for FATP1 protein content.

Intramuscular effectors of lipid activation and oxidation support the effect of the exercise training on lipid oxidation. Indeed, muscle ASCL1 mRNA massively increased in both groups and its fold change after training was strongly associated with both fasting and post-prandial total fat oxidation (Figures 6a, e and f). CPT1 mRNA rose in the lean group only (Figure 6b). The HAD enzyme activity, involved in β-oxidation, was not affected by training in both groups (Figure 6d). Lastly, gene expression of mtGPAT, a key protein involved in muscle TG synthesis, was not affected by training, though a lower trend ($P = 0.07$) was noted in overweight subjects after training. An interesting negative correlation was observed between the fold change in mRNA mtGPAT after training and the change in dietary palmitate oxidation (Figures 6c and f).

## DISCUSSION

The limited weight loss induced by training programs has been interpreted as a spontaneous reduction in non-training AEE (physical activities of daily living not involving sport and recreational activities) to compensate for structured exercise,[30] along with increase in energy intake over long period of time. This observation has questioned the importance of exercise in the regulation of body weight.[31] Evidence are however scarce, given the small number of studies using adequate methodology to test this hypothesis. By using doubly labeled water we showed that sedentary lean male adults maintain their daily non-training AEE in response to training, which results in significant increase in both AEE and TEE. This was not associated with weight loss as we clamped body weight by design to avoid changes in energy balance. By contrast, we demonstrated that overweight male participants responded to prescribed exercise training by a 20% decrease in non-training AEE, which prevented the increase in AEE and TEE. Previous studies showed conflicting results. Out of the

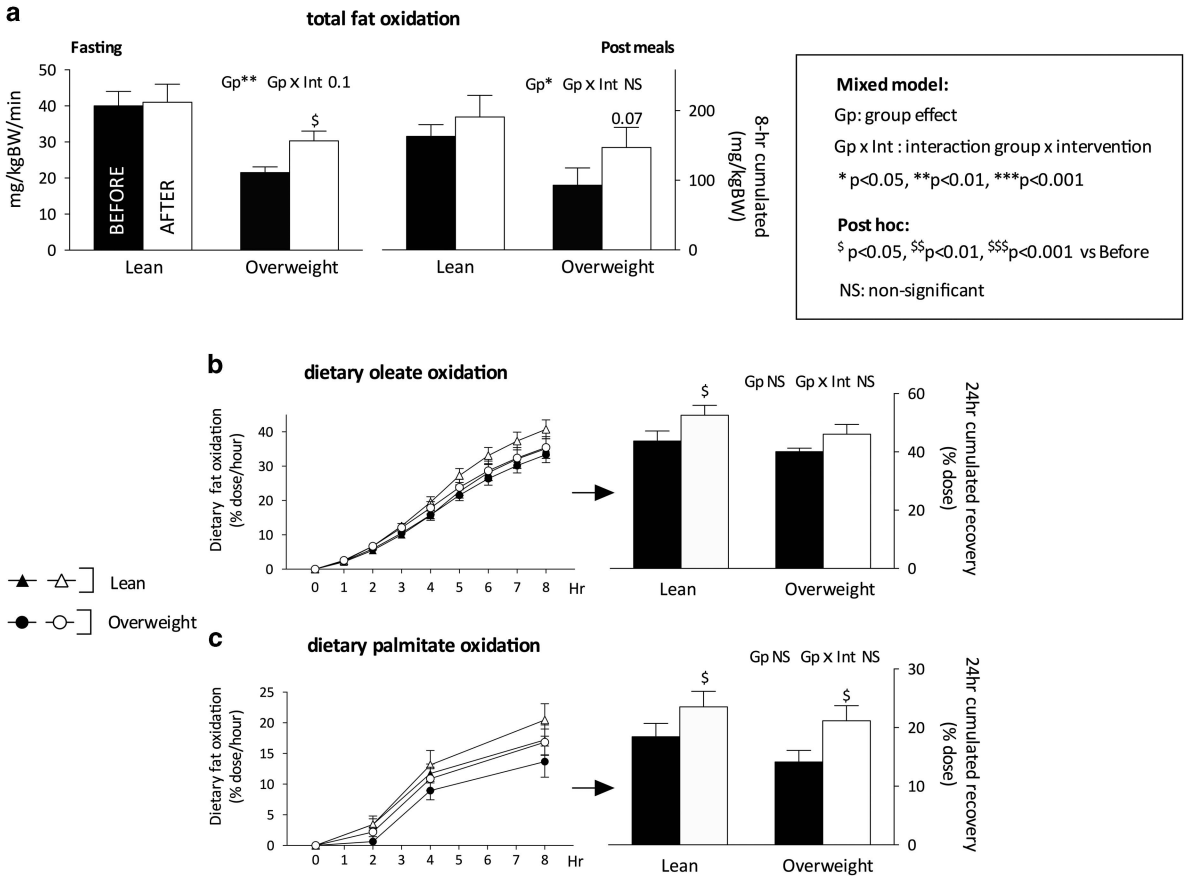


**Figure 4.** Total and dietary fat oxidation. Total fasting (**a**, left) and 8 h cumulated post meals' (**a**, right) fat oxidation, normalized per kg body mass. Eight-hour (hourly kinetics) and 24 h cumulated dietary $^{13}$C-oleate oxidation (**b**) and dietary $D_{31}$-palmitate oxidation (**c**). $n = 10$ in lean and $n = 9$ in overweight. Values are means ± s.e.m.

eight studies that have investigated the compensatory behavioral changes in overweight/obese subjects (see ref. 31 for a review), one study[32] had used the adequate methodological approach and did observe a decrease in non-training AEE after an 8-week moderate walking training protocol. Our study is therefore bringing new evidence feeding the debate. Further studies are needed.

Despite no increase in both AEE and TEE, training conferred metabolic health benefits to the overweight participants. First, our results confirm the efficacy of the current recommendations for improving cardiorespiratory fitness in both lean and overweight individuals. This result provides evidence to keep promoting exercise for health independent of changes in fatness. Second, exercise training had no effect on insulin level in lean men but lowered both fasting and post-prandial plasma insulin concentration in overweight men. The lack of effect in lean men can be explained by the recruitment of healthy young subjects and the use of standard meals instead of the supraphysiological hyperinsulinemic euglycemic clamp. In overweight adults, the improvement in insulinemia concur with previous studies reporting beneficial effects of regular exercise on insulin sensitivity.[33] However, this did not translate into an improved metabolic flexibility. Given the reported relationship between PA and flexibility,[34] this lack of effect may be explained by the absence of increase in AEE. Third, while the oxidative capacity is considered blunted in overweight/obese individuals at rest[35] and during exercise,[36] we showed exercise training elicits an increase in fat oxidation in both lean and overweight adults independent of changes in energy balance. Melanson *et al.*[37] reported no change in nutrient metabolism in both normal weight and overweight adults in response to acute exercise when 24 h energy balance is maintained. On the contrary, we had previously shown that obese male and female display similar increases in total and dietary fat oxidation rates than their lean counterparts in response to acute exercise,[38] but in the presence of a 15% energy deficit. These findings suggest that acute and chronic effects of exercise differ; while the impact of acute exercise seems to be tightly related to energy balance, regular exercise results in physiological adaptations that allow modifying substrate use independently. Such a result brings new data in the open debate of 'fitness versus fatness'. While a negative relationship exists between adiposity and exogenous fat oxidation,[35,39] there is also evidence that individuals with a higher cardiorespiratory fitness have an increased ability to oxidize fats than those with lower cardiorespiratory fitness.[40,41] Our results support that independent of fat mass, improved fitness is associated with greater dietary fat oxidation.

Changes in dietary fat oxidation were not explained by changes in trafficking between lipoproteins and NEFA. Training had no influence on the 8-h post-prandial chylomicrons TG, VLDL-TG and NEFA concentrations, as well as the labeled oleate and palmitate in these pools. The 8-h overall calculations however masked the significant changes we previously reported in the lean subjects when considering the post-breakfast period only (4 h),[17] that is, decreased dietary fat spillover and improved clearance for oxidation. In the overweight group, no changes were observed regardless of the post-meal period considered for calculation (result not shown). The changes in LPL gene expression induced by training in the lean group, but not in the overweight group, may partially explain this between-group difference.

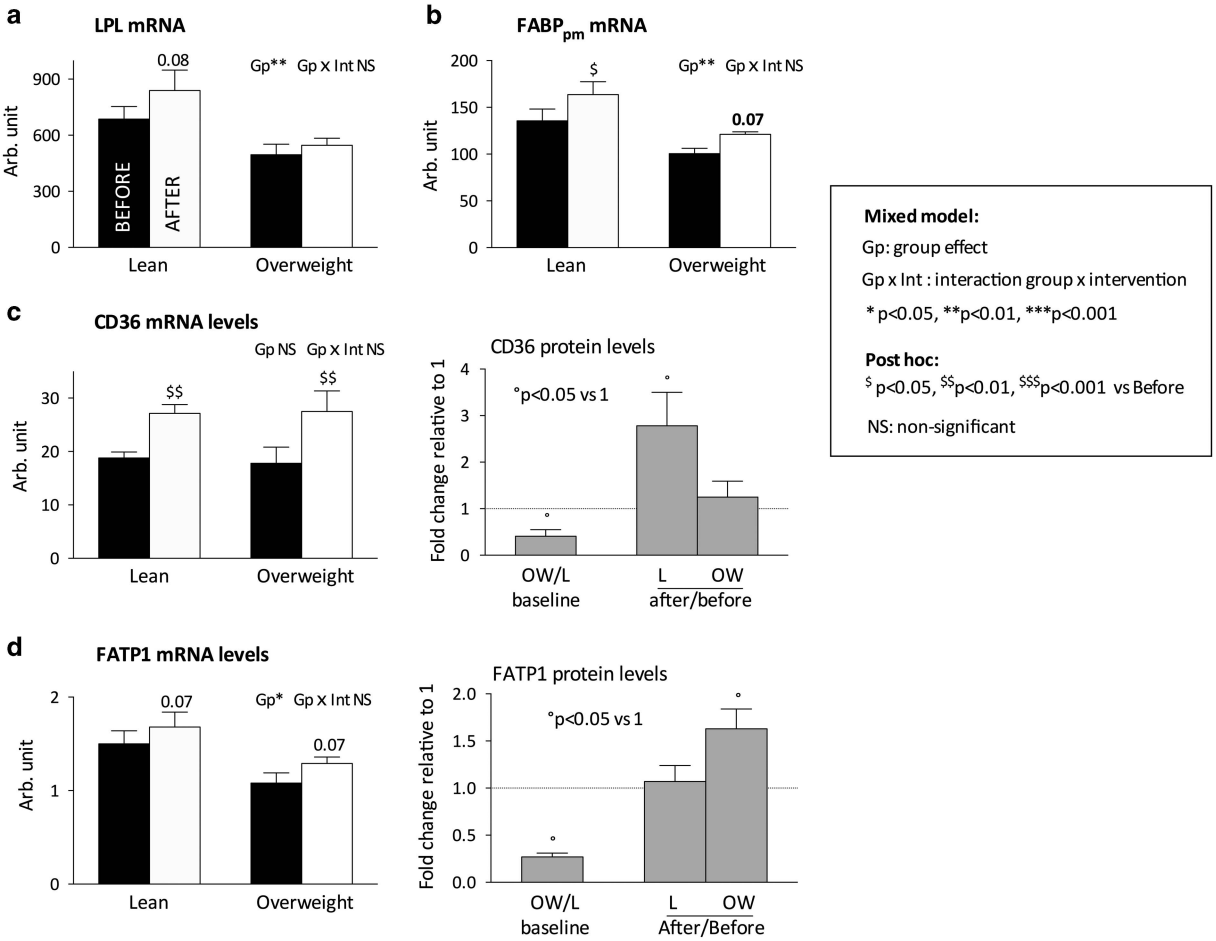


**Figure 5.** Molecular regulation of fat uptake in myocyte. Lipoprotein lipase (**a**, LPL) and plasma membrane fatty acid-binding protein (**b**, $FABP_{pm}$) mRNA levels in the vastus lateralis muscle before and after a 2-month training at current recommendations in lean ($n=10$) and overweight ($n=9$) subjects. Vastus lateralis muscle fatty acid transporter CD36 mRNA (**c**, left; lean $n=10$, overweight $n=9$) and protein (**d**, right; lean $n=4$, overweight $n=4$) levels. Vastus lateralis muscle fatty acid transport protein 1 (**d**, left; FATP1) mRNA (lean $n=10$, overweight $n=9$) and protein (**d**, right; lean $n=4$, overweight $n=4$) levels. FATP1 and CD36 protein levels are presented as the ratio between overweight (OW) and lean (L) values at baseline and as the changes from pre-intervention in each group. In the calculations lean (for before/after comparision) and baseline (for lean/overweight comparison) are mathematically set to 1 for readability. Values are means ± s.e.m.

Numerous data support a key role for CD36, FABPpm and FATP1 in fatty acids translocation from plasma membrane to cytoplasm after LPL action.[40] Training increased skeletal muscle FABPpm, CD36 and FATP1 gene expressions, but these changes did not accordingly translate into changes in proteins contents. Accordingly to our results, Schenk *et al.*[42] also reported no change in the amount of CD36 protein following 10 weeks of endurance training and dieting in obese women. As reviewed by Jayewardene *et al.*,[41] the effect of both acute and chronic exercise is unclear and the role of FATP1 remains controversial. These discrepancies are attributable to the differences in studied populations and training protocols. It is therefore challenging to evaluate the contribution of the fatty acid transport into the myocyte to the greater fat oxidation induced by training in overweight adults.

In the cytosol, fatty acids are acylated to long-chain fatty acyl-CoA by ACSL1 enzyme. We found that ACSL1 protein content increased after exercise training in the overweight group. Interestingly, the fold change in ASCL1 strongly and positively correlated with the fold change in both fasting and post-prandial total fat oxidation. In line with the *in vitro* results from Schneider *et al.*,[43] our results suggest ASCL1 plays a key role in fatty acid uptake, indirectly, by metabolic trapping. Furthermore, although no mechanisms was proposed, ASCL1 gene polymorphism was strongly associated with improvement in $VO_{2peak}$ following the 20-week exercise training program of the HERITAGE family study.[44] Taken altogether these results suggest that ASCL1 may be one of the key cellular proteins involved in the improvement of fitness and fat oxidation in response to aerobic exercise training. Further studies are needed.

In regard of mitochondrial oxidative capacity, no clear changes have been observed in our overweight adults following the 2-month exercise training. The massively depressed gene expression of CPT1 as compared with lean subjects did not improve in post intervention nor did the activity of HAD enzyme. Surprisingly, only few data exist on the effect of exercise on these key proteins. In addition, they are limited to the acute effect of exercise in lean[45] and overweight subjects[46] or to the comparison between sedentary lean and overweight subjects.[47] Although further studies are clearly needed in overweight individuals, the present data suggest that the improved lipid oxidation capacity following training is not explained by changes in CPT1 or HAD activity.

We failed to detect responses of proteins involved in the regulation of fat oxidation. Nevertheless we observed a strong negative relationship between the fold change in mtGPAT, located on the mitochondrial membrane,[48,49] and dietary palmitate oxidation. Very few data in the literature are available in humans, but studies in rodents suggest that it plays a central role in the partitioning between oxidation and storage of saturated fatty

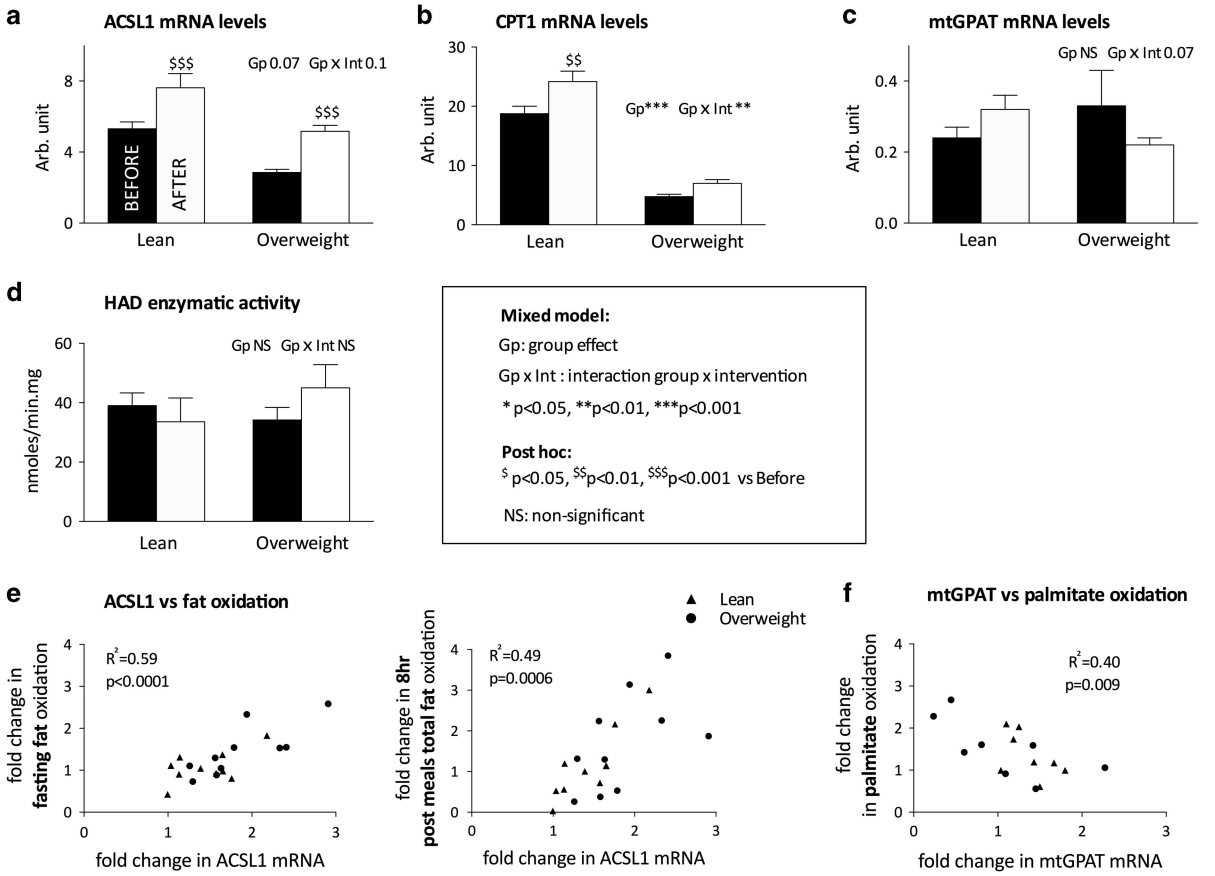


**Figure 6.** Molecular regulation of the oxidative pathway in skeletal muscle. mRNA levels of the vastus lateralis long-chain-fatty acid—CoA synthase 1 (**a**, ACSL1) in the lean ($n=10$) and overweight ($n=9$) groups. The association between the fold changes in mRNA ACSL1 and both the fold changes in total fasting (**e**, left) and post meals (**e**, right) fat oxidation are presented for all subjects. Vastus lateralis muscle carnitine palmitoyl transferase CPT1 mRNA (**b**, lean $n=10$, overweight $n=9$). Mitochondrial glycerol-3-phosphate acyltransferase mtGPAT mRNA levels in vastus lateralis (**c**, $n=10$, overweight $n=9$) and it association (all expressed as fold changes) with dietary palmitate oxidation (**f**). 3-Hydroxyacyl-CoA dehydrogenase (HAD) enzymatic activities in both the lean ($n=4$) and overweight ($n=4$) groups (**d**). Values are means ± s.e.m.

acids.[48,49] As previously reported,[50] our results may suggest that mtGPAT affects the partitioning of saturated fat towards oxidation and away from storage in response to training. Here again further studies are clearly needed at the cellular levels.

Beyond further investigating underlying cellular mechanisms, the main obvious limitation of this study is it was conducted in men only and generalization to women may be erroneous given the growing body of data demonstrating metabolic sex differences.

In conclusion, exercise at current recommendations, independent of energy balance, improves fitness in overweight subjects, even if it fails to restore a lean metabolic phenotype. The results point towards a key role for non-exercise AEE in the regulation of energy expenditure and suggest that training need to be applied in addition to and not in replacement of habitual physical activities.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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