Activity energy expenditure is a major determinant of dietary fat oxidation and trafficking, but the deleterious effect of detraining is more marked than the beneficial effect of training at current recommendations1–4

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ABSTRACT

Background: Previous studies suggested that physical activity energy expenditure (AEE) is a major determinant of dietary fat oxidation, which is a central component of fat metabolism and body weight regulation.

Objective: We tested this hypothesis by investigating the effect of contrasted physical activity levels on dietary saturated and mono-unsaturated fatty acids oxidation in relation to insulin sensitivity while controlling energy balance.

Design: Sedentary lean men (n = 10) trained for 2 mo according to the current guidelines on physical activity, and active lean men (n = 9) detrained for 1 mo by reducing structured and spontaneous activity. Dietary [d31]palmitate and [1-13]C-oleate oxidation and incorporation into triglyceride-rich lipoproteins and nonesterified fatty acid, AEE, and muscle markers were studied before and after interventions.

Results: Training increased palmitate and oleate oxidation by 27% and 20%, respectively, whereas detraining reduced them by 31% and 20%, respectively, whereas detraining reduced them by 31% and 20%, respectively, whereas detraining reduced them by 31% and 20%, respectively, whereas detraining reduced them by 31% and 20%. Training increased palmitate and oleate oxidation by 27% and 20%, respectively, whereas detraining reduced them by 31% and 20%. Changes in AEE were positively correlated with changes in oleate (R² = 0.62, P < 0.001) and palmitate (R² = 0.66, P < 0.0001) oxidation. The d31-palmitate appearance in nonesterified fatty acid and very-low-density lipoprotein pool was negatively associated with changes in fatty acid translocase CD36 (R² = 0.30), fatty acid transport protein 1 (R² = 0.24), and AcylCoA synthetase long chain family member 1 (ACSL1) (R² = 0.25) expressions and with changes in fatty acid binding protein expression (R² = 0.33), respectively. The d31-palmitate oxidation correlated with changes in ACSL1 (R² = 0.39) and carnitine palmitoyltransferase 1 (R² = 0.30) expressions (P < 0.05 for all). Similar relations were observed with oleate. Insulin response was associated with AEE (R² = 0.34, P = 0.02) and oleate (R² = 0.52, P < 0.01) and palmitate (R² = 0.62, P < 0.001) oxidation.

Conclusion: Training and detraining modified the oxidation of the 2 most common dietary fats, likely through a better trafficking and uptake by the muscle, which was negatively associated with whole-body insulin sensitivity. Am J Clin Nutr doi: 10.3945/ajcn.112.057075.

INTRODUCTION

There has been considerable attention paid to the concept of energy balance as a tool to understand and hopefully reverse the global obesity problem (1). This concept can appear trivial: changes in energy stores equal the difference between energy intake and expenditure. However, the physiologic reality of energy balance is complex because changes on one side of the

equation influence the other side of the equation (2). This complexity is illustrated by the difficulties encountered in public health campaigns aimed at preventing weight gain and treating obesity. Part of the difficulty is that energy-balance components (ie, body stores, energy expenditure, and intake) are dynamic in nature, and independent changes in subcomponents (ie, fat, carbohydrates, proteins, activity energy expenditure (AEE)³, resting metabolic rate (RMR), and postprandial thermogenesis) can either mitigate or magnify changes in another component (3, 4).

Energy balance can be further viewed through oxidative balance (1). In macronutrients, fat and its metabolism plays a central role in this interdependent systems because fat storage is more flexible than carbohydrate and protein storage (1). Consequently, energy balance is directly related to fat balance (1, 5, 6). Thus, an understanding of the weight regulation requires an understanding of the factors that affect fat balance. An additional underappreciated observation is that de novo lipogenesis is a negligible metabolic route in humans (7–9), unlike in rodents. This observation implies that dietary fat is the main source of lipids stored in human body and that an understanding of weight regulation requires a delineation of factors involved in the re-partition of dietary fat between oxidation and storage. One of the main interactions between energy balance and fat balance that has been indicated by some animal studies is that physical activity, which is the most variable component of total energy expenditure (TEE), is a determinant of dietary fat oxidation (10). Support for the importance of this relation comes from the observation that the performance of exercise accelerates the adjustment of fat oxidation to match fat intake on high-fat diets in both men (11) and women (12) but also in animals (13).

Of interest, such adaptive changes appear independent of the type of dietary fat ingested when the outcome measure is total fat oxidation by using indirect calorimetry (14) but dependent on the type of fat when the outcome measure was specific fatty acid oxidation by using stable isotope tracers (15). Although acute exercise significantly increases dietary oleate oxidation, it does not modify palmitate oxidation in humans. In contrast, we showed that extreme physical inactivity induced by long-term bed rest decreased total and saturated dietary fat oxidation but not dietary monounsaturated fat oxidation (16, 17). Taken together, the results suggest that physical AEE is a major determinant of dietary fat oxidation, and this relation may differ according to the nature of fatty acids. To directly examine this relation, we investigated the effect of contrasted physical activity levels on dietary fat metabolism in free-living conditions. Dietary oleate and palmitate trafficking (ie, the main MUFA and SFA of the human diet) were studied at the whole-body and molecular levels in sedentary and highly active lean men who were submitted to 2 mo of moderate physical training on the basis of current recommendations and 1 mo of detraining, respectively.

SUBJECTS AND METHODS

Participants and experimental protocol

Twenty-four lean men [mean±SEM age: 26 ± 7 y; 20 ≤ BMI (in kg/m²) ≤ 25] who were free of any chronic known diseases and weight stable were included in the study in 2006–2007. Subjects had no first-degree family history of obesity or type 2 diabetes. A sedentary or active status was defined by using the Monica Optional Study of Physical Activity questionnaire (18), with sedentary subjects reporting sedentary occupations and no structured exercise programs over the 12 mo before enrollment (n = 12) and active subjects reporting ≥2–3 h/wk of physical activity.

![FIGURE 1](image-url)

**FIGURE 1.** Overview of the study design and details of the test day. Cal, indirect calorimetry; TEE, total energy expenditure.
moderate-to-vigorous leisure physical activity \((n = 12)\). The peak oxygen uptake \((\dot{V}O_{2\text{peak}})\) was measured on a cycle ergometer before and after interventions. Training was performed at current recommendations for 2 mo (ie, three 60-min supervised sessions/wk at 50% \(\dot{V}O_{2\text{peak}}\) on a cycle ergometer and one additional session in free-living conditions during weekends). Detraining consisted of suspending all structured physical activities and reducing spontaneous activities of daily living. Adherence was checked by weekly counseling and accelerometer data. The diet was regularly adjusted by a dietician to maintain the energy balance. The study was approved by the Alsace-1 Institutional Review Board. All subjects signed an informed consent. Two sets of identical tests were performed before and after interventions (Figure 1).

### Energy metabolism

TEE was determined before and at the end of interventions by using the doubly labeled water (DLW) method over a 10-d period (19). Subjects ingested a premixed 2 g/kg estimated total body water (TBW) dose of DLW composed of and estimated TBW of \(H_2^{18}O\) and \(\delta^2\)H\(_2\)O of 0.2 and 0.15 g/kg, respectively (CIL). Details on equilibration and endpoint urine cleaning, mass spectrometry procedures, and calculations have been previously published (19, 20). Briefly, TBW and TEE were calculated by using a food tracer II (Datex; GE Health Care) for 1 h after an overnight fast.

Additional urine and breath samples were collected 24 h after the tracer rinsing to assess dietary fat oxidation and trafficking in chylomicrons, VLDL, and nonesterified fatty acid (NEFA). Part of the breakfast was composed of a liquid meal (Renutril Booster; Nestlé) containing 15 mg \(d_{31}\)-palmitic acid/kg of and 10 mg \([1-13\text{C}]\)-oleic acid/kg (both >98% enriched; CIL) homogenized at 65°C. A lunch (70% carbohydrate, 11% fat, and 19% protein; 1.8 MJ) with no tracers was served 4 h after breakfast. Over 8 h, the total substrate oxidation was assessed by hourly indirect calorimetry and nitrogen excretion. Hourly arterialized venous blood, urine, and breath samples were collected to assess dietary fat oxidation and trafficking in chylomicrons, VLDL, and nonesterified fatty acid (NEFA). Additional urine and breath samples were collected 24 h after the dose.

### Body composition

Fat-free mass (FFM) was assessed by hydrometry from the DLW-derived TBW by using a hydration factor of 0.73 (20). Fat mass (FM) was calculated by the difference with body weight.

### Dietary fat trafficking

Subjects were provided standard meals (30% breakfast, 40% lunch, and 30% dinner of the total energy intake) with the same macronutrient composition (55% carbohydrate, 15% protein, and 30% lipid) 36 h before each test. The energy content matched requirements on the basis of activity questionnaires and measured RMR. After the collection of baseline breath, urine, and arterialized venous blood samples, a breakfast was served to subjects (50% RMR as energy, 53% carbohydrates, 14% protein, and 33% fat; 3.6 MJ). Part of the breakfast was composed of a liquid meal (Renutril Booster; Nestlé) containing 15 mg \(d_{31}\)-palmitic acid/kg of and 10 mg \([1-13\text{C}]\)-oleic acid/kg (both >98% enriched; CIL) homogenized at 65°C. A lunch (70% carbohydrate, 11% fat, and 19% protein; 1.8 MJ) with no tracers was served 4 h after breakfast. Over 8 h, the total substrate oxidation was assessed by hourly indirect calorimetry and nitrogen excretion. Hourly arterialized venous blood, urine, and breath samples were collected to assess dietary fat oxidation and trafficking in chylomicrons, VLDL, and nonesterified fatty acid (NEFA). Additional urine and breath samples were collected 24 h after the dose.

### TABLE 1

Subjects characteristics, fasting metabolites, and insulin, energy expenditure, and substrate oxidation before and after interventions on physical activity

<table>
<thead>
<tr>
<th>Variables</th>
<th>Sedentary lean men ((n = 10))</th>
<th>Active lean men ((n = 9))</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anthropometric measures</strong></td>
<td></td>
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<tr>
<td>Body mass (kg)</td>
<td>76.2 ± 3.4</td>
<td>71.7 ± 2.9</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>23.0 ± 0.7</td>
<td>22.3 ± 0.6</td>
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<tr>
<td>FFM (kg)</td>
<td>59.1 ± 2.1</td>
<td>61.2 ± 2.5</td>
</tr>
<tr>
<td>FM (kg)</td>
<td>17.0 ± 1.9</td>
<td>10.5 ± 1.2</td>
</tr>
<tr>
<td>FM (%)</td>
<td>21.9 ± 1.7</td>
<td>14.5 ± 1.5</td>
</tr>
<tr>
<td>(\dot{V}O_{2\text{peak}}) (mL·min(^{-1})·kg FFM(^{-1}))</td>
<td>51.7 ± 2.3</td>
<td>58.7 ± 2.0</td>
</tr>
<tr>
<td><strong>Fasting plasma</strong></td>
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<tr>
<td>Glucose (g/L)</td>
<td>0.89 ± 0.03</td>
<td>0.84 ± 0.01</td>
</tr>
<tr>
<td>Insulin (mU/L)</td>
<td>4.7 ± 0.4</td>
<td>3.7 ± 0.3</td>
</tr>
<tr>
<td>Triglycerides (g/L)</td>
<td>0.94 ± 0.10</td>
<td>0.49 ± 0.04</td>
</tr>
<tr>
<td>NEFA (mol/L)</td>
<td>0.40 ± 0.04</td>
<td>0.41 ± 0.03</td>
</tr>
<tr>
<td><strong>Energy expenditure</strong></td>
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<td></td>
</tr>
<tr>
<td>TEE (MJ/d)</td>
<td>10.7 ± 0.5</td>
<td>14.0 ± 0.6</td>
</tr>
<tr>
<td>REE (MJ/d)</td>
<td>6.7 ± 0.2</td>
<td>6.3 ± 0.2</td>
</tr>
<tr>
<td>AEE (MJ/d)</td>
<td>29.0 ± 0.4</td>
<td>6.3 ± 0.4</td>
</tr>
<tr>
<td>AEE (kJ·d(^{-1})·kg BW(^{-1}))</td>
<td>38.8 ± 5.1</td>
<td>88.9 ± 5.7</td>
</tr>
<tr>
<td><strong>Substrate oxidation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting NPRQ</td>
<td>0.851 ± 0.010</td>
<td>0.872 ± 0.014</td>
</tr>
<tr>
<td>Postprandial oxidations (kJ × 8 h)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipids</td>
<td>444 ± 45</td>
<td>525 ± 70</td>
</tr>
<tr>
<td>Glucids</td>
<td>1743 ± 104</td>
<td>1365 ± 60</td>
</tr>
</tbody>
</table>

\(\dot{V}O_{2\text{peak}}, \text{peak oxygen uptake.}\)
Chylomicrons fractions were prepared by ultracentrifugation 30 min at 80,000 rpm at 12°C (KENDRO S150, rotor S140AT; Thermo Scientific Sorvall) during 30 min as previously described (21). The supernatant fluid that contained the chylomicrons was gently removed. The precipitant was further suspended in a potassium bromide solution. VLDLs were separated by centrifugation for 1 h 10 min at 10°C and 140,000 rpm. Triglyceride concentrations in both fractions were quantified. Lipids were extracted by a Folch procedure, and triglycerides and NEFAs were further separated from plasma by solid-phase extraction before derivitization (17). Heptadecanoic acid and triheptadecanoyl glycerol were added as internal standards.

The isotopic enrichment of d31-palmitic and [1-13C]-oleic acids in chylomicron triglycerides, VLDL triglycerides, and NEFA was measured by using gas chromatography coupled with a mass spectrometer as described previously (17). To assess both unlabeled and labeled fatty acid concentrations in the same run, we designed a dual-acquisition program in a single-ion monitoring mode. mz ratios of 296 and 297 for oleate and 270 and 301 for palmitate were acquired. Concentrations were calculated by multiplying the molar percentage enrichment by the concentration of its corresponding unlabeled compound. NEFA (NEFA-C WAKO kit, SOBIOIDA S.A.S), glucose (GLUCm glucose kit, Beckman Coulter System SYNCHRON), triglycerides (GPO TG kit from Beckman Coulter System SYNCHRON), and insulin (ADVIA Centaur Insulin IRI Siemens) were assessed by using standard tests.

[1-13C]-oleate oxidation was calculated as the instantaneous percentage recovery of 13C in expired CO2 per 1 h over 8 h and measured in triplicate on a continuous-flow inlet system connected to an isoprime isotopic ratio mass spectrometer (GV Instruments). Instantaneous recoveries were corrected for isotope sequestration by a factor of 51% (22), and cumulative oxidation rates were calculated. To measure d31-palmitate oxidation, 2H:1H ratios from urine samples were analyzed, as previously described for the DLW method. Oxidation rates of palmitate were calculated from the cumulative recovery of 2H in TBW and were corrected for the loss in 2H2O that remained from the DLW method by using individual daily deuterium elimination rates. Calculations have been described elsewhere (23).

Expression of genes involved in lipid metabolism in muscles, protein contents, and enzyme activities

Vastus lateralis biopsies were performed by using a Bergström needle at the end of the test day. RNA was extracted by using a mirVana miRNA Isolation Kit (Applied Biosystems). First-strand complementary DNAs were synthesized from 500 ng total RNA in the presence of 100 U Superscript II reverse transcriptase (Invitrogen) by using a mixture of random hexamers and oligo (dT) primers (Promega). Real-time polymerase chain reaction assays were performed by using a Rotor-GenETM 6000 real-time analyzer (Corbett Research). The HPRT messenger RNA (mRNA) concentration was determined in each sample

![FIGURE 2](image-url). Oxidation rates of d31-palmitate (A) and [1-13C]-oleate (C) expressed as the hourly cumulative percentage dose recovery over the 8 h of the inpatient test day in sedentary (n = 10) and active (n = 9) lean men. Bars on the right of panels A and C represent the 24-h cumulative percentage dose recovery. *P < 0.05 compared with baseline (paired t test); †P < 0.05 compared with baseline sedentary men (unpaired t test). Correlation analyses between changes in AEE and changes in d31-palmitate (B) and [1-13C]-oleate oxidations (D). Statistics are presented in the figure. AEE, activity energy expenditure.
and was used as internal standard for normalization of target mRNA expression. The expression of lipoprotein lipase (LPL), CD36, FABPpm/GOT2, FATP1/SLC27A1, AcylCoA synthetase long chain family member 1 (ACSL1), CPT1, and mitochondrial glyceraldehyde 3-phosphate dehydrogenase (mGPAT)/GPAM were studied.

In a subgroup of subjects, proteins were extracted for Western-blot experiments. Anti-CPT1-M, anti-CD36, anti-FATP1, anti-GAPDH (Santa Cruz) and anti-oxidative phosphorylation (OXPHOS) (MS604) (MitoSciences Inc) antibodies were used. The signal was quantified by using the ImageJ program (version 1.45s Java 1.6.0_45; Wayne Rasband, National Institutes of Health) (sum of 5 mitochondrial proteins for OXPHOS) and normalized to GAPDH. Coomassie blue staining was performed to check for equivalent loading (see Supplemental Figure 1 under “Supplemental data” in the online issue for blots).

For 3-hydroxyacyl-CoA dehydrogenase (HAD) (EC 4.1.3.7) enzyme activity, 8 μg proteins were added to an assay buffer containing 50 mmol/L Tris (pH: 7.6), 2 mmol/L EDTA, 50 μmol/L NADH, H+, and 0.01% Triton X-100 (Sigma) (vol:vol). NADH, H+ oxidation was fluorometrically recorded after the addition of 75 μmol/L acetoacetylCoA (24). Measurements of citrate synthase activity were performed as described (25).

Statistical analysis
Four subjects who did not respect the protocol and one subject for whom tracers were not homogenized in the liquid meal were excluded from the analyses. Thus, analyses are presented for 9 active and 10 sedentary subjects. AUCs of the concentration-time curve or substrate oxidations were calculated. Unpaired Student’s t tests were used to compare groups at baseline, and the paired Student’s t test was used to compare before and after the intervention within the same group. To examine effects of physical activity taken as a continuum on dietary fat oxidation, we pooled both groups and looked at the relation between changes (differences between before and after physical activity interventions) of some key variables by using Pearson’s correlations. Data are presented as means ± SEMs. Significance was set up for P < 0.05. Statistics were performed with StatView5.0.1 software (SAS Institute).

RESULTS
At baseline, men in the sedentary and active groups had comparable FFM and BMI; however, a trend for higher body mass was noted in the sedentary group (Table 1). Sedentary subjects also had higher FM, fasting plasma triglycerides, insulin, and respiratory quotient than active subjects did, whereas fasting glycemia and circulating NEFA were similar between groups. Sedentary individuals exhibited lower \( iO_2 \) peak because of a 2.2-fold lower AEE. Dietary palmitate oxidation and oleate oxidation (Figure 2, A and C) were lower in the sedentary group, whereas postprandial insulin (Figure 3B), total triglycerides (Figure 4A), and chylomicrons triglycerides (Figure 4B) were higher. Expressions of LPL, CD36, FATP1, and mGPAT as well as protein contents of CD36 and OXPHOS were significantly lower in the vastus lateralis muscle of the sedentary group, which indicated reduced muscle lipid uptake and decreased oxidative capacity for lipids in this group. This was further supported by the lower HAD enzyme activity in the sedentary compared with physically active groups (Figure 5, A–C).

Body weight, FFM, and FM were not affected by training, whereas detraining decreased FFM and consequently increased the percentage of FM. \( iO_2 \) peak changes went in expected directions after both interventions. The increase in TEE induced by the training of sedentary individuals as well as the drop in

![Figure 3](image-url) Cumulative plasma glucose (A) and insulin (B) concentrations in response to breakfast and lunch ingestions expressed as the AUC over the 480 min in sedentary (n= 10) and active (n = 9) lean men. *P < 0.05 compared with baseline (paired t test); †P < 0.05 compared with baseline sedentary men (unpaired t test). Correlation analyses between changes in insulin response over the 480-min inpatient test day and changes in AEE (C) and \( d_{13} \)-palmitate (D) and \( [1-\text{13C}] \)-oleate oxidations (E). Statistics are presented in the figure. AEE, activity energy expenditure.
TEE induced by detraining of previously trained subjects were mainly explained by changes in AEE (Table 1).

The 2 interventions induced opposite effects on total fat use, but only the drop in fat oxidation after detraining was significant (Table 1). Training increased d31-palmitate and [1-13C]-oleate oxidation by 27% and 20%, respectively, whereas detraining decreased them by 31% and 13%, respectively (Figure 2, A and C). Variations in AEE were strongly associated with changes in both d31-palmitate and [1-13C]-oleate oxidation (Figure 2, B and D). Total fat oxidation variations in response to physical activity interventions were positively correlated with changes in both d31-palmitate (R² = 0.39, P = 0.006; data not shown) and [1-13C]-oleate oxidation (R² = 0.22, P = 0.048; data not shown). Taken together, changes in dietary palmitate and oleate oxidation explained 39% (P = 0.024) of the variations observed in total fat oxidation, suggesting that changes in total fat oxidation were associated with changes in exogenous fat oxidation.

Detraining increased fasting insulin (Table 1) and postprandial insulin (Figure 3B) and glucose (Figure 3A) concentrations, whereas no changes were noted after training of previously sedentary subjects. When subjects of both groups were considered, changes in the insulin response over the test day were associated with changes in AEE and even more strongly with both d31-palmitate and [1-13C]-oleate oxidation (Figure 3, C–E).

Although total triglyceride, chylomicron-triglyceride, VLDL-triglyceride, and NEFA concentrations remained unchanged after training (Figure 4, A–D), detraining significantly increased chylomicron-triglyceride concentrations and tended to increase postprandial total triglycerides. In previously active subjects, detraining also tended to decrease postprandial NEFA (Figure 4D).

The d31-palmitate appearance in chylomicron triglycerides showed opposed responses to training and detraining; training decreased the appearance, whereas detraining increased it. Because isotopic enrichments in chylomicrons triglycerides were not modified by the interventions, these differences in d31-palmitate concentrations could only be explained by changes in palmitic acid concentrations in chylomicrons triglycerides (Figure 4, E).
This further suggested that differences in tracer concentrations are likely explained by changes in chylomicron-triglyceride clearance rather than production. The very same changes in d31-palmitate appearance in the NEFA pool were noted after the respective interventions on physical activity (ie, an increased appearance after detraining and decreased appearance after training). These appearance rates were explained by modifications in enrichments but not by changes in palmitic acid concentrations (Figure 4, G and H). Because NEFA concentrations tended to be lower (Figure 4D) despite higher insulin concentrations (Figure 3B) after detraining, and both NEFA and insulin concentrations did not change after training, these results suggested that none of the interventions affected adipose tissue insulin sensitivity. Although we could not infer the effect of the interventions on adipose tissue mobilization, on the basis of our data, we assumed that changes in d31-palmitate appearance in NEFA were possibly explained by changes in fatty acid uptake after LPL action on chylomicron triglycerides. Although training had no effect, detraining affected the d31-palmitate appearance in the VLDL-triglyceride fraction by increasing it (Figure 4, I and J). This effect was essentially explained by the increase in isotopic enrichment (Figure 4I), which suggested recycling of the d31-palmitate NEFA. The same results were observed for [1-13C]-oleate appearances in chylomicron triglycerides, VLDL-triglyceride, and NEFA fractions (see Supplemental Figure 2 under “Supplemental data” in the online issue). In support of an overall effect of the physical activity level on dietary chylomicron-triglyceride clearance, we observed negative relations between changes in both d31-palmitate and [1-13C]-oleate in chylomicron triglycerides and their respective oxidation (Figure 6, A and B).

At the muscle level, the expression of genes involved in fatty acid uptake (LPL, CD36, and CPT1) and oxidation (ACSL1) increased after training. A similar trend was noted for proteins, but only changes in the OXPHOS content were significant. However, training did not improve HAD or citrate synthase activities. In contrast, only FABPpm expression was decreased after detraining; neither protein contents nor enzymes activities were modified (Figure 5, D–F).

Changes induced by physical activity interventions in several muscle gene expressions were related to changes in dietary fat
plasma trafficking and oxidation in response to detraining and training. Changes in the expression of FABPpm were negatively correlated with d31-palmitate appearance in the VLDL pool (Figure 7B), with a similar trend noted for the appearance in chylomicrons (Figure 7A) and NEFA (Figure 7C) pools. Variations of d31-palmitate in the NEFA pool further negatively correlated with changes in gene expression of the 2 other fatty acid transporters measured in this study [ie, CD36 (Figure 7D) and FATP1 (Figure 7E)] but also with changes in the expression of ACSL1 (Figure 7F). Last, changes in the expression of both ACSL1 (Figure 7G) and CPT1 (Figure 7H) were associated with variations in d31-palmitate oxidation. Similar relations were observed for oleate metabolism, but significance was reached with fewer variables (data not shown).

**DISCUSSION**

Our study focused on the metabolism of dietary fat in response to chronic changes in habitual physical activities while strictly controlling energy balance. We showed that AEE predicted saturated and monounsaturated dietary fat oxidation through coordinated changes in the incorporation of dietary fat into plasma triglyceride-rich lipoproteins and NEFA and muscle handling.

Our data provide mechanistic evidence to support results from Stubbs et al (26) who showed that active individuals oxidize a higher percentage of dietary fat after meal ingestion than do sedentary individuals. Besides the expected observation that sedentary subjects were fatter, had a lower aerobic capacity, higher fasting and postprandial insulin and triglycerides concentrations, and a preference for carbohydrates as a substrate, we noted that both dietary palmitate and oleate oxidation were suppressed compared with their active counterparts. Although changes in adipose tissue metabolism could not be excluded, our results suggested that differences in plasma lipid clearance and mitochondrial oxidative capacity at muscle level likely explained the between-group differences before the interventions. Gene expressions of LPL, CD36 (and its protein), and FATP1 were markedly lower in sedentary individuals, which implied that both the hydrolysis of triglyceride-rich lipoproteins and uptake of the NEFA released from this hydrolysis by the muscle cells were reduced. These differences resulted in a higher incorporation of palmitate and oleate in the plasma NEFA pool, which suggested a greater quantity of NEFA was liberated from chylomicrons triglycerides that escaped the peripheral tissue uptake. Taken with the lower OXPHOS protein content and HAD enzyme activity, our results indicated that fitness affects whole-body dietary fat oxidation. In this context, the higher CPT1 expression observed in the sedentary compared with active groups was unexpected. But this may have only limited functional consequences in sedentary individuals and was not necessarily contradictory to the observed reduced oxidative capacity. Indeed, the higher reliance on carbohydrates as previously observed in sedentary subjects (27) in the context of hyperinsulinemia may result in higher concentrations of malonyl-CoA, which exerts an inhibitory effect on CPT1 activity (28). In support of this possibility, the expression of acetyl-CoA carboxylase (ACC2), which catalyzes the conversion of acetyl-CoA into malonyl-CoA, was 1.7-fold higher in the sedentary than active groups (data not shown).

To our knowledge, our results provide new evidence regarding key mechanisms that likely explain why exercising subjects reach a fat balance more rapidly after shifting to a high-fat diet than do inactive subjects (11, 12). The proportion of dietary fat being oxidized compared with being stored depends on the between-organ trafficking of dietary fatty acids. Trafficking can be inferred from the kinetics of appearance and disappearance of the 13C-oleate and d31-plamitate in triglyceride-rich lipoproteins and NEFA, which involve a coordinated action of both LPL and long-chain fatty acid transporters. Higher levels of physical activity were associated with decreased dietary oleate and palmitate appearance in both chylomicrons and NEFA. The significant correlation between both labeled fatty acids in chylomicrons and their oxidation rates suggested that physical activity affected dietary fat clearance and uptake presumably by the muscle, as shown in rats (13). This possibility was further supported by the negative associations observed between changes in the expression of FABPpm, which is involved in the transport of long-chain fatty acids at both plasma membrane and mitochondria levels (29), and the changes in appearance of dietary palmitate in chylomicrons triglycerides, VLDL triglycerides, and NEFA.

**FIGURE 6.** Pearson’s correlation analyses between changes in dietary d31-palmitate (A) and [1-13C]-oleate (B) appearance in chylomicron-triglyceride fractions and changes in d31-palmitate (A) and [1-13C]-oleate oxidations (B), respectively, in sedentary (n = 10) and active (n = 9) lean men. Statistics are presented in the figure.
Such observations were associated with an increase in the response to training in muscle LPL, CD36, FABPpm, and ACSL1 expressions (with similar trends in the protein content), which favored the uptake and activation of fatty acids to fatty-acyl-CoA within muscle cells. An increase in the expression and activation of these enzymes and transporters in response to exercise training has been well described (30). In contrast, detraining increased the exogenous fatty acid appearance in chylomicrons and NEFA, which suggested a reduced plasma clearance and uptake by peripheral tissues. The resulting increased availability of NEFA from dietary sources to the liver likely explained the 2-fold increase in the incorporation of exogenous 13C-oleate and d31-palmitate into VLDL triglycerides. Our results support previous bed-rest experiments that suggested that physical inactivity decreased dietary fat oxidation independent of measurable energy-balance changes by decreasing the fat uptake primarily at the muscle level, with a resulting increased incorporation of dietary fat into plasma triglycerides (17). Taken together, these findings suggest that the trafficking of dietary fat is a function of the amount of energy spent in physical activity. Although these linear relations may make it seem that physical inactivity or detraining is simply the mirror image of insufficient exercise, our results showed that mechanisms underlying the metabolic effects of detraining and training are likely different. Thus, our study supports the hypothesis of Hamilton et al (31) of the existence of an inactivity physiology that is distinctive from an exercise physiology, which the authors developed on the basis of novel results obtained in rats. Additional data are needed to draw clear-cut conclusions about differences in the mechanisms involved in a inactivity and detraining physiology compared with an exercise and training physiology.

The strong negative correlations between changes in whole-body dietary fat oxidation and postprandial insulin secretion provide support for the data of Rimbert et al (32) that were obtained ex vivo in aged individuals, showing that the muscle’s ability to oxidize fat is a major regulator of insulin sensitivity. The association between dietary fat oxidation and AEE further suggested that AEE is an important mediator between fat oxidation and insulin sensitivity. It was recently shown that an increase in energy expenditure from physical activity is the most
powerful determinant of improved insulin sensitivity in overweight patients (33). Our relations indicated that the mechanisms that underlie the beneficial effect of increased AEE on insulin sensitivity involve an increased oxidative capacity for dietary lipids. A mechanistically lower AEE would favor a low energy turnover of intramuscular triglycerides (IMTGs) leading to cytosolic accumulation of fatty acid metabolites and disruption of the early steps in insulin signal transduction (34). Although future studies will need to directly measure the skeletal muscle lipid fractions, the current study indirectly supports the idea that the turnover of the IMTG pool is sensitive to changes in the physical activity level. Indeed, the expression of mgiPAT, protein esterifying fatty acyl-CoA into triglycerides, was depressed in our sedentary subjects, despite the well-described high IMTG content in this population (35).

Some limitations have to be acknowledged. Although measures of incorporations of d31-palmitate and [1-13C]-oleate into triglyceride-rich lipoproteins and NEFA helped us to understand the relation between AEE and dietary fat oxidation, measures of free fatty acid turnover were lacking to assess changes in fatty acid spillover. In France, infusions are unfortunately not permitted anymore. A complementary molecular biology analysis at the adipose tissue level would have also provided us with evidence regarding the potential synergistic response of adipose tissue and muscle in dietary fat trafficking. Even if skeletal muscle represents only 12–14% of the RMR (36), the strength and number of our correlations suggested that muscle is an important component of the metabolic fate of dietary fat. Because the transport of fatty acids from blood to mitochondria is a highly regulated process that involves numerous intracellular storage vesicles and the recruitment of several receptors (37), the absence of a subcellular fractionation also limited the use of our molecular data. However, we believe that the consistent associations we observed strongly suggested that the relation between AEE and trafficking of fatty acids results from a coordinated response involving fatty transporters and enzymes involved in the mitochondrial oxidative capacity. Finally, our lipid test, because of its duration (ie, 8 h postbreakfast) did not provide data about the eventual compensation in fuel oxidation overnight. However, recent data obtained with a room calorimeter at the University of Colorado (AB, unpublished data, 2012) showed that a single bout of exercise performed during the day led to an increase in total fat oxidation not over the day but at night in sedentary lean adults. This result suggested that the sleep/hight period plays a key role in lipid metabolism regulation. Additional investigations are needed to know if additional metabolic changes occur at night in response to chronic perturbations of habitual physical activity.

In conclusion, this study shows that physical activity is a major determinant of dietary fat trafficking and oxidation, not only in response to severe inactivity but also in the range of physical activities attainable by most free-living individuals through mechanisms that likely involve the transport of fatty acids into muscle cells and mitochondria. These changes, which are associated with the insulin response, suggest that the promotion of physical activity along with the study of physical inactivity must remain major topics in research that aims to understand modern metabolic diseases.

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