Exercise increases fat oxidation at rest unrelated to changes in energy balance or lipolysis

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Exercise increases fat oxidation at rest unrelated to changes in energy balance or lipolysis. Am. J. Physiol. 270 (Endocrinol. Metab. 33): E1009–E1014, 1996.—The hypothesis that exercise increases fat oxidation at rest independently of changes in energy balance, body composition, and/or lipolysis was tested in 21 volunteers. After a period of energy balance, volunteers were randomly allocated to one of four groups: control, overfed (OF), overfed and exercised (OF-EX), and exercised (EX). OF and OF-EX were overfed 50% excess of energy balance calories; OF-EX and EX spent 50% excess of energy balance calories during daily exercise sessions. Exercise increased fat oxidation at rest independently of dietary intake (OF-EX = 122 ± 4.4, EX = 123 ± 1.5 mg/min) and reduced carbohydrate oxidation (OF-EX = −49 ± 6.2, EX = −46 ± 5.4 mg/min). Volunteers in the OF group had an increase in carbohydrate oxidation (85 ± 5.9 mg/min) and a decline in fat oxidation (−33 ± 1.4 mg/min). Protein oxidation did not change in any group. Those changes occurred without a direct relation with changes in lipolysis and persisted even when expressed as a percentage or as an absolute equivalent of resting metabolic rate in calories. Thus exercise, independent of changes in energy intake and body composition and not related to changes in lipolysis, increases fat oxidation at rest, which may explain the beneficial effects of exercise in weight loss programs.

fatty acids; fuel oxidation; body composition

OBESITY IS THE STORAGE in the body of excessive amounts of fat (5). The accumulation and maintenance of stored fat depends on the balance between energy and fat intake and utilization (33). When energy and fat intake are held constant, the loss or maintenance of fat (hence obesity) depends on the oxidative disposal of free fatty acids (FFA-ox) (26, 33). The factors that regulate fat oxidation in humans are not clear.

One common concept is that FFA-ox is simply a linear function of the serum FFA concentration, because uptake of FFA by muscle is linear with the serum concentrations of total (bound and unbound) FFA (16). In this model, availability of FFA for FFA-ox depends on lipolysis and is the main and perhaps only mechanism that governs fat oxidation. However, we and others have reported that the systemic delivery of FFA exceeds FFA-ox at rest (3, 9, 10, 22, 23, 34), during physical activity, and in the recovery period from exercise (37). Furthermore, it has been shown that the uptake of FFA by tissues is a saturable process and is a function of the unbound FFA concentrations (29, 31, 32, 35), compatible with a facilitated process requiring FFA membrane transporter(s). Three putative long chain fatty acid transporter(s) have been isolated or cloned (1, 25, 27), supporting the concept that FFA uptake and FFA-ox are not simple functions of the circulating FFA concentrations but may be regulated by a more complex mechanism.

Recently, knowledge has accumulated demonstrating that β-oxidation in striated muscle is a regulated pathway, responsive to acute changes in malonyl-CoA (13). This compound is the first committed substitute in conversion of glucose to fat and is responsive to glucose and insulin (12) as well as to exercise (14).

Among other things (7), adaptation to endurance training is accompanied by an increase in the enzymatic capacity of striated muscle for FFA-ox (21, 24) and is associated with an enhanced capacity for fatty acid uptake (36). These adaptations have physiological implications. For example, we have shown that aerobic exercise training for 8 wk increases resting whole body fat oxidation in elderly individuals despite unchanged rates of lipolysis and without detectable changes in body composition (22). Because even a single bout of strenuous exercise enhances fat oxidation (6, 24), possibly secondary to glycogen depletion and acute negative energy balance, it is not clear whether the enhanced FFA oxidation observed in this study was secondary to a nondocumented negative energy balance or was exercise-induced per se.

This study was designed to test the hypothesis that exercise increases resting FFA-ox in human subjects independently of changes in energy balance, body composition, and/or lipolysis.

MATERIALS AND METHODS

Subjects

Twenty-two young normal healthy volunteers were recruited from the local community. One of the subjects did not comply with the strict guidelines of the project and was dismissed. All the subjects underwent a thorough physical examination and had normal electrocardiograms (basal and during a stress test). The volunteers had normal routine laboratory tests (including thyroid-stimulating hormone and free thyroxine), normal oral glucose tolerance, and normal lipid profile. None of the subjects was taking medications, and weight had been stable over the last 6 mo within 2 kg. All subjects received explanations and signed the consent form. The study was approved by the Committee on Human Research for the Medical Sciences of the University of Vermont and was conducted in its entirety at the Clinical Research Center (CRC) of the University of Vermont.

Study Paradigm

The data presented in this paper pertain to fatty acid metabolism. Data on energy expenditure have been reported
in a previous publication (15). The subjects were admitted and remained as in-patients for the duration of the study. All volunteers underwent initially a sedentary period of 10 days confined entirely to the CRC of the University of Vermont. This sedentary period was considered as a baseline (basal) period. Five volunteers were randomized to this group; (3) an overfed and exercised (OF-EX) group, in which energy intake was increased by 50%, as in the OF group; however, physical activity was also increased to maintain energy balance; six subjects were assigned to this group; (4) an exercised (EX) group, in which the volunteers increased their level of physical activity to induce a net negative energy balance of 50% of energy requirements established during the basal period; these subjects were not allowed to increase energy intake; this group was composed of four subjects.

Dietary intake during the study. The volunteers were prescribed a weight maintenance diet calculated at 40 kcal/kg fat-free mass, as measured by underwater weight (28) on the first morning of the study. The level of energy intake was thus individualized and kept constant throughout the study. The distribution of calories was 15, 55, and 30% for protein, carbohydrate, and fat, respectively, given in three main meals and a night snack. All meals were consumed at the CRC. For the OF and OF-EX groups, the additional calories were provided in the form of a liquid supplement with the same caloric distribution as the regular food. Further details on the dietary intake are described in the previous publication (15).

Physical activity. Subjects were confined to the CRC during the entire duration of the study, and physical activity (other than the one prescribed to OF-EX and EX) was limited to movements to and from different areas of the CRC, private bathrooms, grooming, and the like. In the OF-EX and EX groups, physical activity was increased. The prescription of exercise was tailored to expend 50% of estimated energy intake during the baseline sedentary period. The energy cost of exercise was measured on the first morning of the treatment period while the subject was pedaling a cycle ergometer at ~50% of maximal aerobic capacity. The pace and workload were then fixed for the remainder of the study, and the time of exercise needed to expend 50% of energy intake was calculated. The energy cost of the exercise sessions was monitored every other day by measuring oxygen consumption (VO2) for 20 min during the exercise session.

Resting Substrate Oxidation

Rates of VO2 and carbon dioxide production (VCO2) were measured with indirect calorimetry by use of the ventilated hood technique with a metabolic cart (Delta Metabolic Monitor, Sensormedics, CA). The measurement began after intravenous lines were placed, continued as long as necessary to obtain stable readings, and then continued for an additional 30 min. An overnight timed urine collection estimated the rate of urinary nitrogen excretion. Substrate oxidation rates were calculated as suggested by Jequier and Felber (19). The resting metabolic rate was measured for 2 consecutive days at the end of each treatment period. One of these measurements coincided with the tracer infusion (performed the last day of treatment). The tests were performed ≥12–14 h after the last bout of exercise (in those individuals assigned to OF-EX and EX groups) and/or the last meal.

Palmitic Acid Kinetics

This test was performed at the end of every period (basal and treatment) in all subjects. The subject was awakened at 0630, and an intravenous line (placed in a forearm vein the night before) was used for infusion of the tracer. A second intravenous line was placed in a dorsal vein of the opposite hand and used for blood sampling by the "hot box" technique (2). After baseline blood samples were taken, a nonprimed constant infusion of [14C]palmitic acid (0.20 μCi/min) was started, which lasted 120 min. Samples for determination of hormones, substrates, and plasma enrichment of palmitic acid were taken every 10 min during the last 30 min of infusion.

Analytic Methods and Calculations

Plasma glucose was measured using the glucose oxidase method in an automated glucose analyzer (YSI Instruments, Yellow Springs, OH). Serum insulin was measured by radioimmunoassay at the core laboratory of the CRC (30). The palmitic acid infusate (tracer) was prepared and measured as previously described in detail (9, 10, 22).

Rates of energy expenditure were calculated from VO2, VCO2, and urinary nitrogen (19). The rate of appearance of palmitic acid was calculated using the isotope dilution principle, as described previously (8, 9, 22), after steady conditions of both tracer and tracee had been verified in plasma with use of a linear model of analysis of variance (ANOVA) and regression analysis (slopes of the concentrations regressed on time equal to zero).

Statistical Analysis

Data were reduced and analyzed in a personal computer with use of the Number Cruncher Statistical System (Jerry L. Hintze, Kaysville, UT) and presented as means ± SE unless otherwise specified. Changes due to treatment (above or below baseline) were analyzed with a full factorial two-way ANOVA model in which the main treatment factors considered were diet and exercise. Each of these factors was studied (as per the experimental design) at two levels, basal and 150%. Fisher's least significant difference post hoc test was used if ANOVA indicated differences among the groups (CO, OF, OF-EX, EX). P < 0.05 was considered statistically significant.

RESULTS

Table 1 lists the clinical characteristics of the subjects at the end of the baseline period, and Table 2 lists those after the treatment period. By design, subjects in the OF group increased their intake by 1,206 ± 61 kcal/day and those in the OF-EX group by 1,109 ± 225. In the OF-EX and the EX groups, the prescribed exercise amounted to 1,288 ± 323 kcal/day. There was no significant difference among the different groups at baseline in serum concentrations of insulin and glucose. The individuals of the EX group were leaner than those in the other groups. Serum insulin decreased in those after the treatment period. By design, subjects in the OF group increased their intake by 1,206 ± 61 kcal/day and those in the OF-EX group by 1,109 ± 225. In the OF-EX and the EX groups, the prescribed exercise amounted to 1,288 ± 323 kcal/day. There was no significant difference among the different groups at baseline in serum concentrations of insulin and glucose. The individuals of the EX group were leaner than those in the other groups. Serum insulin decreased in those after the treatment period.

Body composition did not change in those groups in which energy balance was maintained (CO, OF-EX).
metabolic characteristics

ANOVA model showed a significant increase in the rate of carbohydrate oxidation and a consequent decline in fat oxidation at the end of the treatment period ($P < 0.001$ vs. CO, OF, OF-EX, and EX). Although OF-EX and EX groups were different from the OF and CO groups, we could not demonstrate statistically an interaction of exercise and energy intake. The changes in fat or carbohydrate oxidation did not have any correlation with changes in lipolysis ($r = 0.1$, not significant). Protein oxidation did not change in any of the groups. The differences among groups remained similar when the rates of fat and carbohydrate oxidation were expressed as a percentage or as an absolute equivalent of resting metabolic rate calories (Fig. 2).

**DISCUSSION**

The original finding of this study is that the rate of fat oxidation at rest is increased by physical training independently of energy and substrate balances, not directly related to changes in lipolysis and body composition.

The pattern of changes in fuel oxidation during acute exercise is well established. Initially, there is dependence on carbohydrate oxidation, with a later shift to the utilization of fat (7). These changes are thought to be coordinated by hormonal changes (increase in counterregulatory hormones, decrease in insulin) that enhance lipolysis (7) and to depend on the initial muscle concentrations of glycogen (18). Because the content of glycogen in skeletal muscle depends on energy balance and diet composition (18), fat oxidation, in this model, is considered a function of the carbohydrate and energy balances and is therefore, in itself, not a regulated process. This model supports the concept that the oxidation of fat depends on its availability, which is the result of lipolysis (4). Indeed, fatty acid uptake by muscle is linearly related to the concentration of serum FFA (16).

This is an appealing model. However, there are data in the literature that do not support it. We (3, 9, 10, 22, 34) and others (23, 37) have reported that the rate of systemic delivery of fatty acids is in excess of the rate of lipolysis (4). Indeed, fatty acid uptake by muscle is linearly related to the concentration of serum FFA (16).

### Table 1. Baseline clinical and metabolic characteristics

<table>
<thead>
<tr>
<th></th>
<th>CO</th>
<th>OF</th>
<th>OF-EX</th>
<th>EX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>23 ± 2</td>
<td>26 ± 2</td>
<td>29 ± 2</td>
<td>26 ± 3</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>66 ± 4</td>
<td>75 ± 4</td>
<td>72 ± 4</td>
<td>67 ± 6</td>
</tr>
<tr>
<td>Fat mass, %</td>
<td>15 ± 3</td>
<td>15 ± 3</td>
<td>14 ± 3</td>
<td>9 ± 4^a</td>
</tr>
<tr>
<td>RMR, cal/min</td>
<td>1,722 ± 117</td>
<td>1,786 ± 107</td>
<td>1,701 ± 108</td>
<td>1,656 ± 152</td>
</tr>
<tr>
<td><strong>RER, VCo2/VO2</strong></td>
<td>0.87 ± 0.03</td>
<td>0.88 ± 0.05</td>
<td>0.88 ± 0.05</td>
<td>0.84 ± 0.04</td>
</tr>
<tr>
<td>Fat-ox, mg/min</td>
<td>43 ± 9</td>
<td>40 ± 8</td>
<td>31 ± 8</td>
<td>49 ± 12</td>
</tr>
<tr>
<td>Cho-ox, mg/min</td>
<td>150 ± 25</td>
<td>161 ± 18.7</td>
<td>159 ± 32</td>
<td>115 ± 21</td>
</tr>
<tr>
<td>Prot-ox, mg/min</td>
<td>36 ± 2</td>
<td>44 ± 2</td>
<td>51 ± 3.2</td>
<td>47 ± 1</td>
</tr>
<tr>
<td>Insulin, pmol/ml</td>
<td>61 ± 9</td>
<td>53 ± 10</td>
<td>48 ± 9</td>
<td>41 ± 5</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>91 ± 3</td>
<td>88 ± 4</td>
<td>92 ± 5</td>
<td>88 ± 4</td>
</tr>
<tr>
<td>Rpal, μmol/min</td>
<td>120 ± 21</td>
<td>126 ± 23</td>
<td>78 ± 23</td>
<td>82 ± 18</td>
</tr>
</tbody>
</table>

Values are means ± SE. CO, control; OF, overfed; OF-EX, overfed and exercised; EX, exercised groups. RMR, resting metabolic rate; RER, respiratory exchange ratio; CO2 production (VCo2)/O2 consumption (VO2); Fat-ox, Cho-ox, and Prot-ox, fat, carbohydrate, and protein oxidation; respectively; Rpal, rate of appearance of palmitic acid.

^a $P < 0.05$ vs. CO, OF, and OF-EX.

There was an increase in weight and fat mass in OF subjects and a decrease in the EX group (Table 2).

At baseline the four groups had rates of appearance of palmitic acid (Table 1) that were not statistically different, although a tendency for a lower rate in the OF-EX and EX groups was apparent. The rate of production of palmitic acid increased in both the OF-EX and the EX groups and decreased in the OF group (Table 2).

Rates of substrate oxidation were similar at baseline among the different groups (Table 1). Substrate oxidation rates in the CO group were similar at the end of the treatment period vs. baseline. However, marked differences were found at the end of the treatment period in the OF, OF-EX, and EX groups (Fig. 1 and Table 2). The ANOVA model showed a significant increase in the rate of whole body fat oxidation in volunteers assigned to groups in which an increase in exercise was imposed ($P < 0.001$ vs. CO and OF), with a reciprocal reduction in the rate of total body rate of carbohydrate oxidation ($P < 0.0001$ vs. CO and OF). In contrast, volunteers in

### Table 2. Clinical and metabolic changes after intervention

<table>
<thead>
<tr>
<th></th>
<th>CO</th>
<th>OF</th>
<th>OF-EX</th>
<th>EX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, kg</td>
<td>0.10 ± 0.01^a</td>
<td>1.0 ± 0.9^a</td>
<td>1.0 ± 0.5^a</td>
<td>-1.0 ± 0.4^b</td>
</tr>
<tr>
<td>Fat mass, %</td>
<td>0 ± 0.4^a</td>
<td>2.0 ± 0.1^a</td>
<td>0.1 ± 0.1^a</td>
<td>-1.0 ± 0.8^b</td>
</tr>
<tr>
<td><strong>RER, VCo2/VO2</strong></td>
<td>0.01 ± 0.003</td>
<td>0.071 ± 0.01^b</td>
<td>-0.051 ± 0.01^c</td>
<td>-0.042 ± 0.02^d</td>
</tr>
<tr>
<td>Fat-ox, mg/min</td>
<td>-3 ± 8^a</td>
<td>-33 ± 1^b</td>
<td>22 ± 2^c</td>
<td>23 ± 2^c</td>
</tr>
<tr>
<td>Cho-ox, mg/min</td>
<td>-14 ± 4^a</td>
<td>85 ± 6^b</td>
<td>-49 ± 6^c</td>
<td>-46 ± 5^a</td>
</tr>
<tr>
<td>Prot-ox, mg/min</td>
<td>7 ± 3.3</td>
<td>7 ± 5.2</td>
<td>2 ± 2.2</td>
<td>6 ± 5.1</td>
</tr>
<tr>
<td>Insulin, pmol/ml</td>
<td>-5 ± 2^a</td>
<td>30 ± 2^b</td>
<td>-18 ± 5^c</td>
<td>-22.5 ± 5^a</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>9 ± 2</td>
<td>2 ± 3</td>
<td>1 ± 3</td>
<td>47 ± 19^a</td>
</tr>
<tr>
<td>Rpal, μmol/min</td>
<td>-10 ± 15^a</td>
<td>-74 ± 23^b</td>
<td>13 ± 25^a</td>
<td>47 ± 19^a</td>
</tr>
</tbody>
</table>

Values are means ± SE. Superscript letters indicate changes in means that are similar (2-factorial ANOVA, post hoc Fisher’s least significant differences test, with $P < 0.05$ taken as significant).
Fig. 1. Substrate oxidation changes. Fat oxidation (filled bars) and carbohydrate oxidation rates (open bars) are changes from baseline. Pattern of changes was different in the overfed (OF) group from the overfed and exercised (OF-EX) and exercised (EX) groups, and these 3 groups were different from control. OF-EX and EX display similar patterns of change.

decrease in fat oxidation in women that is a function of the fitness level (8). In addition, we found that 8 wk of aerobic training increased fat oxidation without changing body composition and independent of changes in the rate of production of fatty acids (22). Thus we have consistently found dissociation between the apparent availability of FFA and FFA-ox. Interestingly, Martin et al. (20) demonstrated that, during exercise, trained individuals exhibit a lower rate of production of fatty acids (lipolysis) than individuals who are not physically fit, yet they have higher rates of fat oxidation.

Therefore, there is evidence to support the existence of a regulatory mechanism to increase (or decrease) fat oxidation that is independent of serum FFA concentrations. In this study, fat oxidation at rest increased in those individuals who were engaged in daily sessions of exercise. This increase was independent of energy and substrate balance, because it was present whether or not the exercise-associated energy expenditure was balanced by an increase in dietary intake. In addition, this change in the oxidation of fuels at rest is not merely a function of serum FFA, because the change in oxidation of fat did not correlate with changes in lipolysis. Furthermore, the increase in fat oxidation associated with exercise was not related to changes in body composition, because it was observed in both OF-EX (no change in weight and fat mass) and EX groups (loss of body weight and fat mass). It should be stressed, however, that this was a short study and may not completely dissociate acute effects of exercise from those of adaptations to endurance training. Nevertheless, these results support a role for exercise in programs aimed at decreasing body fat and weight control by inducing a shift in the pattern of fuel utilization at rest.

The basic mechanisms responsible for the shift in fuel utilization at rest are not discernible from this study. The documented increase in the number of mitochondria after chronic endurance training (17) is unlikely to play a role, because our study was for only a few days. Martin et al. (20) proposed an increase in intramuscular lipolysis and oxidation of fatty acids to resolve this paradox. In rodents, it has been shown that striated muscle fatty acid oxidation is activated by low intracellular levels of malonyl-CoA (13), the first committed intermediate in the conversion of glucose to fat. Striated muscle concentrations of malonyl-CoA increase in response to glucose and insulin (12) and decrease during exercise (14). Thus an increase in the \( \beta \)-oxidation pathway, mediated by changes in intracellular concentrations of malonyl-CoA, can certainly provide an explanation for our data. However, this pathway is substrate dependent, which dictates an enhanced
delivery of fatty acids to the intracellular environment as a preliminary step. Thus a possible supplementary mechanism is an exercise-induced increase in the capacity for FFA uptake by muscle. This could be explained by activation of a facilitated system of FFA uptake. Several proteins have been postulated as specific long-chain fatty acid transporters (1, 25, 27). We have published evidence that supports the existence of at least one of these putative transporters in membranes of human skeletal muscle (11). The putative role of these proteins in human muscle physiology and the effects of exercise in the activity or the membrane concentration of these fatty acid transporters remain to be determined and are currently being investigated in our laboratory.

In summary, we present evidence to support the hypothesis that exercise, independent of changes in energy and substrate balances and not directly related to changes in lipolysis, shifts the pattern of fuel utilization at rest toward fat oxidation. This shift may aid in the regulation of weight and avoidance of obesity. The molecular mechanism may involve activation of the \( \beta \)-oxidation pathway in concert with an increase in the activity of putative membrane fatty acid transporters.

The authors express gratitude to the Staff of the Clinical Research Center (CRC) of the University of Vermont, without whom this study would have been impossible. We thank also our volunteers, who endured a most demanding project.

This study was supported by National Institutes of Health Grants K08-01963 (J. Calles-Escandón), RO1-DK-18535 (E. Danforth, Jr.), RO1-DK-401973 (S. Nair), and MO1-RR-00109 (CRC).

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Received 24 July 1995; accepted in final form 19 December 1995.

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