Fat oxidation in response to four graded energy challenges in younger and older women

Kathleen J Melanson, Edward Saltzman, Robert R Russell, and Susan B Roberts

ABSTRACT We examined whether older individuals have an impairment in their ability to oxidize dietary fat, a factor that could help to explain age-associated weight gain. The subjects were 16 healthy younger and older women. Fat oxidation was determined by indirect calorimetry before and after consumption of four different test meals consumed ≥ 5 d apart. The intervention meals contained 0, 1046, 2092, or 4184 kJ (simulating extended fasting, and consumption of a snack, a small meal, and a moderately large meal, respectively), with 35% of energy from fat. The duration of each measurement was the amount of time required for postprandial energy expenditure to return to the premeal fasting value. A total of 96 measurements were obtained, including duplicates for all meal sizes in the younger women (in the follicular and luteal phases of the menstrual cycle). Total postprandial fat oxidation increased in proportion to meal size in the younger subjects, but did not increase above that for the 2092-kJ meal in the older women. In addition, older subjects had significantly lower total fat oxidation after consumption of the 4184-kJ meal (781 compared with 1029 kJ/meal, P < 0.02) and also significantly greater fat deposition (745 compared with 464 kJ/meal, P < 0.02). These findings suggest that, relative to younger women, older women have a reduced ability to oxidize dietary fat when they consume large meals. Am J Clin Nutr 1997;66:860–6.

KEY WORDS Energy metabolism, body composition, energy intake, dietary fat, fat oxidation, age, women

INTRODUCTION

Body fat typically doubles between the ages of 20 and 50–60 y (1–3), even in adults whose weight remains relatively constant; this change has been causally linked to increased risks of several diseases, including cardiovascular disease and non-insulin-dependent diabetes (4–6). The underlying causes of this increase in body fat content are not well understood (7), although such information is needed before the most effective methods for prevention and treatment can be developed.

The ability of the body to oxidize dietary fat is a critical determinant of the success or failure of body energy regulation and hence the risk of obesity (8, 9) because exogenous fat that is not oxidized must be stored. Thus, if fat oxidation can adapt to match the fat content of the diet, there will be no net fat deposition and body adipose stores will remain constant. Conversely, if fat oxidation is less than intake, body fat stores will increase. The potential role of reduced fat oxidation in the development of obesity is seen in the data from epidemiologic studies that link consumption of a high-fat diet to increased body fat (10) and in dietary intervention studies that have shown higher energy intakes with high-fat diets than with low-fat diets matched for palatability (11). More direct evidence is also seen in the prospective studies that have shown a significant association between low fat oxidation, relative to intake, and subsequent weight gain (12).

On the basis of several related observations, we speculated that a decreased ability to use fat as a metabolic fuel might be an important factor contributing to weight gain in older individuals. First, older individuals have a smaller skeletal muscle mass, which is the primary site for fat oxidation (1–3). Second, they perform less vigorous physical activity, which could suppress the extent to which fat is used as a fuel source instead of carbohydrate (13). Finally, national dietary surveys have suggested that the proportion of dietary energy supplied by fat may slightly increase between early adult life and 50–60 y of age (14), thus increasing the dietary load of fat, which has to be oxidized in relation to other energy substrates. The combination of these three factors suggests that a reduced ability to oxidize fat could be an important factor contributing to weight gain during adult life. Currently, however, there is only limited and conflicting information on the effects of age on fat oxidation in the fasting state (15–17), and we are aware of no information on fat oxidation in the quantitatively more important postprandial state.

Approaches to the prevention and treatment of age-associated weight gain would differ according to the nature of the underlying problem. Therefore, the study described here was designed to provide more information on the potential role of impaired fat oxidation in promoting weight gain during adult life. Specifically we tested the hypothesis that older individuals

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have a decreased ability to oxidize fat, a problem that is seen during the consumption of large meals but not during the consumption of small ones.

SUBJECTS AND METHODS

Subjects

Eight normally menstruating younger women and eight older women participated in the study (Table 1), having been recruited by advertisements in the local community. All were in good health, as determined by a routine physical examination, blood test, and psychologic and health-history questionnaires. All had normal glucose tolerance, as determined with a standard oral-glucose-tolerance test during screening (18, 19), and were euthyroid. None of the subjects smoked; took oral contraceptives, postmenopausal hormones, or other medications; or consumed large amounts of caffeine or alcohol. Women with endocrinopathies, digestive problems, eating disorders, or a family history of diabetes were excluded. The protocol was approved by the Human Investigations Review Committee of Tufts University and New England Medical Center, Boston, and informed consent was obtained from all volunteers. The study was conducted in the Metabolic Research Unit at the Jean Mayer US Department of Agriculture Human Nutrition Research Center on Aging.

Protocol

The study consisted of four 2-d residency periods for the older women, with a minimum of 5 d between visits, followed by a fifth 1-d visit for a test of maximal aerobic capacity (VO2max). In the younger women, the four 2-d visits were repeated so that each measurement of fat oxidation could be obtained once in the follicular phase of the menstrual cycle (days 6–11) and once during the luteal phase (days 16–26), with ovulation determined with home ovulation-detector kits (First Response; Carter-Wallace, Inc, New York). Duplicate measurements were not conducted in the older women because of their constant hormonal profiles. Throughout enrollment in the study, with exceptions noted below, all subjects were expected to maintain their normal lifestyle and dietary and activity patterns as well as their usual body weight.

The first day of each visit was considered a preparation day and the second day was the testing day. During the preparation days, subjects consumed food provided by the research unit consisting of the recommended dietary allowance for energy (20), with 15% derived from protein, 25% from fat, and 60% from carbohydrate. The percentage of carbohydrate was chosen to provide each subject with ≥300 g glucose to help normalize glycogen stores among and within the subjects. In addition, subjects were required to perform no strenuous physical activity during the preparation days and to keep all preparation days as similar to each other as possible. Caltrac activity monitors (Muscle Dynamic Fitness Network, Torrance, CA) were worn to verify compliance with the restriction of physical activity (21). Information on body composition and standard anthropometric measurements were obtained in the morning of the first preparation day. The first preparation day was also used to familiarize subjects with the testing procedures.

Subjects were woken at 0600 on the testing day and asked to empty their bladder. Small activity monitors (model 101 Motion Recorder, Timex Kaulins & Willins, Middlebury, CT) were then attached to all four limbs to detect and discourage movement and an intravenous line was inserted into an antecubital vein for collection of blood samples during the measurement period. (No differences in activity were seen between subject groups or test meals; data not shown.) After a 30-min rest period, resting metabolic rate was determined by indirect calorimetry (22) over 40 min under thermoneutral conditions while subjects rested quietly in the supine position. After the measurement of resting metabolic rate, a test meal was presented to the volunteer. The energy contents of the four test meals were 0, 1046, 2092, or 4184 kJ (0, 250, 500, or 1000 kcal) so that a dose-response design could be created. In all meals, which consisted of a peanut butter and jelly sandwich and glass of milk, 48% of the energy was from carbohydrate, 17% was from protein, and 35% was from fat; the food quotient for the meals was 0.858. The order of the meals was randomized, but the 0-kJ test meal always followed the 4184-kJ meal so that the length of the fasting test day could be matched to that of the 4184-kJ day. On the test day with 0 kJ, 224 mL (8 oz) room temperature water was given to the volunteer. The subjects and the investigators conducting measurements were blinded to the size of the meal to be consumed on any given study day.

Subjects were allowed 30 min to consume their meal and then indirect calorimetry was resumed until metabolic rate returned to baseline, or for a maximum of 10 h. The length of testing on the fasting day was matched to that of the day when the subject consumed 4184 kJ. The measurements were taken in 0.5-h blocks, consisting of 20 min of continuous recording of respiratory exchange and 10-min breaks for collection of blood, urine samples, or both, and for assessment of the volunteer’s comfort. Subjects were asked to void all urine at the end of the measurement and all urine produced during the measurement period was mixed and samples frozen at −80 °C before being analyzed for nitrogen (23).

Energy expenditure and fat, carbohydrate, and protein oxidations were calculated from the data on respiratory gas ex-

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Subject characteristics †</th>
<th>Younger women (n = 8)</th>
<th>Older women (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>25.3 ± 1.8</td>
<td>72.3 ± 2.1 ±</td>
<td></td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.61 ± 0.06</td>
<td>1.58 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>57.4 ± 6.9</td>
<td>63.9 ± 14.1</td>
<td></td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>22.1 ± 3.2</td>
<td>25.4 ± 4.3</td>
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<tr>
<td>Body fat (% of wt)</td>
<td>26.4 ± 5.4</td>
<td>39.5 ± 0.2 ±</td>
<td></td>
</tr>
<tr>
<td>Lean body mass (kg)</td>
<td>41.6 ± 3.8</td>
<td>36.0 ± 0.2 ±</td>
<td></td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.79 ± 0.07</td>
<td>0.83 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>Waist-to-thigh ratio</td>
<td>1.51 ± 0.13</td>
<td>1.70 ± 0.20 ±</td>
<td></td>
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<tr>
<td>VO₂ max (mL · kg⁻¹ · min⁻¹)</td>
<td>36.1 ± 7.1</td>
<td>21.0 ± 4.3 ±</td>
<td></td>
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<tr>
<td>Maximum heart rate during</td>
<td>177 ± 11</td>
<td>149 ± 14 ±</td>
<td></td>
</tr>
<tr>
<td>VO₂ max test (beats/min)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Duration of VO₂ max test (min)</td>
<td>7.6 ± 1.3</td>
<td>4.1 ± 0.1 ±</td>
<td></td>
</tr>
<tr>
<td>Average RMR (kJ/d)</td>
<td>5292 ± 233</td>
<td>4989 ± 231 ±</td>
<td></td>
</tr>
<tr>
<td>Average fasting RQ</td>
<td>0.868 ± 0.043</td>
<td>0.865 ± 0.030</td>
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</tbody>
</table>

† ¯x ± SD. VO₂ max, maximal aerobic capacity; RMR, resting metabolic rate; RQ, respiratory quotient.

‡ Significantly different from younger women: *P < 0.001, †P < 0.05, ‡P < 0.01.
change (extrapolated from 20 to 30 min for each block of time) and urinary nitrogen output by using the coefficients described by Livesey and Elia (24). Mean values for energy expenditure and substrate oxidations were calculated for each younger woman from follicular- and luteal-phase data. Substrate balances were calculated as the difference between nutrient intakes and rates of substrate oxidation.

Body-composition analysis

All body-composition testing was conducted after a 12-h overnight resident fast. Hydrostatic weighing was performed on all volunteers (25) and measurements were repeated until at least four were within 1% of each other. The nitrogen-dilution technique was used to correct the results from hydrostatic weighing for each individual’s residual lung volume (26).

Testing of maximal aerobic capacity

\(\text{VO}_2\text{max}\) was determined on the last study day, midmorning, 2–3 h after a light breakfast without caffeine. Subjects exercised on an electronically braked cycle ergometer at a constant 70 rpm with increasingly higher workloads until exhaustion, according to our standard Bruce protocol (27, 28).

Blood analyses

Blood was collected for measurement of fatty acids and triacylglycerol concentrations just before and 60, 120, 180, and 300 min after consumption of the test meals (follicular-phase measurements only in the younger women). Fatty acid concentrations were determined in serum with the Wako enzymatic method (ACS-ACOD method for fatty acids, kit 990–75401; Wako Chemicals USA Inc, Richmond, VA), and triacylglycerol concentrations were measured in plasma with a modified glycerophosphate oxidase method (triacylglycerol kit 44119; Roche Diagnostic Systems Inc, Nutley, NJ). Both measurements were made with automated clinical chemistry analyzers (Cobas Mira and Cobas Fara; Roche Diagnostic Systems Inc). Mean fasting triacylglycerols were calculated by using all time points on the 0-KJ measurement day. Mean postprandial triacylglycerol concentrations (60-, 120-, 180-, and 300-min values) were calculated for the 1046-, 2092-, and 4184-KJ meals for each subject.

Statistical methods

Data are expressed as means ± SEMs unless otherwise specified. Comparisons between younger and older women were performed by using unpaired t tests; statistical significance was accepted at the 0.05 level. Analysis of variance with linear polynomial tests was used to test phase-by-meal size interactions for the dose responses, with protein, carbohydrate, and fat oxidations as dependent variables. Pearson correlation and multiple-regression analyses were performed to determine relations between postabsorptive and postprandial substrate oxidation and independent variables, including age, body composition, fitness level, and blood analytes. In the multiple-regression analyses, all possible combinations of two and three independent variables were examined; larger numbers of variables were not examined because of the relatively small sample size of the study. The statistical program used was SYSTAT for Macintosh version 5.1 (Evanston, IL).

RESULTS

Data on the subjects’ body composition, fitness, and resting metabolic rate are shown in Table 1. Height, weight, and body mass index did not differ significantly between the age groups. However, as expected, older women had a significantly higher percentage of body fat and lower lean body mass. Both waist-to-hip and waist-to-thigh ratios were higher in the older women, but only the waist-to-thigh ratio was significantly so. The older women were also less fit than the younger women, as seen by their lower VO\textsubscript{2}max, shorter endurance during the exercise test, and lower maximum heart rate (though these latter values are normal for the age groups studied). Average resting metabolic rate was significantly lower in the older women than in the younger women.

Substrate oxidation rates in relation to meal size in the younger and older subjects are shown in Figure 1. There was no significant difference between the age groups for any macronutrient, except for fat, which was oxidized at a lower rate in the older subjects than in the younger ones after consumption of the 4184-KJ meal (1.91 ± 0.14 compared with 1.48 ± 0.17 kJ/min, \(P < 0.05\)). A comparable difference between younger and older women was also found for the nonprotein respiratory quotient after consumption of the 4184-KJ meal (0.867 ± 0.008 compared with 0.894 ± 0.010, \(P < 0.05\)). As a result of the decreased fat oxidation after consumption of the 4184-KJ meal in the older subjects, fat balance was significantly more positive (745 ± 79 compared with 464 ± 72 kJ/measurement, \(P < 0.02\)) whereas carbohydrate balance was lower, albeit not significantly so (Figure 2). Protein balances were essentially identical in the younger and the older subjects (Figure 2).

To explore the relation between meal size and fat oxidation in more detail, individual values for total fat oxidation per measurement period were examined in relation to meal size. These fat oxidation values were corrected for individual differences in postprandial energy expenditure to minimize between-subject variability due to differences in energy expenditure. This was done by dividing each volunteer’s total fat oxidation by a correction factor equal to her postprandial energy expenditure expressed as a proportion of the mean postprandial energy expenditure of all subjects for that meal size. Note that there was no significant difference in postprandial energy expenditure between the younger and older subjects and mean values were very similar. As shown in Figure 3, the older women did not show the same linear trend in total fat oxidation with increasing meal size as the younger women, and there was no increase in total fat oxidation above that for the 2092-KJ meal. After consumption of the 4184-KJ test meal, corrected total fat oxidation was significantly lower in the older women than in the younger women (781 ± 75 compared with 1029 ± 58 kJ, \(P < 0.02\)). The uncorrected total fat oxidation after consumption of the 4184-KJ meal was also significantly lower in the older women than in the younger women (1036 ± 256 compared with 734 ± 79 kJ/measurement, \(P < 0.01\)). The 95% CI for reduced fat oxidation in the older women compared with that in the younger women was −451 to −45 kJ.

Regression analyses were performed on factors related to the age-associated reduction in fat oxidation after larger meals. The best single predictor of corrected fat oxidation for all subjects combined was VO\textsubscript{2}max. However, this relation was
FIGURE 1. Rates of fat, protein, and carbohydrate oxidation (absolute values) in relation to meal size in the younger (●) and older (○) women. *Significantly different from younger women, P < 0.05.

not significant for the older subjects when considered separately (Figure 4). Postprandial fat oxidation was significantly correlated with postprandial plasma triacylglycerol concentrations in multiple-regression models in which age group or lean body mass and VO\textsubscript{2}max were used (Figure 5).

Regression analyses were also performed to explore associations between fat oxidation after consumption of the 0-kJ meal and metabolic variables. The best single predictor of fasting-corrected total fat oxidation for all subjects combined was serum fatty acids ($R^2 = 0.762$, $P < 0.01$) followed by VO\textsubscript{2}max ($R^2 = 0.575$, $P < 0.05$). Serum fatty acids were a significant predictor of postabsorptive fat oxidation in the younger women ($R^2 = 0.681$, $P < 0.02$) but not in the older women ($R^2 = 0.050$, $P = 0.593$).

FIGURE 2. Fat, protein, and carbohydrate balances (absolute values) in relation to meal size in the younger (●) and older (○) women. *Significantly more positive after consumption in the older women, $P < 0.02$.

DISCUSSION

Recent research has suggested that a general dysfunction of energy regulation systems within the aging body may underlie age-related changes in body composition. In particular, we and others have shown that aging is associated with a decreased ability to regulate food intake (7, 29) and, in addition, older men appear to be less able to “burn off” surplus energy by increasing resting and postprandial energy expenditure when they overeat (30). In addition to this dysfunction of energy regulation systems, we hypothesized that an age-related reduc-
FIGURE 3. Individual values for fat oxidation (corrected for postprandial energy expenditure) in relation to meal size and study duration in the 16 younger and older women. There was a significant age group-by-meal size interaction ($P < 0.02$) and older women had significantly lower values for fat oxidation after consumption of the 4184-kJ meal than did the younger women ($P < 0.02$). There was no significant difference in study duration between age groups.

FIGURE 4. Relation between maximal aerobic capacity and fat oxidation after consumption of the 4184-kJ meal in younger (●) and older (○) women. There was a significant relation in all subjects combined ($R^2 = 0.733, P < 0.001$) and in younger subjects alone ($R^2 = 0.716, P < 0.01$) but not in the older subjects ($R^2 = 0.085, P = 0.574$).

lower fat oxidations after consumption of a moderately large meal (4184 kJ). Note that fat oxidation values used in this study were corrected for individual differences in postprandial energy expenditure but that similar results and significances were obtained with uncorrected data. In addition, the results were not explainable by impaired absorption of fat from the gastrointestinal tract because circulating triacylglycerol concentrations after consumption of the test meal were higher in the older subjects than in the younger ones. The difference between the groups was apparently due to a combination of a mean increase in the rate of fat oxidation in the younger group (relative to that of a medium-sized meal) combined with a mean decrease in the rate of fat oxidation in the older group, so further studies are needed to confirm the finding. Such studies should consider using additional control groups, matching the young and older individuals for both body composition and fitness level, and

FIGURE 5. Relation between mean plasma triacylglycerol and fat oxidation after consumption of the 4184-kJ meal in the younger (●) and older (○) women. Triacylglycerols ($P < 0.05$) and age group ($P < 0.01$) were significant independent predictors of fat oxidation in a combined multiple-regression model ($R^2 = 0.462, P < 0.02$). Substitution of lean body mass or fitness for age group gave a comparable level of significance.
meals with energy contents proportional to energy requirements in each individual.

In regression analyses, fitness (VO₂ max), lean body mass, and circulating triacylglycerol concentrations explained individual differences in fat oxidation. However, the number of subjects was rather small for this type of analysis and there were several similar models with comparable statistical significance. In addition, the separation of age from body composition and fitness was not possible. In relation to this observation, note that at least some of the loss in lean body mass and fitness with aging is an inevitable consequence of the aging process (27, 31) and also that fitness has been reported to influence postprandial energy expenditure in previous studies (32, 33). Thus, the difference in fat oxidation between younger and older women can be judged to be linked to the aging process, even if mediated in whole or part by changes in fatness and fitness.

Data from two previous studies are available on aging and substrate oxidation in the fasting state: one reported an increase in fasting fat oxidation in older individuals (16) and the other observed a negative correlation between age and fasting fat oxidation (17). Data from the present investigation showed no significant effects of age per se on fasting fat oxidation measured over 10 h but a significant negative effect of body fat in the best-fitting multiple-regression model, which was probably because our fatter subjects were in the older group (perhaps suggesting that body fatness was more reflective of biological age than was chronologic age in our subjects). Thus, our observations on fasting fat oxidation are tentatively consistent with the data of Calles-Escandon et al (17) and lend weight to the suggestion that aging may also be associated with impairments in fasting fat oxidation despite the fact that fat mass, which is positively correlated with fasting fat oxidation in younger subjects, is increased (34).

On the basis of the observation that total fat oxidation in the older women did not increase above the amount observed after our 2092-kJ test, in contrast with the increase observed in older women, and that fat provided only 35% of the energy in the meal, it is likely that impaired fat oxidation occurs routinely in older women taken as a group. Studies are needed to examine potential preventive measures that could be taken to avoid the observed age-associated relative decrease in postprandial fat oxidation. In particular, routine reliance on several modest-sized meals of normal fat content each day rather than fewer large meals could potentially be beneficial, particularly because we observed higher total fat oxidation in the older subjects than in the younger subjects after they consumed a 2092-kJ meal. In addition, it is likely that exercise interventions designed to increase skeletal muscle mass and fitness might increase the capacity for fat oxidation in the fasting state and thereby help to offset any postprandial impairment at high energy intakes. Several previous studies, including one in elderly subjects, have reported increased fasting fat oxidation with improved fitness resulting from increased muscle mass and increased activity of fat oxidative enzymes in muscle (35–38).

In conclusion, we observed that older women with typical values for body composition and fitness have lower fat oxidation and more positive fat balance than younger women after consumption of relatively large meals but not after consumption of smaller meals or snacks. This apparent impairment in fat oxidation may help to explain the vulnerability of older individuals to weight gain and obesity. Further studies are needed to confirm our findings in a larger group of subjects and to examine the relative importance of aging, body composition, and fitness to the observed results.

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