Changes in 24-h substrate oxidation in older and younger men in response to exercise

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Changes in 24-h substrate oxidation in older and younger men in response to exercise. J Appl Physiol 103: 1576–1582, 2007. First published August 23, 2007; doi:10.1152/japplphysiol.01455.2006.—The purpose of this study was to compare 24-h substrate oxidation in older (OM; 60–75 yr, n = 7) and younger (YM; 20–30 yr, n = 7) men studied on sedentary day (Con) and on a day with exercise (Ex; net energy expenditure = 300 kcal). Plasma glucose and free fatty acids were also measured at several time points during the 24-h measurement. Weight was not different in OM and YM (means were also measured at several time points during the 24-h measurement. Weight was not different in OM and YM (means ± SD; 84.8 ± 16.9 vs. 81.4 ± 10.4 kg, respectively), although percent body fat was slightly higher in OM (25.9 ± 3.5 vs. 21.9 ± 9.7%; P = 0.17). Values of 24-h energy expenditure did not differ in OM and YM on the Con (means ± SE; 2,449 ± 162 vs. 2,484 ± 104 kcal/day, respectively) or Ex (2,902 ± 154 vs. 2,978 ± 122 kcal/day) days. Under both conditions, 24-h respiratory quotient was significantly lower and fat oxidation significantly higher in OM. Glucose concentrations were not different at any time point, but plasma free fatty acid concentrations were higher in OM, particularly following meals. Thus, under these controlled conditions, 24-h fat oxidation was not reduced and was in fact greater in OM. We speculate that differences in the availability of circulating free fatty acids in the postprandial state contributed to the observed differences in 24-h fat oxidation in OM and YM.

AGE-RELATED CHANGES IN BODY composition have deleterious effects on health. In particular, age-related increases in fat mass (FM) (10), especially in the abdominal compartment (1, 3, 7, 24), may increase the risk of cardiovascular disease and diabetes in the elderly (6). Because lower rates of fat oxidation predict fat accumulation (22, 28), it could be hypothesized that the age-related increases in FM are due to reductions in the ability to oxidize fat, but previous studies have yielded discordant results. Whole body fat oxidation was reported to be lower in older compared with younger adults after ingestion of a meal (17, 23) and during exercise (2, 18, 25), but the ability to adjust substrate oxidation in response to changes in macronutrient intake is preserved (5). Exercise prevents weight gain in older adults and attenuates age-related gains in FM (11, 19), but nonetheless, body fatness is still greater in regularly exercising older adults compared with their younger counterparts (10). Measurements of 24-h fat oxidation under sedentary and exercise conditions may provide a more complete picture of the responses to exercise. If 24-h fat oxidation is lower in older compared with younger adults under both sedentary conditions and exercise, this would suggest that an age-related reduction in fat oxidation is a potential mechanism by which older men are susceptible to increasing body FM. This contention is supported by the results of a previous room calorimetry study which demonstrated lower 24-h fat oxidation rates in older compared with younger adults on a day that exercise was performed (12). However, subjects were heterogeneous with regard to their levels of habitual physical activity, so it is not clear whether these differences were due to the reduced physical activity levels of older individuals rather than the aging process per se. Thus the primary aim of this study was to compare 24-h fat oxidation in sedentary older men (OM) and younger men (YM) under sedentary (Con) and exercise (Ex) conditions. We hypothesized that fat oxidation would be lower in OM under both conditions. Furthermore, because differences in fuel availability may affect substrate oxidation, the secondary aim was to compare substrate availability over 24 h in YM and OM.

METHODS

Institutional approval. The study was approved by the Colorado Multiple Institutional Review Board and the Scientific Advisory Board of the General Clinical Research Center (GCRC) at the University of Colorado at Denver and Health Sciences Center (UCDHSC).

Subjects. Men were recruited through electronic and paper bulletins on the UCDHSC campus. Respondents were queried about their age, current and past body weight, physical activity patterns, and tobacco and alcohol use during an initial telephone screening. Inclusion criteria were age (YM; 20–30 yr; OM, 60–75 yr), weight stability (<5-lb. change in body weight over the previous 6 mo), and sedentary lifestyle (defined as no participation in regular exercise). Major exclusion criteria were 1) the use of prescription medications for cardiovascular disease, hypertension, diabetes, thyroid conditions, or lipids; 2) current use of tobacco products; or 3) excessive alcohol intake (>2 drinks/day). Volunteers who passed the initial screening provided informed written consent, and were then invited to participate in a health history and physical examination. Body mass index (BMI) was confirmed by measuring height and weight while the subjects were wearing only socks, undergarments, and a hospital gown. Volunteers reporting orthopedic limitations; having a history of chronic disease (e.g., hypertension, cardiovascular disease, diabetes, thyroid disease), use of prescription or over-the-counter medications known to affect appetite, food intake, or energy expenditure (EE; e.g., appetite suppressants, thyroid medications, lithium, antidepressants, dehydroepiandrosterone, testosterone, etc.); or with evidence of hypertension (systolic pressure >140 mmHg or diastolic pressure >90 mmHg) were excluded. A fasted blood sample was then obtained, and any volunteer with a fasting glucose >126 mg/dl was excluded.
Prestudy measurements. Body composition was measured using dual-energy X-ray absorptiometry (Lunar DPX-IQ bone densitometer, Software version 4.38). Resting metabolic rate (RMR) (27) was measured following an overnight fast and 24-h abstention from exercise using standard indirect calorimetry (model 2900 Metabolic Cart, Sensor Medics, Yorba Linda, CA) with the ventilated hood technique. Maximal aerobic capacity (\( \text{V}_{\text{O2peak}} \)) was determined using a progressive exercise test on an electronically braked stationary bicycle ergometer (Excalibur Lodebike Excalibur, Groningen, The Netherlands). Heart rate (monitored using a 12-lead electrocardiogram) and blood pressure were measured every minute. \( \text{V}_{\text{O2peak}} \) was determined from the average of the highest three measurements during the final stage of exercise. To be accepted as valid, the test was required to meet two of the following three criteria: 1) a respiratory quotient (RQ) >1.1; 2) heart rate within 10 beats/min of 85% of age-predicted maximum; and 3) an increase in oxygen consumption (\( \text{V}_{\text{O2}} \)) in response to the final workload of \(<2.0\ \text{mlkg}^{-1}\text{min}^{-1} \). Following the \( \text{V}_{\text{O2peak}} \) measurement, volunteers were permitted time to rest, and then they performed a submaximal exercise test to determine the workload that elicited a \( \text{V}_{\text{O2}} \) corresponding to 60% of \( \text{V}_{\text{O2peak}} \).

Study design. Each subject was studied on three occasions in the whole room indirect calorimeter located in the GCRC at the University of Colorado Hospital. The first study day served as a baseline day, and energy intake on the subsequent experimental days was based on 24-h EE measured during this stay, permitting energy balance to be targeted with greater precision on the experimental day. The studies were performed in a nonrandomized manner, with the Con day occurring during the second stay and the Ex day occurring during the third stay. This was done to confirm the state of energy balance under the nonexercise condition and to make further adjustments to energy intake if necessary (no further adjustments were required). The experimental days (2nd and 3rd calorimeter measurements) were separated by 1–3 wk. To minimize the effects of variations in energy and macronutrient intake, the men consumed a controlled outpatient diet for 5 days before the experimental calorimeter measurements. The daily energy content of the outpatient diet was estimated to meet free-living energy requirements \([1372 \times 23.9\ \text{fat-free mass (FFM)} \times 1.4 – 1.5]\), and the macronutrient composition was 30% fat, 55% carbohydrate, and 15% protein. Breakfast was consumed in the GCRC, and other meals were packaged and taken with the subject. All food was required to be consumed, and no other food was permitted. Two optional food modules (200 kcal each, 30% fat, 55% carbohydrate, 15% protein) were provided in the event that the subject experienced hunger. The calorimeter study was performed on the sixth day. Energy intake in the calorimeter was based on 24-h EE measured during the baseline day, and the macronutrient composition was the same as the outpatient diets.

Calorimeter protocol. Volunteers entered the calorimeter at 0800 and exited at 0700 the following day. The data were extrapolated to 24-h values, based on average minute values. Two bouts of bench-stepping exercise (3 \( \times \) 10 min at 72 steps/min) were performed each day at 1430 and 1830. The purpose of these bouts was to mimic physical activity performed outside the calorimeter. Meals were provided at 0900, 1315, and 1730, and a light snack was provided at 2000. The men were free to move about the calorimeter during other times of the day, but primarily this time was spent in sedentary behavior (reading, writing, watching television). The men were instructed to remain awake and not nap, to not perform any exercise other than that prescribed by the protocol, and to go to bed at the same time during each calorimeter stay. On the exercise day, the men performed a bout of stationary cycling at 1000, and then they remained quietly seated watching television or reading until the lunch meal was served (1315). The exercise session induced a net increase of 300 kcal above resting levels and was performed at the workload corresponding to 60% of \( \text{V}_{\text{O2peak}} \). Stepping and cycling exercises were supervised by the investigator. The power output on the cycle ergometer was controlled external to the calorimeter, and thus it could not be changed by the participant. Energy intake on the exercise day was adjusted (+375 kcal) for the estimated increased EE due to the exercise bout. Based on a previous study (16), we assumed that that level of exercise (300 kcal) would produce an excess postexercise \( \text{VO2} \) equivalent to ~75 kcal.

Venous blood samples (~7.0 ml) were obtained at several time points during the calorimeter session. Because continuous or frequent blood sampling is not possible in the room calorimeter, we selected time points to represent fasting (before entry, after exiting the calorimeter), postprandial (after lunch), premeal (before dinner), and postexercise measurements. The men extended their arm through a leak-free port in the calorimeter wall to provide blood samples. Blood samples were obtained from a forearm vein; therefore, the circulating measurements represent arterial concentrations plus venous drainage from the local muscle bed.

24-h EE and substrate oxidation. Total daily EE and substrate oxidation were determined from \( \text{V}_{\text{O2}} \) and carbon dioxide production measured in the calorimeter. Gas volumes were determined from the flow rate and the difference in carbon dioxide and oxygen concentrations between entering and exiting air using Hartman and Braun (Frankfurt, Germany) oxygen (Magnos 4 G) and carbon dioxide (Uras 3 G) analyzers. Measured gas volumes were expressed as standard temperature pressure dry (STPD). Urine was collected for the duration of the calorimetry stay and analyzed for total nitrogen concentration (26), which was then used to determine 24-h protein oxidation (13). EE and substrate oxidation were calculated from \( \text{V}_{\text{O2}} \) and the RQ based on the equations of Jequier et al. (9). Values for all variables were averaged over 1-min intervals and recorded to a data file. The operation of the calorimeter was controlled, and data were collected minute by minute using a customized computer program. EE and RQ data were also calculated during the exercise period, during the stepping exercise bouts, and during sleep (1 AM to 4 AM).

Blood analyses. Whole blood (2.5 ml) was added to 40 ml of preservative (3.6 mg EDTA plus 2.4 mg glutathione in distilled water) for plasma norepinephrine determination. The remaining sample was allowed to clot, and the serum was separated after spinning. Serum was stored at ~80°C until analyzed. All samples were assayed for glucose, insulin, the free fatty acids (FFA), epinephrine, and norepinephrine concentrations. Glucose concentrations were determined using the hexokinase method (Roche Indianapolis, IN). Insulin concentrations were measured using standard, double-antibody radioimmunoassay (Diagnostic Systems Laboratory, Webster, CT). FFA concentrations (Wako Chemical, Richmond, VA) were determined using direct enzymatic-calorimetric assays (COBAS Mira Plus Chemistry analyzer). Plasma epinephrine and norepinephrine concentrations were determined using HPLC technology (Dionex HPLC System, Sunnyvale, CA).

Statistical analysis. Statistical analyses were carried out using SAS (SAS Institute, Cary, NC). Data were examined for outliers or unusual features and appropriate remedies were used (e.g., double check unusual data values, log non-normally distributed data). Independent-sample \( t \)-tests were used to compare characteristics of the two age groups at baseline and to compare calorimeter measurements for the two age groups. Paired \( t \)-tests were used to compare calorimeter measurements between the exercise and sedentary days. Blood measurements glucose, insulin, FFA, epinephrine, and norepinephrine were analyzed using repeated-measures analysis of variance with with-in-subject factor exercise condition, and between-subject factor age group was used to analyze and compare time profiles between groups, conditions, and times. SAS PROC MIXED with a heterogeneous compound symmetric covariance structure was used. These methods provide valid handling of the occasional observations missing for random reasons. Contrasts were used within these models to carry out particular comparisons of interest. Because the data were not normally distributed, insulin and norepinephrine were log transformed before analysis. All results are presented in the original units (not...
RESULTS

Subject characteristics are presented in Table 1. The groups did not differ in weight and BMI, although percent body fat was slightly higher in OM ($P = 0.17$). Fasted glucose ($93 \pm 9$ vs. $82 \pm 5$ mg/dl) was significantly higher in OM ($P < 0.01$), but based on homeostasis model assessment (HOMA) (14), none of the men were insulin resistant (HOMA $\geq 4.0$). HOMA scores were not different in OM ($1.8 \pm 1.0$) and YM ($1.4 \pm 0.9$). Fasted total cholesterol ($181 \pm 42$ vs. $194 \pm 28$ mg/dl, OM vs. YM), high-density lipoprotein ($48 \pm 9$ vs. $45 \pm 8$ mg/dl), low-density lipoprotein ($111 \pm 32$ vs. $111 \pm 28$ mg/dl), and triglyceride ($112 \pm 47$ vs. $138 \pm 47$ mg/dl) concentrations were also not different in OM and YM, respectively. Exercise-related measurements at baseline are presented in Table 2. As expected, $V_{\text{O2peak}}$ was significantly lower in OM compared with YM. Consequently, the workload corresponding to the target exercise intensity (60% of $V_{\text{O2peak}}$) was lower in OM, and the duration necessary to achieve the target EE (net expenditure of 300 kcal) was longer.

EE. 24-h EE did not significantly differ between OM and YM on either the Con or Ex days (Table 3), but 24-h EE was significantly increased in both groups on the Ex day compared with the Con day ($P < 0.01$). EE during the stepping exercise bouts did not differ in OM or YM on either the Con or Ex days, and it did not differ within groups between the Con and Ex days. Net EE (exercise EE $- \text{RMR}$) was not significantly different in OM ($328 \pm 66$ kcal) and YM ($377 \pm 42$ kcal). Sleep EE did not differ in OM and YM on either the Con or Ex days. However, sleep EE was slightly but significantly ($P < 0.05$) increased on the Ex day in both OM and YM. Energy balance did not differ in OM and YM on either the Con ($65 \pm 62, -22 \pm 182$, respectively) or Ex days ($63 \pm 153, -56 \pm 245$, respectively).

RQ. The 24-h RQ (Table 4) was significantly lower ($P < 0.05$) in OM compared with YM both the Con (95% confidence interval, OM: 0.871, 0.926, YM: 0.809, 0.893) and Ex days (95% confidence interval, OM: 0.895, 0.945, YM: 0.842, 0.906). RQ during the stepping exercise bouts was lower in OM compared with YM on both Con or Ex days, but it was significantly different only during the first stepping exercise bout. Within groups, RQ did not significantly differ between the Con and Ex days. RQ during exercise was not significantly different in OM compared with YM. Sleep RQ did not differ in OM and YM, and it did not differ on the Con and Ex day in both OM and YM. Examination of the 24-h RQ profiles (Fig. 1) suggests that differences in RQ were more pronounced during the waking hours, with the largest differences occurring just before lunch.

24-h Substrate oxidation. Values for 24-h protein oxidation did not differ between groups on either the Con or Ex, and they were not affected by the exercise condition (Fig. 2). In absolute terms (g/day), 24-h carbohydrate oxidation was significantly lower in OM on both the Con ($P = 0.03$) and Ex days ($P = 0.02$), and it significantly increased in both OM and YM on the Ex day. The difference in carbohydrate oxidation between OM and YM on the Con (OM $= 3.10 \pm 0.89$, YM $= 4.42 \pm 1.45 \text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}; P = 0.06$) and Ex days (OM $= 4.47 \pm 0.96$, YM $= 5.84 \pm 1.76 \text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}; P = 0.09$) was no longer significant when expressed relative to total body mass. The same was true when carbohydrate oxidation was expressed relative to FFM (Con, 4.17 $\pm 1.17$ vs. 5.59 $\pm 1.49 \text{g}\cdot\text{kg}^{-1}\cdot\text{FFM}^{-1}\cdot\text{day}^{-1}; P = 0.07$; Ex, 6.05 $\pm 1.42$ vs. 7.40 $\pm 1.44 \text{g}\cdot\text{kg}^{-1}\cdot\text{FFM}^{-1}\cdot\text{day}^{-1}; P = 0.10$, OM and YM, respectively).

Absolute fat oxidation was greater in OM compared with YM on both the Ex day ($P = 0.03$) but not the Con day ($P = 0.10$). Fat oxidation did not significantly increase in either group on the Ex day (Fig. 2). When expressed relative to total body mass, fat oxidation was still greater in OM on the Ex day ($1.21 \pm 0.38$ vs. $0.69 \pm 0.34 \text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}; P = 0.02$), and the comparison on the Con day reached borderline significance ($1.28 \pm 0.58$ vs. $0.79 \pm 0.22 \text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}; P = 0.07$). The same was true when fat oxidation was expressed relative to FFM (Con, 1.74 $\pm 0.80$ vs. 1.02 $\pm 0.29 \text{g}\cdot\text{kg}^{-1}\cdot\text{FFM}^{-1}\cdot\text{day}^{-1}; P = 0.06$; Ex, 1.61 $\pm 0.47$ vs. $0.89 \pm 0.41 \text{g}\cdot\text{kg}^{-1}\cdot\text{FFM}^{-1}\cdot\text{day}^{-1}; P < 0.01$).

Blood measurements. Repeated-measures ANOVA revealed a significant effect of time for blood glucose during both exercise conditions for OM and YM ($P < 0.001$), but there was no differences in the pattern across time for the OM vs. YM or during Ex vs. Con. Post hoc analyses indicated that glucose was significantly higher ($P < 0.0001$) after the lunch meal compared with the other time points, and on average it was slightly higher in OM compared with YM ($P = 0.004$).

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Table 1. Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>Age yrs</th>
<th>BMI, kg/m²</th>
<th>Fat-Free Mass, kg</th>
<th>Fat Mass, kg</th>
<th>Body Fat, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>OM</td>
<td>65±5 (60–73)</td>
<td>26.3±2.8 (22.7–31.4)</td>
<td>60.5±8.9 (49.7–70.6)</td>
<td>20.8±3.4 (17.9–25.7)</td>
<td>25.9±3.5 (21.0–29.9)</td>
</tr>
<tr>
<td>YM</td>
<td>25±5* (21–29)</td>
<td>25.5±4.7 (19.4–32.7)</td>
<td>64.6±5.8 (56.6–72.1)</td>
<td>20.2±11.7 (5.4–35.0)</td>
<td>21.9±9.7 (8.1–33.9)</td>
</tr>
</tbody>
</table>

Values are means ± SD with range in parentheses. OM, older men; YM, younger men; BMI, body mass index. *$P < 0.05$ vs. OM.

Table 2. Maximal aerobic capacity and submaximal workload and duration of exercise performed in the calorimeter on the exercise day

<table>
<thead>
<tr>
<th></th>
<th>$V_{\text{O2peak}}, \text{l/min}$</th>
<th>$V_{\text{O2peak}}, \text{ml\cdotkg}^{-1}\cdot\text{min}^{-1}$</th>
<th>Submaximal Exercise Workload, W</th>
<th>Submaximal Exercise Duration, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>OM</td>
<td>2.1±0.5* (1.7–2.8)</td>
<td>25.6±3.8* (21.8–31.8)</td>
<td>94±35* (35–150)</td>
<td>62±15* (40–75)</td>
</tr>
<tr>
<td>YM</td>
<td>3.4±0.4* (3.0–3.9)</td>
<td>40.7±9.3* (29.1–54.1)</td>
<td>135±26* (90–170)</td>
<td>34±4* (29–40)</td>
</tr>
</tbody>
</table>

Values are means ± SD with range in parentheses. $V_{\text{O2peak}}$, maximal aerobic capacity. *$P < 0.05$ vs. OM.
insulin concentrations were significantly different at all times \((P < 0.01)\) except at the first (baseline) and last (exit) times, and they were highest after the lunch meal compared with the other time points. There was a significant difference in time patterns between OM and YM \((P = 0.004\) for the group \(\times\) time interaction), but these differences were slight. For FFA, there were different patterns over time for the age groups and during the exercise conditions (Fig. 3, \(P < 0.0001\) for both group \(\times\) time and condition time interactions). At the postexercise measurement, plasma FFA concentrations decreased below baseline values in both groups under both conditions. Plasma FFA concentrations remained below baseline values for the remainder of the day in YM. However, in OM, plasma FFA concentrations increased at the postlunch measurement. Examination of intraindividual differences in FFA between Con and Ex at each time showed a difference between Con and Ex of \(-153.4 \pm 122.2 \mu\text{eq/l}\) for OM and \(7.3 \pm 99.6 \mu\text{eq/l}\) for YM at the postlunch measurement. Postlunch FFA were 153 \(\mu\text{eq/l}\) higher \((P = 0.016)\) in OM on the Ex compared with the Con day \((P = 0.86)\), indicating a significant group \(\times\) condition interaction. Stated another way, the difference in FFA obtained at the postexercise time point was significantly different between the groups, with the OM exhibiting a significant increase in postexercise FFA relative to the sample obtained at the same time on the Con day and with the YM showing no difference. Similar interactions at other times were nonsignificant \((P > 0.4)\). Before the dinner meal, plasma FFA were still significantly higher in OM than YM \((P < 0.05)\). Plasma FFA concentrations returned to baseline values on exit from calorimeter and were not different between groups or condition. There was a significant condition \(\times\) time interaction for plasma norepinephrine \((P < 0.0001)\), as well as a significant main effect of group. Post hoc analyses indicated that norepinephrine concentrations were on average significantly higher in OM, and higher at the postexercise measurement on the Ex compared with the Con day (Fig. 4).

**DISCUSSION**

The primary aim of this study was to compare 24-h fat oxidation in OM and YM under sedentary and exercise conditions. Under highly controlled conditions (in terms of energy and macronutrient intake and energy expenditure), we found that 24-h fat oxidation was not reduced in OM compared with YM in either condition. Rather, contrary to our hypothesis, 24-h fat oxidation was greater in OM compared with YM. If fat oxidation had been lower in OM compared with YM, this would have given support to the hypothesis that age-related

### Table 4. Respiratory quotient

<table>
<thead>
<tr>
<th></th>
<th>YM</th>
<th>OM</th>
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<tbody>
<tr>
<td><strong>Nonexercise day</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24-h RQ</td>
<td>0.899±0.030†</td>
<td>0.851±0.045</td>
</tr>
<tr>
<td>First stepping bout*</td>
<td>0.929±0.027†</td>
<td>0.885±0.043</td>
</tr>
<tr>
<td>Second stepping bout*</td>
<td>0.951±0.047</td>
<td>0.895±0.083</td>
</tr>
<tr>
<td>Sleep</td>
<td>0.814±0.049</td>
<td>0.790±0.048</td>
</tr>
<tr>
<td><strong>Exercise day</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24-h RQ</td>
<td>0.920±0.027†</td>
<td>0.874±0.035</td>
</tr>
<tr>
<td>First stepping bout*</td>
<td>0.958±0.055</td>
<td>0.863±0.076</td>
</tr>
<tr>
<td>Second stepping bout*</td>
<td>0.940±0.030</td>
<td>0.904±0.048</td>
</tr>
<tr>
<td>Exercise</td>
<td>0.978±0.035</td>
<td>0.964±0.039</td>
</tr>
<tr>
<td>Sleep</td>
<td>0.800±0.005</td>
<td>0.828±0.065</td>
</tr>
</tbody>
</table>

Values are means ± SD with range in parentheses. RQ, respiratory quotient. *EE from the hour encompassing the 3 \(\times\) 10-min stepping bouts. †\(P < 0.05\) vs. OM.
decreases in fat oxidation contribute to increases in FM in aging individuals. In the present study, we chose to impose a similar load of exercise (in terms of EE) to closely match the EE between the groups. However, to achieve this, OM needed to exercise nearly twice as long as YM. Although it could be argued that this may have confounded the present results because of an increased reliance on fat in the OM (2, 18, 25), no differences were observed in RQ during exercise in OM and YM. Moreover, fat oxidation was higher in the OM on both the Con and Ex days. Thus our findings suggest that other factors (e.g., declines in total daily EE because of decreased physical activity) are a more important determinant of age-related changes in FM.

Our conclusion that 24-h fat oxidation is not impaired in OM differs with those of two previous room calorimetry studies. Levadoux et al. (12) reported lower 24-h fat oxidation rates were in older compared with young adults on a day that exercise was performed. However, these results may have been confounded by differences in habitual physical activity and lack of prestudy control for energy intake or exercise, important considerations to ensure a similar state of energy balance and fuel repletion at the time of testing. It is also unclear whether subjects were in energy balance during the calorimeter measurement. In another study, Davy et al. (5) reported that the ability to adjust substrate oxidation in response to increases in

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Fig. 1. 24-h respiratory quotient (RQ) profiles in older men (OM) and younger men (YM) on the nonexercise control (Con top) and exercise (Ex; bottom) days. Error bars are SE. Data for each individual were averaged for each 60-min interval and then averaged. Upward arrows, time of the meals; downward arrow, start of exercise.

Fig. 2. 24-h Substrate oxidation in OM and YM on the Con and Ex days. Error bars are SE. *YM vs. OM, \( P < 0.05 \). †Con vs. Ex, \( P < 0.05 \).

Fig. 3. Plasma free fatty acid (FFA) concentrations in OM and YM on the Con (top) and Ex (bottom) days. Error bars are SE. *YM vs. OM, \( P < 0.05 \). †Con vs. Ex, \( P < 0.05 \).

Fig. 4. Plasma norepinephrine concentrations in OM and YM on the Con (top) and Ex (bottom) days. Error bars are SE. Post-ex, postexercise. *YM vs. OM, \( P < 0.05 \). †Con vs. Ex, \( P < 0.05 \).
dietary fat intake was similar in older \((n = 6, 63 \pm 3\) yr) and younger \((n = 6, 25 \pm 1\) yr) men. Although there were no significant differences in substrate oxidation between the groups, 24-h RQ tended to be lower in the older men on both the high-carbohydrate and high-fat diets, suggesting an increased utilization of fat in the older men, which is consistent with the results of the present study. Additionally, these authors concluded that factors other than age-related changes in the capacity to oxidize fat contribute to the increases susceptibility of obesity with aging, which is in agreement with the results of the present study.

Although previous studies have reported lower fat oxidation rates in older compared with younger adults (2, 18, 25), those studies were performed in the fasting state. In the present study, men were provided breakfast, lunch, and dinner meals, and thus they spent a substantial portion of the day in the postprandial state. As will be discussed below, we believe that differences in the hormonal milieu in the fasted and postprandial states in older and younger adults contribute to differences in fuel availability and substrate oxidation. Although two previous studies reported a lower postprandial fat oxidation in older men (23) and women (17), this was only observed when subjects were overfed; there were no differences in postprandial fat oxidation after consumption of smaller meals.

It is worth noting that the values observed for 24-h RQ in sedentary YM in the present study are higher than those observed in another group of moderately active (3–5 h exercise/wk) younger men in a previous study conducted by our group (16). We might expect a higher 24-h RQ (and therefore lower 24-h fat oxidation) in the more sedentary individuals. However, 24-h RQ in the more active younger men was similar to that of the sedentary older men in our present study. Thus, if the more active younger men served as our comparison group, we would still conclude that 24-h fat oxidation is not reduced in older men.

A second finding of the present study was that there were differences between groups in plasma FFA availability in OM and YM at different times of the day. Interestingly, the elevation in plasma FFA coincided with the period of time in which RQ was lower in OM (Fig. 1), suggesting a relative increase in fat oxidation at these time points. In humans, increasing FFA availability (e.g., through infusion of intralipid plus heparin, consumption of caffeine) causes an increase in fat oxidation during exercise (8). Thus we speculate that differences in FFA availability contributed the observed differences in 24-h macronutrient oxidation. In apparent contrast to our findings, Sial et al. (25) reported that fat oxidation and the rate of appearance of FFA was lower in older compared with younger men during exercise performed at the same relative intensity (~56% of maximal \(V_{\text{O2}}\)). However, unlike the present study, measurements in that study were made in a fasted state. Penev et al. (21) recently reported that there is a greater catecholamine response following meal consumption in older (50–69 yr) compared with younger men (20–28 yr). Consistent with these results, we observed higher circulating norepinephrine concentrations in OM (Fig. 4) in the postprandial periods (postlunch and predinner measurements). Thus it is possible that higher postprandial catecholamine concentrations in OM promote a greater postprandial availability of plasma FFA, as observed in the present study (Fig. 3). Moreover, OM likely had a greater amount of visceral fat (1, 3, 7), and visceral fat appears to be more resistant to the antilipolytic effects of insulin (15, 20). Under these conditions, we hypothesize that a reduction in insulin-stimulated inhibition in lipolysis in OM combined with greater catecholamine responses to meal ingestion and exercise contributed to a greater availability of FFA and thus fat oxidation. We further hypothesize that this increase in FFA availability overrides any age-related reductions in the maximal ability to mobilize and utilize fat. Because exercise further stimulates catecholamine stimulation, postprandial FFA availability is further increased by the effects of prior exercise, as observed in the present study (Fig. 3). It is interesting to note that RQ was lower in OM during the first stepping exercise bout on both the Con and Ex days, but it did not differ in OM and YM during the second stepping exercise bout. The first stepping bout was performed at 1430, and the second stepping bout was performed at 1830. As shown in Figs. 3 and 4, FFA and norepinephrine were higher in OM after lunch and before dinner. The first stepping period occurred in this period. Although we did not obtain blood samples before or after the stepping bout, we assume that FFA were elevated in OM and therefore contributed to an increased fat oxidation in OM. Indeed, RQ was lower in OM at this time point. Conversely, RQ did not differ during the second stepping bout. Because we did not obtain blood samples after dinner, we can only speculate that FFA concentrations were similar in OM and YM during the evening, perhaps because of a continued declined in catecholamine-stimulated lipolysis.

In agreement with our laboratory’s previous study (16), we observed no difference in 24 h RQ in either OM or YM between the Con and Ex days. The absence of an effect of exercise on 24-h RQ or substrate oxidation suggests that a compensation in substrate oxidation occurred during the remaining portions of the day, i.e., there was an increase in fat oxidation during the nonexercise portion of the day. We examined the sleeping periods, but not detect any differences in sleeping RQ (Table 2). We hypothesize that this compensation is an integrated response, and therefore it may not be detectable when analyzing a small segment of the 24-h record. Importantly, there was no change in 24 h RQ in either OM or YM, suggesting that this compensation occurred in both groups.

There are several limitations to the present study. First, we only studied men to avoid confounding effects of the menstrual cycle and menstrual status (e.g., pre- vs. postmenopausal). Thus studies in women are warranted. Second, blood samples were only obtained at select time points because it is not possible to obtain more frequent samples in the room calorimeter. However, review of the individual data indicated that the responses (e.g., lower RQ, higher postprandial FFA, and higher norepinephrine concentrations throughout the day) were robust and observed in all OM. We also did not measure any postexercise response. Although subjects spent this time primarily engaged in sedentary activities, more rigorous control of physical activity during the postexercise periods would be required for these associations to be meaningful. Finally, the reader should be aware that room calorimeters are designed to provide measurements of integrated responses over extended periods of time. In general, the shorter the measurement period, the more noise in the data, particularly during periods of low EE (e.g., sleep). However, the shortest period of time analyzed in the present study was 60 min (during the stepping period), and the
EE associated with this period was consistent within and between days (Table 3).

In conclusion, under controlled energy-balance conditions, 24-h fat oxidation was greater in OM than YM. In addition, in OM, we observed higher plasma FFA concentrations compared with YM during postexercise period and higher norepinephrine concentrations throughout the day. We speculate that this resistance to the antilipolytic effects of insulin in OM combined with a greater catecholamine response to meal ingestion leads to an increased availability of FFA in the postprandial state. Although there are age-related decreases in the maximal capacity of skeletal muscle to oxidize fat (4), the results of the present study suggest that under physiological conditions, older men will oxidize more fat than younger men. Thus we suggest that factors other than age-related changes in the capacity to oxidize fat contribute to the gain in FM that occurs with aging.

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