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Muscle fatty acid oxidative capacity is a determinant of whole body fat oxidation in elderly people

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Morio, Béatrice, Jean-François Hocquette, Christophe Montaurier, Yves Boirie, Corinne Bouteloup-Demange, Caroline McCormack, Nicole Fellmann, Bernard Beaufère, and Patrick Ritz. Muscle fatty acid oxidative capacity is a determinant of whole body fat oxidation in elderly people. *Am J Physiol Endocrinol Metab* 280: E143–E149, 2001.—In sedentary elderly people, a reduced muscle fatty acid oxidative capacity (MFOC) may explain a decrease in whole body fat oxidation. Eleven sedentary and seven regularly exercising subjects (65.6 ± 4.5 yr) were characterized for their aerobic fitness [maximal O₂ uptake ($\dot{V}O_{2\max}$)/kg fat free mass (FFM)] and their habitual daily physical activity level [free-living daily energy expenditure divided by sleeping metabolic rate (DEE_{FLC}/SMR)]. MFOC was determined by incubating homogenates of vastus lateralis muscle with [¹⁻¹⁴C]palmitate. Whole body fat oxidation was measured by indirect calorimetry over 24 h. MFOC was 40.4 ± 14.7 and 44.3 ± 16.3 nmol palmitate·g wet tissue⁻¹·min⁻¹ in the sedentary and regularly exercising individuals, respectively ($P =$ nonsignificant). MFOC was positively correlated with DEE_{FLC}/SMR ($r = 0.58$, $P < 0.05$) but not with $\dot{V}O_{2\max}/kg$ FFM ($r = 0.35$, $P =$ nonsignificant). MFOC was the main determinant of fat oxidation during all time periods including physical activity. Indeed, MFOC explained 19.7 and 30.5% of the variance in fat oxidation during walking and during the alert period, respectively ($P < 0.05$). Furthermore, MFOC explained 23.0% of the variance in fat oxidation over 24 h ($P < 0.05$). It was concluded that, in elderly people, MFOC may be influenced more by overall daily physical activity than by regular exercising. MFOC is a major determinant of whole body fat oxidation during physical activities and, consequently, over 24 h.

habitual physical activity; endurance training; indirect calorimetry; vastus lateralis muscle

THE INCREASE IN FAT MASS observed during aging (13) results from an imbalance between fat intake and fat utilization. Fat intake does not seem to increase with aging (11, 26); by contrast, fat utilization may decrease with advancing age. Indeed, whole body fat oxidation

was shown to be lower in elderly people compared with young adults after meal ingestion (20), during moderate-intensity exercise (32), and at rest in some studies (7, 8), but not all (5, 16).

Part of the age-related defect in fat oxidation may be explained by decreasing physical activity. In fact, combined results from the literature suggest that regular physical training prevents the changes in age-related fuel metabolism. Hence, the defect in fat oxidation during exercise observed in elderly people compared with young adults almost disappeared after 16 wk of endurance training (33). Moreover, Horber et al. (18) have shown that, compared with young adults, fat oxidation at rest was significantly lower in sedentary elderly men but not in endurance-trained elderly men. Finally, endurance training was shown to stimulate fat oxidation at rest in sedentary elderly people (28), albeit in a time- or intensity-dependent manner (23).

The mechanisms of alterations in fat oxidation are still not clear. Part of the changes in fat oxidation could be explained by changes in muscle mass, e.g., age-related loss (7) or training-induced gain (23, 28). However, differences in fat oxidation still exist when body composition is taken into account (18, 23, 33). This suggests a defect of fat oxidation intrinsic to muscle.

In this respect, it has been suggested that the decrease in fat oxidation during exercise may be caused by a reduced capacity of muscle to oxidize fatty acids (33). This defect has been reported in young sedentary men (compared with young athletes) and has been explained by a reduced long-chain fatty acid entry into the mitochondria (34). However, it has not been shown at rest and/or in elderly subjects.

Changes in the capacity of muscle to oxidize fatty acids have often been assessed using the maximal activity of β -hydroxy-acyl-CoA dehydrogenase (HAD) as an indicator of the mitochondrial β -oxidation pathway (1, 10). However, the maximal activity of a single

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enzyme operating within a complex pathway is unlikely to represent the entire process (3). In fact, other limiting factors, such as the activity of carnitine palmitoyltransferase I (CPT-I) and that of the tricarboxylic acid cycle could modulate muscle fatty acid oxidative capacity (17). For these reasons, we chose, in the present study, to assess the maximal activity of the overall fatty acid oxidation pathway in muscle using an ex vivo method. This technique is based on the incubation of fresh muscle homogenates with [^{14}C]palmitate as a substrate (27, 39).

Thus the present study was aimed at determining 1) whether the muscle fatty acid oxidative capacity of elderly subjects was modulated by physical activity and 2) whether any changes in muscle fatty acid oxidative capacity had significant consequences on the main components of 24-h whole body fat oxidation, e.g., exercise, alert period, and sleep. Two levels of physical activity were taken into account: exercise status and habitual physical activity level. For that purpose, the subjects were recruited based on their self-reported time spent exercising per week, i.e., either they did not participate in any regular endurance exercise program or they regularly exercised by walking, running, cycling, and/or swimming ≥ 5 h/wk. Thereafter, each subject had his or her level of daily physical activity determined over 3 days in free-living conditions.

SUBJECTS AND METHODS

Subjects and General Study Design

Subjects. Eighteen healthy elderly people participated in the study. Eleven subjects (7 men and 4 women, 65.8 ± 4.2 yr) were sedentary, i.e., they did not participate in any regular endurance exercise program. Seven subjects (5 men and 2 women, 65.4 ± 4.5 yr) regularly exercised by walking, running, cycling, and/or swimming ≥ 5 h/wk. All subjects had taken a medical examination; they were nonsmokers, were not suffering from any diagnosed disease, and were under no medication known to influence energy metabolism. All women were postmenopausal. The Medical School Ethics Committee for Biomedical Research approved the study.

General study design. The subjects completed an activity questionnaire and performed a maximal aerobic power test. Kinetics of heart rate were recorded over 3 days (from Friday to Sunday) in free-living conditions. Thereafter, the volunteers were placed on a controlled diet 2 days before and throughout the measurement period. For that purpose, eight daily menus (35% of energy as lipids, 50% as carbohydrates, 15% as proteins) were determined individually according to each subject's basal metabolic rate predicted (4) from his or her body composition [determined from bioelectrical impedance analysis measurements (37)] and daily activities (known from the activity questionnaire) by use of the factorial method (24). Recipes were simple and detailed precisely for the volunteers to cook easily. The following measurements were performed on separate days during the diet-controlled period: body composition [dual-energy X-ray absorptiometry (DEXA) scan; see *Body composition measurements*], muscle biopsy, and 36-h whole body indirect calorimetry.

Subjects Characterization

Activity questionnaire, maximal aerobic power test, and heart rate recordings. The Baecke activity questionnaire (2) was used to calculate the time spent exercising per week and to determine the type of activities performed on a weekly basis.

The maximal aerobic power tests were all performed on the same cycloergometer (Ergomeca, Monark, Sweden) under cardiovascular supervision by a cardiologist, with use of the protocol described recently (23). Maximal oxygen uptake ($\dot{V}\text{O}_{2\text{max}}$) was determined when the following criteria were reached: a plateau in oxygen consumption, a respiratory quotient > 1.1 , and a maximal heart rate close to the theoretical maximal heart rate [$220 - \text{age}$ (yr)].

The physical activity level (including housework and leisure and sports activities) was characterized by the ratio of free-living daily energy expenditure (DEE_{FLC}) over sleeping metabolic rate (SMR; $\text{DEE}_{\text{FLC}}/\text{SMR}$; see Ref. 21). DEE_{FLC} was determined using the heart rate recording method as described recently (24). Briefly, heart rate was recorded minute-by-minute using telemetry (Polar Protrainer, Polar Electro, Oy, Finland) during 3 days, from Friday morning until Sunday night. Energy expenditure was calculated from these recordings by means of individual relationships set up between heart rate and energy expenditure measured in the calorimeters (24). SMR was measured in the calorimeters.

Body composition measurements. Body mass was measured to the nearest 0.1 kg on a SECA 709 scale (SECA, Les Mureaux, France). Height was measured to the nearest 0.2 cm. A total body scan was performed using DEXA (Hologic QDR 4501, Hologic, Waltham, MA) for determination of total and regional (arms, legs, and trunk) body composition. Fat free mass (FFM) was calculated as the sum of lean mass, soft tissue, and bone mineral content (36).

Muscle Biopsy and Assays

Materials. [^{14}C]palmitic acid was purchased from Amer-sham International (Bucks, UK). ATP, NAD^+ , and cytochrome *c* were supplied by Boeringer Mannheim (Meylan, France). Acetyl-coenzyme A, fatty acid-free bovine serum albumin, L-carnitine, palmitic acid, oxaloacetate, L-malate, and coenzyme A were purchased from Sigma (St. Louis, MO). Other chemicals used were of the highest grade commercially available.

Muscle biopsy and assay of palmitate oxidation capacity. Biopsies (60–120 mg) were obtained from the vastus lateralis muscle at 0800 after one night of fasting. Tissue was cut into pieces and cooled in ice-cold buffer consisting of 0.25 M sucrose, 2 mM EDTA, and 10 mM Tris·HCl (pH 7.4). Muscle homogenate (5% wt/vol) was rapidly prepared in the same buffer by hand homogenization with a glass-glass homogenizer (27, 39). Two pestles with different diameters were used (intervening space 0.050 and 0.075 mm). Palmitate oxidation rate was measured using sealed vials in a medium (pH 7.4) containing (in mM): 25 sucrose, 75 Tris·HCl, 10 K_2HPO_4 , 5 MgCl_2 , and 1 EDTA supplemented with 1 NAD^+ , 5 ATP, 0.1 coenzyme A, 0.5 L-malate, 0.5 L-carnitine, and 25 μM cytochrome *c* (39). All assays were performed in triplicate under conditions that were optimal with respect to time and concentration of palmitate and of tissue material (27, 39). After 5 min of preincubation at 37°C with shaking, the reaction was started by addition of 100 μl of 600 μM [^{14}C]palmitate bound to albumin in a 5:1 molar ratio. The final incubation volume was thus 0.5 ml, containing 75 μl of muscle homogenate. The oxidation proceeded for 30 min at 37°C and was stopped by addition of 0.2 ml of 3 M perchloric

acid. The released $^{14}\text{CO}_2$ was trapped in 0.3 ml ethanalamine-ethylene glycol (1:2 vol/vol) and measured by liquid scintillation counting in 5 ml of Ready Safe (Beckman Instruments, Fullerton, CA). After 90 min at 4°C, the acid incubation mixture was centrifuged for 5 min at 10,000 *g*, and the 0.5-ml supernatant containing ^{14}C -labeled perchloric acid-soluble products was assayed for radioactivity by liquid scintillation. Total palmitate oxidation rate was calculated from the sum of $^{14}\text{CO}_2$ and ^{14}C -labeled acid-soluble products (39) and expressed in nanomoles palmitate per gram of wet tissue per minute.

Analytical assays. HAD, cytochrome *c* oxidase, and citrate synthase activities were assayed spectrophotometrically on the above muscle homogenates as described previously (6, 27, 39). One unit of enzyme is defined as the amount that catalyzes the oxidation of 1 $\mu\text{mol}/\text{min}$ of cytochrome *c* for cytochrome *c* oxidase (at 25°C), the liberation of 1 $\mu\text{mol}/\text{min}$ of coenzyme A for citrate synthase (at 25°C), and the disappearance of 1 $\mu\text{mol}/\text{min}$ of NADH for HAD (at 30°C).

Measurements in the Calorimeters

Activity program and food intakes in the calorimeters. The activity program in the calorimeters consisted of four periods of 30 min each of walking at 50% $\dot{V}\text{O}_{2\text{max}}$. Food energy supply was calculated individually using the factorial method (22). For that purpose, daily energy expenditure was calculated from the duration and the energy cost of the various activities in the calorimeters (e.g., walking) (24), and a predicted basal metabolic rate was calculated from each subject's body composition (4). Food energy supply provided 50% of energy as carbohydrates, 35% as lipids, and 15% as proteins.

Indirect calorimetry measurements. Respiratory gas exchanges were measured continuously using two open-circuit whole body calorimetric chambers, as described recently (21–24). Gas analyzers were calibrated upon commencement, after 13 h (evening), and at the end of the 24-h measurement period with the use of standard gas mixtures. Gas exchanges were computed from the minute-by-minute measurement of outlet air flow, differences in gas concentrations, atmospheric pressure, chamber air temperature and hygrometry, and by taking into account the gas analyzer's drifts and the variations of the volumes of CO_2 and O_2 in the chambers. The validity of gas exchange measurements was checked gravimetrically, comparing the amounts of gases (CO_2 , O_2) analyzed and those expected from the weights of gases (CO_2 , N_2) injected into the chambers during a 24-h period (40). The recovery was $96.9 \pm 0.1\%$ for O_2 and $100.1 \pm 1.0\%$ for CO_2 .

Urine was collected over 24 h, partitioned into two periods (alert period, from 0700 to 1100, and sleep, from 2300 to 0700) for the determination of urinary nitrogen excretion. Energy expenditure was calculated using Weir's equation (41) from the minute-by-minute measurement of gas exchanges, corrected for urinary nitrogen excretion. Daily energy balance was calculated from the difference between daily energy intake and daily energy expenditure and was expressed as percent daily energy expenditure.

Four time periods were determined: 1) walking (4×30 min), 2) alert period (from 0700 to 1100), 3) sleep (from 2300 to 0700), and 4) 24 h (from 0700 to 0700). Fat oxidation was calculated from gas exchanges and urinary nitrogen excretion over the periods of interest by use of Ferrannini's equations (12). In particular, respiratory gas exchanges during walking were determined over the last 20 min of each session. Gas exchanges during sleep were computed during the 2nd night in the calorimeters, over 5–6 h after the 2nd h after

which the subjects had gone to bed, as described recently (21–24).

Statistical Analyses

Results are reported as means \pm SD. The Mann-Whitney *U*-test was used for comparison of means between sedentary and regularly exercising subjects' characteristics, body composition, muscle palmitate oxidative capacity, and fat oxidation. Normality of the data was tested using the χ^2 test. Stepwise regressions were performed to determine which variables among daily energy balance, FFM, $\dot{V}\text{O}_{2\text{max}}$, and muscle palmitate oxidative capacity contributed independently to variation in fat oxidation. Correlation coefficients are Pearson product-moment correlations. Results were considered statistically significant at the 5% level.

RESULTS

Subject Characteristics

Subject characteristics, physical capacity, and body composition are given in Table 1. The aerobic fitness ($\dot{V}\text{O}_{2\text{max}}/\text{kg}$ FFM), but not the physical activity level ($\text{DEE}_{\text{FLC}}/\text{SMR}$), was significantly lower in the sedentary subjects than in the regularly exercising subjects ($P < 0.001$). There were no significant differences between sedentary and regularly exercising subjects for age and height. Body mass and fat mass were 12.7 and 32.0% higher in the sedentary group than in the regularly exercising subjects, respectively ($P = 0.10$ and $P < 0.05$). FFM and appendicular muscle mass were similar between the two groups.

Muscle Metabolic Activity

Muscle enzyme activities. The maximal activity of HAD and cytochrome *c* oxidase tended to be higher in regularly exercising subjects than in their sedentary counterparts, but the differences did not reach the level of significance (3.48 ± 0.70 vs. 2.78 ± 0.80 , $P = 0.06$, and 23.9 ± 11.13 vs. 15.09 ± 4.89 , $P = 0.08$, respectively). The maximal activity of citrate synthase was not significantly different between the two groups [4.07 ± 1.92 vs. 3.32 ± 1.07 , $P = \text{not significant (NS)}$].

Table 1. Subject characteristics according to their exercise status

	Subjects	
	Sedentary	Regularly exercising
Age, yr	65.8 ± 4.2	65.4 ± 4.5
$\dot{V}\text{O}_{2\text{max}}$, $\text{ml} \cdot \text{kg FFM}^{-1} \cdot \text{min}^{-1}$	38.5 ± 4.7	$44.5 \pm 3.1^*$
$\text{DEE}_{\text{FLC}}/\text{SMR}$	1.70 ± 0.29	1.73 ± 0.24
Height, cm	171.1 ± 10.7	168.6 ± 7.0
Body mass, kg	77.3 ± 11.0	68.6 ± 7.9
FFM, kg	56.6 ± 12.4	53.0 ± 9.7
Appendicular muscle mass, kg	24.2 ± 6.2	22.6 ± 5.1
Fat mass, kg	20.6 ± 5.6	$15.6 \pm 3.2^\dagger$

Results are means \pm SD for 11 sedentary (7 men, 4 women) and 7 regularly exercising (5 men, 2 women) subjects; $\text{DEE}_{\text{FLC}}/\text{SMR}$, ratio of daily energy expenditure determined in free-living conditions (DEE_{FLC}) over sleeping metabolic rate (SMR), measured in calorimeters; FFM, fat-free mass; $\dot{V}\text{O}_{2\text{max}}$, maximal O_2 uptake. Significantly different from sedentary subjects: * $P < 0.001$, $^\dagger P < 0.05$.

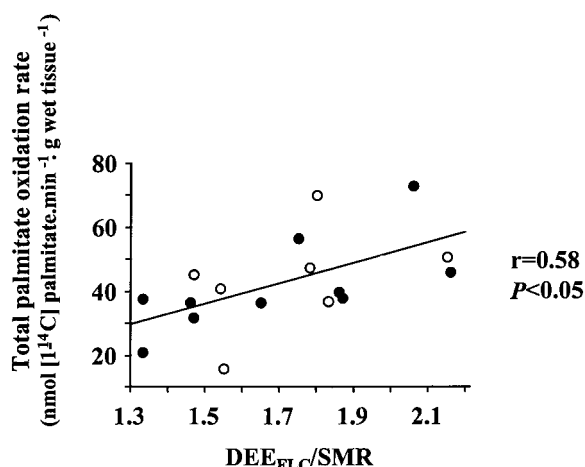


Fig. 1. Correlation between muscle palmitate oxidative capacity and the index of daily physical activity [free-living daily energy expenditure divided by sleeping metabolic rate (DEE_{FLC}/SMR)] in 11 sedentary (●) and 7 regularly exercising (○) elderly people.

Maximal activity of HAD was not significantly correlated to DEE_{FLC}/SMR or to $\dot{V}O_{2\max}/kg$ FFM ($r = 0.20$ and 0.14 , respectively, $P = NS$). Maximal activity of citrate synthase and cytochrome *c* oxidase was positively correlated with $\dot{V}O_{2\max}/kg$ FFM ($r = 0.42$ and 0.57 , respectively, $P < 0.05$) but not with DEE_{FLC}/SMR ($r = -0.18$ and -0.07 , respectively, $P = NS$).

Muscle palmitate oxidative capacity. Muscle palmitate oxidative capacity (i.e., total oxidation rate of palmitate) was 44.3 ± 16.3 and 40.4 ± 14.7 nmol palmitate $\cdot g$ wet tissue $^{-1} \cdot min^{-1}$ in the regularly exercising and sedentary groups, respectively ($P = NS$). Muscle palmitate oxidative capacity was positively correlated with DEE_{FLC}/SMR ($r = 0.58$, $P < 0.05$; Fig. 1) but not with $\dot{V}O_{2\max}/kg$ FFM ($P = NS$).

Whole Body Fat Oxidation

Measurements in the calorimeters. Energy expenditure (kJ/h and $kJ \cdot kg$ FFM $^{-1} \cdot h^{-1}$) and daily energy balance were not significantly different between the two groups. Fat oxidation (mg/h) was not significantly different between the two groups ($P = NS$). When differences in FFM were taken into account, fat oxidation ($mg \cdot kg$ FFM $^{-1} \cdot h^{-1}$) was slightly higher in the regularly exercising subjects than in the sedentary group at all measurement times, but the differences did not reach the level of significance (P ranged from 0.08 to 0.15; Table 2).

Determinants of whole body fat oxidation. Fat oxidation was always significantly correlated to daily energy balance ($r = -0.42$, -0.50 , -0.69 , and -0.62 during walking, alert period, sleep, and over 24 h, respectively, $P < 0.05$), FFM ($r = 0.36$, 0.47 , 0.48 , and 0.53 during walking, alert period, sleep, and over 24 h, respectively, $P < 0.05$ except during walking, $P = NS$), and $\dot{V}O_{2\max}$ ($r = 0.52$, 0.56 , 0.56 , and 0.63 during walking, alert period, sleep, and over 24 h, respectively, $P < 0.05$). Stepwise regressions showed that $\dot{V}O_{2\max}$ was a better determinant of fat oxidation than

Table 2. Results obtained from 24-h measurements in calorimeters

	Subjects	
	Sedentary	Regularly exercising
Fat oxidation, g/h		
During walking	7.16 ± 1.91	9.09 ± 2.84
During alert period	4.39 ± 1.13	5.83 ± 2.56
During sleep	2.67 ± 0.87	3.33 ± 1.78
Over 24 h	3.81 ± 0.88	4.70 ± 1.37
Energy expenditure, kJ/h		
During walking	296.8 ± 54.2	334.4 ± 69.8
During alert period	540.7 ± 68.6	550.9 ± 92.5
During sleep	270.4 ± 45.0	274.1 ± 45.8
Over 24 h	450.6 ± 59.5	458.6 ± 76.2
DEE_{CAL}/SMR	1.67 ± 0.10	1.70 ± 0.10
Daily energy balance (% DEE_{CAL})	-6.9 ± 7.6	-11.3 ± 10.1

Results are means \pm SD for 11 sedentary (7 men, 4 women) and 7 regularly exercising (5 men, 2 women) subjects. EE, energy expenditure; DEE_{CAL} , daily energy expenditure measured in calorimeters.

FFM at all measurement times. The amount of variance in fat oxidation explained by $\dot{V}O_{2\max}$ is presented in Table 3. Muscle palmitate oxidative capacity was the main determinant of fat oxidation during the time periods including physical activity, i.e., during walking and during the alert period (Table 3). It explained 19.7 and 30.5% of the variance in fat oxidation during walking and during the alert period, respectively ($P < 0.05$, Table 3). By contrast, muscle palmitate oxidative capacity was not a significant determinant of fat oxidation during sleep (Table 3). Finally, muscle palmitate oxidative capacity explained 23.0% of the variance of fat oxidation over 24 h ($P < 0.05$, Table 3). These results were confirmed by use of the method described by Ravussin and Bogardus (30). After adjustment for differences in daily energy balance and $\dot{V}O_{2\max}$, fat oxidation residuals were significantly correlated with muscle palmitate oxidative capacity during walking ($r = 0.46$, $P < 0.05$), during the alert period ($r = 0.59$, $P < 0.01$), and over 24 h ($r = 0.54$, $P < 0.05$), but not during sleep ($r = 0.04$, $P = NS$).

DISCUSSION

The effects of physical activity on muscle fatty acid oxidative capacity and the consequences on 24-h whole

Table 3. Significant predictors in fat oxidation

	Total Variance r^2	Variance (%) in Fat Oxidation Explained by:		
		Daily energy balance	$\dot{V}O_{2\max}$	Muscle oxidative capacity
Fat oxidation, g/h				
During walking	0.516*	16.2*	15.7*	19.7*
During alert period	0.634†	20.3*	12.6*	30.5*
During sleep	0.578*	36.6†	17.8*	NS
Over 24 h	0.678‡	26.9†	17.9†	23.0*

r^2 , Correlation coefficient derived from stepwise linear regression between fat oxidation and its predictors (daily energy balance, $\dot{V}O_{2\max}$, and muscle oxidative capacity). Additional results are the amount of variance in fat oxidation explained by each significant predictor. NS, not included in the stepwise regression. Level of significance: * $P < 0.05$, † $P < 0.01$, ‡ $P < 0.001$.

body fat oxidation were examined in elderly people. Two levels of physical activity were taken into account, the exercise status and the habitual physical activity level. For that purpose, the subjects were recruited on the basis of self-reported time spent exercising per week, i.e., either they did not participate in any regular endurance exercise program or they regularly exercised by walking, running, cycling, and/or swimming ≥ 5 h/wk. Thereafter, each subject was characterized by his or her level of daily physical activity in free-living conditions (i.e., DEE_{FLC}/SMR). The capacity of muscle to oxidize fatty acids was assessed *ex vivo* from the maximal activity of the overall fatty acid oxidation pathway by use of palmitate as a substrate. Muscle maximal capacity to oxidize palmitate was not significantly affected by the exercise status. It was, however, positively correlated to DEE_{FLC}/SMR (Fig. 1). Furthermore, muscle palmitate oxidative capacity was the most important predictor of whole body fat oxidation during walking and the alert period, but not during sleep. Consequently, it was a significant predictor of whole body fat oxidation over 24 h. It is possible that the negative energy balance during the experiments stimulated fat oxidation (including muscle palmitate oxidative capacity) in the subjects. However, because the data from the calorimeters showed no difference in energy status between the two groups, they both should have been affected similarly. Hence, the presence of a negative energy state should not change our conclusions.

Muscle maximal capacity to oxidize palmitate was determined using an *ex vivo* method based on the incubation of fresh muscle homogenates with [$1-^{14}C$]palmitate (27, 39). The experiment was conducted under optimal conditions with respect to concentration of palmitate, albumin, and cofactors and to physicochemical parameters (27, 39). Moreover, the diameters of the pestles were adapted to minimize the degradation of the subcellular organelles with respect to peroxisomes and subsarcolemmal and intermyofibrillar mitochondria (27, 39). Therefore, the *ex vivo* technique measured, in a partially preserved cellular environment, the maximal activity of the overall fatty acid oxidation pathway.

Even though peroxisomal β -oxidation of palmitate contributes $\sim 28\%$ to total muscle palmitate oxidative capacity (39), the latter is mainly determined from the oxidative capacity of mitochondria. Physical training has often been associated with increased maximal activity of mitochondrial oxidative enzymes in muscles of young (42) and elderly people (3). In the present study, although we did not show significant differences in the maximal activity of cytochrome *c* oxidase and citrate synthase between the two groups, the maximal activity of these two enzymes was positively correlated to $\dot{V}O_{2\max}/kg$ FFM. Surprisingly, we did not find any connection between exercise status and muscle palmitate oxidative capacity; the latter was similar between the two groups and was not significantly correlated to $\dot{V}O_{2\max}/kg$ FFM. Correlation analyses showed that muscle palmitate oxidative capacity was not deter-

mined by the maximal activity of citrate synthase ($r = 0.05$, $P = NS$) and cytochrome *c* oxidase ($r = 0.25$, $P = NS$), which is in agreement with Van Hinsberg et al. (38). In contrast, it was partially determined by the maximal activity of HAD ($r = 0.49$, $P < 0.05$), which was weakly altered by the exercise status. Other factors, therefore, have to determine the maximal capacity of muscle to oxidize palmitate. For instance, the activity of the enzyme controlling the entry of fatty acids into the mitochondria (CPT-I) has been suggested to be a predominant point of control of muscle fatty acid oxidative capacity (29).

Interestingly, muscle palmitate oxidative capacity was positively correlated to DEE_{FLC}/SMR . This index characterizes the intensity of the overall activity, which includes resting, housekeeping, and leisure and sports activities. DEE_{FLC} has been calculated using the heart rate recording method. This method is based on the relationship between heart rate and energy expenditure, which is calibrated individually from the 24-h measurements in the calorimeters (24). The heart rate recording method has been validated against the doubly labeled water method in free-living elderly people (24). Mean differences in daily energy expenditure were 4–6% between the two methods (24). In the present study, the index of daily physical activity may have been partially biased, because heart rate was measured over only 3 days in free-living conditions. Because within-subject coefficient of variation of DEE_{FLC} was shown to be only 12% in elderly men and 6% in elderly women over 14 days (24), the conclusion is likely to be still valid. Hence, the present results suggest that subjects with low daily physical activities were characterized by low muscle oxidative capacity, even if they exercised ≥ 5 h/wk. It is noteworthy that, although the subjects who exercised regularly had a significantly higher $\dot{V}O_{2\max}$ and a lower fat mass, their daily physical activity was not significantly higher than that of sedentary subjects. In other words, there is no systematic relationship between the level of exercise and the daily physical activity. This is likely due to the fact that some of the regularly exercising subjects decrease their daily activity to compensate for the fatigue caused by exercising. This situation has already been described in sedentary elderly people after an endurance training program (14, 22): the initially sedentary elderly people decreased their energy expenditure during the alert period to compensate for the additional energy cost of the training sessions. Therefore, this result suggests that, in elderly people, the training-induced stimulation of muscle fatty acid oxidative capacity may not be detected if the subjects rest during the remaining time. Conversely, it suggests that muscle fatty acid oxidative capacity may be stimulated when the elderly subjects perform physical activities of moderate intensity over a long time period. To subdivide the subjects according to their daily physical activity level, a cut-off value has to be determined. According to previous studies (22, 24), the DEE_{FLC}/SMR of elderly people averaged ~ 1.8 . A cut-off value could be proposed at 1.7 so that, below this value,

subjects could be considered inactive, whereas above this value, subjects could be considered "normally" active or very active. By use of this cut-off value, muscle palmitate oxidative capacity was 36.8% lower in the less active group than in the more active one (respectively, $DEE_{FLC}/SMR = 1.47 \pm 0.10$ vs. 1.92 ± 0.16 , muscle palmitate oxidative capacity = 32.4 ± 10.0 vs. 51.4 ± 13.2 nmol palmitate·g wet tissue⁻¹·min⁻¹; $n = 9$ in each group, $P < 0.05$). This result needs to be confirmed, however, by comparing two groups of sedentary elderly people differing from each other only by their level of daily physical activity.

As expected (23), FFM was an important determinant of whole body fat oxidation. It was, however, less pronounced than $\dot{V}O_{2,max}$. This is likely due to the fact that $\dot{V}O_{2,max}$, which is strongly related to FFM, also includes the peripheral effects of exercising on the respiratory and cardiovascular systems (15) and maybe on the hormonal control of substrate metabolism (28, 31, 35). Because muscle mass is the largest component of FFM (42% of FFM in both males and females in the present study) and is one of the main tissues oxidizing fatty acids, it may be involved in alterations in whole body fat metabolism (43). We therefore investigated the relationships between variations in muscle fatty acid oxidative capacity and whole body fat oxidation. Implicit in the statistical evaluation (i.e., correlations) that we performed is the assumption that the ex vivo measurement of palmitate oxidation from one muscle is a good predictor of whole body muscle fatty acid oxidative capacity. The fiber composition of the vastus lateralis being mixed, this muscle was assumed to be rather representative of whole body muscle mass. But it should also be remembered that the vastus lateralis is one of the most accessible muscles for sampling in elderly humans, in whom multiple biopsies are not feasible. Hence, we found a positive relationship between muscle palmitate oxidative capacity and whole body fat oxidation during walking. We acknowledge that, since vastus lateralis muscles are solicited during walking, fatty acid oxidation capacity of this specific muscle may have greater consequences on whole body fat oxidation than that of upper-body skeletal muscles. Because substrate and O₂ availability is not rate limiting during low- to moderate-intensity exercise (19), the relationship between muscle palmitate oxidative capacity and whole body fat oxidation suggests that in vivo muscle fatty acid oxidation may be rate limited by muscle-specific metabolic factors during exercise. This relationship still existed during the alert period and over 24 h, probably because the subjects performed only moderate-intensity activities in the calorimeters. These results are in agreement with findings of Zurlo et al. (43), who showed in 14 adults that 24-h respiratory quotient was negatively correlated with the maximal activity of HAD measured on vastus lateralis biopsies. Therefore, because elderly people often practice low- to moderate-intensity activities in free-living conditions (24), these results suggest that their whole body fat oxidation during the alert period may be partly determined by

their muscle fatty acid oxidative capacity. Especially in elderly subjects with low daily physical activities, whole body fat oxidation during the alert period may be blunted because of a low muscle fatty acid oxidative capacity. This may participate in achieving positive fat balance and thus be a factor in the increased fat mass observed with aging.

By contrast, muscle palmitate oxidative capacity was not a predictor of fat oxidation during sleep, although skeletal muscles still contribute to ~43% of whole body fat oxidation in resting conditions (estimation in elderly subjects from Ref. 9). This may be due to the fact that skeletal muscle at rest has a low metabolic rate per mass unit (9), so that its fatty acid oxidation rate is not maximal. In resting conditions, whole body fuel metabolism may be determined more by hormonal control and energy balance. In fact, in the present study, 36.6% of the variance of whole body fat oxidation during sleep was explained by daily energy balance. Moreover, fat oxidation at rest in women, but to a lesser extent in men, has been significantly correlated to insulin and free thyroxine plasma concentrations (25). Finally, fat oxidation at rest may also be modulated by sympathetic nervous system activity and tissue sensitivity to catecholamines (28, 35).

In conclusion, in elderly people, muscle palmitate oxidative capacity may be influenced more by overall daily physical activity than by regular exercise. Furthermore, muscle palmitate oxidative capacity is a major determinant of whole body fat oxidation during moderate-intensity activities and, consequently, whole body fat oxidation during the alert period and over 24 h. However, muscle palmitate oxidative capacity is not a determinant of whole body fat oxidation during sleep. Therefore, our results suggest that a sedentary lifestyle may be associated with a reduced muscle fatty acid oxidative capacity that may participate in decreasing whole body fat oxidation during moderate intensity activity and, consequently, over 24 h.

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