Whole-Body Energy Metabolism and Skeletal Muscle Biochemical Characteristics

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A low metabolic rate for a given body size and a low fat versus carbohydrate oxidation ratio are known risk factors for body weight gain, but the underlying biological mechanisms are poorly understood. Twenty-four–hour energy expenditure (24EE), sleeping metabolic rate (SMR), 24-hour respiratory quotient (24RQ), and forearm oxygen uptake were compared with respect to the proportion of skeletal muscle fiber types and the enzyme activities of the vastus lateralis in 14 subjects (seven men and seven women aged 30 ± 6 years [mean ± SD], 79.1 ± 17.3 kg, 22% ± 7% body fat). The following enzymes were chosen to represent the major energy-generating pathways: lactate dehydrogenase (LDH) and phosphofructokinase (PFK) for glycolysis; citrate synthase (CS) and β-hydroxyacl–coenzyme A dehydrogenase (β-OAC) for oxidation; and creatine kinase (CK) and adenylokinase (AK) for high-energy phosphate metabolism. Forearm resting oxygen uptake adjusted for muscle size correlated positively with the proportion of fast-twitch muscle fibers (IIa: $r = .55$, $P = .04$; IIb: $r = .51$, $P = .06$) and inversely with the proportion of slow oxidative fibers (I: $r = -.77$, $P = .001$). 24EE and SMR adjusted for differences in fat-free mass, fat mass, sex, and age correlated with PFK activity ($r = .56$, $P = .04$ and $r = .69$, $P = .007$, respectively). 24RQ correlated negatively with β-OAC activity ($r = -.75$, $P = .002$). Our findings suggest that differences in muscle biochemistry account for part of the interindividual variability in muscle oxygen uptake and whole-body energy metabolism, ie, metabolic rate and substrate oxidation.

FOR A GIVEN BODY SIZE and body composition and under eucaloric feeding, both the daily metabolic rate and the fuel-mix oxidation are quite variable from one subject to another. The variability in energy expenditure and in 24-hour respiratory quotient (24RQ) aggregates in families,[1-3] and is most probably genetically determined.[4] Prospective studies have provided evidence that a reduced rate of energy expenditure for a given body size and composition and a high RQ (low fat to carbohydrate oxidation ratio) are both risk factors for body weight gain.[2,3,5-7] Because of its relatively low resting energy metabolism,[8-10] skeletal muscle has often been overlooked when trying to explain interindividual differences in metabolic rate. However, Astrup et al[11] showed that skeletal muscle appears to be the principal site of the thermogenic effect of sympathomimetic agents. Indeed, since skeletal muscle accounts for approximately 40% of body weight in nonobese subjects,[10] it can account for 20% to 30% of total resting oxygen uptake.[12,13] Also, we have shown that part of the interindividual variability in resting energy expenditure is explained by differences in resting skeletal muscle oxygen uptake.[13] This study was therefore undertaken to investigate the relationship between skeletal muscle biochemical characteristics (fiber types and enzymes involved in energy-generating pathways) and whole-body metabolism in 14 healthy sedentary white volunteers.

We hypothesized that part of the variability among individuals with respect to whole-body metabolic rate, muscle oxygen uptake, and 24RQ might be related to differences in skeletal muscle fiber type proportions and/or differences in enzymatic activities. Twenty-four–hour energy expenditure (24EE) and its different components were measured in a respiratory chamber.[14] Muscle metabolism was assessed by forearm oxygen uptake, whereas biochemical characteristics were assessed in the vastus lateralis muscle by muscle fiber histochemistry and by measuring activities of six key enzymes involved in energy-generating pathways, as follows: lactate dehydrogenase (LDH) and phosphofructokinase (PFK) in glycolysis; citrate synthase (CS) in the citric acid cycle; β-hydroxyacyl–coenzyme A dehydrogenase (β-OAC) in fatty acid oxidation; and creatine kinase (CK) and adenylokinase (AK) in high-energy phosphate metabolism.

SUBJECTS AND METHODS

Subjects and Experimental Design

Fourteen white subjects were admitted for 7 to 10 days to the clinical research ward of the Clinical Diabetes and Nutrition Section of the National Institutes of Health in Phoenix, AZ. Some of the results of this study have been previously reported.[13,15] Upon admission, all subjects were determined to be in good health by means of medical history, physical examination, electrocardiogram, blood screening, and urine tests. The subjects were not diabetic according to an oral glucose tolerance test.[16] None were taking any medication or had clinical evidence of illness apart from obesity. Subjects were fed a weight-maintenance diet (50% carbohydrate, 30% fat, and 20% protein).[17] Body density was determined by underwater weighing[18] with simultaneous measurement of residual lung volume, and percent body fat was calculated according to the Keys and Brozek equation.[19] After at least 2 full days on the metabolic ward, subjects spent 23 hours in a respiratory chamber where whole-body energy metabolism was measured as previously described.[14] The following morning, after an overnight fast, forearm oxygen uptake was measured.[13] After completion of the forearm measurement, muscle was obtained by biopsies of the vastus lateralis. The protocol was approved by the National

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Institute of Diabetes and Digestive and Kidney Diseases Clinical Research Subpanel, and written, informed consent was obtained. Subject characteristics are listed in Table 1.

Muscle Fiber Typing and Enzyme Assays of Muscle Homogenate

Muscle samples (40 to 60 mg) were obtained by percutaneous biopsies of the vastus lateralis 4 to 5 cm from the midline in the midlateral thigh, using a Bergström needle. Samples were immediately frozen and stored in liquid nitrogen until assayed. A portion of each sample was processed for histochemistry and stained for myofibrillar adenosine triphosphatase activity, following preincubation at pH 4.6, to obtain estimates of the fiber population as described by Dubowitz et al.[20] Individual skeletal myocytes in the muscle biopsies were classified as slow-twitch oxidative (type I, characteristically high in oxidative enzymes and low in glycolytic and high-energy phosphate enzymes), fast-twitch oxidative glycolytic (type IIa, characteristically high in oxidative enzymes and glycolytic and high-energy phosphate enzymes), or fast-twitch glycolytic (type IIb, characteristically low in oxidative enzymes and high in glycolytic and high-energy phosphate enzymes) fibers by dark, light, or intermediate staining intensities, respectively.[21] This technique yields highly reproducible values (coefficient of variation, 0% to 5%) on repeated measurements of the same muscle sample (P.M. Nemeth, unpublised data).

Activities of LDH, PFK, CS, β-OAC, CK, and AK were determined in homogenates of the remainder of each vastus lateralis biopsy. Samples were homogenized at high speed using a motor-driven tissue grinder (Con-Torque, Eberbach, Ann Arbor, MI). The homogenization medium contained 5 mL β-mercaptoethanol, 0.5 mL EDTA, 0.02% bovine serum albumin, and 50% glycerol in 20 mL sodium phosphate buffer, pH 7.4. Studies conducted previously on rabbit and monkey skeletal muscle demonstrated negligible loss of activities of all enzymes assayed during prolonged storage of fresh frozen tissue at −70°C.[22] Enzyme activities were quantified fluorometrically at 25°C using spectrophotometrically determined standards, and were expressed as moles per kilogram protein per hour as previously described in detail.[22] Protein determination was made with a BCA-1 kit (Sigma Chemical, St Louis, MO).

Table 1. Physical Characteristics, Forearm O_2 Uptake, and Whole-Body Energy Expenditure of the 14 Subjects (seven men, seven women)

Variable	Mean ± SD	Range
Physical characteristics		
Age (yr)	30 ± 6	22-41
Height (cm)	173.7 ± 7.3	162.5-188.5
Weight (kg)	79.1 ± 17.3	56.2-109.2
Body fat (%)	22 ± 7	9-37
Waist/thigh circumference ratio	1.46 ± 0.11	1.32-1.63
Forearm O_2 uptake		
mL/L forearm/min	1.29 ± 0.35	0.68-1.81
mL/L forearm muscle/min	2.07 ± 0.56	1.33-3.01
Whole-body energy expenditure		
24EE (kcal/d)	2,127 ± 425	1,534-3,170
SMR (kcal/d)	1,531 ± 208	1,104-1,820
24RQ	0.862 ± 0.018	0.824-0.889

NOTE. Individual values have been previously published.[13]

Forearm Oxygen Uptake

Forearm oxygen uptake was measured as previously described.[13] Briefly, after 40 minutes of complete rest, direct blood flow measurement across the forearm was obtained with a capacitance plethysmograph (model 2560, UFI, Morro Bay, CA). Venous and arterial blood samples were simultaneously collected and immediately analyzed for total blood oxygen content using a co-oximeter (IL482 Co-Oximeter, Instrumentation Laboratory, Lexington, MA). Three determinations were performed in each subject at 40-minute intervals. At the end of the test, the forearm volume between the two pneumatic cuffs was measured by water displacement. Forearm oxygen uptake (mL/min/L forearm volume) was calculated as forearm blood flow (mL/min) × (arterial O_2 content − venous O_2 content [mL O_2/mL])/volume forearm (L). The composition (muscle mass *v* nonmuscle mass) of the dominant forearm was assessed by computerized tomography.[23] Forearm oxygen uptake was also expressed per unit of muscle volume (mL O_2/min/L forearm muscle volume).

Whole-Body Energy Expenditure

After at least 2 full days on the metabolic ward, subjects spent 23 hours in a respiratory chamber where energy expenditure (24EE, basal and sleeping metabolic rate [SMR]) and spontaneous physical activity were measured as previously described.[14] No vigorous exercise was allowed in the chamber. For each subject, a predicted energy expenditure was calculated from a linear regression equation derived from 138 healthy white subjects (86 men and 52 women aged 28 ± 7 years, 92.4 ± 32.3 kg, 26% ± 12% body fat) with fat-free mass, fat mass, age, and sex as covariates. For each subject, the residual between measured and predicted values of energy expenditure was used as an adjusted energy expenditure.

The 24RQ was calculated as the ratio between 24-hour carbon dioxide production and 24-hour oxygen consumption. Since the RQ is influenced by differences in energy balance, percentage of body fat, and sex,[2] values were also adjusted for these three covariates. For each subject, a predicted 24RQ was calculated from a linear regression equation derived from the same 138 healthy white subjects mentioned above.

Calculations and Statistical Analyses

Data are expressed as the mean ± standard deviation. Statistical analyses were performed with the procedures of the SAS Institute, Cary, NC.[24] Correlation coefficients were obtained by Spearman ranked order correlations. Multivariate and partial correlation analyses were performed by the general linear model procedures.

RESULTS

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The proportions of fiber types and enzymatic activities of mixed-fiber homogenates are presented in Table 2. The percentage of type I fibers was 51% ± 16%, ranging from 32% to 80%; the percentage of type IIa fibers was 36% ± 15%, ranging from 8% to 60%; and the percentage of type IIb fibers was 13% ± 9%, ranging from 0% to 32%. Mean enzyme activities (mol/kg protein/h) were 48 ± 23 for LDH, 9 ± 4 for PFK, 5 ± 2 for CS, 7 ± 2 for β-OAC, 113 ± 36 for AK, and 981 ± 330 for CK. The total relative proportion of type II fibers (IIa + IIb) correlated positively with AK activity ($r = .59$, $P = .03$) and tended to correlate with LDH activity ($r = .47$, $P = .09$). Conversely, the propor-

Table 2. Percentage of Muscle Fiber Types and Enzyme Activities of Vastus Lateralis in 14 Volunteers (seven men, seven women)

Subject	Fiber Types (%)*			Enzyme Activities (mol/kg protein/h)					
No./Sex	I	IIa	IIb	LDH	PFK	CS	β-OAC	AK	CK
3/M	32	40	28	56	7	4	6	121	1,083
10/F	32	60	8	48	17	8	10	193	1,322
12/M	32	56	12	70	8	4	7	155	1,184
1/M	40	40	20	45	9	2	3	76	571
4/M	40	52	8	57	4	3	5	102	1,014
14/F	40	40	20	58	10	4	9	131	718
7/M	48	44	8	73	14	5	6	111	1,183
13/F	50	40	10	14	11	3	5	87	573
2/M	52	16	32	97	14	9	10	147	1,333
11/F	60	20	20	22	5	4	4	69	577
9/F	65	30	5	26	9	6	10	120	1,041
5/F	68	32	0	49	7	7	10	111	1,589
6/F	76	20	4	17	5	3	7	72	603
8/F	80	8	12	39	8	8	8	82	936
Mean	51	36	13	48	9	5	7	113	981
SD	16	15	9	23	4	2	2	36	330

NOTE. Subjects are sorted according to increasing percentage of type I fibers; the subject number refers to the number in a previous report.[13]

*Type I = slow-twitch oxidative; type IIa = fast-twitch oxidative glycolytic; type IIb = fast-twitch glycolytic.

tion of type I fibers correlated negatively with AK activity ($r = -.59$, $P = .03$). Percent body fat correlated negatively with CK activity ($r = -.55$, $P = .04$).

Forearm Oxygen Uptake Versus Muscle Characteristics

Individual results of forearm O_2 uptake have been previously published,[13] and mean values are presented in Table 1. Forearm oxygen uptake (mL/min) correlated with forearm total volume and forearm muscle volume ($r = .80$, $P = .0006$ and $r = .75$, $P = .002$, respectively). Values were therefore expressed per volume of forearm or per volume of forearm muscle. SMR adjusted for differences in fat-free mass, fat mass, age, and sex correlated with oxygen uptake per volume of forearm ($r = .59$, $P = .03$). A similar trend was found between the adjusted 24EE and the forearm oxygen uptake ($r = .51$, $P = .06$). Forearm resting oxygen uptake adjusted for muscle mass correlated with the proportion of fast-twitch muscle fibers (IIa: $r = .55$, $P = .04$; IIb: $r = .54$, $P = .05$). As shown in Fig 1, there was a positive correlation between forearm oxygen uptake (mL/L forearm muscle/min) and the combined percentage of fast-twitch IIa and IIb fibers ($r = .77$, $P = .001$); a similar relationship ($r = .65$, $P = .01$) was found when forearm oxygen uptake was expressed per total forearm volume (mL/L forearm volume/min). Conversely, there was a significant negative correlation between forearm oxygen uptake (mL/L forearm volume) and percentages of slow-twitch fibers I alone ($r = -.65$, $P = .01$) or pooled together with the other oxidative fibers, ie, IIa ($r = .74$, $P = .002$). Similar relationships ($r = -.77$, $P = .001$ and $r = -.51$, $P = .06$, respectively) were found when forearm oxygen

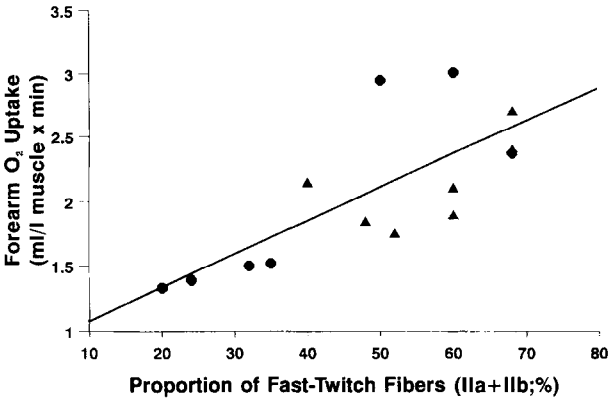

Fig 1. Relationship between forearm oxygen uptake (mL O_2/L forearm muscle/min) and the proportion of total fast-twitch muscle fibers (type IIa + type IIb) in the vastus lateralis in 14 normal white subjects. (▲) Men; (●) women ($r = .77$, $P = .001$).

uptake was expressed per forearm muscle volume (mL/L forearm muscle/min).

Whole-Body Energy Expenditure Versus Muscle Tissue Characteristics

Individual values of 24EE and SMR have already been reported.[13] Mean values are shown in Table 1. The deviation from the predicted energy expenditure (difference between measured energy expenditure and energy expenditure predicted on the basis of fat-free mass, fat mass, age, and sex) varied from −347 to +565 kcal/d for 24EE and from −154 to +235 kcal/d for SMR. The residuals for 24EE correlated with the residuals for SMR ($r = .57$, $P = .03$). SMR and 24EE, adjusted for the above covariates, correlated positively with PFK activity ($r = .69$, $P = .007$ and $r = .56$, $P = .04$, respectively; Fig 2). 24RQ was 0.862 ± 0.018, ranging from 0.824 to 0.889. As shown in Fig 3, 24RQ correlated inversely with the activity of β-OAC ($r = -.75$, $P = .002$), an enzyme involved in fatty acid oxidation. Similar inverse correlations were found between 24RQ and the activities of AK ($r = -.73$, $P = .003$) and CK

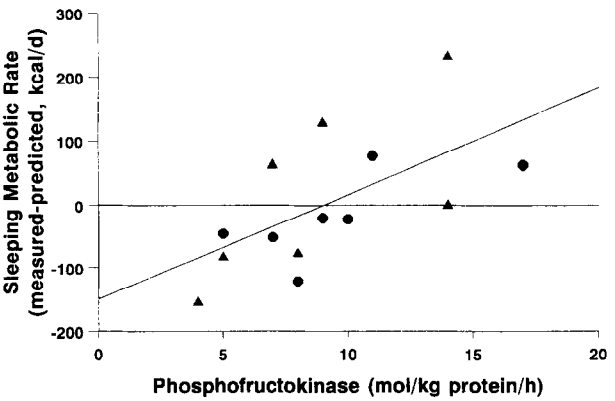

Fig 2. Relationship between the deviation of measured SMR (kcal/d) from predicted SMR and the activity of PFK (mol/kg protein/h) in the vastus lateralis muscle. (▲) Men; (●) women ($r = .69$, $P = .007$).

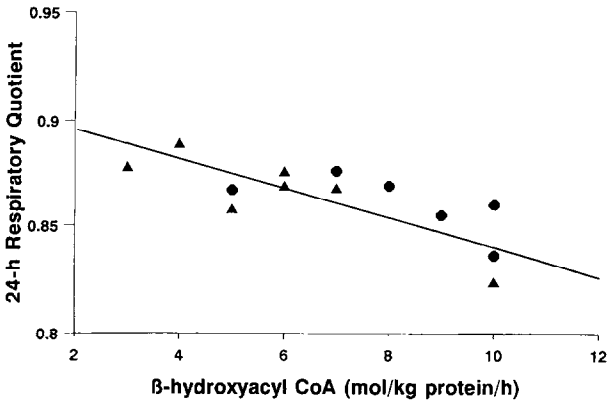

Fig 3. Relationship between the 24RQ and the activity of β-OAC (mol/kg protein/h) in the vastus lateralis muscle. (▲) Men; (●) women ($r = -.75, P = .002$).

($r = -.55$, $P = .04$). When 24RQ was adjusted for differences in energy balance, percent body fat, and sex, it still correlated with β-OAC activity ($r = -.70, P = .005$).

DISCUSSION

The present study shows that in healthy young adults, skeletal muscle biochemical characteristics are related to whole-body energy expenditure and substrate oxidation. Since both of the latter factors have been reported to be involved in body weight gain, these results therefore suggest that skeletal muscle (fiber types and/or enzymatic activities) may play a role in its etiology.

Obesity is a multifactorial syndrome in which both a deficit in sedentary metabolic rate and low rates of fat relative to carbohydrate oxidation (high RQ) have been shown to be important.[2,3,5-7,25-27] Fat oxidation during low-intensity exercise has been found to be related to both fatness and muscle fiber type proportions.[26] This observation has prompted us to test whether significant relationships existed between the metabolic profiles of skeletal muscle (fiber types and/or enzymatic activities) and known risk factors of obesity.

Compared with other tissues, skeletal muscle at rest has a low metabolic rate per mass unit, but represents the body's largest tissue mass. Recently, we have shown that differences in resting skeletal muscle metabolism could account for 40% to 50% of the variability in whole-body metabolic rate observed among individuals.[13] Among factors that might influence the variability in muscle oxygen uptake, we investigated individual differences in muscle fiber types and the activity of the following six muscle enzymes involved in energy-generating metabolism: LDH and PFK for glycolysis; CS, the first enzyme of the citric acid cycle; β-OAC, a key enzyme in fatty acid β-oxidation; and CK and AK, two enzymes of high-energy phosphate metabolism. The skeletal muscle samples of vastus lateralis used in our assays were obtained by percutaneous biopsies.

Several previous studies have shown a wide variation in enzyme activities among individuals in many animal species,[28] as well as in humans.[29] In the present study, we have confirmed the wide variability among individuals in enzyme activities, but we also found a large variability in fiber-population profiles. We found the expected trends between fiber-type populations and mixed-fiber homogenate enzyme activities, ie, high oxidative enzyme activities in subjects with a high proportion of oxidative fibers and low AK activity in subjects with a low proportion of glycolytic fibers. However, some discrepancies between enzyme activities and fiber proportion can be accounted for by interindividual variation[30] and by the use of different samples from the same biopsy for histochemistry and biochemistry determinations. Even if the samples were taken from the same muscle biopsy, they might not be identical because of the variability in enzyme activities within a fiber-type population. Therefore, the histochemical type (myosin adenosine triphosphatase) did not always predict the actual specific enzyme activities, but provided us with a useful indication of oxidative versus glycolytic metabolism. Since fiber typing in numerous muscles is available in only six cadavers, it remains to be determined how representative of the entire skeletal muscle system is the vastus lateralis.[31]

We found a positive correlation between the proportion of fast-twitch muscle fibers (IIa and IIb) and resting forearm oxygen uptake, and a negative correlation of the latter with the proportion of slow-twitch oxidative muscle fibers. This might seem surprising; however, slow-twitch, phasic fibers are responsible for both maintaining posture and performing slow, repetitive movements. These fibers contain many mitochondria, and their myofibrils hydrolyze adenosine triphosphate only very slowly.[32] Our data suggest that a relative decrease of type I slow-twitch fibers and/or a relative increase of type II fast-twitch fibers may favor a "less efficient" use of the energy substrates giving preference to glycolytic pathways. This might be due to the higher rate of fructose-6-phosphate/fructose biphosphate cycling in type II fibers.[33] In support of this hypothesis, we found that SMR and 24EE, adjusted for body size and body composition, correlated positively with the activity of PFK, the rate-limiting enzyme of glycolysis. Similarly, adjusted 24EE also correlated with the activity of AK, an enzyme controlling the adenosine triphosphate flux.

Interestingly, our results are in agreement with studies in athletes in whom a greater proportion of type I muscle fibers is associated with less oxygen uptake during exercise.[34,35] Coyle et al[34] have recently shown that "elite-national class" athletes have a greater percentage of type I muscle fibers, lower LDH activities, and greater muscle capillary density than "good-state class" athletes matched for maximum O_2 consumption and lean body weight. During a 1-hour performance, elite athletes cycled 10% faster and were able to generate 11% more power than the other athletes. The investigators concluded that muscle fiber types, enzyme characteristics, and higher muscle capillary density all contributed to the remarkable endurance in these elite athletes. Park et al[35] studied untrained muscle of world-class runners and also found a greater proportion of type I muscle fibers. They suggested that

greater oxidative capacity does reflect a genetic endowment for physical endurance.

In the present study, we also found a striking inverse correlation between 24RQ and the activity of β-OAC, a key enzyme in the β-oxidation of fatty acids. This finding suggests that an increased capacity for fat oxidation in skeletal muscle is accompanied by a higher fat to carbohydrate oxidation ratio in the whole body at rest, probably due to the fact that most fat oxidation occurs in skeletal muscle. This is in agreement with the observation of Wade et al,[26] who found an inverse relationship between the RQ during mild exercise and the proportion of slow-twitch fibers. However, in our study with gas-exchange measurements performed over an entire day, we did not find such a relationship with fiber types.

Our data suggest that the biochemical characteristics of skeletal muscle are important in determining daily energy expenditure and daily energy substrate oxidation. For instance, subjects with higher β-OAC activity in their skeletal muscle have higher rates of fat to carbohydrate oxidation and are therefore less likely to be in positive fat balance, the major etiological factor of obesity.[25] In conclusion, we suggest that people with more oxidative fibers, ie, with less capacity for substrate cycling, and/or with muscle fibers with reduced capacity for fatty acid oxidation might have a higher risk in sustaining positive energy/fat balance and therefore becoming obese.

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