Skeletal Muscle Characteristics Predict Body Fat Gain in Response to Overfeeding in Never-Obese Young Men

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The associations between skeletal muscle morphological and metabolic properties and the changes in body composition and metabolic rates in response to long-term overfeeding were investigated in 24 healthy young male identical twins (12 pairs). The proportions of muscle fiber types (type I, type IIA, and type IIB) and the activities of creatine kinase (CK), oxoglutarate dehydrogenase (OGDH), and phosphofructokinase (PFK) were determined from biopsies of the vastus lateralis before and after the overfeeding protocol. Body weight, fat mass (FM), fat-free mass (FFM), percent body fat (%FAT), resting metabolic rate (RMR), and thermic effect of a standardized meal (TEM) were also measured before and after 100 days of overfeeding. Type I muscle fiber proportions correlated inversely with the changes of FM and %FAT (r = -0.43, P = .035; r = -0.49, P = .01), and type IIA positively with the same overfeeding-induced changes (r = 0.43, P = .035; r = 0.47, P = .021). Baseline CK and PFK activities correlated negatively with the changes of RMR (r = -0.49, P = .017; r = -0.53, P = .01). OGDH activity at baseline correlated negatively with the changes of FM (r = -0.47, P = 0.02) but the ratio of PFK/OGDH correlated positively with the change of FM (r = 0.46, P = .02). We conclude that overfeeding induced a lower gain of FM in individuals with higher proportions of type I fiber, lower proportions of type IIA fiber, and higher OGDH activities at baseline. CK and PFK activities at baseline were associated with an attenuated increase in RMR when challenged by overfeeding. The significant correlations range from 0.43 to 0.53, and account for 18% to 28% of the variance in the response to overfeeding. The results suggest that an elevated skeletal muscle oxidative capacity plays a protective role in the response to long-term positive energy balance.

OBESITY HAS BEEN characterized as an epidemic and is one of the leading public health concerns in the world.[1] It is a multifactorial disease determined by both genetic predisposition and environmental factors, such as physical inactivity and excessive energy intake.[1,2] Although skeletal muscle has a low resting metabolic rate (RMR) per kilogram, it can account for as much as 30% of total resting oxygen uptake.[3] Moreover, a low capacity for fat oxidation could play a role in the predisposition to obesity.[4] Since skeletal muscle is an important tissue for fat oxidization, a reduced muscle capacity to metabolize lipids could favor the development of obesity.

Several studies have suggested that skeletal muscle metabolism plays a role in the etiology of obesity.[5,6] Percent body fat (%FAT) has been shown to be inversely correlated to the proportion of type I muscle fibers in some studies but not in others.[5,6] A high proportion of type IIB muscle fiber has been considered as a risk factor for obesity in some studies.[7,8] Significant correlations between the activities of muscle enzymes involved in aerobic oxidation and glycolysis with body fat have been reported.[9,10] However, findings are not always consistent and are at times even conflicting.[5-11] It is important to emphasize that the studies reported to date were cross-sectional. Thus, intervention studies could shed some light on these issues. The present study was performed to test the hypothesis that skeletal muscle metabolic and morphological properties are associated with the changes in body composition and metabolic phenotypes in response to long-term overfeeding. To achieve this, 24 healthy young males (12 pairs of identical twins) were evaluated before and after a 100-day overfeeding protocol.

METHODS

Twenty-four sedentary young men (12 pairs of monozygotic twins) participated in this overfeeding experiment.[12] The subjects had been reared together and had been living together before the study. Written informed consent was obtained from each subject, and the study was approved by the Laval University Medical Ethics Committee and the Office for Protection from Research Risks of the National Institutes of Health, Bethesda, MD. The monozygosity of the twins was established on the basis of a questionnaire; their physical appearance; and the similarity of 12 polymorphic red blood cell antigens and enzymes; the A, B, and C loci of the human leukocyte antigen (HLA) system; and 10 polymorphic adipose-tissue proteins visualized by 2-dimensional gel electrophoresis. Their homozygosity has since then been confirmed by a large number of DNA markers. They were healthy and had no history of recent illness, obesity, hypertension, diabetes, hyperlipidemia, or endocrinopathy. Each subject had a normal physical examination. Men whose parents were obese or had diabetes or lipid disorders were not accepted into the study.

Each man stayed in a closed section of a dormitory supervised 24 hours a day for 120 consecutive days: 14 days for baseline testing, 3 days for testing before the period of overfeeding, 100 days for the period of overfeeding, 3 days for testing after the period of overfeeding. Each subject was overfed by 1,000 kcal per day, 6 days a week, for a total of 84 days over a 100-day period. The total caloric surplus that each subject had to consume was 84,000 kcal. A more detailed description of the protocol can be found in Bouchard et al.[12] In addition, data pertaining to the effects of overfeeding on energy expenditure[13]; the lipolytic activity of adipose cells[14]; thyroid hormones[15]; glucose, insulin, and glucagon levels[16]; adrenal and gonadal steroids[17]; and/or the role of selected candidate genes[18] have been published thus far.

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Table 1. Characteristics of the Subjects

Variable	Baseline	Changes
Age (yr)	21 ± 2	—
Body weight (kg)	60.3 ± 8.0	8.1 ± 2.4*
Body mass index (kg/m^2)	19.7 ± 2.0	2.7 ± 0.7*
%FAT	11.3 ± 5.0	6.5 ± 2.4*
FM (kg)	6.9 ± 3.5	5.3 ± 1.9*
FFM (kg)	53.4 ± 6.6	2.8 ± 1.5*
$\dot{V}o_{2max}$ (mL/min)	2.97 ± 0.50	0.19 ± 0.06†
$\dot{V}o_{2max}$ (mL/min/kg FFM)	55.6 ± 5.6	0.3 ± 1.2*

NOTE. Values are expressed as means ± SD; N = 24.

*$P < .0001$; †$P < .05$. Statistical significance was determined by a 2-way ANOVA for repeated measures on 1 factor (time) with the twins nested; see methods for details.

Determinations of Skeletal Muscle Fiber Types

Muscle biopsies were obtained from the middle region of the vastus lateralis muscle (ie, ≈14 cm above the patella) and approximately 2 cm away from the epimysium by the percutaneous needle biopsy technique.[19] Muscle samples were frozen in isopentane cooled to its freezing point (-160°C) with liquid N_2 and stored at –70°C until processing.

Muscle fiber type proportion was determined from 10-μm slices cut at –20°C and stained for myofibrillar actomyosin triphosphatase (mATPase) activity according to the single-step ethanol-modified technique.[20] The 3 major fiber types were designated as type I, type IIA, and type IIB based on the mATPase properties under these conditions. The technical error (SD of repeated measurements on repeated biopsies) associated with the determination of the fiber type proportions in human skeletal muscle samples under such conditions varies from 5% to 7%.[21]

Determinations of the Activities of Enzyme Markers of Different Metabolic Pathways

The enzymatic markers studied were creatine kinase (CK; enzyme in high-energy phosphate metabolism), oxoglutarate dehydrogenase (OGDH; enzyme in aerobic oxidation), and phosphofructokinase (PFK; enzyme in glycolysis) and the ratio of PFK/OGDH (an indication of glycolysis to aerobic oxidation).

A piece of the frozen muscle sample (≈10 mg) was mixed in a small Duall glass homogenizer (Kontes Glass Co, Vineland, NJ) with 39 vol (wt/vol) of extracting medium (0.1 mol/L K, Na-phosphate, 2 mmol/L EDTA, pH 7.2). The muscle sample was homogenized with several passes of the glass pestle and was used for the enzyme activity measurements. Maximal activity of CK, OGDH, and PFK were fluorometrically assayed the day of the biopsy at 25°C (30°C for PFK) according to the procedures described in previous studies.[9,19]

Measurements of RMR and Thermic Effect of a Meal

RMR was measured early in the morning after subjects had fasted for 12 hours. To reduce previous disturbances, subjects sat in a comfortable reclining seat with the head inside a hood system (Beckman Instrument Division, Schiller Park, IL) for 30 minutes, and then RMR was measured over the next 30 minutes.[22] The air fractions of oxygen and carbon dioxide were measured with paramagnetic and infrared analyzers, respectively (Beckman OM-11 and LB-2). Pulmonary ventilation was determined with a turbine Beckman respirometer. The energy equivalent of oxygen was calculated using the Weir formula.[23] After the measurement of RMR, the subject consumed a 1,000-kcal meal with the following composition: 15% protein, 35% lipid, and 50% carbohydrate.[22] The test meal was consumed in 15 minutes, after which the calorimetric measurements were continued for 240 minutes while the subject remained in a semi-reclined position. The thermic effect of a meal (TEM) was calculated as energy expenditure above RMR.

Measurements of Body Compositions

Body mass index (BMI) was calculated as body weight (in kilograms) divided by the height (in meters squared). Body density was determined by the underwater weighing method,[24] and fat mass (FM) and fat-free mass (FFM) were calculated from %FAT with a standard equation.[25] Pulmonary residual volume was measured by the helium-dilution technique.[26]

Statistical Analyses

Results are presented as means and standard deviations (SD). The effect of overfeeding on CK, OGDH, PFK, and PFK/OGDH was assessed with a 2-way analysis of variance (ANOVA) for repeated measures with twins rested. One factor was the twin pairs and the other was the overfeeding treatment.[12] Because the distributions of the skeletal muscle data were skewed, nonparametric Spearman correlation coefficients were calculated to determine the associations between the proportion of skeletal muscle fiber types or enzyme activities and changes with overfeeding defined as the postoverfeeding value minus before overfeeding for body weight, FM, FFM, %FAT, RMR, and TEM.

The mean value of each twin pair and the 24 individual scores were both used to calculate the Spearman correlation coefficients in all analyses. The results obtained with the 2 methods were compared and were generally similar. The differences and similarities are highlighted in the tables.

RESULTS

The basic physical characteristics of subjects are listed in Table 1. The body composition changes caused by the overfeeding protocol have been described previously.[12] The mean body weight gain was 8.1 kg. Subjects gained more adipose tissue than lean tissue.[12] As a result of the gain in body mass, the maximal oxygen uptake of the 24 subjects increased from 2.97 (SD = 0.50) at baseline to 3.14 (SD = 0.48) L O_2/min ($P < .05$) after overfeeding. However, on a per kilogram of body mass basis, VO_{2max} remained constant throughout the protocol (≈55 mL O_2/kg/min) as reported previously.[15] RMR (kJ/kg FFM) increased significantly, but TEM did not change.[22] Table 2 shows the changes in skeletal muscle fiber type distribution and enzyme activities with the 100-day overfeeding protocol. No significant change in skeletal muscle fiber type proportion was found. The activity of skeletal muscle CK

Table 2. Skeletal Muscle Fiber Type Distribution and Activities of Enzymes of Energy Metabolism Before and After 100 Days Overfeeding (mean ± SD)

	Before Overfeeding	After Overfeeding	*P**
Type I (%)	44.8 ± 10.4	47.1 ± 11.2	.40
Type IIA (%)	37.5 ± 11.6	37.8 ± 9.6	.73
Type IIB (%)	17.7 ± 8.9	15.1 ± 6.5	.15
CK (U/g wet wt)	200 ± 66	222 ± 40	**.01**
OGDH (U/g wet wt)	0.80 ± 0.39	0.68 ± 0.32	**.04**
PFK (U/g wet wt)	112 ± 35	120 ± 36	.16
PFK/OGDH	199 ± 189	243 ± 211	**.0009**

*Statistical significance was determined by a 2-way analysis of variance for repeated measures on 1 factor (time) with the twins nested; see methods for details.

Table 3. Correlation Between the Proportions of Skeletal Muscle Fiber Types and Body Composition, RMR, and TEM at Baseline

	Before Overfeeding					
	Type I		Type IIA		Type IIB	
Before Overfeeding	*r*	*P*	*r*	*P*	*r*	*P*
BMI (kg/m^2)	−0.02	NS	−0.02	NS	0.13	NS
FM (kg)	0.13	NS	−0.08	NS	−0.01	NS
FFM (kg)	−0.08	NS	−0.05	NS	0.13	NS
%FAT	0.18	NS	−0.06	NS	−0.10	NS
RMR (kJ/kg FFM)	0.26	NS	−0.11	NS	−0.22	NS
TEM (KJ)	−0.00	NS	−0.02	NS	0.08	NS

NOTE. N = 24 for all variables except RMR (n = 23).
Abbreviation: NS, not significant.

increased, whereas OGDH activity decreased significantly with overfeeding. Overfeeding had no effect on PFK activity. Hence, the PFK/OGDH ratio increased significantly compared with baseline value.

No significant correlation was found between the baseline proportion of skeletal muscle fiber types and baseline body composition and metabolic phenotypes (Table 3).

However, baseline CK activity correlated negatively with baseline %FAT and RMR, and positively with TEM (Table 4). A significant negative correlation was found between baseline OGDH activity and %FAT and RMR. PFK activity did not correlate with any body composition and metabolic phenotypes at baseline. Finally, the PFK/OGDH ratio correlated positively with baseline %FAT and RMR (Table 4).

The correlations between baseline proportion of skeletal muscle fiber types and the overfeeding-induced changes in body composition and metabolic phenotypes are shown in Table 5. The baseline proportion of type I muscle fiber correlated negatively with the gains in FM and %FAT (Fig 1), while the baseline proportion of type IIA muscle fiber showed a positive correlation with the increases in FM and %FAT. No significant correlation was found between pre-overfeeding type IIB and changes in body composition and metabolic variables. A significant negative correlation was detected between baseline skeletal muscle CK activity and changes in RMR (Table 6). Baseline OGDH activity was negatively correlated with changes in FM (Fig 2). Baseline PFK activity demonstrated a negative correlation with the changes in RMR. Finally, the baseline PFK/OGDH ratio correlated positively with the gain in FM.

Table 4. Correlation Between Selected Skeletal Muscle Enzyme Activities and Body Composition, RMR, and TEM at Baseline

	Before Overfeeding							
	CK		OGDH		PFK		PFK/OGDH	
Before Overfeeding	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
BMI (kg/m^2)	0	NS	0.19	NS	−0.05	NS	−0.04	NS
FM (kg)	−0.30	NS	−0.25	NS	−0.03	NS	0.33	NS
FFM (kg)	0.39	NS	0.35	NS	−0.07	NS	−0.21	NS
%FAT	−0.48	**.02**	−0.43	**.04**	−0.07	NS	0.44	**.03**
RMR (kJ/kg FFM)	−0.65	**.0008**	−0.52	**.01**	−0.06	NS	0.46	**<.03**
TEM (kJ)	0.41	**.05**	0.34	NS	−0.00	NS	−0.27	NS

NOTE. N = 24 for all variables except RMR (n = 23). *P* values for the correlations between CK and RMR, CK and TEM, OGDH and RMR, and PFK/OGDH and %FAT are .01, .02, .05, and .02 when the mean of each pair is used for the computation.

Table 5. Correlation Between the Proportions of Skeletal Muscle Fibers and the Changes in Body Composition, RMR, and TEM in Response to 100-Day Overfeeding

	Before Overfeeding					
	Type I		Type IIA		Type IIB	
Changes With Overfeeding	*r*	*P*	*r*	*P*	*r*	*P*
ΔWeight (kg)	−0.25	NS	0.33	NS	−0.13	NS
ΔFM (kg)	−0.43	**.035**	0.43	**.035**	−0.01	NS
ΔFFM (kg)	0.16	NS	−0.03	NS	−0.18	NS
Δ%FAT	−0.49	**.01**	0.47	**.021**	0.01	NS
ΔRMR (kJ/kg FFM)	0.08	NS	−0.00	NS	−0.12	NS
ΔTEM (kJ)	−0.09	NS	−0.00	NS	0.13	NS

NOTE. Changes are post-overfeeding minus pre-overfeeding. N = 24 for all variables except RMR (n = 23). *P* values for the correlations between type I skeletal muscle fibers and Δ%FAT, and type IIA skeletal muscle fibers and Δ%FAT are .03 and .01 when the mean of each pair is used.

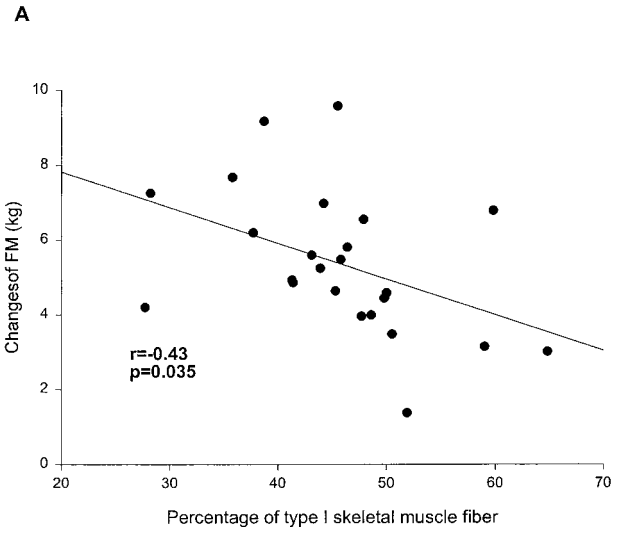

Fig 1. Correlation between the baseline proportions of skeletal muscle fiber type I and the changes of (A) FM and (B) %FAT after 100 days of overfeeding (N = 24 cases).

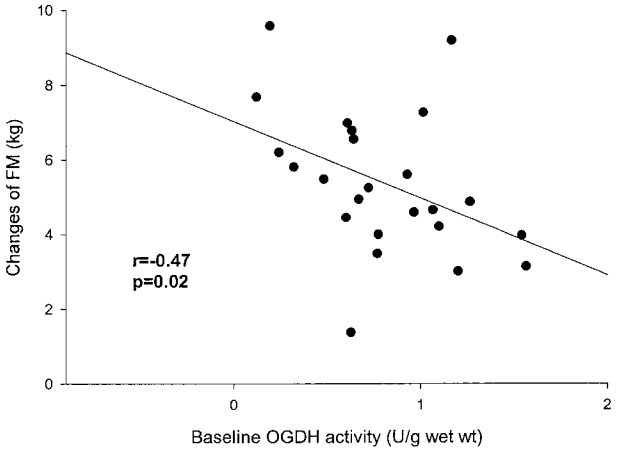

Fig 2. Correlation between the baseline OGDH activity and the changes of FM after 100 days of overfeeding (N = 24 cases).

DISCUSSION

Obesity is the consequence of long-term positive energy balance. Liver, brain, heart, kidneys, and skeletal muscle are the most energy-consuming tissues at rest with skeletal muscle accounting for about 20% to 30% of the total resting oxygen uptake.[3] The present study focused on the relationship between baseline levels of skeletal muscle fiber types and key enzymes in relation to the overfeeding-induced changes in body composition and metabolic rates.

The results showed that the type I skeletal muscle fiber type proportion was inversely correlated with the overfeeding-induced changes in FM and %FAT, whereas the proportions of type IIA fiber showed positive correlations with the same phenotypes. The results suggest that the proportions of muscle fiber type in the vastus lateralis do associate with the changes in adiposity when people are challenged by a long period of energy surplus. OGDH was used as a marker of the Krebs cycle and the oxidative pathway. OGDH activity level decreased significantly with overfeeding, which suggests that long-term energy surplus reduced skeletal muscle oxidative capacity. It had been shown previously that the ratio of PFK to citrate synthase (CS) was increased in obese individuals, in large part due to decreased CS activity.[27] The present study showed that

Table 6. Correlation Between the Baseline Skeletal Muscle Enzyme Activities and the Changes of Body Composition, RMR, and TEM in Response to 100-Day Overfeeding

Changes With Overfeeding	Before Overfeeding							
	CK		OGDH		PFK		PFK/OGDH	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
ΔWeight (kg)	−0.19	NS	−0.39	NS	−0.04	NS	0.30	NS
ΔFM (kg)	−0.23	NS	−0.47	**.02**	−0.10	NS	0.46	**.02**
ΔFFM (kg)	−0.04	NS	−0.10	NS	−0.26	NS	−0.02	NS
Δ%FAT	−0.22	NS	−0.29	NS	0.17	NS	0.21	NS
ΔRMR (kJ/kg FFM)	−0.49	**.017**	0.12	NS	−0.53	**.01**	−0.35	NS
ΔTEM (kJ)	0.20	NS	0.13	NS	0.40	NS	0.13	NS

NOTE. Changes are post-overfeeding minus values of pre-overfeeding. N = 24 for all variables except RMR (n = 23). *P* values for the correlations between OGDH and ΔWeight, OGDH and ΔFM, and PFK and ΔRMR are .002, .03, and .03 when the mean of each pair is used.

baseline OGDH activity correlated negatively with baseline %FAT, which indicates that, even among normal-weight subjects, adiposity is negatively associated with the muscle oxidative profile. Although it has been documented that enzyme markers of oxidative metabolism in skeletal muscle of obese subjects were lower compared with normal-weight subjects,[28] it is not known whether this is the result of the obese state or of a pre-existing condition that may have contributed to obesity. That a low baseline OGDH correlated negatively with both baseline %FAT and changes of FM following long-term overfeeding in the present study suggests that a low skeletal muscle oxidative capacity is in fact a potential contributor to the development of an obese state. Hence, the more active the oxidative pathway, the less adiposity gained under positive energy balance. The positive correlation between the ratio of PFK to OGDH with the changes in FM also supports this notion, a high baseline PFK/OGDH ratio favoring FM gain.

PFK is the key enzyme of the glycolytic pathway. The pre-overfeeding skeletal muscle PFK activity was correlated negatively with the changes in RMR. A low RMR for a given body size and composition is thought to be a risk factor for weight gain,[29] although there is some contradictory evidence. A recent meta-analysis has shown that formerly obese subjects had a 3% to 5% lower mean relative RMR than control subjects.[30] Our result supports the hypothesis that the more active the glycolytic pathway as indicated by a high PFK activity at baseline, the smaller the subsequent increase in RMR in response to overfeeding. It is consistent with the result from a previous study, in which high skeletal muscle anaerobic and glycolytic capacities were shown to be associated with obesity in a cross-sectional design.[28] Approximately 30% to 35% of postprandial thermogenesis over 6 hours occurs in skeletal muscle.[31] There was a positive correlation between PFK and the changes in TEM, although the correlation did not reach statistical significance. Overall, correlations of the magnitude observed here between baseline PFK activity and overfeeding-induced changes in RMR (r = -0.53) and TEM (r = -0.40) suggest that skeletal muscle glycolytic rates may be of considerable importance for human metabolic rates.

CK is the key enzyme in the rapid resynthesis of adenosine triphosphate (ATP). The CK activity was significantly enhanced after the overfeeding protocol. Baseline skeletal muscle CK activity had negative correlations with both baseline RMR and the changes in RMR with overfeeding. Thus, a high baseline CK activity may be indicative of a diminished capacity to increase RMR when exposed to overfeeding.

The physical activity level of the subjects was fully controlled during the entire study. No significant difference in VO_{2max} per kg body weight was observed as a result of the overfeeding protocol. This suggests that the cardiorespiratory fitness of the subjects was unchanged, indicating that the skeletal muscle alterations were caused by overfeeding rather than by a decrease in fitness.

The results of the present study need to be replicated with a larger sample size. Moreover, the subjects were young men who were lean at baseline. The role of skeletal muscle fiber type composition and metabolism could be different in overweight or obese individuals.

In summary, the associations between human skeletal muscle properties and the changes in body composition and metabolic rate phenotypes were investigated in 24 young healthy males (12 pairs of identical twins) in response to 100 days of overfeeding. The proportion of type I skeletal muscle fiber correlated inversely with the overfeeding-induced changes in FM and %FAT, while type IIA fiber proportion correlated positively with the same changes. Baseline skeletal muscle oxoglutarate dehydrogenase activity correlated negatively with both baseline %FAT and the changes in FM. A positive correlation between the baseline ratio of activities of PFK/OGDH and the change in FM was observed in response to overfeeding. Moreover, baseline PFK activity demonstrated a negative correlation with the changes in RMR. The results suggest that an elevated skeletal muscle oxidative capacity plays a protective role in the response to long-term positive energy balance.

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