Skeletal muscle enzymes as predictors of 24-h energy metabolism in reduced-obese persons[1–4]

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ABSTRACT

Background: Little is known about the effects of weight loss on the relation between skeletal muscle enzymes and energy metabolism.

Objective: This study was performed retrospectively to investigate the relation between skeletal muscle enzymes and 24-h energy metabolism in obese persons before and after weight loss.

Design: Ten women and 9 men [with body mass indexes (in kg/m^2) > 30] underwent a 15-wk weight-loss program (-700 kcal/d). Body weight and composition, 24-h energy metabolism (whole-body indirect calorimetry), and maximal activities of phosphofructokinase (EC 2.7.1.11), creatine kinase (CK; EC 2.7.3.2), citrate synthase (CS; EC 4.1.3.7), 3-hydroxyacyl-CoA dehydrogenase (HADH; EC 1.1.1.35), and cytochrome-*c* oxidase (COX; EC 1.9.3.1) were determined from biopsy samples of the vastus lateralis taken before and after weight loss.

Results: Before weight loss, fat-free mass (FFM) was the only predictor of 24-h energy expenditure ($R^2 = 0.70$, $P < 0.001$), whereas the cumulative variance in sleeping metabolic rate explained by FFM and fat mass (FM) was 83% ($P < 0.001$). After weight loss, CS ($r = 0.45$, $P = 0.05$) and COX ($r = 0.65$, $P < 0.01$) were significantly associated with 24-h energy expenditure, whereas CK ($r = 0.53$, $P < 0.05$), CS ($r = 0.45$, $P < 0.05$), COX ($r = 0.64$, $P < 0.01$), and HADH ($r = 0.45$, $P = 0.05$) were all significant correlates of sleeping metabolic rate. After weight loss, FFM, FM, and COX explained 84% ($P < 0.01$) of the variance in 24-h energy expenditure, whereas FFM, FM, and CK all contributed to the cumulative variance in sleeping metabolic rate explained by this model ($R^2 = 0.82$, $P < 0.05$).

Conclusion: Maximal activities of key skeletal muscle enzymes contribute to the variability in 24-h energy metabolism in reduced-obese persons.   *Am J Clin Nutr* 2003;78:430–5.

KEY WORDS   Obesity, weight loss, energy expenditure, skeletal muscle enzymes

INTRODUCTION

The fact that energy expenditure (EE) is related to body weight and even more closely to fat-free mass (FFM) is well recognized (1). Evidence also supports the independent contribution of fat mass (FM) to the variance in EE (2, 3). In this sense, it is not surprising that changes in body weight and composition induce proportional changes in EE (3). Despite this, it is now becoming apparent that factors other than body composition per se can influence the changes in EE that occur in response to weight loss. In fact, it has been reported that the variations in EE that occur in response to body weight loss are greater than what would be expected from the corresponding changes in body weight and composition (4, 5) and that formerly obese subjects typically have lower EEs than do never-obese control subjects (6, 7). Factors such as changes in leptin (8), thyroid hormones (9), catecholamine excretion (9), sympathetic nervous system activity (10), and even organochlorine compounds (11) may all contribute to the variability in the changes in EE that occur in response to fluctuations in body weight.

Skeletal muscle is important in the regulation of EE (12) and constitutes an important site for the utilization of both carbohydrates and lipids (13). Because the synthesis and resynthesis of ATP within skeletal muscle is the result of 5 energy-generating pathways, ie, glycolysis, β-oxidation, the citric acid cycle, the respiration chain, and high-energy phosphate metabolism, and because there are rate-limiting enzymes in each of these pathways, it could be speculated that these enzymes influence EE. Along these lines, it has been shown that the maximal activities of hydroxyacyl-CoA dehydrogenase (HADH; EC 1.1.1.35) and phosphofructokinase (PFK; EC 2.7.1.11) in skeletal muscle contribute independently to the variability in 24-h EE and fat oxidation in weight-stable humans (14).

Another interesting issue pertains to the effects of body weight loss on these key enzymes. Whereas some investigators have reported that weight loss does not significantly decrease skeletal muscle enzyme activity (15, 16), others have shown otherwise (17, 18). As such, the effects of weight loss on the activity of key skeletal muscle enzymes remain to be clarified. Because of the considerable interindividual variability in the response of these enzymes to weight loss and because of their crucial role in the resynthesis of ATP, changes in the activities of key skeletal muscle enzymes brought about by weight loss may contribute to the variance in EE observed in reduced-obese persons. This also remains to be determined.

We thus hypothesized that interindividual differences in the decrease in key enzymes involved in EE might be partly responsible for the changes in energy metabolism that occur in response to weight loss. More precisely, the maximal activities of key skeletal muscle enzymes (determined from biopsies of the vastus lateralis) were correlated with variables of 24-h energy metabolism before and after weight loss and with the changes brought about by the intervention in obese men and women. A secondary objective of this study was to clarify the effects of body weight loss on changes in the activities of key skeletal muscle enzymes.

SUBJECTS AND METHODS

Twenty-six subjects (14 men and 12 women) volunteered to participate in this whole-body calorimeter substudy. The subjects were recruited from a larger group of 46 subjects who underwent weight loss by drug therapy (60 mg fenfluramine/d) or placebo treatment coupled to nonmacronutrient-specific energy restriction (-700 kcal/d) for 15 wk, as previously described (8). Of the 26 subjects who accepted to participate in this substudy (9 from the placebo group and 17 from the fenfluramine group), 19 subjects (9 men and 10 women, 7 from the placebo group and 12 from the fenfluramine group) completed the weight-loss program, and their data are analyzed in the present article. The subjects gave their written consent to participate in this study, which received the approval of the Laval University Medical Ethics Committee.

After the suspension of fenfluramine and dexfenfluramine because of a potential association with disturbances in cardiac valvular function (19), all subjects (including those in the placebo group) underwent an echocardiogram. After this assessment, a detailed analysis of cardiac valvular function was performed by cardiologists, who detected no abnormalities in response to the use of fenfluramine under the conditions described in this study (20).

Anthropometric measurements

Body weight was measured on a standard beam scale, and body density was determined by hydrodensitometry (21). The closed-circuit helium dilution method was used to assess residual lung volume (22). The Siri formula (23) was used to estimate the percentage of body fat from body density, whereas FM and FFM were estimated from the derived percentage of body fat and total body weight.

Muscle biopsies

Muscle samples were obtained 3 wk before the start of the weight-loss intervention and 4–6 wk after the end of the energy restriction. Both pre- and post-weight-loss muscle samples were obtained in a state of weight stability. Subjects who were recruited to participate in this study had been weight stable (body weight variation < 2 kg) for ≥6 mo before being randomly assigned to a study group. As well, between 2 to 4 wk elapsed between the end of the intervention and the post-weight-loss measurements. Subjects were instructed to maintain weight stability during this period, and their mean body weight change was 0.8 ± 2.3 kg (from 92.0 ± 12.2 kg at the end of the intervention to 91.2 ± 12.3 kg at the time of measurement; NS). Biopsy samples were taken from the middle region of the vastus lateralis (15 cm above the patella) and 2 cm away from the fascia by the use of the percutaneous needle biopsy technique described by Evans et al (24) and regularly used in our laboratory (25). Muscle samples were divided into 2 parts: one was frozen in liquid nitrogen for subsequent determination of muscle enzyme activities and the other was trimmed,

mounted, and frozen in isopentane, cooled on liquid nitrogen, and stored at $-80\,°C$ for histochemical analysis.

Activities of skeletal muscle enzymes

Muscle samples were kept at $-80\,°C$ until assayed for maximal enzyme activities. Small pieces of the muscle sample (10 mg) were homogenized in a glass-glass homogenizer with 39 volumes of ice-cold extracting medium (0.1 mol Na-K-phosphate/L, 2 mmol EDTA/L, pH 7.2), and maximal enzyme activities were measured as previously described (26). Key enzymes were chosen to represent every major energy-yielding pathway. PFK was chosen for glycolysis, citrate synthase (CS; EC 4.1.3.7) for the citric acid cycle, cytochrome-*c* oxidase (COX; EC 1.9.3.1) for the respiration chain, HADH for β-oxidation, and creatine kinase (CK; EC 2.7.3.2) for high-energy phosphate metabolism. The ratio of PFK to CS (PFK:CS) was also used as an indicator of the glycolysis to aerobic oxidation capacity. Maximal enzyme activities are expressed as micromoles of substrate consumed per minute per gram of tissue ($\mu mol \cdot min^{-1} \cdot g^{-1}$). The intraindividual reproducibility for these measurements was reported previously (27).

Twenty-four–hour whole-room indirect calorimetric measurements

Twenty-four–hour EE and sleeping metabolic rate (SMR) were measured by using a whole-body calorimeter. The testing protocol and the reproducibility of measurements in the respiratory chamber were previously reported (28). Subjects were fed a mixed diet (food quotient = 0.85) ad libitum during their stay in the calorimeter (29). SMR was calculated as the mean of 2 consecutive hours representing the lowest values of EE obtained during sleep and then extrapolated over 24 h. Because EE and substrate oxidation are influenced by the state of energy balance, and because a slightly greater positive energy balance was observed before weight loss, energy metabolism variables were adjusted for energy balance [energy intake (kcal) $-$ energy expenditure], and regression analyses were performed on residual scores.

Statistical analysis

JMP software (version 3.1.6.2; SAS Institute Inc, Cary, NC) was used for all analyses. Two-way analysis of variance [considering treatment (placebo or fenfluramine) and sex (male or female) as the between-subject factors] for repeated measures (considering time as the within-subject factor) was performed for all dependent variables. No significant effects of treatment (drug compared with placebo) and no interactions between sex and treatment, time and treatment, or sex and treatment and time were observed for any of the variables reported here. Partial correlation analyses (adjusted for sex, FM, and FFM) were performed between maximal activities of skeletal enzymes and 24-h energy metabolism variables (adjusted for energy balance) before and after body weight loss and for the changes in these variables (after weight loss $-$ before weight loss). Stepwise multiple regression analyses were then performed to identify predictors of 24-h energy metabolism before and after weight loss and for the changes in these variables (after weight loss $-$ before weight loss). Variables entered in these models included maximal activities of PFK, CK, CS, HADH, and COX; PFK:CS; sex; and body composition (FM and FFM). Probability to enter the models was set at $P \leq 0.05$. Data are expressed as means $\pm$ SDs, and effects were considered significant at $P \leq 0.05$.

RESULTS

The characteristics of the subjects before and after the weight-loss program are presented in **Table 1**. A significant effect of time

TABLE 1
Subject characteristics before and after body weight loss[1]

| | Weight loss | | Effect[2] | | |
	Before	After	Sex	Time	Sex × time
Body weight (kg)	101.6 ± 12.3[3]	92.0 ± 12.2	NS	<0.001	NS
Fat mass (kg)	44.6 ± 9.7	36.5 ± 12.4	<0.001	<0.001	NS
Fat-free mass (kg)	57.0 ± 11.7	55.5 ± 12.0	<0.001	NS	NS
BMI (kg/m²)	35.9 ± 4.1	32.7 ± 4.9	0.006	<0.001	NS

[1] $n = 9$ men and 10 women.

[2] Two-way ANOVA (with sex and treatment as between-subject factors) for repeated measures (with time as a within-subject factor). There were no significant effects of treatment (fenfluramine compared with placebo) and no significant sex-by-treatment, time-by-treatment, or sex-by-treatment-by-time interactions.

[3] $\overline{x} \pm$ SD.

was observed for body weight (-9%; $P < 0.001$), FM (-19.4%; $P < 0.001$), and body mass index. Although FFM was slightly reduced in response to this program, this difference was not significant. The men and women did not differ significantly in age (44.1 ± 2.0 compared with 39.8 ± 1.9 y, respectively; $P = 0.14$).

As expected, 24-h EE was significantly reduced after the weight-loss program (2379 ± 345 kcal/d before compared with 2134 ± 315 kcal/d after; $P < 0.001$; **Table 2**). After the intervention, SMR values were significantly lower than before weight loss (1761 ± 245 kcal/d before compared with 1617 ± 236 kcal/d after; $P < 0.01$). Both the 24-h respiratory quotient (RQ) and the RQ SMR tended to decrease in response to the intervention, although not significantly so. However, a significant sex-by-time interaction was observed for RQ SMR, indicating different trends in men (0.89 ± 0.06 before compared with 0.91 ± 0.10 after) and women (0.97 ± 0.06 before compared with 0.87 ± 0.04 after) for this variable. In addition, the subjects tended to be in a slightly greater positive energy balance before weight loss than after weight loss (941 ± 453 compared with 793 ± 511 kcal/d, respectively; NS).

Variations in maximal activities of key skeletal muscle enzymes in response to weight loss are shown in **Table 3**. No significant effect of time was observed for any of the enzymes reported here. A significant effect of sex was observed for CS ($P < 0.05$), outlining slightly higher maximal activity for this enzyme in men. To confirm these observations, we compared the maximal activities of these enzymes before and after weight loss in all subjects (22 women and 21 men) for whom we had values (values of 24-h energy metabolism, however, were available only for the 19 subjects presented in this article). The results from these analyses confirmed that this weight-loss program did not cause any significant reductions in the maximal activities of the key enzymes reported.

Partial correlation analyses adjusted for sex and body composition (FM and FFM) were performed between 24-h energy metabolism variables and maximal activities of skeletal muscle enzymes before and after weight loss and on the changes that occurred during the intervention (after weight loss − before weight loss). No significant correlations were observed in weight-stable obese subjects before weight loss. Post-weight-loss partial correlation coefficients are shown in **Table 4**. Twenty-four–hour EE was associated with CS ($r = 0.45$, $P < 0.05$) and COX ($r = 0.65$, $P < 0.01$), whereas CK ($r = 0.53$, $P < 0.05$), CS ($r = 0.45$, $P = 0.05$), COX ($r = 0.64$, $P < 0.01$), and HADH ($r = 0.45$, $P = 0.05$) were all associated with SMR at the end of the intervention. Similarly, significant associations between changes in the maximal activities of key enzymes and changes in 24-h energy metabolism were noted. Changes in 24-h EE were significantly associated with changes in PFK:CS ($r = -0.47$, $P < 0.05$). Changes in PFK were also associated with changes in 24-h RQ ($r = 0.57$, $P = 0.01$), whereas changes in SMR were associated with changes in CS ($r = 0.60$, $P < 0.01$), HADH ($r = 0.48$, $P < 0.05$), COX ($r = 0.43$, $P = 0.06$), and PFK:CS ($r = -0.47$, $P < 0.05$).

Stepwise multiple regression analyses were performed to further document the contribution of skeletal muscle enzyme activity to the variance in 24-h energy metabolism. The results of these analyses are presented as the cumulative variance explained by these models and are shown in **Tables 5–7**. The only significant predictor of 24-h EE before weight loss was FFM ($R^2 = 0.70$, $P < 0.001$; Table 5). Before weight loss, sex entered the model as a significant predictor of 24-h RQ ($R^2 = 0.50$, $P < 0.001$) and RQ SMR ($R^2 = 0.45$, $P < 0.01$). Predictors of pre-weight-loss SMR included FFM and FM ($R^2 = 0.83$, $P < 0.001$).

Shown in Table 6 are results of the stepwise multiple regression analyses performed after the weight-loss program. FFM, FM,

TABLE 2
Energy metabolism values before and after body weight loss[1]

| | Weight loss | | Effect[2] | | |
	Before	After	Sex	Time	Sex × time
24-h EE (kcal/24 h)	2379 ± 345[3]	2134 ± 315	0.009	0.001	NS
24-h RQ	0.88 ± 0.05	0.86 ± 0.05	NS	NS	NS
24-h SMR (kcal/24 h)	1761 ± 245	1617 ± 236	NS	0.009	NS
24-h RQ SMR	0.93 ± 0.07	0.89 ± 0.08	NS	NS	0.02

[1] $n = 9$ men and 10 women. EE, energy expenditure; RQ, respiratory quotient; SMR, sleeping metabolic rate.

[2] Two-way ANOVA (with sex and treatment as between-subject factors) for repeated measures (with time as a within-subject factor). There were no significant effects of treatment (fenfluramine compared with placebo) and no significant sex-by-treatment, time-by-treatment, or sex-by-treatment-by-time interactions.

[3] $\overline{x} \pm$ SD.

TABLE 3
Skeletal muscle enzyme activity before and after body weight loss[1]

	Weight loss		Effect[2]		
	Before	After	Sex	Time	Sex × time
	$\mu mol \cdot min^{-1} \cdot g^{-1}$				
PFK	58.3 ± 10.5^3	56.2 ± 8.7	NS	NS	NS
CK	431.5 ± 43.8	399.3 ± 69.4	NS	NS	NS
CS	9.4 ± 1.7	8.9 ± 2.2	0.04	NS	NS
COX	7.6 ± 1.4	6.9 ± 2.0	NS	NS	NS
HADH	15.4 ± 2.5	14.8 ± 3.3	NS	NS	NS
PFK/CS	6.4 ± 1.4	6.5 ± 1.4	NS	NS	NS

[1] n = 9 men and 10 women. PFK, phosphofructokinase; CK, creatine kinase; CS, citrate synthase; COX, cytochrome c-oxidase; HADH, 3-hydroxyacyl-CoA dehydrogenase.

[2] Two-way ANOVA (with sex and treatment as between-subject factors) for repeated measures (with time as a within-subject factor). There were no significant effects of treatment (fenfluramine compared with placebo) and no significant sex-by-treatment, time-by-treatment, or sex-by-treatment-by-time interactions.

[3] $\overline{x} \pm$ SD.

and COX together explained 84% ($P < 0.01$) of the cumulative variance in 24-h EE. CK was the only significant predictor of 24-h RQ ($R^2 = 0.24$, $P < 0.05$). FFM and FM explained 74% ($P < 0.01$) of the variance in post-weight-loss SMR, and CK increased the cumulative explained variance of the model to 82% ($P < 0.05$).

Changes in FM, FFM, and PFK:CS together explained 67% of the variance in the changes in 24-h EE, whereas changes in FM ($P < 0.01$) and in PFK ($P < 0.05$) explained 59% of the variance in changes in 24-h RQ (Table 7). Similarly, changes in FM, FFM, and CS explained 78% of the cumulative variance in changes in SMR. Finally, sex was a significant predictor of changes in RQ SMR ($R^2 = 0.34$, $P < 0.01$).

DISCUSSION

The main finding of the present study was that maximal activities of key skeletal muscle enzymes become independent predictors of 24-h energy metabolism after body weight loss. In addition, the present results support previous reports that an ≈10% weight loss is not sufficient to significantly alter the oxidative potential of skeletal muscle.

TABLE 4
Partial correlations, adjusted for sex, fat mass, and fat-free mass, between skeletal muscle enzyme activity and adjusted scores of 24-h energy expenditure (EE), sleeping metabolic rate (SMR), and respiratory quotient (RQ) after body weight loss[1]

	24-h EE	24-h RQ	SMR	RQ SMR
PFK	0.21	−0.27	0.24	−0.24
CK	0.41	−0.32	0.53^2	−0.30
CS	0.45^2	−0.30	0.45^2	−0.40
COX	0.65^3	0.06	0.64^3	−0.04
HADH	0.34	−0.20	0.45^2	−0.22
PFK:CS	−0.32	0.14	−0.35	0.28

[1] n = 9 men and 10 women. PFK, phosphofructokinase; CK, creatine kinase; CS, citrate synthase; COX, cytochrome c-oxidase; HADH, 3-hydroxyacyl-CoA dehydrogenase.

[2] $P \leq 0.05$.

[3] $P \leq 0.01$.

TABLE 5
Stepwise multiple regression analysis for predictors of 24-h energy metabolism before body weight loss[1]

Dependent variable	Step no.	Predicting variable	R^2	P
24-h EE	1	FFM	0.7	<0.001
24-h RQ	1	Sex	0.50	<0.001
SMR	1	FFM	0.63	<0.001
	2	FM	0.83	<0.001
RQ SMR	1	Sex	0.45	0.002

[1] n = 9 men and 10 women. EE, energy expenditure; RQ, respiratory quotient; SMR, sleeping metabolic rate; FFM, fat-free mass; FM, fat mass.

It was intriguing to observe that the relation between EE and enzymatic activity emerged only after body weight loss because Zurlo et al (14) showed previously that 24-h energy metabolism was independently associated with some key enzymes in a cross-sectional study of 14 weight-stable men and women. In the present study, we observed no such associations before body weight loss. Although it is speculative, it is nonetheless possible that the increased gradient of substrate associated with obesity homogenizes the variance in the enzymatic profile, thus blurring such relations. In addition, even if no significant changes in the enzymatic profile had been observed after weight loss, some persons did indeed display a marked decrease in maximal activities, thus making the relation between EE and the enzymatic activity of skeletal muscle more apparent. Some results partly support this idea. It was shown that postobese women have lower maximal activities of key skeletal muscle enzymes than do normal-weight subjects (30) and that formerly obese subjects typically have lower resting energy expenditure values than do matched lean control subjects (6, 7).

It is obvious that we cannot conclude a causal relation from this design. Nonetheless, these results suggest that marked variations in the enzymatic profile of skeletal muscle can contribute to the variability in 24-h energy metabolism in reduced-obese men and women. It could be concluded that the reduction in EE associated with the reduction in body weight results in a decrease in key enzymatic activities. The decrease in skeletal muscle activity could in turn be interpreted as a result of the decrease in EE and not as a causative factor. It is clear that we cannot disregard this possibility, and that it might partially explain the associations between skeletal enzyme activity and 24-h energy metabolism during and after weight loss. Nonetheless, we tend to believe that other factors could affect enzymatic activities after weight loss.

TABLE 6
Stepwise multiple regression analysis for predictors of 24-h energy metabolism after body weight loss[1]

Dependent variable	Step no.	Predicting variable	R^2	P
24-h EE	1	FFM	0.59	<0.001
	2	FM	0.72	0.02
	3	COX	0.84	0.005
24-h RQ	1	CK	0.24	0.03
SMR	1	FFM	0.34	0.009
	2	FM	0.74	<0.001
	3	CK	0.82	0.02

[1] n = 9 men and 10 women. EE, energy expenditure; RQ, respiratory quotient; SMR, sleeping metabolic rate; FFM, fat-free mass; FM, fat mass; COX, cytochrome c-oxidase; CK, creatine kinase.

TABLE 7
Stepwise multiple regression analysis for predictors of change (Δ) in 24-h energy metabolism before body weight loss[1]

Dependent variable	Step no.	Predicting variable	R^2	P
Δ24-h EE	1	ΔFM	0.4	0.004
	2	ΔFFM	0.57	0.02
	3	ΔPFK:CS	0.67	0.05
Δ24-h RQ	1	ΔFM	0.38	0.005
	2	ΔPFK	0.59	0.01
ΔSMR	1	ΔFM	0.37	0.006
	2	ΔFFM	0.69	<0.001
	3	ΔCS	0.78	0.02
ΔRQ SMR	1	Sex	0.34	0.009

[1] n = 9 men and 10 women. EE, energy expenditure; RQ, respiratory quotient; SMR, sleeping metabolic rate; FM, fat mass; FFM, fat-free mass; CS, citrate synthase; PFK, phosphofructokinase.

Recent results from our group have highlighted the possibility that the rise in plasma organochlorine concentration that results from weight loss is independently associated with changes in the enzymatic profile of skeletal muscle (31). This factor and others could thus contribute to a more pronounced decrease in the enzymatic activity of skeletal muscle in some persons that could in turn introduce large interindividual variability in energy metabolism in reduced-obese persons.

Contrasting results exist in terms of the effects of body weight loss on the maximal activity of key oxidative enzymes. Whereas some researchers have shown no changes in the activities of HADH and CS (15, 16), others have shown a significant decrease in COX and HADH in response to a weight-loss program (17, 18). These differences might be partly explained by the greater decrease in body weight (-15%) in the studies by Kelley et al (17) and Simoneau et al (18), and it might only be under such circumstances that changes in key enzyme activities occur in a greater proportion of subjects. In the present study, as in others in which a similar amount of body weight loss was observed (15, 16), no significant reduction in key enzyme activities was observed after weight loss, even if some trends were noted for most enzymes.

We were unable to show any consistent relations between the enzymatic profile of skeletal muscle and whole-body RQ (an indicator of substrate partitioning), either before or after body weight loss. Our results agree with those of Blaak et al (15), who reported no significant associations between changes in HADH and CS and changes in resting fat oxidation, even though they found evidence of a relation between variations in cystolic fatty acid binding protein and changes in resting fat oxidation during weight loss. In contrast, Zurlo et al (14) reported that HADH was negatively associated with 24-h RQ. One of the characteristics of the latter report was that lean, weight-stable humans were measured during energy balance. Because the obese subjects in the present study were measured while in positive energy balance, and because the RQ is responsive to this situation (32), it is possible that the association was blurred by this condition, even if a statistical correction was applied to take this factor into account. Another important aspect that must be discussed is that although whole-body RQ is not systematically different between lean and obese persons, leg RQ, as measured by arteriovenous differences in oxygen and carbon dioxide values, is considerably higher in obese persons and this difference persists even after body weight loss (17). These observations could complicate the comparison of the effects of skeletal muscle enzyme activity on substrate oxidation between lean and obese persons. In any event, the association between oxidative potential and whole-body fat oxidation, if any, will need to be investigated further.

Recent results have shown that skeletal muscle ultrastructure and metabolism can play a significant role in the fat deposition observed in response to prolonged positive energy balance. Persons with an increased proportion of type 1 fibers have a lesser FM gain at the end of 100 d of overfeeding, whereas persons with a high ratio of PFK to oxoglutarate dehydrogenase seem predisposed to greater fat deposition under such conditions (33). Although it remains to be determined whether this is also the case for body weight and fat regain after prolonged negative energy balance, these latter results suggest that the role of skeletal muscle in the regulation of energy balance is robust enough to potentiate or attenuate the effects of positive energy balance on body fat gain.

The role of physical activity as a possible therapeutic agent favoring weight maintenance after weight loss has received much attention (34). More specifically, persons who weekly expend large amounts of energy ($\approx$2800 kcal/wk) through vigorous physical activity can remain weight stable after weight loss (35). Beyond the contribution of this amount of EE to the maintenance of energy balance in a reduced-obese state, it is also possible that part of the effects of vigorous exercise in the maintenance of weight stability are exerted through effects on the oxidative potential of skeletal muscle. Indeed, previous results showed that high-intensity intermittent training induces a greater weight loss than does a continuous aerobic-type exercise regimen, despite the lower energy cost of the higher-intensity exercise program (36). The authors of that study concluded that part of this effect may be attributed to the effects of this program on the oxidative potential. We can only give a partial answer to this question. We previously showed that EE is increased in reduced-obese men after an 18-wk exercise regimen (37). Moreover, by the end of the exercise follow-up in 6 of these men, the activities of HADH, CS, and COX had increased by 14% ($P = 0.08$), 56% ($P < 0.05$), and 37% ($P < 0.01$), respectively. Unfortunately, because too few subjects were sampled for both biopsies and calorimetric measurements at that time, we were unable to corroborate these observations with correlation analyses. Although it is clear that more studies will be needed to confirm this hypothesis, it is possible that physical activity favors weight maintenance through its probable effects on the oxidative potential of skeletal muscle in some persons.

In summary, the results of the present study suggest that changes in the activities of key skeletal muscle enzymes after weight loss may be sufficient to complicate the maintenance of energy balance in some reduced-obese persons. Future studies will be needed to investigate whether an altered skeletal muscle enzyme profile after weight loss predisposes to weight regain and whether the effects of a physical activity program favor body weight maintenance partially through effects on these enzymes.

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