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Research Article

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Relationship between Skeletal Muscle Lipoprotein Lipase Activity and 24-hour Macronutrient Oxidation

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Abstract

A low ratio of whole-body 24-h fat/carbohydrate (CHO) oxidation has been shown to be a predictor of subsequent body weight gain. We tested the hypothesis that the variability of this ratio may be related to differences in skeletal muscle metabolism. Since lipoprotein lipase (LPL) plays a pivotal role in partitioning lipoprotein-borne triglycerides to adipose (storage) and skeletal muscle (mostly oxidation), we postulated that a low ratio of fat/CHO oxidation was associated with a low skeletal muscle LPL (SMLPL) activity. As an index of substrate oxidation, 24-h RQ was measured under sedentary and eucaloric conditions in 16 healthy nondiabetic Pima males. During a 6-h euglycemic, hyperinsulinemic clamp, muscle biopsies were obtained at baseline, 3, and 6 h. Heparin-elutable SMLPL activity was 2.92 ± 0.56 nmol free fatty acids/g $\cdot$ min (mean $\pm$ SD) at baseline, was unchanged (2.91 ± 0.51) at the third hour, and increased significantly ($P < 0.05$) to 3.13 ± 0.57 at the sixth hour of the clamp. The mean (of baseline and 3-h) SMLPL activity correlated inversely with 24-h RQ ($r = 0.57$, $P < 0.03$) but not with body size, body composition, or insulin-mediated glucose uptake. Since SMLPL activity is related to the ratio of whole body fat/CHO oxidation rate, a decreased muscle LPL activity may, therefore, predispose to obesity. (*J. Clin. Invest.* 1993. 92:441–445.) Key words: lipoprotein lipase • 24-h respiratory quotient • euglycemic, hyperinsulinemic clamp • skeletal muscle • human

Introduction

24-h RQ, an index of the ratio of carbohydrate (CHO)¹/fat oxidation rate, has been reported to vary widely between individuals under eucaloric conditions (1). Since family membership explained 28% of the variability in 24-h RQ, genetic factors may play a role in determining an individual's capacity to oxidize and therefore partition dietary energy. More impor-

tantly, a decreased ratio of fat/CHO oxidation rate was found to be a predictor of subsequent body weight gain (1, 2). Therefore, attention should be focused on the possible mechanisms underlying fat balance and nutrient partitioning.

Skeletal muscle is the largest tissue in the body, accounting for 20–30% of oxygen consumption while resting and up to 90% during exercise (3). The heterogeneity of human skeletal muscle with respect to the composition of muscle fiber types may account for differences in muscle metabolism and possibly for the variability in metabolic rate and whole-body substrate utilization rates. The RQ of skeletal muscle at rest is close to 0.70 with lipid, primarily fatty acids (FA), accounting for $\geq 80\%$ of substrate oxidation (4). Most FA entering muscle cells are esterified and enter intracellular pools with slow resting turnover rates (5, 6). During exercise, turnover is more rapid and this lipid reserve is an important source of energy until mobilization of FA from adipose tissue increases sufficiently to meet demand (7, 8). Besides plasma-free FA, triglycerides in lipoproteins, i.e., VLDL and chylomicrons, are an important source of FA for oxidation in muscle (9). Lipoprotein lipase (LPL) is the rate-limiting enzyme hydrolyzing lipoprotein-borne triglycerides into FA and glycerol at the endothelial/luminal interface of capillaries (10, 11). Uptake of triglyceride-derived FA occurs by diffusion into subjacent tissues and is directly related to LPL activity (12). LPL activity is known to be regulated by nutritional and hormonal factors in a tissue-specific manner and therefore is directly involved in energy substrate partitioning (13).

Wade et al. (14) showed that the combustion of FA during light exercise is related to muscle fiber-type proportions. It is also recognized, from studies in both humans and animals, that muscle LPL activity is higher in muscles composed predominantly of high-oxidative slow twitch fibers and low in muscle with greater content of low-oxidative fast twitch fibers (15–18). We hypothesized that a high 24-h RQ, i.e., a low fat/CHO oxidation ratio, is associated with low skeletal muscle LPL activity. Since a high 24-h RQ is a risk factor for body weight gain, a low muscle LPL activity might represent a mechanism leading to decreased muscle lipid oxidation and, consequently, increased lipid storage in adipose tissue. We, therefore, studied fasting and insulin-stimulated muscle LPL activities and their relationships to 24-h substrate oxidation in 16 nondiabetic Pima Indian males.

Methods

Subjects. 16 male Pima Indians were admitted for 6–7 d to the metabolic ward of the Clinical Diabetes and Nutrition Section of the National Institute of Diabetes and Digestive and Kidney Disease (NIDDK; Phoenix, AZ). The studies were approved by the ethical committee of the NIDDK and by the tribal leaders of the Gila River Indian Community and subjects gave informed consent. All the subjects were in good health, as assessed by medical history; physical exami-

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1. Abbreviations used in this paper: CHO, carbohydrate; CV, coefficient of variation; EMBS, estimated metabolic body size; FA, fatty acids; KRP, Krebs-Ringer phosphate; LPL, lipoprotein lipase; SMLPL, skeletal muscle LPL.

nation; electrocardiography; and routine hematological, biochemical, and urine tests.

During the entire admission period, the subjects were fed a weight maintenance diet composed of 30% fat, 50% carbohydrate, and 20% protein. The energy content was first calculated on the basis of body weight and sex and then adjusted to maintain a constant weight ($\pm 1\%$). After receiving this diet for ≥ 2 d, an oral glucose tolerance test was performed (19). Four subjects were found to be glucose intolerant but none had diabetes mellitus. Body composition was determined by underwater weighing with simultaneous determination of residual lung volume (20). Percent body fat was calculated from body density using the Siri formula (21). Circumferences of the waist (at the level of the umbilicus) and thigh (at the gluteal fold) were measured supine and standing, respectively. The ratio of waist/thigh circumference was an index of body fat distribution. The characteristics of the subjects are shown in Table I.

Energy expenditure measurements. After ≥ 3 d on the metabolic ward, 24-h energy expenditure and RQ were measured in a respiratory chamber as previously described (22). Measurements were performed for 23 h and then extrapolated to 24 h. The 24-h RQ is the ratio between 24-h carbon dioxide production and 24-h oxygen consumption and is used to calculate energy substrate oxidation (23).

Euglycemic hyperinsulinemic clamp. After ≥ 5 d on the metabolic ward and after a 12-h overnight fast, a euglycemic hyperinsulinemic clamp was performed. At 0400, after the subjects had voided, an intravenous catheter was placed in an antecubital vein for infusion of insulin, glucose and [$3\text{-}^3\text{H}$]glucose. Another catheter was placed retrograde in a dorsal vein of the contralateral hand for blood sampling. To arterialize the blood, the hand was kept in a warming box at 70°C . A primed ($30\text{ }\mu\text{Ci}$) continuous ($0.3\text{ }\mu\text{Ci}/\text{min}$) infusion of [$3\text{-}^3\text{H}$]glucose was administered from 0400 until the end of the procedure. After 1.5 h, four plasma samples were obtained during a 20-min period for plasma insulin levels and [$3\text{-}^3\text{H}$]glucose-specific activity determinations. After 2 h, a primed continuous infusion of purified pork insulin (Velosulin; Nordisk-USA, Bethesda, MD) ($60\text{ mU}/\text{m}^2$ per min) was started. Immediately after, a variable 20% glucose infusion was started to maintain the plasma glucose concentration at approximately the

basal glucose level for the entire 6 h of hyperinsulinemia. [$3\text{-}^3\text{H}$]Glucose tracer was added to the exogenous glucose infusate to minimize the underestimation of the endogenous glucose production rate (24). Samples for plasma glucose concentrations were obtained every 5 min throughout the test. Samples for plasma insulin and [$3\text{-}^3\text{H}$]glucose-specific activity were obtained every 10 min from 140 to 180 min and again from 320 to 360 min. Mean plasma insulin concentrations (mean $\pm$ SEM) were $99 \pm 6\text{ }\mu\text{U}/\text{ml}$ (coefficient of variation [CV] = $13 \pm 3\%$) and $99 \pm 7\text{ }\mu\text{U}/\text{ml}$ (CV = $14 \pm 5\%$) at 3 and 6 h, respectively. The corresponding plasma glucose concentrations were 93 ± 0.9 and 95 ± 0.9 (CV = $2.7 \pm 0.3\%$ and $2.7 \pm 0.2\%$, respectively).

Muscle biopsies. Biopsies of the vastus lateralis muscle were obtained via an 8-mm incision through skin and fascia 1–2 inches from the midline in the midlateral thigh using a Bergström needle. The patient was supine, with the lower limb slightly internally rotated, and the needle was directed vertically. Biopsies were performed at the end of the baseline period before insulin infusion, and then after 180 and 360 min of insulin/glucose infusion. All biopsies were quickly blotted in gauze, frozen in liquid nitrogen, and stored at -70°C .

Calculations. The glucose disappearance rate (R_d) was calculated from [$3\text{-}^3\text{H}$]glucose-specific activities, the specific activity of the glucose infusate, and the glucose infusion rate using a modification of Steel's non-steady state equations during two 40-min periods (140–180 and 320–360 min) (24). The endogenous glucose production rate was calculated as the difference between the glucose R_d and the exogenous glucose infusion rate. In eight subjects, endogenous glucose production rate was negative but considered totally suppressed. Whole-body glucose disposal rate was considered the greater of either R_d or exogenous glucose infusion rate. To account for differences in metabolic size among individuals, glucose disposal rates were divided by the estimated metabolic body size (EMBS; fat-free mass + 14), as described elsewhere, and then adjusted for steady state plasma glucose and insulin concentrations (25, 26). The adjusted glucose disposal rates represent the index of insulin action (M value).

LPL assay. The LPL assay used in these studies was modified from the method of Eckel et al. (27). Frozen muscle tissue was thawed in cold Krebs–Ringer phosphate (KRP) buffer (pH = 7.4) containing $2\text{ }\mu\text{g}/\text{ml}$ aprotinin (Sigma Chemical Co., St. Louis, MO). Tissue was minced in cold buffer, blotted on filter paper, and weighed. Skeletal muscle LPL (SMLPL) activity was eluted from tissue pieces (40–50 mg) by incubation for 45 min at room temperature in $400\text{ }\mu\text{l}$ KRP buffer containing $2\text{ }\mu\text{g}/\text{ml}$ aprotinin and $15\text{ }\mu\text{g}/\text{ml}$ heparin (Fisher Scientific Co., Pittsburgh, PA). Enzyme activity was measured as hydrolyzed ^{14}C -labeled fatty acids after incubation of 0.1 ml of eluted enzyme with 0.1 ml of substrate for 45 min at 37°C . The substrate was prepared with $2.5\text{ }\mu\text{Ci}$ [^{14}C]triolein (Amersham Corp., Arlington Heights, IL), 5 mg triolein (Sigma Chemical Co.), and 0.24 mg L- α -lecithin (Calbiochem Corp., La Jolla, CA), to which 1.9 ml distilled water, 1.0 ml 2 M Tris (pH-8.2), 0.8 ml 10% FA-free albumin (Sigma Chemical Co.), and 0.3 ml human serum were added. Emulsification of the substrate (final volume, 4 ml) was performed with a sonicator (Heat Systems-Ultrasonics, Inc., Farmingdale, NY) for 100 s on ice at a setting of 34 (74.8 W). The reaction was stopped by the addition of 3.4 ml of an organic mixture containing chloroform/methanol/heptane (1.25:1.41:1.00) and FA were extracted with a bicarbonate buffer (pH = 10). After mixing and centrifugation, an aliquot of the top layer containing the extracted FA was counted in a beta scintillation counter (Beckman Instruments, Inc., Fullerton, CA). Data were expressed as nanomoles of free fatty acids released per minute per gram of tissue (nmol FFA/g $\cdot$ min). The intraassay CV for replicates of muscle from the same biopsy was 11%.

A test for specificity was performed by assessing the ability of a polyclonal goat anti-rat LPL antibody to inhibit LPL activity. The anti-LPL antisera was diluted (1:10 or 1:100) in KRP buffer containing heparin-released LPL. For comparison, nonimmune goat serum at the same dilutions was mixed with heparin-released LPL in KRP buffer. After incubation for 1.5 h at 4°C , LPL activity was assayed as usual. Specific activity was reduced by $> 85\%$ in the sample mixed with anti-LPL antisera compared with control.

Table I. Physical Characteristics, 24-h RQ, and Insulin-mediated Glucose Disposal Rate of 16 Nondiabetic Subjects

Subject	Weight	Height	Waist/thigh ratio	Body fat	24-h RQ	Glucose disposal
	kg	cm		%		mg/kg EMBS* $\cdot$ min
1	193.7	183	1.91	48	0.866	2.51
2	85.6	174	1.38	14	0.860	8.29
3	97.2	173	1.60	29	0.823	4.53
4	129.1	171	1.89	44	0.885	6.30
5	82.6	176	1.68	29	0.857	5.24
6	168.1	184	1.81	47	0.875	2.24
7	82.0	169	1.68	28	0.858	4.45
8	85.1	169	1.65	34	0.868	4.78
9	61.7	166	1.81	30	0.830	10.97
10	58.8	161	1.52	21	0.895	7.17
11	83.3	170	1.61	28	0.904	6.12
12	77.8	171	1.46	27	0.831	8.27
13	152.1	182	1.67	44	0.832	5.67
14	97.8	163	1.67	43	0.864	3.01
15	103.5	174	1.84	36	0.827	3.93
16	92.2	177	1.55	24	0.869	4.02
Mean	103.2	173	1.67	33	0.859	5.52
SD	38.2	7	0.15	10	0.025	2.40

* EMBS, estimated metabolic body size (fat-free mass [kg] + 14); (see reference 18).

Statistical analyses. All analyses were performed using the programs of the SAS Institute (Cary, NC), including Spearman rank correlations and Wilcoxon signed rank test. The individual changes in LPL activity during the clamp were subjected to analysis of variance for repeated measures and then, because of a significant time effect, a paired *t* test was performed to evaluate the effect of insulin on LPL activity.

Results

The mean±SD basal SMLPL activity was 2.92 ± 0.56 nmol FFA/g·min, ranging from 2.08 to 4.07. Basal SMLPL was not correlated with body weight, percent body fat, or rate of insulin-mediated glucose uptake, but correlated with the waist/thigh ratio ($r_s = 0.54$, $P < 0.03$).

Individual muscle LPL activity during the euglycemic hyperinsulinemic clamp is shown in Table II and Fig. 1 *a*. SMLPL activity was unchanged after 3 h (2.91 ± 0.51) but increased significantly at 6 h postinsulin infusion to 3.13 ± 0.57 nmol FFA/g·min ($P < 0.05$) (Fig. 1 *b*). Neither the SMLPL activities at 3 and 6 h nor the changes in activity over baseline correlated with body weight, percent body fat, or the rate of insulin-mediated glucose uptake.

To further evaluate the effect of insulin on SMLPL activity, we divided the subjects into two subgroups of seven insulin-resistant (3.6 ± 1.0 mg/kg EMBS·min) and seven sensitive subjects (8.0 ± 1.8 mg/kg EMBS·min), matched for body weight (117 ± 44 vs. 93 ± 35 kg, $P = 0.2$) and percent body fat ($37 \pm 9\%$ vs. $30 \pm 11\%$, $P = 0.3$). After 3 h of insulin infusion, the change in SMLPL activity from baseline was similar in both subgroups but was significantly higher at 6 h in the insulin-resistant subjects (0.26 ± 0.23 vs. 0.13 ± 0.34 nmol FFA/g·min, $P < 0.03$).

The mean 24-h RQ was 0.859 ± 0.025 , ranging from 0.823 to 0.904. 24-h RQ did not correlate with body weight, body

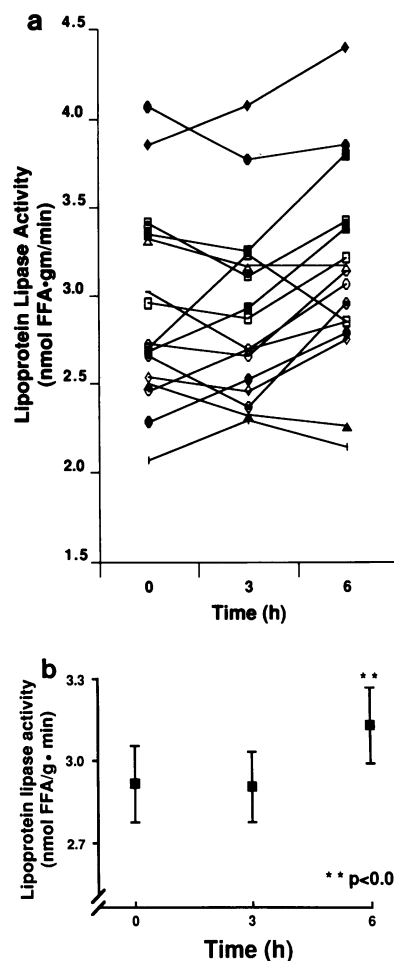


Figure 1. (a) Time course of SMLPL activity during insulin infusion. SMLPL activity for each individual during the euglycemic hyperinsulinemic clamp is shown connected by a solid line. LPL activity is expressed as nmol FFA/g·min. (b) Each point represents the group mean±SEM SMLPL activity. The mean value at 6 h is significantly higher than baseline ($P < 0.05$).

Table II. SMLPL Activity at Baseline, 3-, and 6-h Postinsulin Infusion

Subject	Postinsulin Infusion			Mean LPL activity*
	Baseline	3-h	6-h	
1	2.96	2.87	3.22	2.92
2	2.47	2.79	3.07	2.59
3	2.69	2.93	3.39	2.81
4	4.07	2.78	2.86	3.93
5	3.41	3.12	3.42	3.27
6	2.66	2.37	2.96	2.52
7	3.02	2.70	2.86	2.86
8	2.08	2.30	2.15	2.19
9	3.32	3.17	3.17	3.25
10	2.50	2.33	2.27	2.42
11	2.54	2.47	2.75	2.51
12	3.86	4.08	4.41	3.97
13	2.71	3.24	2.86	2.98
14	2.73	2.66	3.14	2.70
15	3.35	3.26	3.80	3.31
16	2.29	2.53	2.79	2.41
Mean	2.92	2.91	3.13	2.91
SD	0.56	0.51	0.57	0.52

* Means of baseline and 3-h postinsulin infusion.

composition, waist/thigh ratio, or the rate of insulin-mediated glucose uptake. However, 24-h RQ correlated inversely with SMLPL activity at baseline ($r_s = -0.42$, $P = 0.1$), 3 h ($r_s = -0.64$, $P < 0.01$), 6 h ($r_s = -0.54$, $P < 0.03$), and with the mean of basal and 3-h postinsulin SMLPL activities ($r_s = -0.57$, $P < 0.03$) (Fig. 2). The relationships between basal, 3- and 6-h insulin-stimulated skeletal muscle LPL activity and 24-h RQ were all stronger ($r = -0.65$, $P < 0.01$; $r = -0.86$, $P < 0.0001$; $r = -0.74$, $P < 0.002$, respectively) when subject 4 was deleted (24-h RQ = 0.885, mean LPL activity = 3.93).

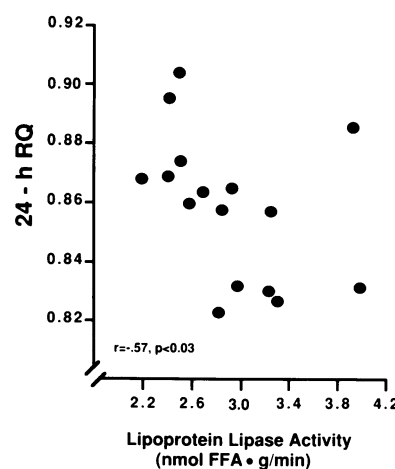


Figure 2. Relationship between 24-h RQ and skeletal muscle LPL activity. Each value represents the mean LPL activity (of baseline and 3-h postinsulin infusion) for each individual, expressed as nmol FFA/g·min. Muscle LPL activity is inversely correlated with 24-h RQ (Spearman rank correlation coefficient: $r = -0.57$, $P < 0.03$).

Discussion

Our investigation of the relationship between 24-h RQ and SMLPL activity is a unique approach to the study of whole-body energy substrate utilization rates. 24-h RQ is an integrative measure of substrate oxidation over 24 h, including both fasting and postprandial metabolism, and reflects the inverse ratio of daily whole-body CHO/fat oxidation rate. Large differences in 24-h RQ exist among weight-stable individuals under eucaloric conditions (1). In the present study, we show that differences in substrate oxidation are associated with differences in SMLPL activity. More specifically, we find that whole-body fat/CHO oxidation is directly proportional to SMLPL activity as shown by the inverse relationship between 24-h RQ and SMLPL activity. We have chosen to express the activity as the mean of fasting and an LPL activity that approximates postprandial metabolism, i.e., 3-h postinsulin LPL activity, as an integrative measure of muscle LPL activity. There is a significant but small effect on SMLPL activity after prolonged insulin stimulation and no relationship was found between basal or insulin-stimulated SMLPL activities and insulin action.

Wade and colleagues (14) have already underscored the preeminent role of skeletal muscle metabolism during light exercise in determining the relative rates of fat/CHO oxidation. The finding of an inverse correlation between 24-h RQ and SMLPL activity supports the hypothesis that LPL-mediated hydrolysis of lipoprotein triglycerides is related to the ratio of whole-body fat/CHO oxidation rate and suggests a role of skeletal muscle in determining daily whole-body substrate utilization rates under sedentary conditions. A reduced ratio of fat/CHO oxidation rate, a risk factor for the development of obesity, may be determined in part by reduced skeletal muscle fat oxidation.

Whether SMLPL activity is regulated by insulin remains controversial. In our study, neither basal nor insulin-stimulated SMLPL activities were associated with insulin-mediated glucose uptake at 3 or 6 h. By pooling data from four metabolically distinct groups, Pollare et al. (28) found a positive correlation between basal SMLPL activity and insulin sensitivity. Different conclusions, however, can be drawn by analyzing each group separately; for example, in the insulin-resistant groups, basal SMLPL activity appeared to correlate inversely with insulin action. Yet, only the fasting enzyme was measured, not the response of SMLPL to insulin. During insulin infusion, a direct and linear relationship between the decrease in SMLPL activity and increase in glucose uptake across the limb has been reported (29). Farese et al. (30) found no correlation between SMLPL activity and rates of glucose infusion during sustained hyperinsulinemia in lean Caucasian subjects, although they did report a small decrease in SMLPL activity ($\approx 8\%$ from basal) after 6-h insulin/glucose infusions. Neither our study nor that of Farese and colleagues (30) found dramatic changes in LPL activity after prolonged insulin stimulation, in contrast to the response of adipose tissue LPL activity to insulin. Of interest, however, the Pima Indians in this study tended to have an increase rather than decrease in SMLPL after insulin administration. Perhaps this response helps to meet the fuel need of muscle in the setting of insulin resistance (31). The greater insulin-mediated increase in SMLPL in more insulin-resistant subjects is consistent with the above hypothesis. Preliminary data from insulin-resistant obese Caucasian women also support such a stimulating effect of insulin on SMLPL (Eckel, R. H., unpublished results).

Since a reduced proportion of type I, high-oxidative muscle fibers has been implicated in the etiology of obesity (14) and both animal and human studies have established the relationship between muscle fiber type and muscle LPL activity (15–18), SMLPL activity would be predicted to be low before or during the development of the obese state. In the study of Taskinen et al. (32), SMLPL was lower in obese subjects, whereas Reitman et al. (33) found no difference between lean and obese. In the present study, no significant relationship was found between percent body fat and SMLPL activity. Surprisingly, basal SMLPL activity correlated positively, albeit weakly, with the waist/thigh ratio, an index of body fat distribution. The significance of this finding is unclear since the fat distribution index was not correlated with 24-h RQ. Further studies will be necessary to clarify whether degree of body fatness or body fat distribution are associated with fasting and/or insulin-regulated muscle LPL activity, and to elucidate the relationship between skeletal muscle fiber types and SMLPL activity in humans.

The Pima Indians of the southwest United States are an obesity-prone population with marked insulin resistance and paradoxically low levels of serum lipoproteins (34). Insulin sensitivity, a low relative metabolic rate, and a low ratio of fat/CHO oxidation (high 24-h RQ) are risk factors for body weight gain (35). Insulin resistance is postulated to be an adaptive mechanism for preventing further weight gain in the obese state (36). Under this circumstance, it is conceivable that augmentation of muscle lipase activity by insulin limits further weight gain by channeling more fat towards muscle for oxidation and less fat towards adipose tissue for storage. Alternatively, skeletal muscle LPL activity may not be the rate-limiting step determining muscle substrate oxidation rates. FA flux in skeletal muscle, including the activity of SMLPL, could be dependent on mitochondrial oxidative metabolism, the key determinant of the intracellular lipid pool turnover rate. Our finding of a direct relationship between muscle LPL activity and the ratio of daily fat/CHO oxidation rate suggests that skeletal muscle metabolism plays a role in determining the ratio of whole-body fat/CHO oxidation rate and therefore supports previous studies implicating skeletal muscle metabolism in the etiology of obesity. Future studies should be focused on elucidating mechanisms underlying the regulation of nutrient partitioning and the variability of fat oxidation rates.

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