Exercise induces human lipoprotein lipase gene expression in skeletal muscle but not adipose tissue

RICHARD L. SEIP, THEODORE J. ANGELOPOULOS, AND CLAY F. SEMENKOVICH
Division of Atherosclerosis, Nutrition and Lipid Research, Department of Medicine, and Department of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, Missouri 63110

Seip, Richard L., Theodore J. Angelopoulos, and Clay F. Semenkovich. Exercise induces human lipoprotein lipase gene expression in skeletal muscle but not adipose tissue. *Am. J. Physiol.* 268 (*Endocrinol. Metab.* 31): E229–E236, 1995.—Lipoprotein lipase (LPL) is regulated by exercise in humans, but the effects of exercise on LPL expression in different tissues and the molecular mechanisms involved are unclear. We assessed the effects of 5–13 consecutive days of supervised exercise on tissue LPL expression as well as fasting plasma lipids and lipoproteins in 32 sedentary, weight-stable adult men. In skeletal muscle, exercise training increased the mean LPL mRNA level by 117% ($P = 0.037$), LPL protein mass by 53% ($P = 0.038$), and total LPL enzyme activity by 35% ($P = 0.025$). In adipose tissue, mean LPL mRNA, protein mass, and activity did not change. Exercise decreased triglycerides [from 172 ± 4.3 to 127 ± 3.2 (SE) mg/dl, $P = 0.002$], total cholesterol (from 188 ± 1.2 to 181 ± 1.0 mg/dl, $P = 0.011$), and very low-density lipoprotein-cholesterol (from 30.1 ± 0.9 to 22.0 ± 0.8, $P = 0.004$) and increased high-density lipoprotein cholesterol (HDL-C; from 43.4 ± 0.35 to 45.0 ± 0.37, $P = 0.030$) and HDL_2-C (from 6.6 ± 0.21 to 7.7 ± 0.19, $P = 0.021$). Changes in muscle but not adipose tissue heparin-releasable LPL activity were inversely correlated ($r = -0.435$, $P < 0.034$) with changes in triglycerides. These data suggest the existence of an exercise stimulus intrinsic to skeletal muscle, which raises LPL activity in part by pretranslational mechanisms, a process that contributes to the improvement in circulating lipids seen with physical activity.

messenger ribonucleic acid; insulin; plasma triglycerides; plasma high-density lipoprotein-cholesterol

ONE OF THE FUNCTIONS of lipoprotein lipase (LPL), a protein bound to the capillary endothelium of most tissues, is to metabolize triglycerides in circulating lipoproteins. Free fatty acids produced by this action are available for uptake by peripheral tissues. Most studies report that aerobic exercise increases LPL enzyme activity in postheparin plasma (20), but the tissue sources of this activity and the mechanisms involved in its induction are unclear.

Major sites of LPL activity include skeletal muscle, where lipolysis yields fatty acids that can be oxidized as an energy source, and adipose tissue, where fatty acids liberated from circulating lipoproteins by LPL are reesterified and stored as triglyceride. Studies in animals have generally shown increases in skeletal muscle LPL and decreases in adipose LPL with exercise, but human studies are less consistent with some showing exercise-induced increases in LPL activity in both tissues (20). Longitudinal training studies show that exercise increases LPL enzyme activity in muscle (17), and exercise-induced increases in muscle can be more robust than those in fat (35). Muscle is probably the major site of triglyceride removal in humans (18, 28, 37), and if LPL is central to this process, LPL-induced lipolysis in muscle may also be a major factor in the generation of high-density lipoprotein-cholesterol (HDL-C). In support of this hypothesis, LPL enzyme activity, the catabolism of triglyceride-rich lipoproteins, and the generation of HDL-C and HDL_2-C are all increased in exercising human muscle (12).

There is also evidence that favorable fasting lipid changes that occur in response to exercise are mostly due to the induction of adipose tissue LPL activity, which accompanies weight loss. At least in the very obese, weight loss is associated with increased LPL expression in adipose tissue (10). If similar mechanisms operate in normal-weight adults, decreasing fat cell size below a predestined "set point" through exercise-induced weight loss may induce LPL activity and increase HDL-C levels (38).

Previous studies have usually evaluated the effects of exercise on LPL enzyme activity in elite athletes and long-distance runners, and little is known about the molecular mechanisms responsible for the observed changes in LPL activity in human tissues. Because most individuals in Western populations are sedentary and exercise is considered a first-line therapy for improving lipids, we conducted the current study to answer the question, how does exercise affect LPL gene expression in healthy, sedentary adults?

METHODS

Subject characterization. The participants were 32 healthy male nonsmokers between the ages of 19 and 72 yr. Women were also recruited in accordance with the National Institutes of Health (NIH) policy, but their responses are not reported here. We specifically included older individuals because age alone is not a contraindication to exercise. Subjects were recruited based on their willingness to undergo biopsies before and after at least 5 days of vigorous exercise. All subjects gave informed consent to participate in studies of exercise training approved by the Washington University Human Studies Committee, and all were financially compensated for their participation. Most (25 of 32) participants were not involved in regular exercise training (defined as 30 min of aerobic activity at least twice per week). Seven individuals were recreationally active, but all stopped exercising before participating in the study. Older subjects (arbitrarily defined as > 55 yr old) were evaluated through medical history, physical examination, oral glucose tolerance testing, SMA-12, chest X-ray, resting electrocardiogram (ECG), and a Bruce treadmill exercise test with ECG and blood pressure monitoring. Subjects were excluded if they had impaired glucose tolerance, other active medical

problems known to affect lipid metabolism, or musculoskeletal disorders that would interfere with daily exercise.

Maximal oxygen uptake ($\dot{V}O_{2\,max}$) was measured during a graded exercise test using treadmill walking, jogging, or cycling. The treadmill protocol was designed to increment exercise intensity by 3–4 ml $O_2 \cdot kg^{-1} \cdot min^{-1}$ and elicit fatigue in 6–12 min. Cycle testing was conducted using a cycle ergometer protocol, beginning at a work rate 30% of maximal, followed by increases in work rate of 40–70 W every 2 min. Cardiorespiratory data were collected at 30-s intervals with a computerized system that included a Parkinson-Cowan CD-4 dry gas meter, O_2 (Applied Electrochemistry S3-A) and CO_2 (Beckman LB-2) gas analyzers, and a 5-liter mixing chamber. $\dot{V}O_{2\,max}$ was taken as the highest $\dot{V}O_2$ recorded over two consecutive 30-s periods. To ensure that $\dot{V}O_{2\,max}$ had been attained, at least two of the following criteria were satisfied: plateau in $\dot{V}O_2$, heart rate within 10 beats/min of age-predicted maximum, and respiratory exchange ratio > 1.10.

Percent body fat was estimated from underwater weight taken at partial exhalation. Lung residual volume was measured outside the hydrostatic weighing tank using the oxygen dilution procedure, and body density was converted to percent body fat. Skinfold thicknesses were measured in duplicate by an experienced technician using Lange calipers.

Experimental design. Initial assessments, including maximal work capacity, body fat, and anthropometric measurements, were completed before training. Five or more days of complete abstention from exercise (inactivity) followed, after which daily exercise began. Plasma samples were obtained, and tissue biopsies were performed in the morning following an overnight fast after the inactive phase and during the exercise training phase. Subjects were counseled to increase caloric intake during training to maintain body weight with the help of a registered dietician. All participants ate the same evening meal 12 h before both biopsies.

All subjects engaged in 5–13 consecutive days of supervised aerobic exercise training, which consisted of daily uphill walking, jogging, and/or stationary cycling for ≥60 min per bout at an intensity ranging from 60 to 75% of $\dot{V}O_{2\,max}$. Based on $\dot{V}O_2$ data and assuming 5 kcal/1 O_2 consumed, daily bouts usually required 400–900 kcal, depending on the size and maximal exercise capacity of each subject. Previous studies have suggested that an energy expenditure during exercise of 1,200 to 2,000 kcal per week is necessary to elicit changes in fasting HDL-C (40). To maximize the possibility for a rise in HDL-C and a fall in triglycerides, participants were required to exercise until a minimum of 2,000 kcal was expended during the 5 days immediately preceding the posttraining biopsies. Some subjects gradually increased exercise over 13 days to meet this requirement.

Skeletal muscle and adipose tissue biopsies. Samples were obtained under local anesthetic from the ventral aspect of the thigh, 12–15 cm proximal to the patella. A small incision (1–1.5 cm) was made in the skin, and subcutaneous adipose tissue samples were obtained by blunt dissection. Samples were washed with 0.9% sterile saline, blotted dry, and quickly frozen in separate aliquots in liquid nitrogen. Additional lidocaine was administered intramuscularly, a small incision was made in the fascia covering the vastus lateralis, and muscle tissue samples were obtained using a Bergstrom needle. Muscle samples were quickly dissected free of visible fat before freezing.

For the posttraining biopsy, exercise preceded tissue sampling by 14–18 h. This time interval was chosen because plasma triglycerides fall to their lowest level 18–24 h postexercise (36) and postheparin plasma lipase activity is known to be high 18 h after exercise (29).

Determination of LPL and actin mRNA levels. Tissue samples were homogenized in a buffer containing 4 M guanidinium isothiocyanate and total RNA isolated as previously described (30). LPL message was quantitated in skeletal muscle and adipose tissue by ribonuclease (RNase) protection. An ~386 nucleotide human LPL NcoI-AatII cDNA template (including nucleotides 412 to 797 as numbered in Ref. 39) was subcloned into the vector pGEM5Z, and the authenticity of the recombinant was verified by sequencing. The vector was linearized with NcoI, gel-purified, and an antisense RNA probe was transcribed using T-7 RNA polymerase and [^{32}P]CTP. In preliminary experiments, this probe recognized human LPL 3.3 and 3.7-kb mRNA species in RNA from human skeletal muscle and adipose tissue on Northern blots. Probes were hybridized with total RNA, mixtures were digested with RNases, and protected fragments were collected on Whatman GF/C filters (16). Message was quantitated by comparing counts of unknowns to counts from standard curves generated using antisense RNA probe hybridized to in vitro transcribed sense cRNA in 10, 20, 50, 100, and 250 pg amounts. All assays were done under conditions of probe excess, and final message levels were calculated by correcting for size differences between mature message and the probe. γ-Actin mRNA levels were also quantitated by RNase protection using an RNA probe previously described (16). For a given individual, pre- and postexercise samples were analyzed in the same assay.

Determination of LPL activity. Tissue LPL activity was determined as the ability of samples to hydrolyze radiolabeled fatty acids from a phospholipid-stabilized triolein emulsion as described by Iverius and Ostlund-Lindqvist (9). Undiluted unknown, either heparin eluate or tissue homogenate, was assayed in a vol/vol ratio (undiluted sample/total assay mixture volume) of 1/5. Pre- and postexercise training samples for a given individual were always analyzed in the same assay. Tissue samples were incubated in heparin (1 mg/ml) for 30–60 min after preliminary experiments showed these conditions to result in the maximal release of LPL activity. Heparin-releasable activity was considered as the LPL activity found in triplicate determinations of the heparin eluate. For heparin-nonreleasable activity, after treatment with heparin, muscle and adipose fragments were homogenized and processed in a buffer (9) containing 0.223 M tris(hydroxymethyl)aminomethane-HCl (pH 8.5), 0.25 M sucrose, sodium deoxycholate (2 mg/ml), Triton X-100 (0.08 mg/ml), heparin (0.05 mg/ml), and bovine serum albumin (10 mg/ml).

Determination of LPL mass. Frozen samples were homogenized using the same buffer (2% wt/vol ratio) described above for the activity assay, except for the omission of albumin and heparin, and the addition of 1 mM phenylmethylsulfonyl fluoride, 1 mM EDTA, and 0.05 mM aprotinin. Tissue homogenates were centrifuged, and 2.5 µg protein from the supernatant (muscle) or infranatant (adipose tissue) were electrophoresed per lane on 8% sodium dedecyl sulfate-polyacrylamide gel electrophoresis slab gels. Proteins were transferred to Immobilon-P membranes by Western blotting. The LPL protein was detected using a monospecific rabbit polyclonal antibody (a gift from Gus Schonfeld) raised against human LPL. In preliminary experiments, this antibody did not inhibit LPL enzyme activity when added to samples in vitro. The Immobilon-P membranes were incubated overnight under standard conditions with anti-human LPL antibody diluted 1:1,000. The blots were then washed, incubated with ^{125}I-labeled goat anti-rabbit immunoglobulin G, washed again, dried, and subjected to autoradiography. The position of the band detected by Western blotting in human tissues was identical to that seen in lanes loaded with LPL protein purified from unpasteurized bovine milk. The same band in human

tissues was also detected with an antibody (a gift from Gunilla Bengtsson-Olivecrona) raised in chickens against bovine milk LPL (21). Bands corresponding to LPL were quantitated by image processing analysis using JAVA software (Jandel Scientific, San Raphael, CA). Pre- and postexercise samples for a given individual were analyzed on the same blot.

Plasma determinations. Samples were obtained by venipuncture immediately before the skeletal muscle and adipose tissue biopsies were performed. Sampling followed an overnight fast and, in the case of posttraining samples, was 14–18 h after the most recent exercise bout. Plasma lipoproteins were measured in the Washington University Lipid Research Clinic Core Laboratory, which participates in the lipid standardization program of the Centers for Disease Control. For any given subject, pre- and posttraining samples were analyzed in the same assay. Apolipoproteins (apo) AI and B were measured by immunonephelometry (Behring Diagnostics, Somerville, NJ). Insulin levels were determined by radioimmunoassay at the Washington University General Clinical Research Center.

Statistical analyses. Student's paired t-test was used to test for differences before and after training. For calculation of Pearson correlation coefficients, triglyceride values were $\log_{10}$ transformed, which reduced skewness and kurtosis. Data were stored, and analyses using SAS software (SAS Institute, Cary, NC) were performed on the VAX computer system housed at the General Clinical Research Center at Washington University School of Medicine.

RESULTS

Subject characteristics are shown in Table 1. As a group, participants were overweight (mean body mass index = 25.7 kg/m^2) and of average fitness (mean $\dot{V}O_{2max}$ = 40.6 ml·kg^{-1}·min^{-1}), both of which are consistent with a sedentary lifestyle.

Exercise training induced significant changes in plasma lipids as shown in Table 2. The mean fasting plasma triglyceride level decreased significantly by 26% (from 171.6 to 126.6 mg/dl), total cholesterol decreased by 3.9% (from 187.8 to 180.5 mg/dl), and very low-density lipoprotein-cholesterol decreased by 27% (from 30.1 to 22.0 mg/dl). There were significant mean increases in plasma HDL-C (1.7 mg/dl) and HDL$_2$-C (1.0 mg/dl). These potentially favorable changes in lipids occurred despite no change (-0.18 ± 0.2 kg, $P = 0.34$) in mean body weight during the study. Levels of low-density lipoprotein-cholesterol, apo B, apo AI, and insulin did not change with training.

Table 1. *Subject characteristics*

Age, yr	41.5 ± 3.4
Body wt, kg	79.7 ± 2.1
Body mass index, kg/m^2	25.7 ± 0.64
Body fat, %	19.6 ± 1.6
Fat-free mass, kg	63.4 ± 1.3
Fat mass, kg	16.2 ± 1.6
Sum of skinfolds, mm	143 ± 11
Thigh skinfold, mm	14 ± 1
Triceps skinfold, mm	11 ± 1
Waist to hip ratio	0.88 ± 0.01
$\dot{V}O_{2max}$, ml·kg^{-1}·min^{-1}	40.6 ± 2.3
$\dot{V}O_{2max}$, l/min	3.15 ± 0.15

Values are means ± SE; $n = 32$ subjects. $\dot{V}O_{2max}$, maximal O_2 uptake.

Table 2. *Plasma changes with training*

	Exercise		Δ	P
	Before	After		
TG	171.6 ± 4.33	126.6 ± 3.18	−45.0 ± 2.41	0.002*
TC	187.8 ± 1.15	180.5 ± 1.04	−7.3 ± 2.71	0.011*
VLDL-C	30.1 ± 0.92	22.0 ± 0.81	−8.1 ± 0.46	0.004*
LDL-C	112.3 ± 0.95	111.9 ± 0.90	−0.5 ± 0.48	0.859
HDL-C	43.4 ± 0.35	45.0 ± 0.37	+1.7 ± 0.12	0.030*
HDL$_2$-C	6.6 ± 0.21	7.7 ± 0.19	+1.0 ± 0.07	0.021*
HDL$_3$-C	36.7 ± 0.19	37.4 ± 0.18	+0.5 ± 0.11	0.650
Apo B ($n = 27$)	109.0 ± 1.04	106.3 ± 0.89	−2.6 ± 0.42	0.280
Apo AI ($n = 27$)	129.6 ± 0.69	129.5 ± 0.71	−0.1 ± 0.42	0.982
Insulin ($n = 28$)	10.17 ± 0.18	9.14 ± 0.16	−1.03 ± 0.15	0.240

Values are means ± SE in mg/dl; $n = 32$ subjects except as noted. TG, triglycerides; TC, total cholesterol; VLDL-C, very low-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol; Apo, apolipoprotein. *Indicates statistical significance.

To determine whether changes in plasma lipids were associated with changes in LPL gene expression, LPL mRNA, protein mass, and enzyme activity were determined in skeletal muscle and adipose tissue, tissues containing the majority of LPL enzyme. LPL message was quantitated by RNase protection. With use of a human LPL RNA probe, gel electrophoresis showed a single discrete protected fragment of the predicted size (~ 378 nucleotides) in both skeletal muscle and adipose tissue (Fig. 1, arrowheads). As a negative control, no bands were seen when the probe was hybridized with transfer RNA (tRNA).

Because the LPL probe protected a single discrete fragment, subsequent mRNA analyses were done by filter assay. To validate the filter assay, increasing amounts of total RNA from human muscle and adipose tissue were analyzed for LPL mRNA content (Fig. 2). For both tissues, the assay was linear over the range of total RNA analyzed from study subjects.

LPL protein content was determined by densitometric scans of Western blots. The anti-LPL antibody detected a single band in both muscle and adipose tissue corresponding to the known size of LPL (Fig. 3, *insets*). To validate the LPL protein assay, increasing amounts of total protein from human muscle and adipose tissue were blotted, and for both tissues the assay was linear over the range of protein analyzed from study participants (Fig. 3).

Skeletal muscle LPL enzyme activity, mRNA, and protein responses to exercise training are shown in Fig. 4. Mean total LPL activity ($n = 24$) rose significantly by 35% from 2.76 ± 0.51 after inactivity to 3.72 ± 0.61 μmol free fatty acid (FFA)·mg^{-1}·h^{-1} after exercise ($P = 0.025$). This change was mostly due to an increase in the heparin-nonreleasable pool, which increased by 63% from 1.39 ± 0.39 after inactivity to 2.26 ± 0.46 μmol FFA·mg^{-1}·h^{-1} after exercise. There was no significant difference in the activity of the heparin-releasable pool (1.37 ± 0.42 after inactivity vs. 1.46 ± 0.43 μmol FFA·mg^{-1}·h^{-1} after exercise).

In muscle LPL message and protein also increased significantly in response to exercise. LPL mRNA (Fig. 4B; $n = 27$) increased by 117% from 119 ± 58 after

inactivity to 258 ± 113 pg/μg total RNA after exercise ($P = 0.037$). Actin mRNA ($n = 10$) did not change with exercise (18.5 ± 6.8 after inactivity vs. 19.9 ± 7.8 pg/μg total RNA after exercise, $P = 0.869$). There were no significant training-associated differences in the yield of total RNA from muscle samples whether expressed per initial wet weight or per milligram protein. Tissue LPL immunoreactive mass (Fig. 4C; $n = 11$) expressed as radiographic density units per microgram total protein increased by 53% with exercise ($P = 0.038$).

Individuals >55 yr old were deliberately included, which raises the possibility that exercise responses for the group were blunted by the inclusion of older subjects. When muscle LPL mRNA responses for older (>55) and younger subjects were assessed by group, the same trends toward an increase with exercise were seen in both groups, although differences compared with the inactive state were not statistically significant by paired t-test for either group (data not shown).

In adipose tissue (Fig. 5), LPL enzyme activity ($n = 24$), mRNA ($n = 29$), and protein ($n = 12$) were not significantly affected by exercise. There were no significant differences in mean total LPL activity (Fig. 5A; 2.58 ± 0.57 after inactivity vs. 2.39 ± 0.41 μmol FFA·mg^{-1}·h^{-1} after exercise) or heparin releasable activity (1.21 ± 0.37 after inactivity vs. 1.00 ± 0.18

Fig. 2. LPL mRNA RNase protection assay is linear over a suitable range of human RNA. Total RNA samples from human muscle (A) and adipose tissue (B) were hybridized with a human LPL riboprobe and treated with RNases as described in Fig. 1 except products were collected on filters. LPL cRNA mass was determined by comparing protected counts from tissue RNA with counts from known amounts of sense RNA transcribed in vitro.

μmol FFA·mg^{-1}·h^{-1} after exercise). Adipose tissue LPL mRNA (Fig. 5B) was 200 ± 75 after inactivity vs. 231 ± 92 pg/μg total RNA after training ($P = 0.66$). Actin mRNA in adipose tissue ($n = 15$) was 103 ± 38 after inactivity vs. 102 ± 38 pg/μg total RNA after training ($P = 0.95$), and as with muscle, there were no exercise-associated differences in total RNA yield from fat.

For skeletal muscle there was a significant inverse correlation between training-induced responses of heparin releasable LPL activity and changes in triglycerides ($r = -0.435; P < 0.034$; Fig. 6A). No significant correlation was detected between changes in adipose tissue heparin releasable LPL activity and changes in triglycerides (Fig. 6B). During inactivity triglyceride levels were inversely related to total ($r = -0.558$, $P = 0.007$) and heparin releasable ($r = -0.547$, $P = 0.008$) LPL activity in adipose tissue, but there was no significant correlation between triglycerides and skeletal muscle LPL activity in the inactive state.

DISCUSSION

In this study we show that short-term, supervised exercise increases LPL mRNA, immunoreactive mass,
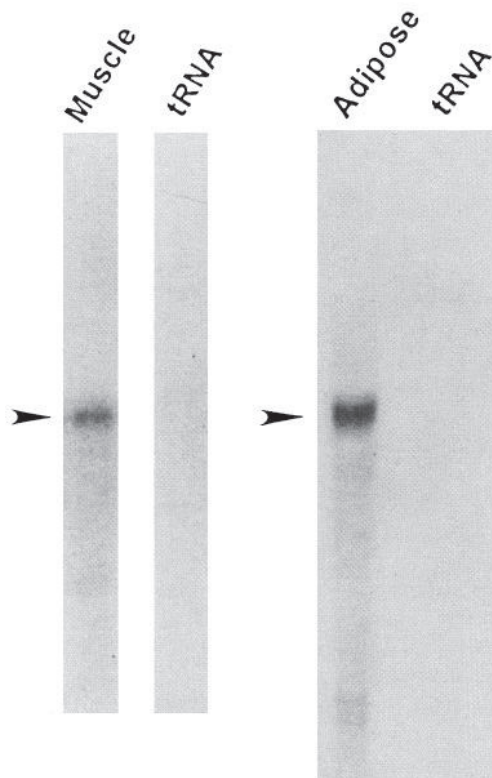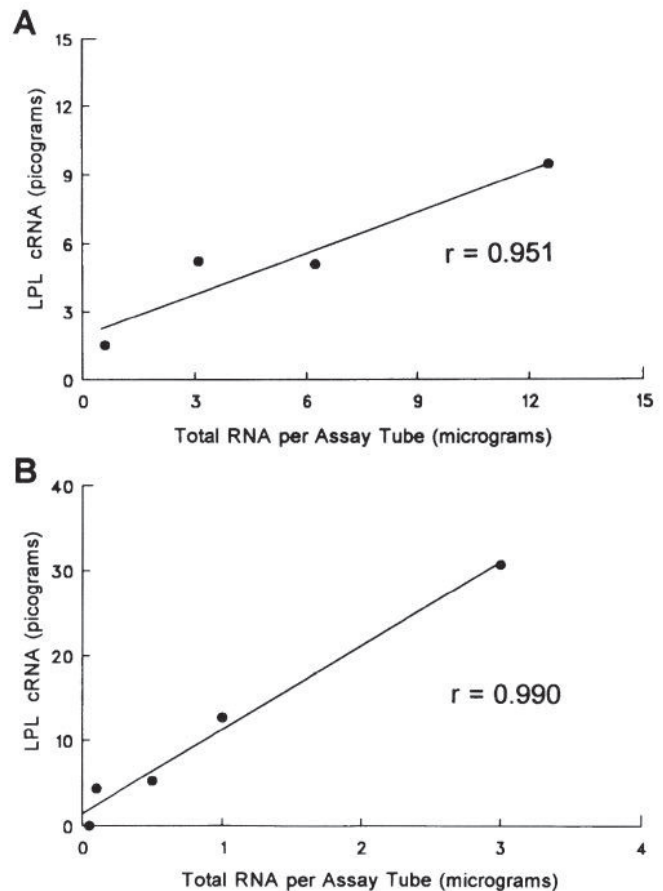
Fig. 1. Detection of lipoprotein lipase (LPL) mRNA by ribonuclease (RNase) protection in human skeletal muscle and adipose tissue. RNA was isolated from biopsies of human tissues, hybridized with a uniformly labeled antisense RNA probe, treated with RNases, and the products subjected to electrophoresis and then autoradiography. Protected fragments of the predicted size (~378 nucleotides, arrowheads) were seen in both muscle and fat. No bands were seen on the same gels when probes were hybridized with transfer RNA (tRNA) as a negative control.

and enzyme activity in the skeletal muscle of previously sedentary adults. No significant effects were seen in subcutaneous adipose tissue. Significant changes in plasma lipids and lipoproteins were also observed, consistent with the concept that muscle lipase activity, upregulated by exercise training, contributes to potentially favorable changes in circulating lipoproteins.

Exercise increases LPL enzyme activity in skeletal muscle (12, 13, 17, 20), but the mechanisms involved are uncertain. In adipose tissue under different conditions, LPL regulation can be transcriptional (24), translational (34), or posttranslational (32). Our data suggest that in previously sedentary humans, exercise regulation of LPL expression in muscle is pretranslational, i.e., LPL mRNA is increased either through increased transcription of the LPL gene, enhanced processing or transport of the LPL message, or stabilization of the message. Recent animal studies have also demonstrated pretranslational regulation of LPL expression by exercise (15).

This conclusion appears to conflict with that of a recent study of detraining in marathoners, which re-
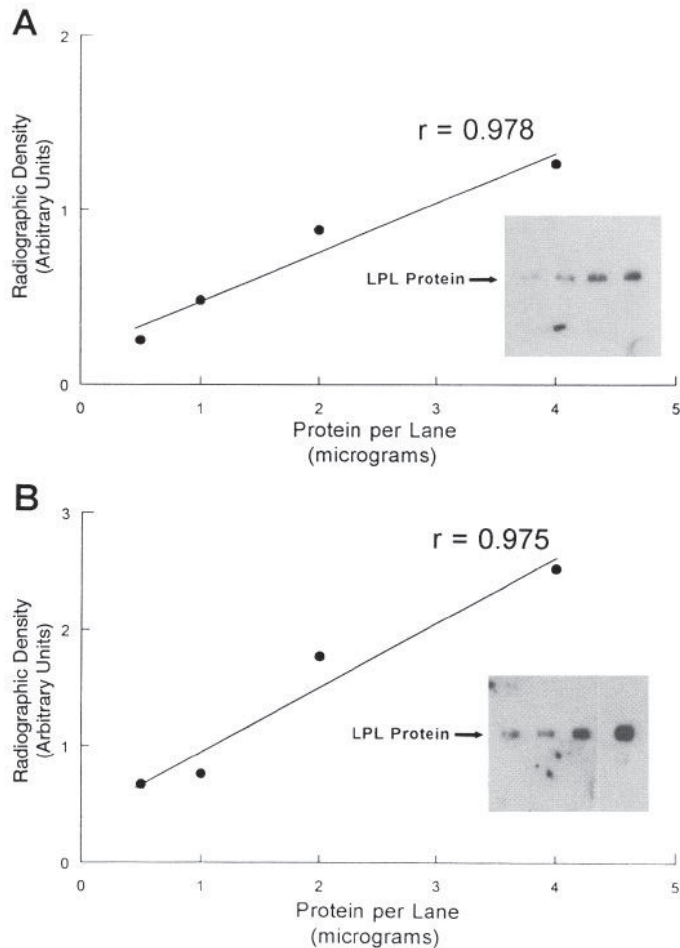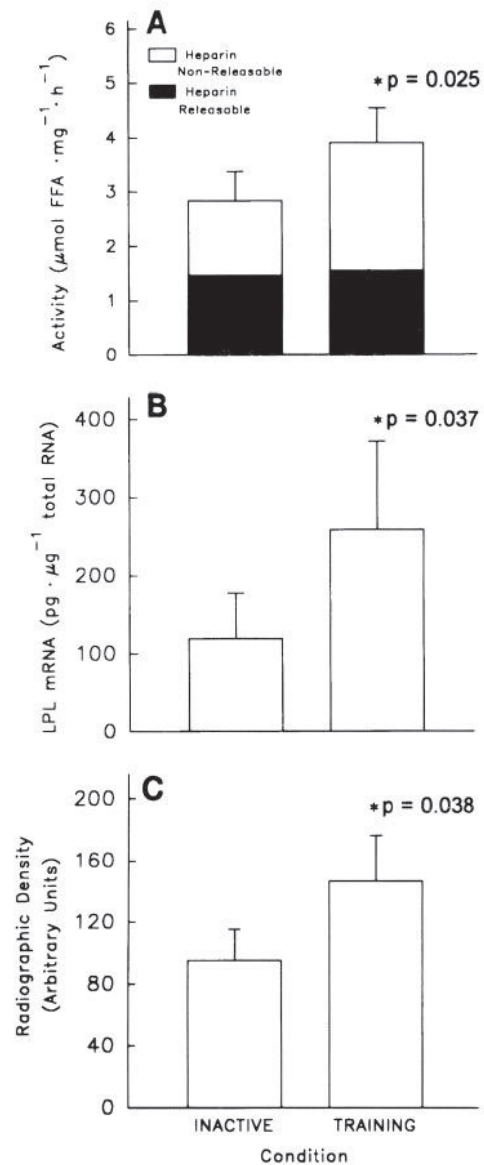

Fig. 4. Skeletal muscle LPL responses to exercise. Vastus lateralis muscle biopsies were performed before (inactive) and after (training) 5–13 days of supervised exercise and analyzed for LPL enzyme activity (A, $n = 24$), mRNA (B, $n = 27$), and protein mass (C, $n = 11$).

Fig. 3. LPL protein mass assay is linear over a suitable range of human protein. Protein samples from human muscle (A) and adipose tissue (B) were subjected to Western blotting as described in METHODS using a rabbit polyclonal antibody raised against human LPL. LPL protein mass was determined by densitometry. *Insets*, blots from which these data were derived. Arrows, position of LPL protein ($\sim$55–60 kDa).

ported changes in average muscle LPL activity without detecting changes in average LPL mRNA or protein (33). However, this conflict is probably more apparent than real. Some individuals in that report showed proportional changes in LPL mRNA and enzyme mass (33, see Fig. 4), suggesting that exercise-mediated regulation of LPL expression can be pretranslational. There was also a great deal of variability in the LPL mRNA response in our study as evidenced by relatively wide standard error bars (Fig. 4). The Simsolo et al. (33) study utilized marathoners biopsied at 24 h postexercise, and we studied sedentary adults biopsied 14–18 h postexercise, making direct comparisons between reports difficult. Nevertheless, the results of both studies highlight the possibility that the mechanism of regulation of muscle LPL by exercise may not be uniform.
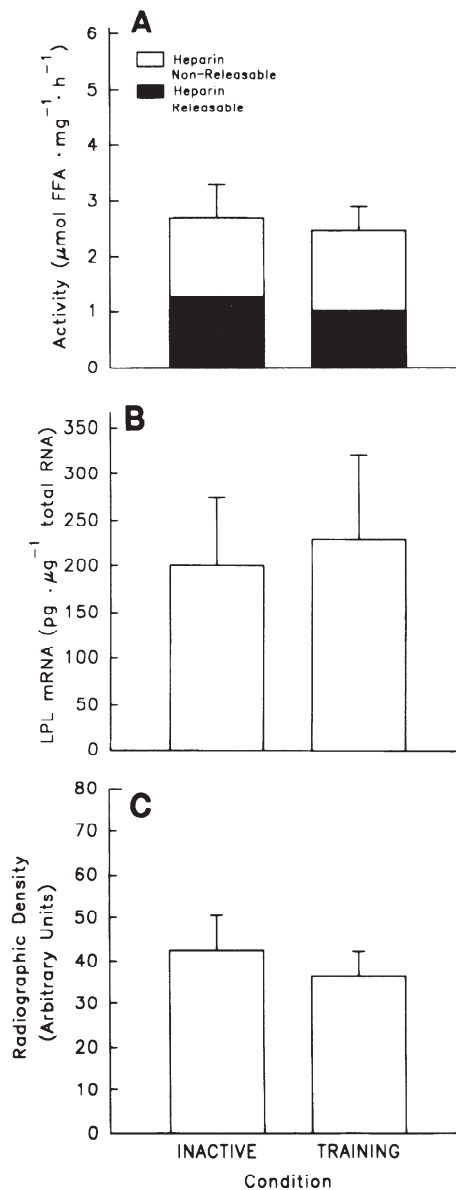
Fig. 5. Adipose tissue LPL responses to exercise. Lateral thigh subcutaneous fat biopsies were performed before (inactive) and after (training) 5–13 days of supervised exercise and analyzed for LPL enzyme activity (A, $n = 24$), mRNA (B, $n = 29$), and protein mass (C, $n = 12$).

How does exercise increase LPL mRNA levels in muscle? Although some information is available about pretranslational control of LPL gene expression in adipocytes (3, 5, 24, 25), almost nothing is known about LPL gene expression in muscle. Exercise in the intensity range we studied is associated with striking fluxes in hormone and nutrient concentrations, which might be implicated in LPL regulation. Catecholamines rise during exertion, and their urinary excretion rates correlate with exercise-induced changes in muscle LPL activity (17). Catecholamines increase adenosine 3',5'-cyclic monophosphate (cAMP) levels, and sequences related to the cAMP-responsive element are present in the human LPL promoter (14). Although catecholamines may affect LPL expression at posttranscriptional steps (22), isoproterenol has been shown to decrease LPL transcription in

adipocytes (25). Because LPL regulation in muscle and fat is usually reciprocal, it is not unreasonable to speculate that catecholamines increase LPL transcription in human muscle. Arguing against a major role of catecholamines in this process is the observation that administration of reserpine to animals does not block exercise-induced increases in muscle LPL (20).

Insulin could mediate the exercise responses. LPL activity is increased in adipose tissue and decreased in muscle (13) by insulin. In rat adipocytes, insulin effects can be pretranslational and may be due to changes in LPL mRNA stability (25). At the time of biopsy, we did not detect differences in fasting serum insulin levels with training (Table 2). However, levels could have been lower at other times critical to LPL regulation. For example, insulin responses to meals might have been attenuated, and subsequent lower overall insulinemia with training could result in less inhibition of muscle LPL expression. Levels of nutrients such as glucose and free fatty acids change with exercise (27) and could also affect LPL mRNA levels in muscle. Glucose alone is known to regulate gene expression in other systems through a variety of mechanisms, including the stimulation of transcription (19) and the alteration of message stability (31). Fatty acids have also been shown to have specific effects on gene expression (4).

A major determinant of LPL gene expression may be muscle contraction itself. Electrical stimulation of muscle increases message levels for hexokinase and the mitochondrial proteins citrate synthase and cytochrome-c (1). Vigorous physical exertion is known to deplete stores of high-energy phosphates, increase concentrations of oxygen-derived free radicals, lower intracellular pH, and increase intracellular calcium concentrations, all potential mediators of LPL gene expression. Phosphorylation is important for the activity of transcription factors, including the cAMP response-element binding protein (7), consistent with the concept that exercise-induced depletion of high-energy phosphate stores could alter gene expression. Oxidative stress induces a human protein-tyrosine phosphatase (11), and the induction of related proteins in muscle in response to exercise could affect the phosphorylation status of transcription factors critical for LPL expression.

Regardless of the nature of the molecular mediator responsible for increasing LPL message with exercise, it is possible that the same factor increases the messages for other genes involved in providing energy substrates for muscle. We have recently shown that short-term exercise in animals induces GLUT-4 gene expression (26). We speculate that the same exercise-specific stimulus increases message levels for both GLUT-4 and LPL in muscle, thus ensuring a ready supply of both glucose and fatty acids to replenish glycogen and triglyceride stores depleted by exercise.

The ultimate goal of increased LPL gene expression is increased enzyme activity at the appropriate tissue site. LPL enzyme activity is thought to exist in anatomically distinct pools. Heparin releasable activity presumably reflects enzyme at the capillary endothelium accessible to circulating lipoproteins. Activity remaining in tissue extracts after treatment with heparin may represent a
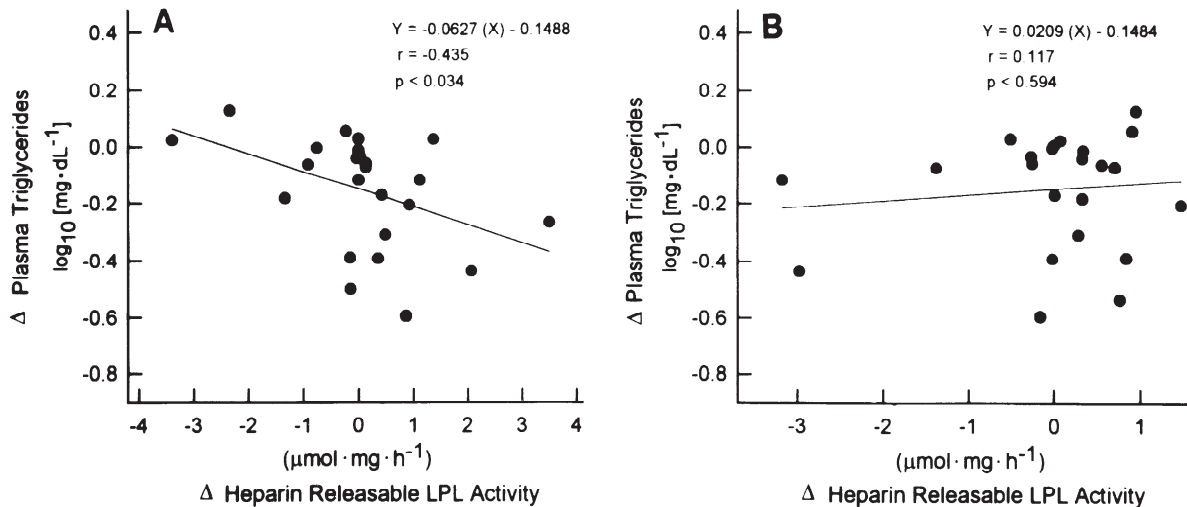
Fig. 6. Correlations between exercise-induced changes in triglyceride levels and changes in heparin-releasable LPL activity in skeletal muscle (A) and adipose tissue (B). Triglyceride values were $\log_{10}$ transformed, and differences between transformed values in the inactive and trained state are shown on the vertical axis. Pearson correlation coefficients were determined, and the equation represented by the line in each panel is presented as an *inset*.

pool of active enzyme, which can be recruited to the endothelium when heparin releasable enzyme is exhausted. Exercise in the current study increased the intracellular pool in muscle, consistent with studies in animals (23). Although muscle heparin releasable activity for the group was not significantly increased by training (Fig. 4), exercise-induced changes in skeletal muscle heparin releasable activity were inversely correlated with changes in triglycerides (Fig. 6), consistent with the view that heparin releasable LPL activity is the fraction most reflective of functional enzyme in vivo. It is also likely that the total flux of active LPL enzyme from intracellular to heparin releasable sites in muscle was increased with training. The larger intracellular pool of active LPL enzyme induced by exercise would readily replenish LPL at the capillary endothelium and ultimately result in enhanced triglyceride lipolysis. The heparin nonreleasable fraction is the predominant form of LPL activity in muscle (2), suggesting that it is this fraction that is most susceptible to exercise-mediated regulation.

Training-induced changes in muscle heparin releasable activity were inversely correlated with training-induced changes in triglycerides, but the two variables were not correlated in the inactive state. However, adipose tissue heparin releasable activity was correlated with triglycerides in the inactive state (see RESULTS). One interpretation of these results is that adipose tissue LPL activity determines triglyceride removal during periods of inactivity, which should promote obesity, but skeletal muscle activity is responsible for triglyceride removal with exercise, which should protect against obesity by promoting fatty acid oxidation (6). There may be other benefits of increased LPL expression in muscle. One of the adaptive responses to exercise is an increase in the ability of muscle to utilize fatty acids as a source of energy (8). The induction of LPL expression in muscle would provide a source of fatty acids for oxidation and may protect against fatigue, allowing the performance of more work and perhaps an improved sense of well-being.

We did not observe significant changes in adipose tissue LPL expression with short-term exercise, which

conflicts with some previous studies reporting higher activity in adipose activity with exercise (20). However, the recent study of detraining in marathon runners (33) showed that exercise was associated with lower adipose tissue LPL activity. Discrepancies between studies might be explained by the study of different adipose tissue sites and the fact that differences in the timing of biopsies after exertion may yield different results.

The major finding of our study is that exercise regulation of muscle LPL expression in humans can be pretranslational. This raises fundamental physiological questions about the time course of the LPL message response after exercise and the intensity of exercise required to elicit this response. We speculate that more vigorous skeletal muscle contraction will result in proportionally more LPL expression, suggesting that for the induction of muscle LPL gene expression, as with most things in life, harder work will be associated with greater benefits.

REFERENCES

1. **Booth, F. W., and D. B. Thomason.** Molecular and cellular adaptation of muscle in response to exercise: perspective of various models. *Physiol. Rev.* 71: 541–585, 1991.

2. **Borensztajn, J.** Heart and skeletal muscle lipoprotein lipase. In: *Lipoprotein Lipase*, edited by J. Borensztajn. Chicago, IL: Evener, 1987, p. 133–148.

3. **Currie, R. A., and R. H. Eckel.** Characterization of a high affinity octamer transcription factor binding site in the human lipoprotein lipase promoter. *Arch. Biochem. Biophys.* 298: 630–639, 1992.

4. **Distel, R. J., G. S. Robinson, and B. M. Spiegelman.** Fatty acid regulation of gene expression: transcriptional and post-transcriptional mechanisms. *J. Biol. Chem.* 267: 5937–5941, 1992.

5. **Enerback, S., B. G. Ohlsson, L. Samuelsson, and G. Bjursell.** Characterization of the human lipoprotein lipase (LPL) promoter: evidence of two *cis*-regulatory regions, LP-α and LP-β, of importance for the differentiation-linked induction of the LPL gene during adipogenesis. *Mol. Cell. Biol.* 12: 4622–4633, 1992.

6. **Ferraro, R. T., R. H. Eckel, D. E. Larson, A.-M. Fontvieille, R. Rising, D. R. Jensen, and E. Ravussin.** Relationship between skeletal muscle lipoprotein lipase activity and 24-hour macronutrient oxidation. *J. Clin. Invest.* 92: 441–445, 1993.

7. **Gonzalez, G. A., and M. R. Montminy.** Cyclic AMP stimulates somatostatin gene transcription by phosphorylation of CREB at serine 133. *Cell* 59: 675–680, 1989.

8. **Holloszy, J. O.** Biochemical adaptations in muscle. Effects of exercise on mitochondrial oxygen uptake and respiratory enzyme activity in skeletal muscle. *J. Biol. Chem.* 242: 2278–2282, 1967.

9. **Iverius, P.-H., and A.-M. Ostlund-Lindqvist.** Preparation, characterization, and measurement of lipoprotein lipase. *Methods Enzymol.* 129: 691–704, 1986.

10. **Kern, P. A., J. M. Ong, B. Saffari, and J. Carty.** The effects of weight loss on the activity and expression of adipose-tissue lipoprotein lipase in very obese humans. *N. Engl. J. Med.* 322: 1053–1059, 1990.

11. **Keyse, S. M., and E. A. Emslie.** Oxidative stress and heat shock induce a human gene encoding a protein-tyrosine phosphatase. *Nature Lond.* 359: 644–647, 1992.

12. **Kiens, B., and H. Lithell.** Lipoprotein metabolism influenced by training-induced changes in human skeletal muscle. *J. Clin. Invest.* 83: 558–564, 1989.

13. **Kiens, B., H. Lithell, K. J. Mikines, and E. A. Richter.** Effects of insulin and exercise on muscle lipoprotein lipase activity in man and its relation to insulin action. *J. Clin. Invest.* 84: 1124–1129, 1989.

14. **Kirchgessner, T. G., J.-C. Chuat, C. Heinzmann, J. Etienne, S. Guilhot, K. Svenson, D. Ameis, C. Pilon, L. D'Auriol, A. Andalibi, M. C. Schotz, F. Galibert, and A. J. Lusis.** Organization of the human lipoprotein lipase gene and evolution of the lipase gene family. *Proc. Natl. Acad. Sci. USA* 86: 9647–9651, 1989.

15. **Ladu, M. J., H. Kapsas, and W. K. Palmer.** Regulation of lipoprotein lipase in muscle and adipose tissue during exercise. *J. Appl. Physiol.* 71: 404–409, 1991.

16. **Levy, R. A., R. E. Ostlund, Jr., G. Schonfeld, P. Wong, and C. F. Semenkovich.** Cholesteryl ester storage disease: complex molecular effects of chronic lovastatin therapy. *J. Lipid Res.* 33: 1005–1015, 1992.

17. **Lithell, H., M. Cedermark, J. Froberg, P. Tesch, and J. Karlsson.** Increase of lipoprotein lipase activity in skeletal muscle during heavy exercise. Relation to epinephrine excretion. *Metabolism* 30: 1130–1134, 1981.

18. **Lithell, H., F. Lindgarde, K. Hellsing, G. Lundqvist, E. Nygaard, B. Vessby, and B. Saltin.** Body weight, skeletal muscle morphology, and enzyme activities in relation to fasting serum insulin concentration and glucose tolerance in 48-year-old men. *Diabetes* 30: 19–25, 1981.

19. **McClain, D. A., A. J. Paterson, M. D. Roos, X. Wei, and J. E. Kudlow.** Glucose and glucosamine regulate growth factor gene expression in vascular smooth muscle cells. *Proc. Natl. Acad. Sci. USA* 89: 8150–8154, 1992.

20. **Nikkila, E. A.** Role of lipoprotein lipase in metabolic adaptation to exercise and training. In: *Lipoprotein Lipase*, edited by J. Borensztajn. Chicago, IL: Evener, 1987, p. 187–199.

21. **Olivecrona, T., and G. Bengtsson.** Immunochemical properties of lipoprotein lipase. Development of an immunoassay applicable to several mammalian species. *Biochim. Biophys. Acta* 752: 38–45, 1983.

22. **Ong, J. M., B. Saffari, R. B. Simsolo, and P. A. Kern.** Epinephrine inhibits lipoprotein lipase gene expression in rat adipocytes through multiple steps in posttranscriptional processing. *Mol. Endocrinol.* 6: 61–69, 1992.

23. **Oscai, L. B., R. A. Caruso, and A. C. Wergeles.** Lipoprotein lipase hydrolyzes endogenous triacylglycerols in muscle of exercised rats. *J. Appl. Physiol.* 52: 1059–1063, 1982.

24. **Previato, L., C. L. Parrott, S. Santamarina-Fojo, and H. B. Brewer, Jr.** Transcriptional regulation of the human lipoprotein lipase gene in 3T3-L1 adipocytes. *J. Biol. Chem.* 266: 18958–18963, 1991.

25. **Raynolds, M. V., P. D. Awald, D. F. Gordon, A. Gutierrez-Hartmann, D. C. Rule, W. M. Wood, and R. H. Eckel.** Lipoprotein lipase gene expression in rat adipocytes is regulated by isoproterenol and insulin through different mechanisms. *Mol. Endocrinol.* 4: 1416–1422, 1990.

26. **Ren, J.-M., C. F. Semenkovich, E. A. Gulve, J. Gao, and J. O. Holloszy.** Exercise induces rapid increases in GLUT4 expression, glucose transport capacity and insulin-stimulated glycogen storage in muscle. *J. Biol. Chem.* 269: 14396–14401, 1994.

27. **Romijn, J. A., E. F. Coyle, L. S. Sidossis, A. Gastaldelli, J. F. Horwitz, E. Endert, and R. R. Wolfe.** Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration. *Am. J. Physiol.* 265 (*Endocrinol. Metab.* 28): E380–E391, 1993.

28. **Rossner, S.** Studies on an intravenous fat tolerance test. Methodological, experimental and clinical experiences with intralipid. *Acta Med. Scand.* S564: 7–24, 1974.

29. **Sady, S., P. D. Thompson, E. M. Cullinane, M. A. Kantor, E. Domagala, and P. N. Herbert.** Prolonged exercise augments plasma triglyceride clearance. *J. Am. Med. Assoc.* 256: 2552–2555, 1986.

30. **Semenkovich, C. F., S.-H. Chen, M. Wims, C.-C. Luo, W.-H. Li, and L. Chan.** Lipoprotein lipase and hepatic lipase mRNA tissue specific expression, developmental regulation and evolution. *J. Lipid Res.* 30: 423–431, 1989.

31. **Semenkovich, C. F., T. Coleman, and R. Goforth.** Physiologic concentrations of glucose regulate fatty acid synthase activity in HepG2 cells by mediating fatty acid synthase mRNA stability. *J. Biol. Chem.* 268: 6961–6970, 1993.

32. **Semenkovich, C. F., M. Wims, L. Noe, J. Etienne, and L. Chan.** Insulin regulation of lipoprotein lipase activity in 3T3-L1 adipocytes is mediated at posttranscriptional and post-translational levels. *J. Biol. Chem.* 264: 9030–9038, 1989.

33. **Simsolo, R. B., J. M. Ong, and P. A. Kern.** The regulation of adipose tissue and muscle lipoprotein lipase in runners by detraining. *J. Clin. Invest.* 92: 2124–2130, 1993.

34. **Simsolo, R. B., J. M. Ong, B. Saffari, and P. A. Kern.** Effect of improved diabetic control on the expression of lipoprotein lipase in human adipose tissue. *J. Lipid Res.* 33: 89–95, 1992.

35. **Taskinen, M.-R., and E. A. Nikkila.** Effect of acute vigorous exercise on lipoprotein lipase activity of adipose tissue and skeletal muscle in physically active men. *Artery* 6: 471–483, 1980.

36. **Thompson, P. D., E. Cullinane, L. O. Henderson, and P. N. Herbert.** Acute effects of prolonged exercise on serum lipids. *Metabolism* 29: 662–665, 1980.

37. **Vessby, B., I. Selinus, and H. Lithell.** Serum lipoprotein and lipoprotein lipase in overweight, type II diabetics during and after supplemented fasting. *Arteriosclerosis* 5: 93–100, 1985.

38. **Williams, P. T.** Weight set-point theory and the high-density lipoprotein concentrations of long-distance runners. *Metabolism* 39: 460–467, 1990.

39. **Wion, K. L., T. G. Kirchgessner, A. J. Lusis, M. C. Schotz, and R. M. Lawn.** Human lipoprotein lipase complementary DNA sequence. *Science Wash. DC* 235: 1638–1641, 1987.

40. **Wood, P. D., W. L. Haskell, S. N. Blair, P. T. Williams, R. M. Krauss, F. T. Lindgren, J. J. Albers, P. H. Ho, and J. W. Farquhar.** Increased exercise level and plasma lipoprotein concentrations: a one-year, randomized, controlled study in sedentary, middle-aged men. *Metabolism* 32: 31–39, 1983.