Predictors of Adipose Tissue Lipoprotein Lipase in Middle-Aged and Older Men: Relationship to Leptin and Obesity, But Not Cardiovascular Fitness

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The effects of long-term endurance exercise training, body composition, and cardiovascular fitness ($\dot{V}o_2max$) on the activity of adipose tissue lipoprotein lipase (AT-LPL) and lipoprotein lipids were examined in 66 healthy age-matched middle-aged and older men (mean ± SE, 61 ± 1 years). We compared subcutaneous abdominal (ABD) and gluteal (GLT) heparin-elutable AT-LPL activity in 19 master athletes ($\dot{V}o_2max$ > 40 mL/kg/min) and 20 lean sedentary men ($\dot{V}o_2max$ < 40 mL/kg/min) versus 27 obese sedentary men ($\dot{V}o_2max$ < 40 mL/kg/min; body fat > 27%). Fasting insulin and leptin levels were similar in master athletes and lean sedentary men, but were lower than in obese sedentary men. There were no differences in fasting values for total cholesterol or low-density lipoprotein cholesterol (LDL-C) among the groups, but master athletes had lower triglyceride (TG) values ($P < .05$) and higher high-density lipoprotein cholesterol (HDL-C) and HDL_2-C ($P < .05$) than obese and lean sedentary men. There were no regional (ABD *v* GLT) differences in the activity of AT-LPL in these groups, but obese sedentary men had higher levels of ABD AT-LPL (2.1 ± 0.3 nmol/10^6 cells · min) than lean sedentary men (0.8 ± 0.2) and master athletes (0.5 ± 0.1, $P = .01$). Similar results were observed for GLT AT-LPL. Both ABD and GLT AT-LPL activity correlated positively with percent body fat ($r = .46$ to $.54$, $P < .001$), fasting insulin ($r = .37$ to $.45$, $P < .001$), and leptin ($r = .61$ to $.65$, $P < .0001$), but not with $\dot{V}o_2max$. In stepwise multiple regression analysis, leptin was the main independent predictor of ABD ($R^2 = .43$, $P < .0001$) and GLT ($R^2 = .40$, $P < .0001$) AT-LPL activity. Plasma TG correlated positively ($r = .32$, $P < .01$) and HDL-C correlated negatively ($r = -.32$, $P = .02$) with ABD AT-LPL activity, but these relationships were not significant after controlling for percent body fat or leptin. The results of this study indicate that in healthy middle-aged and older men, the major determinants of AT-LPL activity are obesity and its major associated hormones, leptin and insulin, not cardiovascular fitness, and also suggest that the higher HDL-C levels observed in endurance-trained men are not associated with increased AT-LPL activity.

LIPOPROTEIN LIPASE (LPL), an enzyme bound to the capillary endothelium of most tissues, is found predominantly in adipose tissue (AT) and skeletal muscle. The enzyme has a key role in the catabolism of triglyceride (TG)-rich lipoproteins and the production of high-density lipoprotein 2 cholesterol (HDL_2-C).[1] Fatty acids released as a result of the lipolytic action of LPL on circulating TG can be oxidized as an energy source in skeletal muscle, while they are reesterified and stored as TG in AT. The regulation of LPL activity is site-specific.

Studies examining the effect of exercise training on AT-LPL show discrepant results. Higher AT-LPL activity was found in young male and female champion-class long-distance runners, and there was a positive correlation between AT-LPL activity and serum HDL-C levels.[2] Increases in AT-LPL activity also were observed in normal-weight middle-aged men after 15 weeks of a moderate training program[3] or after 6 weeks of intensive training, and the increase in AT-LPL activity correlated with HDL-C levels.[4] Conversely, a decrease in the activity of AT-LPL was observed after a 6-month endurance-training program in obese women,[5] and endurance-trained non-obese premenopausal women had lower levels of abdominal AT-LPL activity compared with sedentary controls.[6] Furthermore, detraining in young long-distance runners was associated with an increase in AT-LPL activity,[7] and in one study, there was no change in AT-LPL activity after 2 weeks of aerobic exercise training in a group of normal-weight young and older men.[8]

The effect of endurance exercise training on the activity of AT-LPL in older men is not known. On the other hand, several studies show that exercise training increases the activity of skeletal muscle LPL in normal-weight young males and females,[2,9] as well as older men.[8] These changes were observed in champion-class long-distance runners[2] and also after 2 weeks of aerobic exercise training in sedentary men.[8] The decrease in skeletal muscle LPL activity after deconditioning in young runners is consistent with these findings.[7]

AT-LPL activity is increased in genetic and diet-induced obesity, suggesting that the enzyme may act to preserve adipose cell size.[10-12] Furthermore, insulin, which is also increased in obesity, is an important regulator of AT-LPL activity.[11] The level of leptin, the protein product of the *ob* gene secreted by adipocytes, correlates well with percent body fat and is increased in obesity.[13,14] Although recent in vitro studies show that treatment with leptin increases the expression of LPL mRNA,[15] the relationship between AT-LPL and leptin in middle-aged and older men is not known.

The purpose of the present study was to determine (1) the relationship of AT-LPL activity to body composition and

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$\dot{V}O_2$max in older men and (2) whether the high HDL-C levels in healthy endurance-trained older male athletes are associated with higher levels of AT-LPL activity versus their sedentary peers. We addressed these questions by performing a cross-sectional comparison of these variables in healthy master athletes and lean sedentary and obese sedentary older men of comparable age.

SUBJECTS AND METHODS

Subjects

Healthy nonsmoking white men aged 47 to 76 years from the Baltimore/Washington, DC Metropolitan area provided written consent to participate in the study according to the approved guidelines of the Institutional Review Boards of the University of Maryland School of Medicine and Johns Hopkins Bayview Medical Center. All subjects were weight-stable (± 2.5 kg) for at least 6 months before enrollment and were accepted to participate in the study after completing a medical evaluation that included a medical history, physical examination, and fasting blood profile. No subject had evidence of cardiovascular disease, diabetes (fasting glucose >140 mg/dL), hypertension (BP > 160/90 mm Hg), hyperlipidemia, or liver, renal, or hematologic disease. A graded exercise test (GXT) according to a modified Bruce protocol[16] was performed to exclude subjects with an abnormal cardiovascular response to exercise.

The master athletes were recruited from participants of the Maryland Senior Olympics and athletic clubs in the Baltimore-Washington area.[17] They were long-distance runners who ran an average of 57 km/wk and trained an average of 5 d/wk. Most competed at the local and state levels. On average, the athletes had been training for longer than 15 years. The sedentary men (obese and lean) were recruited as part of an overall recruitment of older healthy sedentary men interested in exercise training and/or weight reduction programs. They were active but had not engaged in regular aerobic exercise (>15 minutes of exercise three times per week at a continuously elevated heart rate) for at least 1 year before enrollment.

Body Composition, Fat Distribution, and $\dot{V}O_2$max

The body mass index (BMI) was calculated by dividing the weight by the height squared. The waist to hip ratio (WHR) was measured as the ratio of the minimal waist circumference to the hip circumference at the maximal gluteal protuberance. Body density was determined by underwater weighing, and percent body fat was calculated after correcting for residual lung volume as previously described.[18,19] Fat-free mass (FFM) was calculated as body weight minus fat mass. $\dot{V}O_2$max was measured as previously described on a motor-driven treadmill using a progressive modified Balke protocol to voluntary exhaustion.[18] A valid $\dot{V}O_2$max fulfilled at least two of the following three criteria: (1) maximal heart rate greater than 90% of age-predicted maximal heart rate (220 bpm − age), (2) respiratory exchange ratio of at least 1.10, and (3) plateau in $\dot{V}O_2$ with increasing work rate. If these criteria were not achieved, the test was repeated.

Experimental Protocol

To avoid the confounding effects of differences in diet among the subjects, the men were counseled on the principles of an American Heart Association (AHA) phase 1 diet[20] for at least 4 weeks, and compliance was verified by analysis of 7-day food records by registered dietitians. Nutrient intake was controlled for at least 3 days prior to metabolic testing by providing each subject with a weight-maintaining diet composed of 50% to 55% carbohydrate, 15% to 20% protein, and 30% fat with 300 to 400 mg cholesterol and a polyunsaturated to saturated fat ratio of 0.6 to 0.8, prepared in the General Clinical Research Center kitchen at Johns Hopkins Bayview Medical Center. Based on the $\dot{V}O_2$max and percent body fat, the men were categorized into one of three groups: master athletes ($\dot{V}O_2$max > 40 mL/kg/min, n = 19), lean sedentary men ($\dot{V}O_2$max < 40 mL/kg/min, n = 20) with body fat less than 24%, and obese sedentary men ($\dot{V}O_2$max < 40 mL/kg/min, n = 27) with body fat greater than 27%. Sedentary men had no recent history of regular aerobic exercise training and participated in physical activity for less than 20 minutes two times per week. The master athletes were all long-distance runners who competed at local races and trained intensively almost continuously for the previous 15 years at least 5 d/wk, averaging 57 km/wk. At the time of study, the master athletes were training for at least 5 years. Research testing was performed after a 12-hour overnight fast, 24 to 36 hours after the last bout of exercise in the case of the master athletes, whereas sedentary subjects maintained their sedentary life-style before testing.

Glucose Metabolism and Leptin

Venous blood samples for measurement of glucose, insulin, and leptin levels were taken in duplicate after a 12-hour fast prior to a 2-hour oral glucose tolerance test (OGTT) with measurement of plasma glucose (Beckman, Fullerton, CA) and insulin[21] levels. Aliquots of plasma for determination of insulin and leptin (radioimmunoassay kit; Linco, St Louis, MO) were frozen at −70°C until measurement.

Plasma Lipoprotein Lipids

Venous blood samples for measurement of lipoprotein lipids were taken after a 12-hour fast on 2 separate days, and the mean of the two measurements is reported. Plasma TG and total cholesterol levels were measured enzymatically[22,23] on plasma separated from fasting venous blood samples drawn into chilled tubes containing EDTA (1 mg/mL blood).[22] The HDL-C level was measured in the supernatant after precipitation of apoprotein B–containing lipoproteins with dextran sulfate, and LDL-C was calculated using the Friedewald equation.[24] A second precipitation with high–molecular-weight dextran separated the HDL_2-C and HDL_3-C subspecies, and HDL_2-C was calculated as HDL-C − HDL_3-C.[25]

AT-LPL Activity

After an overnight fast, subcutaneous AT was obtained under local anesthesia (1% xylocaine) from both the abdominal (ABD) and gluteal (GLT) regions by aspiration with a 16-gauge needle.[26] The mean fat cell diameter, measured from a fixed frozen section of AT,[27] was used to calculate fat cell weight and surface area.[27,28] Heparin-releasable AT-LPL activity was measured in duplicate 30- to 50-mg fragments of AT as previously described[29,30] on 4 μCi 1-^{14}C-glycerol triolein (specific activity, 63 mCi/mmol; Amersham, Arlington Heights, IL) substrate containing 5 mg unlabeled triolein and 240 μg lecithin in 4 mL 0.5-mol/L Tris buffer, pH 8.2, with 2% fatty acid–free bovine serum albumin and 0.3 mL fasting serum. The labeled FFAs generated were quantified by liquid scintillation counting, and AT-LPL activity was expressed as nanomoles of FFA produced per minute by 10^6 cells.

Statistics

Data are reported as the mean ± SE. Statistical analyses were performed using Jump Software (SAS Institute, Cary, NC). Plasma TG, leptin, insulin, and AT-LPL activity values were logarithmically transformed to yield a normal distribution before parametric analyses. Characteristics of the three groups were compared using ANOVA with post hoc testing by the Tukey-Kramer test. A paired *t* test was used to compare ABD and GLT values in the same individual. Pearson's product-moment correlation coefficient was used to quantify associations between two variables. Stepwise multiple regression analyses were used to calculate partial correlation coefficients to determine the major predictors of AT-LPL activity. Significance was accepted at a *P*

level less than .01 in bivariate analysis and P less than .05 in multivariate analysis.

RESULTS

Subject Characteristics

The master athletes, lean sedentary men, and obese sedentary men were comparable in age, but the body weight, BMI, and percent body fat were highest in the obese sedentary men > lean sedentary men > master athletes, and there were no differences in FFM among the three groups (Table 1). The obese sedentary men had a larger waist circumference and WHR than lean sedentary men or master athletes. The master athletes had a significantly higher $\dot{V}O_2$max than the lean and obese sedentary men when values were expressed in liters per minute, but when $\dot{V}O_2$max was normalized for body mass (mL/kg/min), $\dot{V}O_2$max was higher in master athletes > lean sedentary men > obese sedentary men ($P < .05$). In the entire population, $\dot{V}O_2$max (L/min) correlated negatively with percent body fat ($r = -.32$, $P < .007$), waist circumference ($r = -.25$, $P < .03$), and WHR ($r = -.33$, $P < .005$). Age correlated negatively with $\dot{V}O_2$max (L/min) ($r = -.37$, $P < .002$) and FFM ($r = -.42$, $P < .0002$).

Glucose Metabolism and Leptin

The master athletes and lean sedentary men had similar values for fasting glucose, glucose at 2 hours of the OGTT, and fasting insulin and leptin, but these levels were significantly lower ($P < .05$) than the levels in obese sedentary men (Table 2). The difference in leptin levels among the groups disappeared after controlling for percent body fat (obese, 6.9 ± 1.1; lean, 5.2 ± 0.8; athletes, 4.2 ± 1.0 ng/mL; P = NS). Within the three groups, fasting insulin, fasting glucose, 2-hour glucose, and leptin correlated positively with percent body fat ($r = .53$ to .82, $P < .0001$), waist ($r = .45$ to .76, $P < .0001$), and WHR ($r = .40$ to .52, $P < .0001$) and negatively with $\dot{V}O_2$max (L/min) ($r = -.40$, $P < .002$, except for fasting glucose). Neither the glucose metabolism parameters nor leptin correlated with age.

Lipoprotein Lipids

Plasma total cholesterol, TG, and lipoprotein metabolic profiles of these older men were in the normal range, and there were no differences in the fasting values for total cholesterol or LDL-C among the groups. The master athletes had lower TG levels ($P < .01$) and higher HDL-C and HDL_2-C ($P < .01$) than the obese and lean sedentary men (Table 2). The difference in TG values disappeared when the means were adjusted for percent body fat (obese, 87 ± 12; lean, 110 ± 9; and athletes, 91 ± 12 mg/dL). On the other hand, controlling for percent body fat did not affect the mean values for HDL-C (athletes, 48 ± 2; lean, 38 ± 2; obese, 36 ± 2 mg/dL) and HDL_2-C (athletes, 11 ± 1; lean, 5 ± 1; obese, 3 ± 1 mg/dL). Plasma HDL-C and HDL_2-C levels correlated negatively with percent fat ($r = -.52$), waist ($r = -.59$), WHR ($r = -.36$), leptin ($r = -.55$), and fasting insulin ($r = -.51$) and positively with $\dot{V}O_2$max (L/min) ($r = .40$) ($P < .001$). Plasma TG correlated directly with percent body fat ($r = .49$), waist ($r = .44$), WHR ($r = .38$), leptin ($r = .56$), and fasting insulin ($r = .50$) and inversely with HDL-C ($r = -.54$) and $\dot{V}O_2$max ($r = -.51$) ($P < .0001$). The regression slope (mean ± SE) of the relationship between HDL-C levels and percent body fat became nonsignificant when adjusted for group status (−0.09 ± 0.21, NS), and conversely, the regression slope of the relationship between TG levels and percent body fat remained significant after controlling for group status (2.92 ± 1.18, $P = .01$). Thus, in these middle-aged and older men, $\dot{V}O_2$max is the main predictor of HDL-C levels and obesity is the main predictor of TG levels. Lipoprotein metabolism parameters were not related to age.

Table 1. Physical Characteristics of the Subjects

Characteristic	Obese (n = 27)	Lean (n = 20)	Athletes (n = 19)
Age (yr)	61 ± 2	61 ± 1	63 ± 2
Body weight (kg)	92 ± 2[a]	76 ± 2[b]	69 ± 1[c]
BMI (kg/m^2)	30.9 ± 0.5[a]	25.7 ± 0.5[b]	22 9 ± 0.3[c]
Body fat (%)	31.9 ± 0.7[a]	19.2 ± 0.9[b]	15.4 ± 1.1[c]
Fat mass (kg)	29.6 ± 1.1[a]	14.7 ± 0.9[b]	10.7 ± 0.7[c]
FFM (kg)	63 ± 1	62 ± 2	59 ± 1
Waist (cm)	101.6 ± 1.6[a]	89.3 ± 1.4[b]	81.7 ± 0.7[c]
Hip (cm)	108.3 ± 1.1[a]	98.6 ± 1.1[b]	92.7 ± 1.2[c]
WHR	0.97 ± 0.01[a]	0.90 ± 0.01[b]	0.84 ± 0.01[b]
$\dot{V}O_2$max (L/min)	2.7 ± 0.1[a]	2.5 ± 0.1[a]	3.4 ± 0.1[b]
$\dot{V}O_2$max (mL/kg/min)	28.4 ± 0.8[a]	32 9 ± 0.9[b]	48.0 ± 1.0[c]

NOTE. Data are the mean ± SE. Different superscripts distinguish values that differ significantly ($P < .05$).

Table 2. Glucose, Leptin, and Lipid and Metabolic Profiles

Parameter	Obese (n = 27)	Lean (n = 20)	Athletes (n = 19)
Fasting glucose	102 ± 2[a]	94 ± 1[b]	90 ± 1[b]
2-h glucose	146 ± 5[a]	123 ± 6[b]	101 ± 7[b]
Fasting insulin	97.0 ± 7.4[a]	49.9 ± 4.6[b]	28.2 ± 3.1[b]
Leptin	9.5 ± 1.0[a]	4.3 ± 0.5[b]	2.1 ± 0.1[b]
TG	112 ± 8[a]	98 ± 10[a]	68 ± 4[b]
Cholesterol	175 ± 5	189 ± 5	180 ± 6
LDL-C	117 ± 5	131 ± 4	118 ± 5
HDL-C	35 ± 1[a]	39 ± 1[a]	49 ± 2[b]
HDL_2-C	4 ± 1[a]	4 ± 1[a]	10 ± 1[b]

NOTE. Data are the mean ± SE. Different superscripts distinguish values that differ significantly ($P < .05$). All data are in mg/dL except for insulin (pmol/L) and leptin (ng/mL). Leptin measurements were performed in 21 obese men, 19 lean men, and 18 athletes.

AT-LPL, Body Composition, $\dot{V}O_2$max, and Lipoprotein Lipids

The size of both ABD and GLT subcutaneous adipocytes was significantly larger in obese sedentary men versus lean sedentary men and master athletes. There were no regional (ABD *v* GLT) differences in fat cell size in obese or lean sedentary men, but GLT adipocytes were significantly ($P \leq .01$) larger than ABD cells in the master athletes. Since fat cell size differed among the groups, AT-LPL activity was expressed per cell (nmol FFA/10^6 cells · min). There were no regional differences in the activity of AT-LPL in these groups, and obese sedentary men had higher levels of ABD and GLT AT-LPL activity per cell than lean sedentary men and master athletes ($P = .01$) (Table 3). There was a positive relationship between ABD or GLT fat cell size and the corresponding AT-LPL activity expressed per cell (ABD, $r = .62$, $P < .0001$; GLT, $r = .47$, $P < .0001$) or per

Table 3. Regional AT-LPL Activity

Parameter	Obese (n = 27)	Lean (n = 20)	Athletes (n = 19)
ABD fat cell size (μg TG/cell)	0.52 ± 0.03[a]	0.31 ± 0.02[b]	0.22 ± 0.02[b]
GLT fat cell size (μg TG/cell)	0.50 ± 0.02[a]	0.34 ± 0.02[b]	0.27 ± 0.01[b]
ABD AT-LPL (nmol/10^6 cells · min)	2.1 ± 0.3[a]	0.8 ± 0.2[b]	0.5 ± 0.1[b]
GLT AT-LPL (nmol/10^6 cells · min)	2.7 ± 0.7[a]	0.9 ± 0.2[b]	0.8 ± 0.1[b]

NOTE. Data are the mean ± SE. Different superscripts distinguish values that differ significantly ($P < .05$).

Table 4. Pearson Correlation Coefficients for AT-LPL and Measurements of Body Fatness and Fitness in All Subjects

Parameter	ABD AT-LPL (nmol/10^6 cell · min)	GLT AT-LPL (nmol/10^6 cell · min)
Weight (kg)	.32†	.32†
BMI (kg/m^2)	.40*	.31*
Waist (cm)	.40*	.41*
Hip (cm)	.40*	.37†
WHR	.21	.18
Body fat (%)	.50*	.37†
Insulin (pmol/L)	.45*	.32†
Leptin (ng/mL)	.65*	.63*
$\dot{V}o_2$max (L/min)	−.19	−.16

*$P < .001$.
†$P < .05$.

gram of fat (ABD, $r = .24$, $P = .07$; GLT, $r = .30$, $P = .01$). Age was not related to either ABD or GLT AT-LPL activity.

Both ABD and GLT AT-LPL activity related positively to body fatness (Fig 1A and Table 4) but did not correlate with $\dot{V}o_2$max, and similar results were obtained when AT-LPL was expressed per unit of cell surface area (data not shown). To address the role of obesity independently of cardiovascular fitness, we grouped all sedentary men, ie, obese and lean, who differed in percent body fat but had comparable $\dot{V}o_2$max levels. To assess the role of cardiovascular fitness independently of obesity, we grouped all lean men, ie, lean sedentary and master athletes, who had comparable percent body fat and a wide range of $\dot{V}o_2$max levels. The positive associations between AT-LPL activity at either site and percent body fat and waist remained significant when sedentary subjects (obese sedentary and lean sedentary) were grouped, but not when lean subjects (sedentary lean and master athletes) were grouped (data not shown). Therefore, in these middle-aged and older men, AT-LPL activity is mainly related to body fatness, not to $\dot{V}o_2$max.

In these subjects, ABD and GLT AT-LPL activity were positively related to fasting insulin and leptin levels (Fig 1B and Table 4). Since measures of percent body fat, leptin, and insulin were highly interrelated ($r = .63$ to $.82$, $P < .0001$), stepwise multiple regression analysis was performed with these variables in a model to determine the best independent predictors of ABD and GLT AT-LPL activity. There was no independent effect of group on the model. Leptin was the main independent predictor of ABD ($R^2 = .43$, $P < .0001$) and GLT ($R^2 = .40$, $P < .0001$) AT-LPL activity, and neither percent body fat nor insulin contributed significantly to the prediction of ABD or GLT AT-LPL activity beyond that predicted by leptin. Adjustment of ABD and GLT AT-LPL for differences in leptin levels, as well as percent body fat or insulin, eliminated the differences in AT-LPL levels between the groups.

There was no relationship between total cholesterol, LDL-C, or HDL_2-C and AT-LPL activity, but plasma TG correlated positively ($r = .32$, $P < .01$) and HDL-C correlated negatively

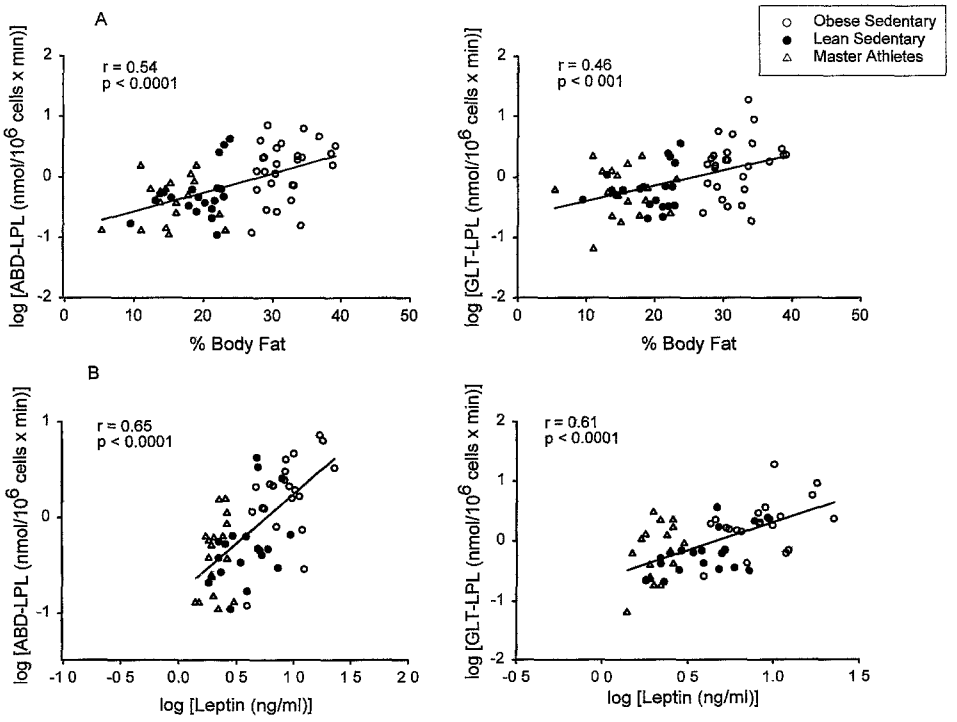

Fig 1. Relationship of ABD AT-LPL (ABD-LPL) and GLT AT-LPL (GLT-LPL) to % body fat (A, N = 66) and leptin levels (B, n = 58) in the subjects.

with ABD AT-LPL ($r = -.32$, $P < .01$) (Fig 2). A similar relationship was observed for plasma TG and GLT AT-LPL activity ($r = .30$, $P = .01$), but no relationship was observed between GLT AT-LPL activity and HDL-C. However, these relationships were not independent of percent body fat, leptin, or insulin, and became nonsignificant after controlling for these variables.

DISCUSSION

The results of this study show that in healthy middle-aged and older men, the major determinants of AT-LPL activity are obesity and its hormonal markers, leptin and insulin, not cardiovascular fitness. Contrary to what is observed in younger men, endurance-trained older men have AT-LPL activity similar to their lean sedentary peers. Despite this, they have lower plasma TG and higher HDL-C levels. The finding of increased AT-LPL activity in older obese men compared with older lean men is similar to the finding in young and middle-aged obese and lean men,[12] and suggests that in this older population AT-LPL activity may also act to preserve obesity.[1] However, in contrast to findings in young sedentary and trained males, there was no difference in the activity of AT-LPL between endurance-trained master athletes and their sedentary lean peers.

Endurance exercise training has a beneficial impact on most of the metabolic abnormalities associated with ABD obesity and the development of atherosclerosis. Particularly, exercise training decreases plasma TG, increases HDL-C, and increases insulin sensitivity and glucose tolerance in both young and older males.[2,31,32] In younger long-distance runners, AT-LPL activity is higher than in sedentary controls and is directly related to HDL-C levels.[2,33] This suggests that the increased AT-LPL activity and concomitant enhanced TG clearance may mediate, at least partially, the higher HDL-C levels found in physically conditioned younger men and women. However, 6 months of endurance exercise training in obese premenopausal women reduced the activity of AT-LPL in both ABD and femoral subcutaneous fat, and there was no relationship between the increase in $\dot{V}O_2max$ and changes in the lipid-lipoprotein profile or AT-LPL activity.[5] Furthermore, in another study, there was an increase in subcutaneous ABD AT-LPL activity after a 2-week period of detraining in younger male and female athletes.[7] The negative relationship between plasma TG levels and $\dot{V}O_2max$, as well as the positive relationship between HDL-C or HDL_2-C and $\dot{V}O_2max$, in our older subjects suggest that endurance exercise training is related to the lower levels of plasma TG and the higher levels of HDL-C and HDL_2-C observed in older master athletes. Thus, contrary to what is described in younger trained men and women,[2,4,33] our results do not support a major role for AT-LPL in mediating the lower TG and higher HDL-C lipoprotein-lipid profile in older endurance-trained men. In our subjects, AT-LPL activity correlated positively with plasma TG levels and negatively with HDL-C levels, but these relationships disappeared after controlling for body fatness and there was no relationship between AT-LPL activity and $\dot{V}O_2max$.

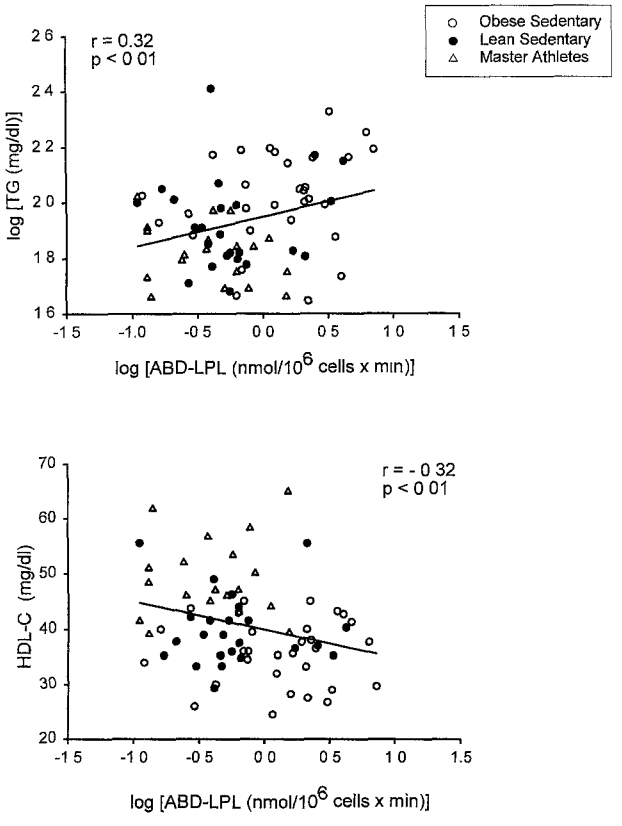

Fig 2. Relationship of plasma TG and HDL-C levels with ABD AT-LPL (ABD-LPL) in all subjects (N = 66).

Recently, Mauriège et al[6] suggested that the lower ABD AT-LPL activity in endurance-trained non-obese premenopausal women compared with sedentary controls was related to the smaller ABD adipose cell size in endurance-trained women, not endurance training per se. In our older subjects, AT-LPL activity expressed per cell or per gram of fat correlated positively with fat cell size. Hence, the similar AT-LPL activity observed in master athletes and lean sedentary controls could be attributed mainly to the similar size of their adipocytes rather than a direct effect of endurance exercise training on AT-LPL activity. Since our older athletes continued training during the study and underwent biopsy 24 to 36 hours after their last exercise session, we cannot exclude the residual effects of exercise as one possible reason for the lower AT-LPL in the older (compared with younger) athletes. However, the activity of AT-LPL was higher and there was a positive correlation between AT-LPL activity and HDL-C levels in young runners with a biopsy the day after their regular training program,[2] as well as in endurance-trained normal-weight middle-aged men with a biopsy 48 to 72 hours after a training session.[4]

Endurance exercise training is associated with an increase in muscle LPL activity,[2,8,9] which may decrease the AT to muscle LPL activity ratio, thereby reducing lipid accumulation in AT and promoting lipid uptake and oxidation as an energy source for exercising muscle.[7] LPL is the rate-limiting enzyme in the clearance of TG-enriched lipoproteins from the circulation, and these adaptations would divert FFAs from storage in fat tissue to oxidation in skeletal muscle. Although we did not measure LPL activity in skeletal muscle, it is possible that improvements in the lipid-lipoprotein profile observed in healthy endurance-trained older men might be mediated mainly by an increase in skeletal muscle LPL, not in AT-LPL activity. However, we

cannot exclude the possibility that other factors also might mediate the less atherogenic lipid profile observed in these older master athletes. The activity of hepatic TG lipase, which hydrolyzes lipids in HDL particles and accelerates the catabolism of HDL, is reduced in male and female runners compared with sedentary controls.[33,34] In addition, the activity of cholesteryl ester transfer protein, which catalyzes the transfer of cholesteryl esters from HDL to other lipoproteins, also is lower in marathon runners compared with sedentary controls.[35] Collectively, these metabolic adaptations may interact to improve the lipid-lipoprotein profile in endurance exercise–trained individuals.

The results of this study confirm the strong relationship between leptin and percent body fat previously described,[13,14] and suggest that leptin may be the best independent surrogate for obesity in this older population. Leptin levels in master athletes tended to be lower than in lean sedentary men, most likely due to the lower percent body fat in the master athletes, since the differences among groups disappeared after controlling for percent body fat. This suggests that endurance exercise training in middle-aged and older men does not alter leptin levels, a finding similar to that described in young and middle-aged men after 12 or 20 weeks of endurance exercise training.[36,37] To the best of our knowledge, this is the first time a significant positive relationship between the activity of AT-LPL and leptin levels is reported.

In summary, our results demonstrate that in healthy middle-aged and older men, obesity is associated with increased AT-LPL activity, and moreover, it is the major predictor of AT-LPL activity. In this older population, AT-LPL activity does not seem to play a role in mediating the favorable HDL-C lipoprotein and TG adaptations to aerobic exercise, since these endurance-trained older individuals had AT-LPL activity comparable to their lean sedentary peers. Further studies are needed to elucidate the cellular mechanisms underlying the apparent age-related differences in AT-LPL responses to endurance exercise training in older men and the metabolic adaptations by which exercise training improves lipoprotein metabolism in healthy older men.

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