Lipoprotein Lipase Activity in Skeletal Muscle Is Related to Insulin Sensitivity

T. Pollare, B. Vessby, and H. Lithell

The relative effects of obesity, alone or in combination with insulin resistance and hyperinsulinemia (with or without diabetes), on lipoprotein concentrations, blood pressure, and other risk factors for cardiovascular disease were investigated in 28 men (mean age, 63 years). Special attention was given to lipoprotein lipase (LPL) activity in tissues and to postheparin plasma LPL activity and hepatic lipase activity and their relation to insulin resistance. The 28 men fulfilled the entrance criteria of the study so that they could be allocated to one of the four groups (seven in each group): 1) normal body weight, normal fasting insulin level, and normal glucose tolerance (controls); 2) the same as in group 1 but with moderate obesity; 3) the same as in group 2 but with fasting hyperinsulinemia; 4) the same as in group 3 but with non–insulin-dependent diabetes mellitus. Glucose infusion rate for the control group was 8.1±2.1 mg/kg body wt/min (mean±SD) at an insulin infusion rate of 56 milliunits/m²/min. The average values in groups 2, 3, and 4 were 6.0±0.7, 3.2±0.5, and 1.9±1.0 mg/kg body wt/min, respectively. Concentrations of very low density lipoproteins as well as blood pressure and urate concentrations were highest and those of high density lipoproteins were lowest in the two hyperinsulinemic groups (groups 3 and 4). Skeletal muscle LPL activity was 46 ±23, 41 ±25, 23 ±6, and 31 ±13 milliunits/g wet wt (mean±SD) in the four groups, respectively. There was a positive correlation between glucose infusion rate and muscle LPL activity (r=0.58, p<0.0001). The hepatic lipase activity was positively correlated with the insulin area under the curve of the intravenous glucose tolerance test (r=0.35, p=0.02). Furthermore, blood pressure, free fatty acid concentration, liver enzymes, and urate concentrations were significantly correlated with glucose infusion rate at the clamp test. These data give further support for insulin resistance as an important factor behind the observed lipoprotein abnormalities and blood pressure elevations as part of the insulin resistance syndrome characteristic for obese and diabetic patients. (Arteriosclerosis and Thrombosis 1991;11:1192–1203)

Insulin resistance has been described as the common underlying mechanism that causes much of the hypertension, dyslipidemia, and impaired glucose tolerance in the population. Elevated levels of triglyceride (TG)-rich lipoproteins are a characteristic of diabetes mellitus and impaired glucose tolerance. A decreased capacity for elimination of TGs may well play a role in the development of hypertriglyceridemia, but the possible underlying mechanisms have only partly been studied.

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To ensure the uptake of fatty acids from the TG-rich lipoproteins in the circulation, the enzyme lipoprotein lipase (LPL) is present in the endothelial wall of the capillaries in most tissues of the body as well as in the endothelium of large arteries. LPL activity is a rate-limiting factor in TG uptake and consequently has a key function in the regulation of the concentrations of TG-rich lipoproteins in the circulation.

In a recent study we reported that in four groups of men characterized by 1) normal body weight and normal insulin concentration, 2) obesity and normal insulin concentration, 3) obesity and hyperinsulinemia, or 4) obesity and non–insulin-dependent diabetes mellitus (NIDDM), adipocytes were not characterized by insulin resistance except for those from the diabetic men. This finding indicated that insulin resistance must prevail in tissues other than adipose tissue because serum insulin concentrations were increased in group 3. Furthermore, LPL activity in adipose tissue did not differ among the four groups.
and thus could not explain the difference in TG levels that existed among the groups.

In other studies it was found that muscle LPL activity was inversely related to body weight and serum insulin concentration and was lower in diabetic than in normoglycemic persons. This finding may indicate that muscle LPL activity is related to insulin resistance and that low muscle LPL activity may be part of the explanation for elevated TGs in diabetes and obesity. Therefore, the primary aim of the present study was to investigate to what extent groups of men with widely differing body weight and insulin and glucose concentrations differed with regard to lipoprotein concentrations and their dependence on LPL activity, as expressed not only in muscle tissue but also in adipose tissue, and LPL activity and hepatic lipase activity in plasma after heparin injection. Because insulin concentrations do not reflect the degree of insulin resistance in diabetic patients, a euglycemic hyperinsulinemic clamp test was included to further elucidate the relations between insulin resistance and carbohydrate and lipoprotein metabolism. Other aims of the study were to record blood pressure, glucose tolerance, liver enzymes, and urate levels in blood plasma; to investigate whether the groups differed in these respects; and to determine to what extent any differences were related to insulin concentrations and insulin resistance.

**Methods**

A total of 28 men were selected for the present study; subjects were drawn mainly from a large health survey (with 2,300 male participants) in Uppsala, a medium-sized town in Sweden. In about 1,600 of these subjects, an intravenous glucose tolerance test (IVGTT) had been performed. The majority of the 28 men had been investigated some years ago. The men were selected so as to fit into one of four categories: 1) men of normal weight (body mass index [BMI, weight \( \text{kg} \) divided by height squared \( \text{m}^2 \)] \(<27\)), normal intravenous glucose tolerance, and normal fasting insulin concentration; 2) moderately obese men (BMI \( >27\)) with normal fasting glucose and intravenous glucose tolerance and a fasting insulin concentration similar to that in the normal-weight group (within the mean+3SDs of the normal group); 3) men with moderate obesity and normal glucose tolerance but with a fasting insulin concentration above the mean+3SDs of that in the normal-weight groups; 4) men with moderate obesity and NIDDM (fasting blood glucose >7 mmol/l) and with fasting insulin concentrations above the mean+3SDs of the control group.

Seven men were allocated to each group. The clinical characteristics of these study groups are presented in Table I. Twelve were smokers, who were distributed in all four groups. There was no significant difference in physical activity among the groups according to a four-point scale.

All participating subjects were free from other diseases, as determined by medical history and a thorough physical examination, and were not being treated with any drugs. There was no clinical or laboratory evidence of hepatic, renal, or thyroid dysfunction. Informed consent was obtained from all subjects after the nature, purpose, and possible side effects of the study had been fully explained. The study protocol was approved by the Ethics Committee of the University Hospital of Uppsala.

**Experimental Procedure**

All subjects were ambulatory at the time of their examination in the outpatient department of the hospital, which took place at 8 AM after an overnight (12-hour) fast. Their mean age was 63 years, ranging from 56 to 67 years.

Adipose and muscle tissue biopsies, an intravenous fat tolerance test (IVFTT), and an IVGTT were performed, and postheparin plasma lipolytic activity and insulin sensitivity were evaluated on four separate...
days, with an interval of 2–3 days between each investigation. Dietary history had revealed a fat intake of 32–38% of energy and of carbohydrates of 42–52% of energy. For this particular study each participant was instructed to adhere to his regular diet but to maintain an intake of at least 250 g carbohydrates daily and to refrain from extreme physical exercise or inactivity for at least 5 days before the investigation. Smokers were instructed not to smoke on the morning of the investigation. No other instructions regarding diet or exercise were given during the study. Blood samples were drawn, and urine specimens were collected after an overnight (12-hour) fast.

**Blood pressure and heart rate.** Blood pressure was measured by mercury sphygmomanometry three times in the supine position after a rest of 10 minutes and twice after 1 minute of standing. The mean of each of these sets of values was used in the analyses. A large cuff was used when appropriate. The pulse rate was recorded before each blood pressure measurement.

**Intravenous glucose tolerance test.** The IVGTT was performed, as recently described in detail, by injection of 300 mg glucose/kg body wt. Plasma glucose was measured by the glucose oxidase method. Immuno-reactive insulin was assayed in plasma by use of a commercial radioimmunoassay kit (Phadeseph Insulin RIA, Pharmacia, Uppsala, Sweden). Glucose tolerance was expressed as the k value, as described by Ikkos and Luft. The peak insulin response was defined as the mean of the values obtained at 2, 4, and 6 minutes, and the insulin index was defined as the ratio between the peak and basal plasma insulin values (mean of values at −10, −5, and 0 minutes). The areas under the curves for glucose and insulin during the IVGTT were calculated as the deviations from the basal value integrated over the sampling time. The average fasting plasma insulin and glucose concentrations were calculated from four samples taken on two separate days. The glycosylated form of the major component of adult hemoglobin fraction Aβ (HbAβ) was measured by fast-performance liquid chromatography (normal range, 3.5–6.0%).

**Clamp studies.** The euglycemic hyperinsulinemic clamp technique, as introduced by DeFronzo et al, and was used and has recently been described in detail. The insulin (Actrapid, Human, Novo, Denmark) infusion rate was 56 milliunits/m²/min in all subjects, resulting in a mean plasma insulin concentration of 102 milliunits/l (range, 79–144 milliunits/l) during the clamp studies. The target level of plasma glucose during the clamp study was 5.3 mmol/l, and this level was maintained by measuring plasma glucose (Beckman glucose Analyzer II, Beckman Instruments, Fullerton, Calif.) every 5 minutes and adjusting the infusion rate of the 20% glucose solution accordingly. In all diabetics the desired plasma glucose level was attained within 60 minutes from the start of the insulin infusion, except in two diabetic patients in whom the clamp test was performed at a glucose level of about 5.7 mmol/l. Their results are included in all calculations. Under steady-state conditions of euglycemia, the glucose input (consisting of exogenous glucose infusion and endogenous glucose production) must equal the glucose use by all tissues of the body.

Endogenous (hepatic) glucose production was quantified in persons from all four groups. Clamp studies were then performed in combination with tritiated D-glucose (D[3-3H]glucose) to measure the effect of hyperinsulinemia on hepatic glucose production. The priming dose was 40 µCi for diabetic patients and 20 µCi for normal subjects, and it was given as a 20-ml bolus injection. The continuous infusion provided 0.40 µCi/min for diabetic patients and 0.20 µCi/min for normal subjects. The duration of the D[3-3H]glucose insulin before the start of insulin infusion was 120 minutes in normal subjects and 180 minutes in diabetic patients. Plasma samples for determination of D[3-3H]glucose specific activity were obtained every 5 minutes during the last 30 minutes of the equilibration period, after which time the insulin infusion was begun. A steady state of glucose-specific activity was achieved by this method in each subject. During the insulin clamp study plasma samples for D[3-3H]glucose specific activity were obtained at 15-minute intervals until the last 30 minutes, at which time they were obtained at 5-minute intervals. D[3-3H]glucose activity was determined after deproteinization with Ba(OH)2–ZnSO4 and evaporation of water. The specific activity of glucose was calculated by dividing the [3-3H]glucose activity by the plasma glucose concentration. D[3-3H]glucose (5 Ci/mmol) was purchased from The Radiochemical Centre, Amersham, England.

**Lipoprotein and Lipid Analyses**

TG and cholesterol concentrations in serum and lipoproteins were determined by enzymatic methods (Boehringer Mannheim, Mannheim, FRG) in a Mutistat III F/LS (Instrumentation Laboratories, Lexington, Mass.). The analytical errors for the two methods were 1.4% and 1.7%, respectively. Very low density lipoproteins (VLDLs) were separated by ultracentrifugation at a density of 1.006 g/ml. An MgCl2/phosphotungstic acid technique was applied to precipitate low density lipoproteins (LDLs) from the bottom fraction after ultracentrifugation. The supernatant (high density lipoprotein, HDL) was analyzed for its contents of TG and cholesterol. HDL subclass 3 (HDL3) was separated from other lipoproteins by ultracentrifugation of serum at a density of 1.120 g/ml. The cholesterol and HDL concentrations were measured in HDL3 (d>1.20 g/ml) and the contents of these lipids in HDL3 were calculated as the difference between those in total HDL and HDL3. The lipid concentrations in LDL were calculated as the difference between those in the bottom fraction after ultracentrifugation at a density of 1.006 g/ml and those in HDL. Serum free fatty acids (FFAs) were determined by an enzymatic colorimetric method supplied in a commercial kit (Wako Chemicals GmbH, Neuss, FRG) adapted for analysis.
in the Multistat III centrifugal analyzer. Free glycerol was measured with a TG kit (Boehringer Mannheim), with omission of the saponification step after adaptation for use in the Multistat III.

**Analyses of Lipoprotein Lipase Activities and the Intravenous Fat Tolerance Test**

Specimens of subcutaneous adipose tissue were obtained from the region lateral to the umbilicus (abdominal site) by use of the technique described by Hirsch and Goldrick. Biopsy samples of skeletal muscle tissue were taken from the lateral vastus muscle with an instrument described by Bergström. The tissue samples were stored in liquid N₂ until analyzed. Each sample was weighed in the frozen condition and then incubated in a reaction medium based on a glycine buffer (2.1 mol/l, pH 8.4) containing heparin (240 IU/ml), a triolein–phospholipid emulsion, albumin (7.5 mg/ml), and serum (3%). The procedures have been described in detail elsewhere. The activity is expressed in milliunits (1 milliunit = 1 nmol fatty acid released/min) per gram wet weight of tissue.

Plasma samples for determination of lipase activities were drawn 40 minutes after intravenous administration of 100 IU heparin (KabiVitrum, Stockholm, Sweden) per kilogram body weight. The activities of hepatic lipase and LPL were determined by specific methods, with the use of glycerol tri[9,10(n)-³H]oleate as the substrate. All samples were stored at −70°C before analysis. The elimination rate of exogenous TG was determined by the IVFTT, as described by Carlson and Rossner, with injection of 1 ml 10% Intralipid (KabiVitrum) per kilogram body weight.

**Routine Hematology, Biochemistry, and Urinalysis**

All other tests (sedimentation rate, hemoglobin concentration, white blood cell count, platelet count, aspartate aminotransferase [ASAT], alanine aminotransferase [ALAT], glutamyltransferase [GT], electrolyte balance, albumin, creatinine, urate concentration in serum, and routine urinalysis) were performed at the Department of Clinical Chemistry of the University Hospital, Uppsala.

**Calculations**

The total amount of glucose infused serves as a measure of the subject's sensitivity to the prevailing plasma insulin concentration. The glucose infusion rate during the glucose clamp test is expressed per kilogram of body weight and adjusted for the mean insulin concentration during the clamp test (mg glucose/kg body wt/min/milliunit/1×100). The calculation of insulin sensitivity is based on the assumption that endogenous hepatic glucose production is entirely suppressed (see "Results"). Urinary glucose losses were negligible under euglycemic conditions. Hepatic glucose production in the basal state (the last 30 minutes of the pre-insulin-infusion period) was calculated by dividing the rate of d[3°H]glucose infusion (cpm/min) by the steady-state level of glucose specific activity (dpm/mg). During the glucose and insulin infusions when glucose kinetics were not in a steady-state condition, the glucose appearance rate was calculated from Steele’s equations in their derivative form, with a value of 0.65 for the pool fraction. The rate of endogenous (hepatic) glucose production was estimated by subtracting the exogenous glucose infusion rate from the total body glucose appearance rate, as calculated by the isotopic tracer technique.

**Statistical Analyses**

Data are presented as mean±SD. An analysis of variance was performed, and all statistical comparisons among the four groups were calculated with Tukey’s studentized range test. Pearson correlation coefficients were calculated. A stepwise regression analysis (maximum R² improvement method) was executed for some dependent variables, for example, postheparin plasma LPL and hepatic lipase activity and LPL activity in muscle and adipose tissue, with insulin sensitivity measures, insulin and glucose values, and anthropometric data as independent variables. In other series different lipoprotein and lipid concentrations were used as dependent variables, and the lipase activities were used as independent variables.

**Results**

The BMI and waist to hip ratio were significantly increased in all the obese study groups compared with the normal-weight controls. In the groups of obese diabetic subjects, a significant increase in the fasting glucose concentration and HbA₁c value and a reduction in glucose tolerance were noted (Table 1). The fasting insulin levels in the obese hyperinsulinemic and the obese diabetic groups did not differ from one another (p=0.15) but were significantly higher than in the other groups (p<0.001) (Table 1). Blood pressure, both systolic and diastolic, in both the supine and standing positions was highest in the obese hyperinsulinemic group (Table 2).

As shown in Table 3, there was a gradual increase in the cholesterol concentration in the VLDL fraction and a decrease in the HDL fraction from the control group to the obese diabetic group. The TG concentrations in serum, VLDL, HDL, and HDL₃ were significantly increased in both the obese hyperinsulinemic and the obese diabetic group compared with the control group (p<0.05). The FFA and plasma glycerol concentrations in plasma were significantly higher in the obese diabetic group than in all the other groups (Table 3).

**Results of Clamp Tests**

During the euglycemic hyperinsulinemic clamp test, plasma glucose was kept constant (mean for all subjects, 5.1±0.6 mmol/l, close to the chosen level and with a coefficient of variation of 3.9±1.1%), and there was no significant difference among the groups (p>0.2).
The steady-state levels of insulin that were reached during the euglycemic clamp test reflect the metabolic clearance rate of insulin. In our study as in others,29 the mean steady-state insulin concentrations were higher in the obese hyperinsulinemic and diabetic groups (116±15 and 124±20 milliunits/l, respectively) than in the normoinsulinemic groups (85±15 and 87±18 milliunits/l, respectively), which is consistent with a lower rate of metabolic insulin clearance in the two hyperinsulinemic groups.

To evaluate the degree to which hepatic glucose production was inhibited with the present hyperinsulinemic clamp procedure in this kind of subject, we studied hepatic glucose levels in two representatives from each group. The hepatic glucose output during the last hour of the clamp study and the percentage suppression of hepatic glucose production are shown for each group separately. In normal-weight controls (n=2), these values were 2.02±0.15 and 0.11±0.04 mg/kg body wt/min and 95%, respectively; in the obese normoinsulinemic group (n=2), 2.29±0.10, 0.14±0.04, and 94%; in the obese hyperinsulinemic group (n=2), 2.62±0.20, 0.20±0.03, and 92%; and in the obese diabetic group (n=3), 2.87±0.10, 0.34±0.02,
Insulin sensitivity index

**FIGURE 1.** Bar graph showing glucose infusion rate (mg/kg body wt/min/milliniunit×100; adjusted for prevailing insulin concentration) during the last hour of the clamp test in the four study groups: 1) normal-weight, normoinsulinemic, normoglycemic control group; 2) obese, normoinsulinemic, normoglycemic group; 3) obese, hyperinsulinemic, normoglycemic group; and 4) obese, hyperinsulinemic diabetic group. **p<0.01; ***p<0.001.

and 88%. This finding indicates that during the last hour of the insulin clamp test, were there not only slight differences among the four groups with regard to hepatic glucose production but also that it was suppressed to as much as 88% in the most resistant groups, very similar to the 95% suppression in the control group. Hepatic glucose production was thus inhibited to a large extent in all the men tested. However, we do not know the degree of hepatic glucose inhibition in all subjects, and therefore we describe the results in terms of glucose infusion rate in all cases adjusted for the prevailing insulin concentration during the test.

During the euglycemic hyperinsulinemic clamp test the glucose infusion rate during the last hour of the clamp test was significantly (p<0.01) decreased in all groups of obese men compared with the nonobese group. This was also the case after adjustment for the prevailing insulin concentration (in controls, 9.7±2.9 mg/kg body wt/min/milliniunit/×100; in obese normoinsulinemic men, 6.8±1.7; in obese hyperinsulinemic men, 2.8±0.6; and in obese diabetics, 1.5±0.7) (Figure 1). Expression of the glucose infusion rate per square meter of body surface area did not change the main findings.

The obese hyperinsulinemic group had significantly (p<0.001) increased fasting, peak, and late insulin values during the IVGTT compared with the other groups (Figure 2). The area under the curve for insulin during the IVGTT was more than twice as large (4,412; p<0.001) in the obese hyperinsulinemic group as in the other groups (in controls, 898; in obese normoinsulinemics, 1,933; in obese diabetics, 1,048). The obese diabetic group showed increased fasting plasma insulin values (p<0.001) and significantly lower peak insulin values in response to a glucose load but increased plasma insulin at the end of the IVGTT compared with the normoinsulinemic groups (Figure 2).

**Lipoprotein Lipase Activities and Intravenous Fat Tolerance**

The postheparin plasma LPL and hepatic lipase activities did not differ significantly among the groups. There was a gradual decrease from the control

**FIGURE 2.** Plasma insulin (mU/l; left panel) and glucose (mmol/l; right panel) response curves during an intravenous glucose tolerance test in the four study groups: 1) normal-weight, normoinsulinemic, normoglycemic control group; 2) obese, normoinsulinemic, normoglycemic group; 3) obese, hyperinsulinemic, normoglycemic group; and 4) obese, hyperinsulinemic diabetic group. *p<0.05; **p<0.01; ***p<0.001.
Lipoprotein Lipase Activity in Skeletal Muscle

Skeletal muscle lipoprotein lipase (LPL) activity was negatively correlated with blood glucose concentration (partial $R^2=13.5\%$). However, the diabetic group had not deteriorated more in any of these variables than the obese hyperinsulinemic group.

Postheparin Plasma Hepatic Lipase Activity

Hepatic lipase activity was strongly negatively correlated with LDL TG ($r=0.58$, $p=0.001$) and also with the TG concentration in HDL and HDL$_3$ (Table 5). Hepatic lipase activity was positively correlated with the insulin area under the curve during the IVGTT ($r=0.41$, $p=0.03$).

Postheparin Plasma Lipoprotein Lipase Activity

LPL activity in postheparin plasma was inversely correlated with TG concentration in serum and VLDL and positively with HDL$_3$ cholesterol concentration (Table 5).

In a stepwise regression model, 51% of the variation in postheparin plasma LPL was explained, the largest contributions coming from the glucose infusion rate during the clamp test (partial $R^2=19\%$) and fasting blood glucose concentration (partial $R^2=14\%$).

Lipoprotein Lipase Activity in Adipose Tissue

Adipose tissue LPL activity was not significantly correlated with any lipoprotein lipid variable (Table 5). It was negatively correlated with HbA$_1c$ ($r=-0.32$, $p=0.033$).

Lipoprotein Lipase Activity in Skeletal Muscle

Muscle LPL activity was negatively correlated with TG concentrations in serum and VLDL and positively with LDL cholesterol concentration and intravascular fat tolerance (Table 6).

Muscle LPL activity was positively correlated with the glucose infusion rate during the clamp test (Table 6 and Figure 4) and negatively with insulin concentration (Figure 4), ALAT, GT, and urate. In a stepwise regression model, 42% of the variation in muscle LPL activity was explained by the glucose infusion rate (partial $R^2=28.5\%$) and fasting glucose concentration (partial $R^2=13.5\%$). When these measures were accounted for, the addition of other variables did not contribute significantly to an improvement in the degree of explanation, indicating, for example, that urate concentration, blood pressure, ALAT, and GT (Table 6) were not directly related to muscle LPL activity but to glucose infusion rate (see below). Neither did the addition of serum TG concentration as an independent variable contribute significantly ($p>0.6$) to the model in the presence of glucose infusion rate and fasting insulin concentration.

Fractional Removal Rates

The $k_2$ values of the IVFTT were negatively correlated with the serum TG. The $k_2$ value was positively correlated with glucose infusion rate during the clamp test and skeletal muscle LPL activity (Table 6). A stepwise regression analysis was performed with $k_2$ as the dependent variable and LPL activities as the independent variables. Twenty-six percent of the variation in $k_2$ was explained by skeletal muscle LPL activity. No other lipase activity improved the degree of explanation significantly. However, when glucose infusion rate was added, the degree of explanation increased significantly to 39%.

Serum Triglycerides

Serum TG levels were inversely correlated with postheparin plasma LPL and skeletal muscle LPL activities and with glucose infusion rate during the clamp test (Table 5) and positively with ALAT (Table 6). A stepwise regression analysis was performed, with serum TG as the dependent variable and the different LPL activities, hepatic lipase activity, FFA concentration, and glucose infusion rate during the clamp test as the independent variables. Thirty-five percent of the variation in serum TG was explained by LPL activity in postheparin plasma, LPL activity in muscle tissue, and hepatic lipase activity, but when the effect of these variables was accounted for, neither adipose tissue LPL activity nor FFA concentration contributed significantly to an improvement in the degree of explanation.
TABLE 5. Correlation Coefficients Between Several Variables for the Study Population

<table>
<thead>
<tr>
<th>Variable</th>
<th>PHP LPL activity</th>
<th>PHP HL activity</th>
<th>Muscle LPL activity</th>
<th>AT LPL activity</th>
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<tbody>
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<td>Serum TG r</td>
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<td>p</td>
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<td>0.88</td>
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Correlation coefficients (\(r\)) between some variables for the study population (\(n=28\)). Statistical significance is indicated below each \(r\) value.

PHP, postheparin plasma; LPL, lipoprotein lipase; HL, hepatic lipase; AT, adipose tissue; TG, triglycerides; VLDL, very low density lipoprotein; chol, cholesterol; LDL, low density lipoprotein; HDL, high density lipoprotein.

Free Fatty Acids

Levels of FFAs were significantly inversely correlated with glucose infusion rate and positively with HbA₁c, ALAT, and GT values (Table 6). The mean value in the diabetic group was significantly higher than in the other three groups.

Diastolic Blood Pressure

Diastolic blood pressure was significantly positively correlated with insulin concentration and inversely with glucose infusion rate during the clamp test (Table 6). Fifty-two percent of the interindividual variation of diastolic blood pressure taken while subjects were in the supine position was explained by three factors together: insulin at 90 minutes (partial \(R^2=34\%\)), glucose infusion rate (partial \(R^2=6\%\)), and the k value at the IVGTT (partial \(R^2=12\%\)).

Serum Alanine Aminotransferase and Glutamyltransferase Concentrations

Serum ALAT and GT concentrations were highest in the obese hyperinsulinemic group. The obese diabetic men (ALAT of 0.66±0.08 \(\mu\)mol) and the obese hyperinsulinemic men (ALAT of 0.73±0.21 \(\mu\)mol) had higher values than the other groups (\(p<0.001\)). The ALAT values were strongly inversely related to the glucose infusion rate (Table 6 and Figure 5). The obese hyperinsulinemic group had the highest serum urate concentration (434±155 \(\mu\)mol/l).

Discussion

Hypertension, obesity, and impaired glucose tolerance often appear together in different combinations\(^2\,30\) and also with lipoprotein abnormalities, in particular hypertriglyceridemia in combination with
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<table>
<thead>
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<th>Variable</th>
<th>Muscle LPL activity</th>
<th>k$_2$ (IVFTT)</th>
<th>Serum TG</th>
<th>Plasma FFAs</th>
<th>Serum ALAT</th>
<th>DBP</th>
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Correlation coefficients (r) between some variables for the study population. Statistical significance is indicated below each r value.

LPL, lipoprotein lipase; IVFTT, intravenous fat tolerance test; TG, triglycerides; FFAs, free fatty acids; ALAT, alanine aminotransferase; DBP, diastolic blood pressure; GT, glutamyltransferase; HbA1c, the glycosylated form of the major component of adult hemoglobin fraction A1; BMI, body mass index; WHR, waist to hip ratio.

low HDL cholesterol. It has been recently suggested that hyperinsulinemia is a common denominator for hypertension, obesity, and impaired glucose tolerance. Hyperinsulinemia is the reflection and consequence of an insulin-resistant state except for the diabetic condition, in which insulin secretion does not match the pronounced insulin resistance and "normal" or low insulin concentration may prevail. The insulin-resistant state thus may be regarded as the basis of a metabolic angiopathic syndrome, "syndrome X."

In the present investigation for most of the factors investigated, for example, blood pressure, lipoprotein concentrations, liver enzyme activities, urate concentration, or glucose infusion rate during the clamp test, there was no clear distinction between the nonobese and obese subgroups, between the normoinsulinemic and hyperinsulinemic subgroups, or between the normoglycemic and hyperglycemic subgroups. The only exception was FFA concentration, which was significantly more elevated in the hyperglycemic group than in the other two obese groups. In the diabetic state adipocytes are insulin resistant, and insufficient inhibition of lipolysis in the adipocytes may explain the higher concentration of FFAs. An elevated portal vein concentration of fatty acids is associated with an increased insulin resistance in the liver, which may explain the reduced inhibition of the glycogenolysis that causes hyperglycemia. Other variables were linearly related to measures of insulin resistance and are not particularly associated with any of the group characteristics.

Diastolic blood pressure was inversely correlated with the glucose infusion rate ($r = -0.55, p = 0.003$) and with the fasting insulin concentration ($r = 0.53, p = 0.004$), and as much as 52% of the interindividual variation in diastolic blood pressure could be explained by insulin concentration, glucose infusion rate, and glucose tolerance. Several mechanisms linking insulin resistance to blood pressure regulation have been demonstrated. In a prospective study the plasma insulin concentration was an independent risk factor for development of hypertension in late-middle-aged men during a 10-year period, which is the first
population-based support implicating insulin as a factor contributing to the development of hypertension.

Increased levels of ALAT and urate were found in the groups with the most pronounced insulin resistance in the present study and may be regarded as new characteristics of the insulin-resistance syndrome.

The VLDL secretion rate is related to the prevailing insulin concentration. In the diabetic state the FFA concentration is the most important factor determining the VLDL secretion rate. Both insulin and FFA concentrations are related to insulin resistance. The results in the present study also show that the capacity for removal of circulating TGs is, to a large extent, related to insulin resistance. The removal capacity was tested in a direct way by use of the IVFFT, which has been validated to reflect the catabolism of endogenous TG-rich lipoproteins.

Only muscle but not adipose tissue LPL activity contributed to an explanation of the interindividual variation in fat tolerance (by 26%), and together with the glucose infusion rate during the clamp test, 39% of the variation was explained. In the fasting state about half of the hydrolysis during the IVFFT takes place in skeletal muscle. Our data demonstrating that muscle LPL activity has a key role in intravenous fat tolerance are therefore in agreement with those findings. This suggestion is further supported by the demonstration that 35% of the interindividual variation of serum TG concentration was explained by postheparin plasma LPL, muscle LPL, and hepatic lipase activity together. The variations in both postheparin plasma LPL and muscle LPL activities were to a large degree explained by glucose infusion rate during the clamp test.

Also, other results indicate that the regulation of muscle LPL activity may be related to insulin action. For instance, the decrease in insulin concentrations during physical exercise and the increases during diet experiments were associated with the reverse changes in muscle LPL activity. Use of a euglycemic hyperinsulinemic clamp test significantly decreased muscle LPL activity after 4 hours of moderate hyperinsulinemia. This demonstrates that insulin itself (or the increased glucose uptake in muscle cells during hyperinsulinemia) downregulates muscle LPL activity. This may be interpreted that the regulation of muscle LPL activity is not insulin resistant and therefore, in such a state when hyperinsulinemia prevails, LPL activity is downregulated.

Adipose tissue LPL activity was not significantly different among the four obese and the normal-weight control groups. However, we did not measure cell size in adipose tissue. If that had been done, it may be assumed that the three obese groups would have had larger adipose cell sizes and would have expressed higher LPL activity per 10^6 cells. This is supported by a finding from a previous study in which there were significant correlations between heparin-releasable acetone ether powder LPL activity expressed per cell and different indexes of obesity. This correlation did not persist when acetone ether powder LPL activity...
was expressed per gram. However, it may be questioned that LPL activity should be expressed per 10⁸ cells because we analyzed the heparin-releasable (extracellular) part. Thus, by analogy, muscle LPL activity should have been expressed per 10⁶ muscle fibers because muscle fiber size is also related to obesity. However, we regard the significant correlations between TG concentration in serum and muscle LPL activity expressed per gram as an indication of the importance of muscle LPL and as an indication that this way of expressing the LPL activity is physiologically relevant.

Hepatic lipase activity is known to be regulated by sex steroids. For example, during treatment with estrogens, activity of this enzyme is decreased, but when estrogen is combined with a gestagen with androgenic sex steroids. For example, during treatment with es-
concentration in the fasting state and intravenous fat muscle LPL activity is correlated with the serum TG and HDL cholesterol concentrations and blood

In conclusion, this study has demonstrated that muscle LPL activity is correlated with the serum TG concentration in the fasting state and intravenous fat tolerance, indicating a role for impaired hydrolysis of circulating TGs in the hypertriglyceridermia of obesity and diabetes. Furthermore, muscle LPL activity was related to glucose infusion rate, suggesting that the hyperinsulinemia associated with insulin resistance may have a downregulating effect on muscle LPL activity. The present results demonstrate that several risk factors for coronary heart disease, such as serum TG and HDL cholesterol concentrations and blood pressure, are correlated with insulin resistance, supporting the concept that insulin resistance is an important underlying pathogenic condition.

References

3. Reaven GM, Grenfeld S: Diabetic hypertriglyceridermia: Eviden-
tice for three clinical syndromes. Diabetes 1982;30(suppl 2):66–75
15. Siegler L, Wu TW: Separation of serum high-density lipopro-
tein for cholesterol determination: Ultracentrifugation vs pre-
spiration with sodium phosphotungstate and magnesium chlo-
25. Carlson LA, Rössner S: A methodological study of an intra-

**KEY WORDS** • lipoprotein lipase activity • insulin sensitivity • insulin • lipids • obesity • diabetes
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