Factors Affecting Lipoprotein Lipase in Hypertensive Patients

Teodoro Marotta, Liberato Aldo Ferrara, Lucrezia Di Marino, Marcello Mancini, Giovanni Annuzzi, Ornella Russo, Gennaro D'Orta, Carmela Lucarelli, and Francesco Rossi

Arterial hypertension is frequently associated with serum lipid abnormalities. Lipid metabolism can also be affected by antihypertensive treatment, possibly via an interference with lipoprotein lipase (LPL) activity. The aims of this study were to investigate the metabolic and hemodynamic factors that can interfere with plasma postheparin LPL activity in a sample of 13 patients with mild, uncomplicated arterial hypertension. The effects of vasodilator administration (prazosin and hydralazine) alone or in combination with a β-blocker (propranolol) were also studied. A direct correlation between serum insulin levels and LPL activity was found during placebo treatment. This was confirmed by multiple regression analysis, which also showed a positive correlation of LPL activity with aortic flow velocity and plasma adrenaline (F significance = 0.0007, R^2 = .905). Serum insulin was also directly correlated with cholesterol in high-density lipoproteins (HDLs) and in the HDL_2 subfraction. A significant decrease in LPL activity was observed during the addition of propranolol to vasodilators as compared with vasodilators alone. A positive correlation was found between LPL and adrenaline changes induced by the combined treatment. These data suggest that LPL may play a role in the pathophysiologic connections between insulin action, the adrenergic nervous system (ANS), and lipid metabolism.

EPIDEMIOLOGIC OBSERVATIONS have indicated that the concomitant occurrence of high blood pressure (BP) and abnormalities of glucose and lipid metabolism cannot be explained by a casual association.[1-4] This finding has stimulated pathophysiologic studies designed to shed light on the reciprocal interactions between mechanisms underlying BP regulation, adrenergic nervous system (ANS) activity, and glucose and lipid metabolism; thus, an integrated view in which these elements are simultaneously present has now been accepted. Some models have been proposed: Reaven has named "syndrome X" a cluster of events that starts from insulin resistance and leads to hypertension and serum lipid abnormalities,[5] and Ferrannini has outlined a network where genetic and environmental factors link together insulin, BP, and lipid homeostasis.[6]

A central role in lipid metabolism is played by lipoprotein lipase (LPL), the enzyme that catabolizes the triglyceride-rich lipoproteins, leading to the shift of cholesterol from the atherogenic apolipoprotein (apo) B–containing molecules to the nonatherogenic high-density lipoproteins (HDLs).[7] A link of LPL with the elements of syndrome X has been hypothesized,[8-10] but the reciprocal relationships between these elements and the activity of the enzyme remain to be elucidated.

LPL is physiologically bound to the luminal surface of capillary endothelium.[7] Therefore, another link between this enzyme and the elements of syndrome X may be found in the hemodynamic features of hypertension: vasoconstriction might affect LPL action through a decrease in the vascular surface available for the enzyme, or through a reduced delivery of substrate.[11]

Antihypertensive treatment adds further strength to the connection between hypertension, glucose metabolism impairment, and hyperlipidemia.[1] The influence of antihypertensive drugs—particularly those affecting the ANS—on glucose and lipid metabolism has been widely studied. The favorable and unfavorable changes in serum lipid pattern observed during administration of α_1- and β-adrenolytic agents, respectively, are well known.[12-13] These changes may be attributed either to the hemodynamic effects of the drugs[11] or directly to their interference with adrenergic receptors.

The aims of the present study were (1) to investigate the factors affecting LPL activity in arterial hypertension by studying some markers of glucose and lipid metabolism, of ANS activity, and of the hemodynamic state in hypertensive patients; and (2) to assess the effect of vasodilating agents with or without α_1-adrenolytic action and of β-blockers on the parameters investigated.

SUBJECTS AND METHODS

Thirteen patients with mild arterial hypertension (12 men and one woman with a mean age of 50 ± 6 years), entered the study after providing informed consent. Patients with hypertriglyceridemia (> 170 mg/dL), diabetes mellitus, overweight (> 20% of ideal body weight), or chronic diseases other than hypertension were not included. Other exclusion criteria were as follows: presence of target organ damage (history or ECG signs of myocardial infarction, signs of cardiac failure, history or signs of stroke, signs of peripheral arteriopathy, or clinical or laboratory signs of renal failure), orthostatic hypotension, bradycardia, disturbances of atrioventricular conduction, asthma, alcoholism, intolerance to any drug included in the experimental design, and premenopausal age (for women). Visits were performed in a quiet, warm, silent room of our Outpatient Clinic.

All patients discontinued previous antihypertensive medications and all drugs able to affect the investigated parameters for a 3-week washout period. An isocaloric (average caloric intake in the group, 2,200 kcal) balanced diet (50% carbohydrates, 20% proteins, and 30% fat, with 230 mg cholesterol and 60 g fiber) without salt restriction was prescribed for the duration of the study. After the washout period, height, body weight, BP, and heart rate (HR)

From the Institute of Internal Medicine and Metabolic Diseases, Federico II University, Naples; and the Institute of Pharmacology and Toxicology, 2nd University of Naples, Naples, Italy.

Submitted July 21, 1993; accepted October 28, 1994.

Address reprint requests to Teodoro Marotta, MD, PhD, Istituto di Medicina Interna e Malattie Dismetaboliche, Facoltà di Medicina e Chirurgia, Università degli Studi di Napoli "Federico II," Via Sergio Pansini 5, 80131 Napoli, Italia.

0026-0495/95/4406-0006$03.00/0

were measured. Thereafter, the following three treatments were administered for 4 weeks each in a double-blind, randomized sequence: placebo, 1 tablet twice daily; prazosin, 1 mg twice daily; and slow-release hydralazine, 50 mg twice daily. A test dose of prazosin (0.5 mg) was administered on the first day of the prazosin period before bedtime, to avoid first-dose orthostatic hypotension.

At the midpoint of each treatment period, patients were seen for assessment of compliance and dose titration. When diastolic BP was greater than 90 mm Hg and/or systolic BP was greater than 160 mm Hg, the dose of prazosin was increased to 2 mg twice daily and that of hydralazine to 100 mg in the morning and 50 mg in the evening; placebo was increased to 2 tablets twice daily.

At the end of the third treatment period, propranolol 40 mg twice daily was added for 4 weeks to the last drug administered (placebo for five patients, prazosin for four, and hydralazine for the remaining four). If systolic BP at this point was less than 140 mm Hg and diastolic BP was less than 85 mm Hg, the dose of prazosin administered with propranolol did not exceed 1 mg twice daily, hydralazine 50 mg twice daily, or placebo 1 tablet twice daily. At the midpoint of this last treatment period, patients were seen for assessment of compliance and detection of side effects. No patient required dose titration.

At the end of each study period, the following procedures were performed in the fasting state (14 to 16 hours of fasting): measurement of body weight and calculation of body mass index (BMI) as body weight in kilograms divided by height in meters squared; measurement of BP and HR twice in the supine position and after 2 and 5 minutes of standing, by an automatic device (Nippon Colin, Osaka, Japan); cannulation of a forearm vein and perfusion with saline with the patient in the supine position. After 30 minutes, blood samples were obtained for measurement of serum lipids, serum glucose and insulin, and plasma catecholamines. Thereafter, heparin 50 U/kg body weight was injected. After 10 minutes, a blood sample was drawn from the opposite arm and collected in tubes containing Na_2EDTA as anticoagulant, for measurement of plasma LPL and hepatic lipase (HL) activities; and measurement of blood flow velocity in the ascending aorta by Doppler procedure.

Two to five days after these procedures, while the treatment was ongoing, an intravenous fat tolerance test (IVFTT) was performed in the fasting state as follows: A bolus of a 10% fat emulsion (Intralipid; Kabi Vitrum, Uppsala, Sweden) was injected at a dose of 1 mL/kg body weight. Blood samples were collected in heparinized tubes at 5-minute intervals from the opposite arm, up to 40 minutes. Plasma was separated by centrifugation performed twice at 60 × *g*, and light scattering was measured by nephelometry after a 1:100 dilution with saline. The fractional removal rate (k_2) was calculated by plotting the light-scattering index against time on a semilogarithmic scale.[14]

BP, HR, and blood flow velocity measurements, blood collections, and IVFTT were performed in the morning during a 1- to 3-hour interval from the last dose of active drug or placebo. Doctors and technicians performing biochemical and hemodynamic measurements were blinded as to the ongoing treatment.

Biochemical Measurements

Serum glucose, cholesterol, and triglyceride levels were measured with Boehringer Biochemia (Mannheim, Germany) kits,[15-17] using the COBAS MIRA (Roche, Basel, Switzerland) autoanalyzer. For measurement of cholesterol levels in HDLs, precipitation of low-density lipoproteins and very–low-density lipoproteins was obtained by adding 50 μL 2-mol/L $MgCl_2$ and 50 μL 2% dextran sulfate (molecular mass, 500,000 d) to 1 mL serum.[18] Cholesterol level was also measured in the HDL_3 subfraction isolated after serum ultracentrifugation at 105,000 × *g* and density 1.125 g/mL for 48 hours.[19] Apo A_1 levels were measured by the radial immunodiffusion method[20] in whole serum in the HDL and HDL_3 fractions. Apo B levels were measured in whole serum by the analog method.[21] Lipid and apolipoprotein concentrations in the HDL_2 subfraction were calculated from the difference between HDL and HDL_3 values. HDL to total cholesterol and HDL_2 to total cholesterol ratios were also calculated. Serum insulin level was measured by radioimmunoassay.[22]

LPL and HL activities in postheparin plasma were measured selectively using the method reported by Nilsson-Ehle,[23] with sonicated [^{3}H]-trioleoylglycerol emulsions as substrate. Specific measurement of LPL activity was obtained at pH 8.0 and 0.15 mol/L NaCl in the presence of serum; HL activity was assayed at pH 9.0 and 1 mol/L NaCl using a substrate sonicated in the presence of albumin. Both enzyme activities are expressed as milliunits per milliliter. One milliunit of enzymatic activity represents the release of 1 nmol fatty acid/min at 37°C. The interassay variation coefficient was 9.9% for LPL and 5.9% for HL. Nilsson-Ehle reported variation coefficients of 7.8% and 5.2%, respectively[23]; however, in this study, determinations of enzymatic activities for each patient were performed in the same rack.

Plasma adrenaline and noradrenaline levels were measured by high-performance liquid chromatography.[24]

Hemodynamic Evaluation

Blood flow velocity was measured by a Doppler instrument operating at a frequency of 2 MHz (Vingmed SD100, Horten, Norway).[25] The probe was placed in the suprasternal position and angled until the flow signal in the ascending aorta was obtained by continuous mode. Thereafter, the pulse mode was used to record signals from aortic valve movements. Maximal flow velocity ($\dot{V}max$) was measured immediately above the aortic orifice as the mean of 10 consecutive beats and expressed in centimeters per second. On a spectral display, the signal had to be characterized by a narrow band of frequencies with few low ones. The outline of the velocity-time complex represented the highest flow velocity recorded at any instant. The complexes were approximately triangular, and the apex represented peak aortic velocity in the midstream and midsystole; the base length was the duration of the left ventricular ejection. The area within the velocity-time complex has the dimensions of a distance and was recorded in centimeters as stroke distance. This parameter was an index of stroke volume, which might be calculated as the product of stroke distance times cross-sectional area of the aortic root. However, we did not measure this area and assumed that it was constant in each patient. Stroke distance multiplied by HR gives a minute distance (MD), representing an index of cardiac output. An index of total peripheral resistance was calculated as (mean BP/MD) × 1,000, where mean BP was diastolic BP + (systolic BP − diastolic BP)/3.

Statistical Analysis

Data are expressed as the mean ± SD. Simple Pearson two-tail correlation and multiple regression analyses were performed. Data from each treatment period were compared by one-way ANOVA and Student's *t* test for paired data. The IBM (New York, NY) Statistical Package for Social Sciences was used for calculations.[26]

RESULTS

Placebo

Analysis of data obtained during the placebo treatment showed the expected correlation between lipid parameters. In particular, serum triglyceride was positively correlated with total cholesterol and inversely correlated with both the HDL to total cholesterol and HDL_2 to total cholesterol

ratios. IVFTT k_2 was inversely correlated with serum triglyceride in a double-logarithmic fashion ($r = .550$, $P < .05$) (Table 1).

An expected, a positive correlation was found between HDL_3 cholesterol and HL activity ($r = .754$, $P < .01$). HL was also positively correlated with apo A_1 levels in HDL, more strongly, HDL_3 ($r = .849$, $P < .002$).

Plasma LPL activity exhibited a direct correlation with serum insulin, as shown in Fig 1. A stepwise multiple regression analysis was performed to examine the strength of the main factors expected to affect LPL activity. BMI, insulin, adrenaline, supine diastolic BP, and $\dot{V}$max were included in the analysis (Table 2). Diastolic BP, insulin, $\dot{V}$max, and adrenaline were included in the equation with a positive slope value and accounted for 90.5% of LPL variability, as shown by the adjusted R^2; BMI was out of the final regression equation. The strongest correlation was found between LPL and insulin ($P < .0005$).

Simple correlation analysis showed a direct relationship of insulin with HDL cholesterol ($r = .591$, $P < .05$) (Fig 2) and HDL_2 cholesterol ($r = .648$, $P < .03$).

Drug Treatment

Prazosin, hydralazine, and propranolol were effective in reducing BP as compared with pretreatment levels, as shown in Fig 3. The addition of propranolol significantly reduced BP and HR in comparison to placebo and vasodilators, as shown by ANOVA of pooled data (Fig 3) and by *t* test performed on each treatment subgroup (propranolol + placebo, propranolol + prazosin, and propranolol + hydralazine). No change was observed in body weight.

Metabolic and hemodynamic effects of monotherapy are listed in Tables 3 and 4. No changes in metabolic and hemodynamic parameters were detected by ANOVA during administration of vasodilators in comparison to placebo.

The addition of propranolol to placebo (five patients) induced a non–statistically significant decrease in both LPL activity (54.4 ± 3 to 50.9 ± 7 mU/mL) and IVFTT k_2 (2.26 ± 0.68 to 2.01 ± 0.93%/min). During administration of propranolol + placebo, plasma adrenaline decreased from 67 ± 8 to 51 ± 9 pg/mL ($P < .03$); plasma noradrenaline was 235 ± 76 pg/mL during placebo and 180 ± 38 during addition of the β-blocker (NS). Fasting serum insulin was not affected by propranolol + placebo (10.95 ± 1.3 to 11.83 ± 2.1 μU/mL). The other metabolic

Table 1. Correlations Between Lipid Metabolism Parameters During Placebo Treatment

Variables			
y	x	*r*	*P*
TG	C	+.686	.010
TG	HDL C	−.528	.064
TG	HDL/total C	−.821	.001
TG	HDL_2/total C	−.733	.007
Apo B	C	+.895	.001
Apo B	HDL/total C	−.691	.009
Apo B	TG	+.621	.023
Log TG	Log IVFTT k_2	−.550	.050

Abbreviations: TG, triglyceride; C, cholesterol.

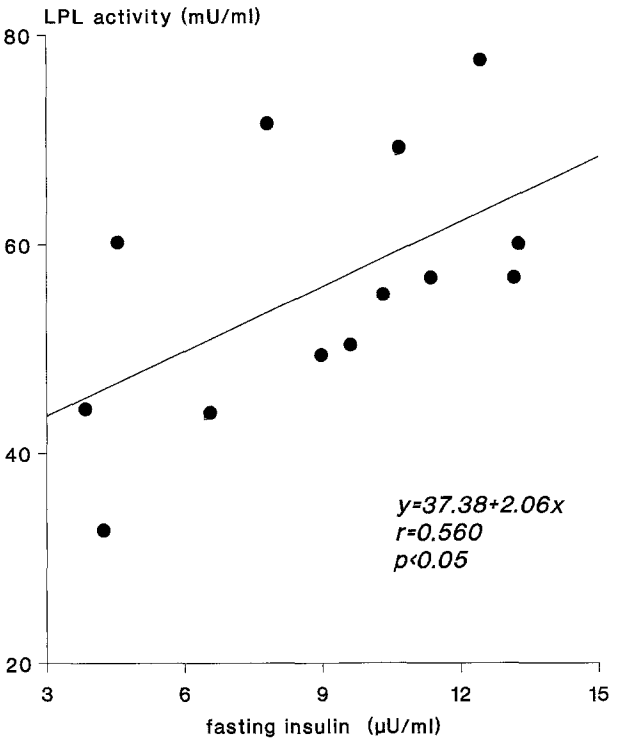

Fig 1. Correlation between fasting serum insulin levels and plasma postheparin LPL activity.

and hemodynamic variables also did not exhibit any change in these patients, apart from a decrease in MD (1,211 ± 371 to 1,047 ± 265 cm/min), which did not reach statistical significance.

A significant decrease in IVFTT k_2 was observed when propranolol was added to prazosin (four patients) or to hydralazine (four patients) (from 2.39 ± 0.40 to 2.11 ± 0.42 and from 2.49 ± 0.68 to 2.19 ± 0.57 %/min, respectively, $P < .05$). LPL activity showed a significant decrease in the propranolol + hydralazine subgroup, as compared with hydralazine alone (64.0 ± 14 to 57.7 ± 13 mU/mL, $P < .03$), whereas the decrease in this parameter did not reach statistical significance in the propranolol + prazosin patients (from 60.6 ± 20 mU/mL during monotherapy to

Table 2. Stepwise Multiple Regression Analysis (dependent variable, LPL) of Data Obtained During Placebo Treatment

Independent Variables	b	SE(b)	T Significance
In the equation			
Insulin	3.397	0.478	.0004
$\dot{V}$max	0.289	0.089	.017
Adrenaline	0.233	0.091	.043
Supine diastolic BP	0.585	0.317	.114
Out of the equation			
BMI			.454
Constant	−79.184	21.629	.011
$r = .971$			
$R^2 = .943$		F significance = 0.0007	
Adjusted $R^2 = .905$			

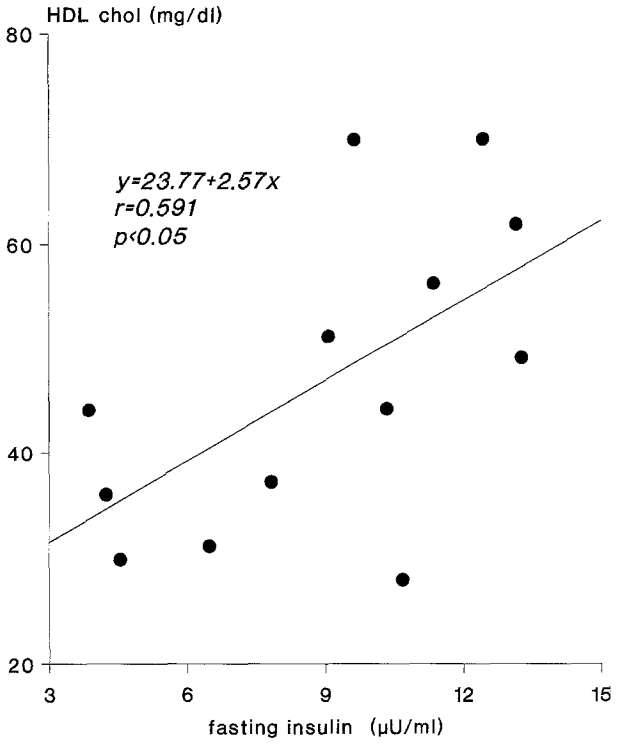

Fig 2. Correlation between fasting serum insulin levels and HDL cholesterol (chol).

55.9 ± 17 during association). Changes in serum insulin, plasma catecholamines, and other metabolic and hemodynamic variables did not reach statistical significance.

We also pooled the data of the eight patients to whom propranolol had been given in addition to vasodilators (prazosin or hydralazine). When these two subgroups were analyzed together, the paired t test showed a significant reduction of LPL activity (62.3 ± 15 to 56.8 ± 14 mU/mL, $P < .005$), IVFTT k_2 (2.44 ± 0.54 to 2.15 ± 0.47 %/min, $P < .001$), and the HDL_2 to total cholesterol ratio (17.5% ± 9.6% to 13.8% ± 8.0%, $P < .02$) with an increase in serum apo B level (106 ± 26 to 117 ± 30 mg/dL,

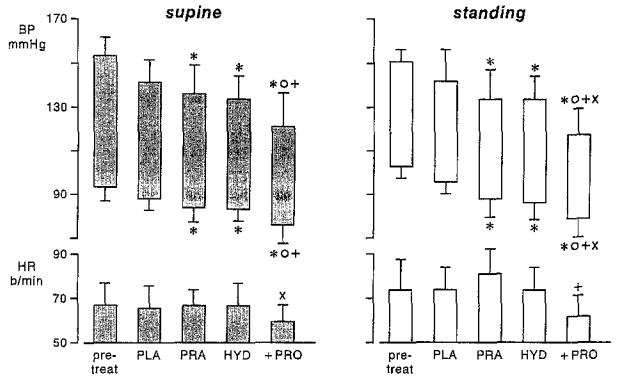

Fig 3. BP and HR in the supine position (■) and standing position (□) before treatment (pre-treat), during placebo (PLA), during monotherapy with prazosin (PRA) or hydralazine (HYD), and during addition of propranolol (+PRO). *$P < .05$ v pretreatment; °$P < .05$ v placebo; †$P < .05$ v prazosin; ˣ$P < .05$ v hydralazine.

Table 3. Age, BMI, BW, and Metabolic and Hemodynamic Parameters in the Whole Group (13 patients) During Placebo and Treatment With Vasodilators (mean ± SD)

Variable	Placebo	Prazosin	Hydralazine
Age (yr)	50 ± 6		
BMI (kg/m²)	26.6 ± 2.1		
BW (kg)	76.3 ± 7.4	76.7 ± 6.8	76.5 ± 6.4
Insulin (μU/mL)	8.95 ± 3.3	9.57 ± 4.3	8.72 ± 1.9
Glucose (mg/dL)	85 ± 9	90 ± 10	85 ± 9
ADR (pg/mL)	71 ± 15	75 ± 14	69 ± 17
NADR (pg/mL)	267 ± 82	274 ± 70	261 ± 60
Vmax (cm/s)	127 ± 20	128 ± 26	128 ± 24
SD (cm)	18.9 ± 4	19.2 ± 5	18.3 ± 5
MD (cm/min)	1,189 ± 277	1,195 ± 266	1,211 ± 389
PRI $\left(\frac{\text{mm Hg} \cdot \text{min}}{\text{cm} \times 1{,}000}\right)$	97.2 ± 17	92.5 ± 20	92.6 ± 31

Abbreviations: BW, body weight; ADR, plasma adrenaline; NADR, plasma noradrenaline; SD, stroke distance; PRI, total peripheral resistance index.

$P < .05$) during addition of the β-blocker as compared with monotherapy. Serum insulin (7.46 ± 1.8 to 8.75 ± 4.1 μU/mL) and plasma catecholamines (adrenaline, 71 ± 14 to 58 ± 10 pg/mL; noradrenaline, 237 ± 54 to 200 ± 41 pg/mL) showed no significant trends to increase or decrease, respectively, during the combination treatment. A trend to decrease was also observed in MD (1,151 ± 273 to 1,089 ± 143 cm/min). Plasma LPL activity and plasma adrenaline changes during the addition of propranolol to vasodilators were positively correlated ($r = .902$, $P < .01$).

DISCUSSION

In this sample of mildly hypertensive subjects without signs of impairment in glucose and lipid metabolism, the expected reciprocal correlations between lipid parameters were observed (Table 1). In particular, serum triglyceride levels were inversely correlated with HDL to total cholesterol and HDL_2 to total cholesterol ratios, despite the low-normal range of triglyceridemia in these subjects (80 ± 30 mg/dL). This was expected, since LPL hydrolyzes the triglycerides of apo B–containing lipoproteins, leading

Table 4. Lipid Metabolism Parameters in the Whole Group (13 patients) During Placebo and Treatment With Vasodilators (mean ± SD)

Variable	Placebo	Prazosin	Hydralazine
C (mg/dL)	186 ± 47	180 ± 47	178 ± 40
TG (mg/dL)	80 ± 30	72 ± 29	74 ± 27
HDL-C (mg/dL)	47 ± 15	47 ± 12	49 ± 14
HDL/total C (%)	26.6 ± 9.9	28.7 ± 10.8	28.9 ± 9.9
HDL_2-C (mg/dL)	23.4 ± 13.5	26.1 ± 14.7	29.1 ± 12.5
HDL_2/total C (%)	13.4 ± 7.6	15.5 ± 9.8	17.2 ± 8.4
Apo A_1 (mg/dL)	140 ± 23	135 ± 19	142 ± 20
HDL apo A_1 (mg/dL)	139 ± 22	132 ± 17	137 ± 18
HDL_2 apo A_1 (mg/dL)	50 ± 17	47 ± 13	53 ± 12
Apo B (mg/dL)	115 ± 36	109 ± 28	107 ± 26
LPL (mU/mL)	55.9 ± 13	56.5 ± 14	53.6 ± 13
HL (mU/mL)	265.5 ± 129	252.0 ± 128	250.9 ± 119
IVFTT k_2 (%/min)	2.28 ± 0.63	2.52 ± 0.89	2.26 ± 0.95

Abbreviations: C, serum cholesterol; TG, serum triglyceride.

as a final event to an increase in HDL cholesterol levels and to the shift from HDL_3 to HDL_2 subfractions.[7,27-28] The inverse correlation previously observed between log triglyceride and log IVFTT k_2, which is an expression of the catabolism of triglyceride-rich lipoproteins,[14,29] was also confirmed.

The first aim of this study was to evaluate the independent contribution of metabolic and hemodynamic factors presumably affecting LPL activity. Therefore, a stepwise multiple regression analysis was performed. In the final equation, supine diastolic BP, insulin, Vmax, and adrenaline were independently and directly correlated with LPL activity, explaining most of the variability (Table 2). The highest T value was reached by insulin (T significance < .0005). This finding confirms the result of the simple correlation analysis (Fig 1) and is in agreement with the positive correlation observed between serum insulin and products of the metabolic cascade induced by LPL (HDL and HDL_2 cholesterol) (Fig 2).

A stimulating effect of insulin on LPL synthesis has been demonstrated in rat adipose tissue.[30,31] Accordingly, insulin infusion performed in humans under euglycemic conditions (euglycemic clamp) induced a threefold increase in LPL activity in adipose tissue, whereas the simultaneous reduction in skeletal muscle was only an average of 14%.[32] Our present finding of a direct, strong, independent correlation between serum insulin and plasma LPL activity suggests that also in fasting conditions insulin level plays a role in LPL regulation.

Some investigators[33-36] have reported an association of low HDL cholesterol and high serum insulin levels. However, the present finding of a direct correlation between fasting insulin levels and LPL activity and HDL and HDL_2 cholesterol is not comparable to previous observations, since none of them were limited to normoinsulinemic subjects. As a matter of fact, these studies either compared low insulin groups with high insulin groups[33,35] or included in their analysis subjects with a wide range of serum insulin levels.[34,36] It is known that high insulin levels are an expression of insulin resistance,[37] which leads to increased release of free fatty acids from adipose tissue[5] and increased hepatic synthesis of very-low-density lipoproteins.[38] This could enhance the delivery of triglyceride for the lipid exchange between apo B– and apo A_1–containing lipoproteins catalyzed by the cholesteryl ester transport protein[27,39]: the final result is a decrease in HDL cholesterol.

Our finding of a positive correlation between fasting serum insulin levels and cholesterol in HDL and HDL_2 is in keeping with the observation that a redistribution of particles from HDL_3 to HDL_2 subfractions—due to increased LPL activity—takes place during insulin therapy in diabetic patients.[40] However, the correlation between LPL activity and cholesterol in HDL and HDL_2 did not reach statistical significance in our sample, possibly because the distribution of serum lipids was not normal due to the exclusion of hypertriglyceridemic patients.

The direct, independent relationship between LPL activity and Vmax in the aortic root, which is an expression of blood flow, is particularly notable, since LPL is located at the luminal surface of vascular endothelium. This correlation supports the view that the hemodynamic state is important in the regulation of the amount of LPL available for its physiologic function and not only for the delivery of substrate to it. It is not possible to formulate a definite conclusion for diastolic BP, since this variable, although included in the final regression equation, did not reach a statistically significant correlation with LPL ($P = .114$).

Plasma adrenaline was tested in the multiple regression analysis as an expression of ANS activity. Adrenaline induces the intracellular inactivation of LPL in rat adipose tissue, while increasing LPL activity in skeletal muscle and heart.[7,30,31] The ANS plays a definite role in arterial hypertension,[41,42] and its activity is stimulated by euglycemic insulin infusion in humans.[43] The independent relationship of plasma adrenaline and LPL activity in our sample confirms the role of the ANS in the constellation of factors regulating LPL activity in fasting conditions.

BMI, although directly correlated with LPL activity and insulin in the simple correlation analysis, was out of the final equation of multiple regression analysis ($P = .454$).

Our second aim was to assess the effect of antihypertensive medications on the investigated parameters, keeping in mind the relevant contribution of drug treatment to the interaction between high BP and lipid abnormalities. Vasodilator agents are of theoretic interest, since their hemodynamic action might interfere with LPL.[11] We have therefore tested two vasodilators, one providing selective α_1-adrenolytic activity (prazosin) and the other devoid of it (hydralazine). In a recent multicenter study,[44] we observed an increase in HDL cholesterol and the HDL to total cholesterol ratio in a large group (111 subjects) of hypercholesterolemic hypertensive patients during administration of doxazosin, an α_1-adrenolytic agent similar to prazosin. However, in the present study, no change in serum lipids was detected by ANOVA during the vasodilating treatment. It is possible that the normal lipid pattern of our sample did not allow any effect of drugs.

Prazosin and hydralazine were able to reduce BP to less than 140/90 mm Hg in the supine and standing position, with a significant decrease as compared with pretreatment levels. However, the difference as compared with placebo did not reach statistical significance. It is probable that the mild degree of hypertension, together with the quiet and comfortable conditions in which the visits were made, may have yielded a partial (although not statistically significant) hypotensive effect for the placebo, as already observed in relevant epidemiologic surveys.[1,45] The greater reduction in BP observed during the combination treatment with propranolol seemed to parallel a decrease in MD, which is an expression of cardiac output ($r = .836$, $P < .04$ for orthostatic diastolic BP in patients treated with vasodilators + propranolol).

The effects of β-blockers on serum lipids have been widely studied.[12,46] They are often used in association with vasodilators to obtain a reciprocal blunting of their side

effects. We added propranolol to the last treatment in the randomized sequence. A significant decrease in BP was detected by ANOVA during the addition of propranolol to any treatment, in comparison to placebo and vasodilators alone. Administration of propranolol induced a significant decrease in plasma adrenaline in comparison to placebo; this finding is in keeping with previous observations.[47] A trend for a decrease of adrenaline was also observed after addition of propranolol to vasodilators.

Paired *t* test versus monotherapy with vasodilators showed the unfavorable effect of addition of a β-blocker on lipid metabolism (decrease in LPL activity and IVFTT k_2, with the ensuing reduction in the HDL_2 to total cholesterol ratio and an increase in apo B). The decrease in IVFTT k_2 confirms previous observations.[48,49] This change, as well as the parallel decrease in LPL activity, was also observed during propranolol plus placebo. However, statistical significance was not reached in the latter condition, probably due to the smaller size of this subsample.

The effect of propranolol on lipid metabolism might be due to various mechanisms.[12,49] It is of particular interest that the change in plasma LPL activity during the association of propranolol and vasodilators (as compared with monotherapy) and the change in plasma adrenaline between the same conditions were positively correlated in our patients. Since plasma adrenaline and LPL activity were independently correlated during placebo (Table 2), it is possible that interference with the adrenergic drive contributed to the β-blockade–induced LPL decrease. Moreover, the correlation between changes in plasma adrenaline and LPL activity during combination treatment suggests that plasma adrenaline concentrations have a relevant role in determining the level of LPL activity in basal conditions.

In conclusion, our data indicate that LPL represents a key point in the pathophysiologic connection between insulin action, ANS activity, and lipid metabolism in the mildly hypertensive, minimally overweight, normolipidemic, normoinsulinemic patient. A role for insulin in the setting of fasting plasma LPL level is proposed: the activity of the enzyme and the products of its action, particularly HDL_2 cholesterol, seem to be a direct function of serum insulin levels.

A statistical correlation does not necessarily indicate a cause-and-effect relationship; however, our findings of an independent, highly significant, direct correlation between serum insulin and plasma LPL activity on one hand and between LPL and adrenaline changes during β-blocker + vasodilator on the other hand should stimulate investigations aimed at confirming the hypothesis that both insulin and adrenaline are significant determinants of fasting LPL activity. It is possible that our recruitment criteria, which excluded patients with diabetes, hyperlipidemia, target-organ damage, and frank overweight, selected a subset of hypertensives in whom the insulin regulation of LPL is not disturbed.

ACKNOWLEDGMENT

Professors Mario Mancini, Gabriele Riccardi, and Paolo Rubba, Dr Paolo Pauciullo, and Dr Angela Rivellese are gratefully acknowledged for their useful contribution in revising the manuscript. Dr Lidia Patti has provided important assistance in implementing the ultracentrifuge technique. Her generous collaboration is gratefully acknowledged. The authors thank Rosanna Scala for her kind, useful help in revising the English form of the manuscript to English, and Pasquale Di Giuseppe for the figures.

REFERENCES

1. Reader R, Bauer GE, Doyle AE, et al: The Australian Therapeutic Trial in Mild Hypertension. Lancet 1:1261-1270, 1980

2. Assmann G: The Prospective Cardiovascular Münster (PROCAM) Study: Prevalence of hyperlipidemia in persons with hypertension and/or diabetes mellitus and the relationship to coronary heart disease. Am Heart J 116:1713-1724, 1988

3. Stamler J, Westworth D, Neaton JD, et al: Factor Intervention Trial Research Group: Is the relation between serum cholesterol and risk of premature death from coronary heart disease continuous and graded? Findings in 356222 primary screenes of the Multiple Risk Factor Intervention Trial (MRFIT). JAMA 256:2823-2828, 1986

4. Laurenzi M, Mancini M, Menotti A, et al: Multiple risk factors in hypertension: Results from the Gubbio study. J Hypertens 8:S7-S12, 1990 (suppl 1)

5. Reaven GM: Role of insulin resistance in human disease. Diabetes 37:1595-1607, 1988

6. Ferrannini E: Metabolic abnormalities of hypertension. A lesson in complexity. Hypertension 18:636-639, 1991

7. Borensztajn J (ed): Lipoprotein Lipase. Chicago, IL, Evener, 1987

8. Foster DW: Insulin resistance—A secret killer? N Engl J Med 320:733-734, 1989

9. Slater EE: Insulin resistance and hypertension. Hypertension 18:I108-I114, 1991 (suppl)

10. Pollare T, Vessby B, Lithell H: Lipoprotein lipase activity in skeletal muscle is related to insulin sensitivity. Arterioscler Thromb 11:1192-1203, 1991

11. Sacks FM, Dzau VJ: Adrenergic effects on plasma lipoprotein metabolism: Speculation on mechanism of action. Am J Med 80:71-81, 1986 (suppl 2A)

12. Weidmann P, Uehlinger DE, Gerber A: Antihypertensive treatment and serum lipoproteins. J Hypertens 3:297-306, 1985

13. Thaulow E, Nitter-Hauge S: Antihypertensive therapy and blood lipids: Alpha blockers. Scand J Clin Lab Invest [Suppl 99] 50:45-48, 1990

14. Rössner S: Studies on an intravenous fat tolerance test. Methodological, experimental and clinical experience with Intralipid. Acta Med Scand [Suppl 564] 1-24, 1974

15. Hyelm M: Enzymatic determination of hexoses in blood and urine. Scand J Invest [Suppl 192] 18:85-98, 1966

16. Siedel J, Schlumberger H, Klose S, et al: Improved reagent for enzymatic determination of serum cholesterol. J Clin Chem Clin Biochem 19:838-839, 1981

17. Wahlefeld AW: Triglycerides determination after enzymatic hydrolysis, in Bergemeyer HU (ed): Methods of Enzymatic Analysis, vol 4 (ed 2). New York, NY, Academic, 1974, pp 1831-1974

18. Kostner GM: Enzymatic determination of cholesterol in high density lipoprotein fractions prepared by polyanion precipitation. Clin Chem 22:695-698, 1976

19. De Lalla O, Gofman JW: Ultracentrifugal analysis for plasma lipoproteins, in Glick P (ed): Methods of Biochemical Analysis, vol 1. New York, NY, Interscience, 1954, pp 459-478

20. Marcovina S, Di Cola G, Rapetto G, et al: Development of a radial immunodiffusion technique employing monoclonal antibodies for apoprotein B determination in human plasma. Clin Chim Acta 147:117-125, 1985

21. Marcovina S, Di Cola G, Catapano AL: Radial immunodiffusion assay of human apoprotein A1 with use of two monoclonal antibodies combined. Clin Chem 32:2155-2159, 1986

22. Debuquois B, Aurbach GD: Use of polyethylene glycol to separate free and antibody bound peptide hormones in radioimmunoassay. J Clin Endocrinol Metab 33:732-738, 1971

23. Nilsson-Ehle P: Measurements of lipoprotein lipase activity, in Borensztajn J (ed): Lipoprotein Lipase. Chicago, IL, Evener, 1987, pp 59-77

24. Foti A, Kimura S, De Quattro V, et al: Liquid-chromatographic measurement of catecholamines in plasma and urine. Clin Chem 33:2209-2213, 1987

25. Coats AJS: Doppler ultrasonic measurement of cardiac output: Reproducibility and validation. Eur Heart J 11:49-66, 1990 (suppl 1)

26. Nie NH, Hull CH, Jenkins JG, et al: Statistical Package for Social Sciences (ed 2). New York, NY, McGraw-Hill, 1975

27. Patsch JR, Prasad S, Gotto AM Jr, et al: High density lipoprotein$_2$. Relationship of the plasma levels of this lipoprotein species to its composition, to the magnitude of postprandial lipemia, and to the activities of lipoprotein lipase and hepatic lipase. J Clin Invest 80:341-347, 1987

28. Levy E, Deckelbaum RJ, Thibault RL, et al: In vitro remodelling of plasma lipoproteins in whole plasma by lipoprotein lipase in primary and secondary hypertriglyceridaemia. Eur J Clin Invest 20:422-431, 1990

29. Marotta T, Ferrara LA, Pasanisi F, et al: Enhancement of exogenous triglyceride removal following calcium channel blockade. Artery 16:312-326, 1989

30. Eckel RH: Lipoprotein lipase. A multifunctional enzyme relevant to common metabolic diseases. N Engl J Med 320:1060-1068, 1989

31. Ashby P, Bennett DP, Spencer IM, et al: Post-translational regulation of lipoprotein lipase activity in adipose tissue. Biochem J 176:865-872, 1978

32. Farese RV, Yost TJ, Eckel RH: Tissue-specific regulation of lipoprotein lipase activity by insulin/glucose in normal-weight humans. Metabolism 40:214-216, 1991

33. Zavaroni I, Bonora E, Pagliara M, et al: Risk factors for coronary artery disease in healthy persons with hyperinsulinemia and normal glucose tolerance. N Engl J Med 320:702-706, 1989

34. Zavaroni I, Dall'Aglio E, Alpi O, et al: Evidence for an independent relationship between plasma insulin and concentration of high density lipoprotein cholesterol and triglyceride. Atherosclerosis 55:259-266, 1985

35. Stalder M, Pometta D, Suenram A: Relationship between plasma insulin levels and high density lipoprotein cholesterol levels in healthy men. Diabetologia 21:544-548, 1981

36. Capaldo B, Rivellese A, Santoro D, et al: Relationship between insulin response to intravenous glucose and plasma lipoproteins in healthy men. Artery 13:108-126, 1985

37. Laakso M: How good a marker is insulin level for insulin resistance? Am J Epidemiol 137:959-965, 1993

38. Frayn KN: Insulin resistance and lipid metabolism. Curr Opin Lipidol 4:197-204, 1993

39. Miesenböck G, Patsch JR: Postprandial hyperlipidemia: The search for the atherogenic lipoprotein. Curr Opin Lipidol 3:196-201, 1992

40. Taskinen M-R, Kuusi T, Helve E, et al: Insulin therapy induces antiatherogenic changes of serum lipoproteins in noninsulin-dependent diabetes. Arteriosclerosis 8:168-177, 1988

41. Tuck ML: Obesity, the sympathetic nervous system, and essential hypertension. Hypertension 19:I67-I77, 1992 (suppl)

42. Mathias CJ: Role of sympathetic efferent nerves in blood pressure regulation and in hypertension. Hypertension 18:22-30, 1991 (suppl 3)

43. Rowe JW, Young JB, Minaker KL, et al: Effect of insulin and glucose infusions on sympathetic nervous system activity in normal man. Diabetes 30:219-225, 1981

44. Ferrara LA, Di Marino L, Russo O, et al: Doxazosin and captopril in mildly hypercholesterolemic hypertensive patients. The Doxazosin-Captopril in Hypercholesterolemic Hypertensives Study. Hypertension 21:97-104, 1993

45. Medical Research Council Working Party: MRC trial of treatment of mild hypertension: Principal results. Br Med J 291:97-104, 1985

46. Mancini M, Ferrara LA, Strazzullo P, et al: Metabolic disturbances and antihypertensive therapy. J Hypertens 9:S47-S50, 1991 (suppl 3)

47. Maling TJB, Ferrara LA, Mucklow JC, et al: Blood pressure and plasma noradrenaline during single high-dose beta adrenoceptor blockade. Eur J Clin Pharmacol 15:375-379, 1979

48. Day JL, Metcalfe J, Simpson CN: Adrenergic mechanism in control of plasma lipid concentrations. Br Med J 284:1145-1148, 1982

49. Murphy MB, Sugrue D, Trayner I, et al: Effects of short term beta adrenoreceptor blockade on serum lipids and lipoproteins in patients with hypertension or coronary artery disease. Br Heart J 51:589-594, 1984