Increased High-Density Lipoprotein-3 Binding to Leukocytes Following Weight Loss and Improved Glycemic Control in Type 2 Diabetic Patients

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This study evaluates the effect on high-density lipoprotein (HDL) binding activity in cultured granulocytes before and after metabolic control of non–insulin-dependent diabetes mellitus ([NIDDM] type 2 diabetes) patients. In 20 type 2 diabetic patients, diabetic control was accomplished by administration of oral antidiabetic agents and dietary restrictions. Adequate metabolic control was reflected by a decrease in the fasting glucose, glycosylated hemoglobin (HbA_{1c}), mean insulin, and body mass index (BMI). After control of the diabetes, the mean HDL_3 cholesterol was increased from 0.918 ± 0.05 to 1.008 ± 0.05 mmol/L ($P < .05$) and apolipoprotein AI (apo AI) was increased from 103 ± 5.8 to 115 ± 5.1 mg/dL ($P < .01$). The HDL_3 maximum specific binding was higher after versus before diabetic control, 77 ± 6 versus 122 ± 8 ng/mg cell protein ($P < .01$). This increase was related to an increase in maximum binding ([B_{max}] from 4.97×10^{-10} to 8.3×10^{-10} mol/L, $P < .001$), and no significant changes were observed in the K_d (from 1.47×10^{-7} v 2.04×10^{-7} mol/L). These results suggest that the metabolic control of type 2 diabetes increases HDL_3 binding activity.

PATIENTS with type 2 diabetes mellitus have a marked increase in the risk to develop atherosclerosis, and coronary heart disease (CHD) is the leading cause of mortality in this population.[1] These patients frequently have increased serum levels of very–low-density lipoprotein (VLDL) and a decrease in both high-density lipoprotein (HDL) cholesterol and apolipoprotein AI (apo AI).[2] These abnormalities in lipid metabolism may play a role in the accelerated atherosclerosis associated with diabetes.[3] In addition, most non–insulin-dependent diabetes mellitus ([NIDDM] type 2 diabetes) patients present with resistance to the peripheral insulin glucose-lowering effect, which results in higher plasma insulin levels.[4] Hyperinsulinemia is also considered a risk factor for the development of CHD in otherwise healthy subjects,[5,6] patients with impaired glucose tolerance, and type 2 diabetics.[7,8]

The mechanism(s) by which hyperinsulinemia may favor atherosclerosis is not clear. However, insulin appears to be an important predictor of the HDL cholesterol level, and in vitro insulin decreases the binding of HDL_3 to its receptor.[9] Since HDL binding to its cell receptor is essential for the removal of excess cholesterol from extrahepatic cells,[10,11] an alteration in HDL binding may be partly responsible for the increase in atherogenesis observed in diabetic patients. Taking these facts into consideration, the present study was designed to evaluate the effect of metabolic control in type 2 diabetic patients on HDL_3 binding activity.

SUBJECTS AND METHODS

Patients

This study included 20 consecutive patients newly diagnosed with NIDDM (type 2 diabetes) defined according to World Health Organization criteria,[12] with a glycosylated hemoglobin (HbA_{1c}) level greater than 7.5% and fasting C-peptide exceeding 0.33 nmol/L. The patients (8 men and 12 women) had a mean age of 54 years (range, 34 to 64), and their body weight was 77 ± 2.3 kg and body mass index (BMI) 30.3 ± 0.7 kg/m^2. All subjects were screened by clinical history, physical examination, and routine chemical analyses. Exclusion criteria were as follows: a history of ketonuria, alcohol consumption more than 10 g/d, cigarette smoking, uncorrected thyroid disorder, liver or kidney disease (aspartate aminotransferase and alanine aminotransferase >120% of upper-normal limit, alkaline phosphatase and bilirubin >150% of upper-normal limit, and serum creatinine >175 μmol/L), and use of diuretics, steroids, β-blockers and lipid-lowering medication.

Study Design

Patients were evaluated before and 3 months after establishing strict metabolic control of their diabetes. Basal caloric requirements were calculated using the Harris-Benedict formula, and all patients were instructed to consume a diet containing 27% of calories as fat and a caloric reduction of about 600 ± 150 kcal/d. All patients were started on glibenclamide at a dose of 2.5 mg/d. Diabetic treatment included regular weekly visits to a diabetes nurse educator to receive information about diet and home blood-glucose monitoring. At each visit, plasma glucose, blood pressure, and weight were determined and the dose of glibenclamide was adjusted accordingly. The aim of treatment was to maintain fasting plasma glucose less than 6.5 mmol/L and premeal glucose less than 7.5 mmol/L. The maximum amount of glibenclamide prescribed was 15 mg/d. Venous blood samples were obtained from patients after a 12-hour overnight fast before and after 3 months of treatment. Blood was collected in tubes containing EDTA (1 mg/mL), followed by centrifugation to separate the granulocytes used for HDL_3 binding assay and serum samples used for biochemical determinations.

Biochemical Determinations

The plasma glucose level was measured by the glucose oxidase method (GOD-PAP; Boehringer, Mannheim, Germany). Automated methods were used for the measurement of cholesterol and triglycerides (CHOD-PAP and GPO-PAP, respectively; Boehringer). Plasma HDL and the subfractions of HDL_2 and HDL_3 cholesterol were determined by a dextran sulfate–magnesium precipitation procedure that corresponds closely to separation by ultracentrifugation.[13] Apo AI and B levels were determined by an immunoturbidimetric method supplied in a commercial kit (Boehringer) adapted for analysis in the BM/Hitachi 911 analyzer.[14] Total glycosylated hemoglobin was determined in fresh

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samples by high-performance liquid chromatography using a Hi-Auto A_1C HA8121 (normal range, 3.4% to 5.8%; interassay coefficient of variation [CV], 7.34% ± 0.34%; CV, 4.6%; Menarini, Kyoto, Japan). Insulin concentrations in plasma were determined by radioimmunoassay ([RIA] 3 to 270 UI/mL; intraassay coefficient of variation, 4.2%; bioMérieux, Marcy, France). Plasma C-peptide content was also measured by RIA (lower limit, 0.003 ng/mL; BYK Sangtec, Dietzenbach, Germany).

Lipoprotein Isolation and Subfractionation

Triglycerides, phospholipids, and cholesterol in the different lipoprotein classes were determined in serum samples drawn after a 12-hour overnight fast. Lipoprotein samples were prepared on the same day or within 72 hours of collection. The VLDL subfraction was separated by centrifugation of the serum samples at density 1.006 (105,000× *g* for 18 hours). This was followed by separation of intermediate-density lipoprotein ([IDL] d = 1.006 to 1.019), low-density lipoprotein ([LDL] d = 1.019 to 1.063), and HDL (d = 1.063 to 1.210) by single ultracentrifugation.[15]

HDL Receptor Assay

Cells. Before and after 3 months of intervention, leukocytes were isolated from a 30-mL blood sample using a Ficoll-Hypaque technique to separate granulocyte and mononuclear cell populations.[16] The granulocyte pellet was resuspended in 8.3 g/L NH_4Cl and 10 mmol/L HEPES buffer (pH 7.4) and incubated at room temperature to remove residual erythrocytes by hypotonic lysis. The number of granulocytes obtained was recorded and subsequently diluted in phosphate-buffered saline (PBS) with 5 g bovine serum albumin (BSA) per liter to obtain a final concentration of 1×10^6 cells/mL per dish. Cell protein was determined by a Bio-Rad Protein Assay (Bio-Rad Laboratories, München, Germany). The mean cellular protein content was 0.31 ± 0.04 mg/dish, and the amount of protein was constant in incubated cells isolated before and after treatment.

Lipoproteins. Lipoproteins for HDL_3 binding assay were isolated from the pooled plasma of normolipidemic subjects by sequential ultracentrifugation. The lipoproteins were separated according to density as follows: LDL (d = 1.019 to 1.063), HDL (d = 1.063 to 1.21), and subfraction HDL_3 (d = 1.125 to 1.21). Densities were adjusted by adding solid KBr followed by centrifugation at 50,000 rpm for 24 hours at 4°C in a Beckman 50 Ti rotor (Beckman Instruments, Palo Alto, CA). HDL_3 lipoproteins were centrifuged at 1,210 g/mL at least twice; the isolated fractions of HDL_3 were dialyzed against 0.15 mol/L NaCl and 5 mmol/L NaEDTA, pH 7.4, at 4°C and directly iodinated using Iodo-Gen reagent (Pierce Chemical, Rockford, IL).[17] Briefly, the iodination reaction contained 100 µg Iodo-Gen, 500-µL aliquots of HDL_3 (5 mg/mL HDL protein in PBS, pH 7.4), and 0.5 mCi carrier-free ^{125}I-Na (Amersham International, Buckinghamshire, UK; 2 mCi/20 µL) and was incubated for 10 minutes at room temperature. The unbound iodine was removed by chromatography on a Sephadex G-25 column equilibrated with PBS buffer. Labeled HDL_3 particles were extensively dialyzed against PBS (0.15 mol/L NaCl, 9 mmol/L Na_2HPO_4, and 1.6 mmol/L KH_2PO_4). As determined by trichloroacetic acid, the precipitable activity of ^{125}I associated with the protein fraction was greater than 98%, and less than 5% of the radiolabel was extractable by ethanol/ether treatment. The specific radioactivity usually obtained was 100 to 300 cpm/ng protein, as measured in a γ-radiation counter. As determined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, this method showed that 86% of ^{125}I-HDL_3 radioactivity was associated with apo AI (64%) and apo AII (22%). The apo E content determined by enzyme-linked immunosorbent assay and expressed as a percentage of total protein was 4.88%. Apo B was not detected. The within-assay coefficient of variation was 5.6% for apo AI, 4.3% for apo AII, and 6.7% for apo E. The coefficient of variation for sample to sample was 8.4% for apo AI, 5.2% for apo AII, and 6.3% for apo E. Finally, each "pool" of ^{125}I-HDL_3 lipoprotein obtained was stored at 4°C and used in binding assays within 2 weeks at most.

Binding experiments of ^{125}I-HDL_3 to cells. The freshly isolated granulocytes were washed with a PBS-albumin mixture (0.2 g/L KCl, 0.2 g/L KH_2PO_4, 8.0 g/L $Na_2HPO_4 \cdot 7H_2O$, and 2.0 g/L BSA, pH 7.4). The cells were used in suspension with 1×10^6 cells/dish, chilled on ice, and incubated at 4°C with cold PBS-BSA (albumin 2 mg/mL, pH 7.4) and increasing amounts of ^{125}I-HDL_3. After an incubation period of 2 hours, the cells were washed 5 times with cold PBS without BSA. The washed pellet was then digested using 0.1 mol/L NaOH. An aliquot was tested for ^{125}I radioactivity, and the rest was used for protein determinations.

The affinity constants for specific binding of HDL_3 to the high-affinity cell-surface binding sites were determined from data obtained in binding studies. The amount of specific receptor-bound lipoprotein was determined at each lipoprotein concentration by subtracting the quantity of non–specifically bound lipoprotein from the total cell-bound lipoprotein. Nonspecific binding was determined in the presence of a 20-fold excess of unlabeled HDL_3 at levels that saturate the high-affinity receptors. The equilibrium dissociation constant (K_d) was determined by plotting the ratio of receptor-bound and free lipoprotein against receptor-bound lipoprotein according to the Scatchard method. When the bound to free ratio is plotted (Scatchard plot) against the bound lipoprotein for a single class of noninteractive sites, a straight line with a slope equal to $-1/K_d$ is obtained and the intercept on the abcissa is the total receptor concentration or maximum lipoprotein bound (B_{max}).[18]

To calculate the data for radioligand binding, a computer program was used which is able to calculate the elapsed time (in days) between the date of manufacture and the use of the isotope and then corrects the specific activity of the ligand using the respective half-life.[19]

To calculate the precision (intraassay variance) of the HDL_3 binding assays, we performed replicate experiments in granulocytes from 5 normal healthy subjects in a single assay. HDL_3 binding was performed at 4°C and 2-hour incubation using medium containing increasing amounts of ^{125}I-HDL_3 with and without a 20-fold excess of unlabeled HDL_3. The mean specific binding at the saturation point (50 µg ^{125}I-HDL_3) was 114.6 ± 9.96 and 117.8 ± 9.73 ^{125}I-HDL_3 ng/mg cell protein. Pearson's correlation coefficient (r) was .891 (P = .043) and the coefficient of variation for this study was 8.1%. The reproducibility (interassay variance) of the HDL_3 binding experiments was explored by a duplicate experiment 3 weeks later in the same 5 normal healthy subjects. In these duplicate assays, different ^{125}I-HDL_3 preparations were used from those employed for previous experiments. The mean specific binding was 114.6 ± 9.96 and 121.8 ± 19.01 ^{125}I-HDL_3 ng/mg cell protein. Pearson's correlation coefficient was .878 (P = .05) and the coefficient of variation for this study was 12.3%.

Studies of competitive inhibition of ^{125}I-HDL_3 binding to granulocytes. To assess the specificity of HDL_3 binding, we performed competition studies with nonlabeled LDL and native HDL_3 isolated from the pooled plasma of normolipidemic subjects in granulocytes obtained from normal nondiabetic subjects. Granulocytes were separated by density-gradient centrifugation, and the cells were washed and incubated for 2 hours at 4°C. Each incubation mixture contained 1×10^6 cells/dish, with a saturating concentration of 50 µg ^{125}I-HDL_3/mL but varying amounts of unlabeled LDL, HDL_3, and HDL_3 + LDL in a total volume of 1 mL with serum-free culture medium containing 2.0 mg/mL albumin. These experiments demonstrated that incubation with a 4-fold molar excess of native unlabeled HDL_3 causes an 83% suppression of the saturable specific component of HDL_3 binding (from 136 ± 14 to 24 ± 6 ng/mg cell protein), while a 4-fold molar excess of native LDL causes only 10% suppression of the binding of ^{125}I-HDL_3 (from 136 ± 14 to 102 ± ng/mg cell protein). The addition to the incubation mixture of LDL + HDL_3 showed only a slight ability of LDL to compete with HDL_3 for ^{125}I-HDL_3 specific binding (from 136 ± 14

to 19 ± 6 ng/mg cell protein). The results of this study suggest that an ultracentrifugally isolated subfraction of HDL_3 shows high-affinity specific binding at its specific binding sites and this presents only very little interaction with LDL particles.

Data Analysis

The normal distribution of the data was demonstrated using the Kolmogorov-Smirnov test. The results are presented as the mean ± SEM. A paired Student's *t* test was used to compare parameters obtained before and after treatment. The relationship between variables was estimated by Pearson's correlation coefficient. A *P* value less than .05 was considered significant.

RESULTS

Clinical and biochemical data for the subjects before and after 3 months of diabetic treatment are shown in Table 1. Fasting blood glucose and HbA_{1c} were decreased after treatment from 11.6 ± 0.48 to 6.9 ± 0.1 mmol/L ($P < .001$) and from 9.2% ± 1% to 6.1% ± 0.4% ($P < .001$), respectively. The improvement in blood glucose control was associated with a decrease in plasma insulin from 351 ± 21 to 236 ± 7.17 pmol/L ($P < .01$). The mean body weight and BMI decreased after metabolic control of diabetic patients from 77 ± 2.3 to 73 ± 2.4 kg ($P < .001$) and from 30.3 ± 0.7 to 28.7 ± 0.7 kg/m^2 ($P < .001$), and this was accompanied by an improvement in the control of blood pressure.

The effect of diabetic control on serum lipid and lipoprotein composition is listed in Table 2. After diabetic control, serum triglyceride levels decreased from 1.60 ± 0.09 to 1.29 ± 0.11 mmol/L ($P < .05$). In contrast, HDL_3-C and apo AI increased from 0.92 ± 0.05 to 1.01 ± 0.05 mmol/L ($P < .05$) and from 103 ± 26 to 115 ± 23 mg/dL ($P < .01$), respectively. No significant changes were observed in serum total cholesterol and apo B levels. The observed decrease in triglycerides was mainly due to a diminution of the VLDL subfraction. The concentration of VLDL phospholipid also decreased after metabolic control ($P < .05$). No significant differences were found for the cholesterol concentration of the various lipoprotein subclasses.

The specific binding of ^{125}I-HDL_3 to cell-surface receptor before and after diabetic control is shown in Fig 1 and indicates that after treatment of type 2 diabetic patients, the high-affinity binding of HDL_3 was increased at each concentration in the assay. The maximum binding increased after control by 58%, from 0.77 ± 0.06 to 1.22 ± 0.08 ^{125}I-HDL_3 ng/μg cell protein ($P < .01$), and Pearson's correlation in this point of binding assay was .851 ($P = .002$). The mean of the Scatchard plots

Table 1. Clinical and Biochemical Data From Type 2 Diabetic Patients

Parameter	Before Treatment	After Treatment
Weight (kg)	77 ± 2.3	73 ± 2.4*
BMI (kg/m^2)	30.3 ± 0.7	28.7 ± 0.7*
Blood pressure (mm Hg)		
Systolic	144 ± 3	138 ± 3†
Diastolic	81 ± 2	76 ± 4
Mean§	101 ± 2.3	94.5 ± 2.9‡
Fasting glucose (mmol/L)	11.6 ± 0.48	6.9 ± 0.1*
HbA_{1c} (%)	9.2 ± 0.3	6.2 ± 0.1*
Fasting C-peptide (nmol/L)	1.38 ± 0.19	1.09 ± 0.09
Fasting insulin (pmol/L)	351 ± 21	236 ± 7.17†

NOTE. Data are the mean ± SE. Mean blood pressure = (systolic − diastolic/3) + diastolic.
*$P < .001$.
†$P < .01$.
‡$P < .05$.

Table 2. Plasma Lipids and Apolipoproteins Before and After Metabolic Control of Type 2 Diabetic Patients

Parameter	Before Treatment	After Treatment
Total cholesterol (mmol/L)	5.14 ± 0.20	4.99 ± 0.18
VLDL	0.46	0.38
IDL	0.26	0.20
LDL	3.31	3.18
HDL	1.12	1.21
HDL_2	0.19 ± 0.09	0.19 ± 0.10
HDL_3	0.92 ± 0.05	1.01 ± 0.05†
Triglycerides (mmol/L)	1.60 ± 0.09	1.29 ± 0.11†
VLDL	0.82	0.60†
IDL	0.13	0.08
LDL	0.33	0.30
HDL	0.31	0.30
Phospholipids (mmol/L)	71.00 ± 2	68.70 ± 2.4
VLDL	8.0	5.76†
IDL	3.74	2.88
LDL	34.56	33.92
HDL	25.50	25.12
Apo AI (mg/dL)	103 ± 5.8	115 ± 5.1*
		109 ± 5.1
Apo B (mg/dL)	114 ± 6	

NOTE. Data are the mean ± SE.
*$P < .01$.
†$P < .05$.

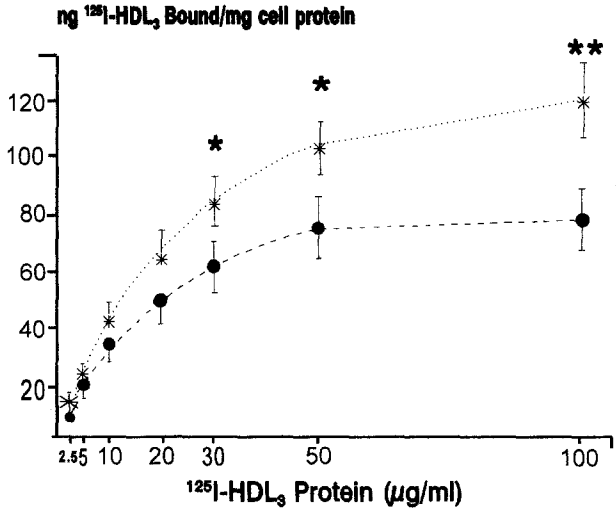

Fig 1. Specific binding of HDL_3 to freshly isolated granulocytes before and after 3 months of metabolic control in type 2 diabetic patients. Binding of ^{125}I-HDL_3 was performed at 4°C and 2-hour incubation using medium containing increasing amounts of ^{125}I-HDL_3 with and without a 20-fold excess of unlabeled HDL_3. Specific binding was calculated as the difference between total and nonspecific binding. Data points represent the mean for 20 patients. HDL_3 binding was higher after treatment of diabetes (*) *v* before (●). *$P < .05$, **$P < .01$.

indicates that the observed increase in HDL_3 binding was due to an elevation in the maximum binding (B_{max}) from 4.97×10^{-10} to 8.3×10^{-10} mol/L ($P < .001$). No significant change was observed in the K_d from 1.47×10^{-7} to 2.04×10^{-7} mol/L. These results are similar when a Scatchard plot is obtained considering each point as the mean of the whole binding assay. This plot is presented in Fig 2, and its analysis demonstrated that the B_{max} increased by 62% after control of diabetic patients, whereas the K_d did not change.

The relationship between changes in the insulin level and changes in the B_{max} of specific HDL_3 binding is shown in Fig 3. These results indicate that the changes observed in insulin after metabolic control of diabetic patients were inversely and significantly correlated with the changes in maximum HDL_3 binding activity to freshly isolated granulocytes observed after treatment of type 2 diabetic patients ($r = -.50$, $P < .05$). In contrast, changes in the insulin level did not correlate with the change in K_d.

Pearson's correlation coefficients between saturated specific HDL_3 binding (50 μg/mL HDL_3, point-of-binding assay) and other variables were explored. There was a negative correlation between changes in HDL_3 binding and changes in the BMI ($r = -.32$, $P < .5$), changes in HbA_{1c} ($r = -.37$, $P < .05$), and changes in fasting insulin ($r = -.32$, $P < .05$).

DISCUSSION

In NIDDM (type 2 diabetes) patients, an inverse correlation between HDL cholesterol and insulin resistance and hyperinsulinemia has been demonstrated, although the precise causes remain undefined. The present study addresses the hypothesis that poorly controlled type 2 diabetes and hyperinsulinemia is associated with a decrease in high-affinity HDL_3 binding activity. The treatment of our patients with a combination of caloric restriction and titrated doses of glibenclamide was associated with significant decreases in the BMI, fasting serum

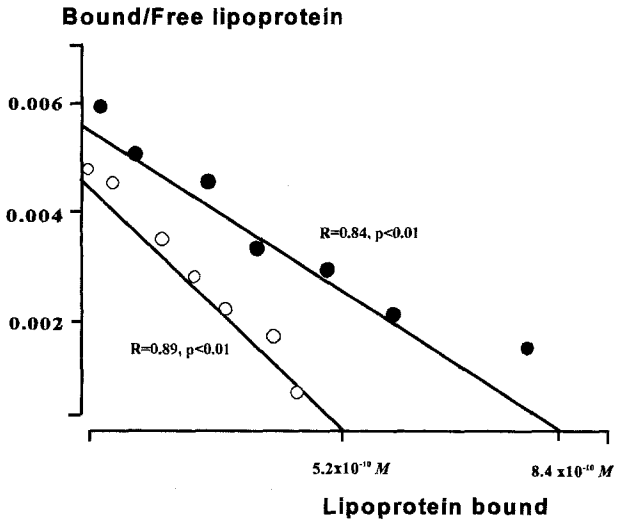

Fig 2. Scatchard analysis of HDL_3 specific binding. Scatchard plot was obtained considering each point as the mean of whole binding assays performed in type 2 diabetic patients before (○) and after (●) treatment. After diabetic control, the B_{max} increased from 5.2×10^{-10} to 8.4×10^{-10} mol/L (62%); however, the K_d did not change significantly (1.15×10^{-7} v 1.52×10^{-7} mol/L).

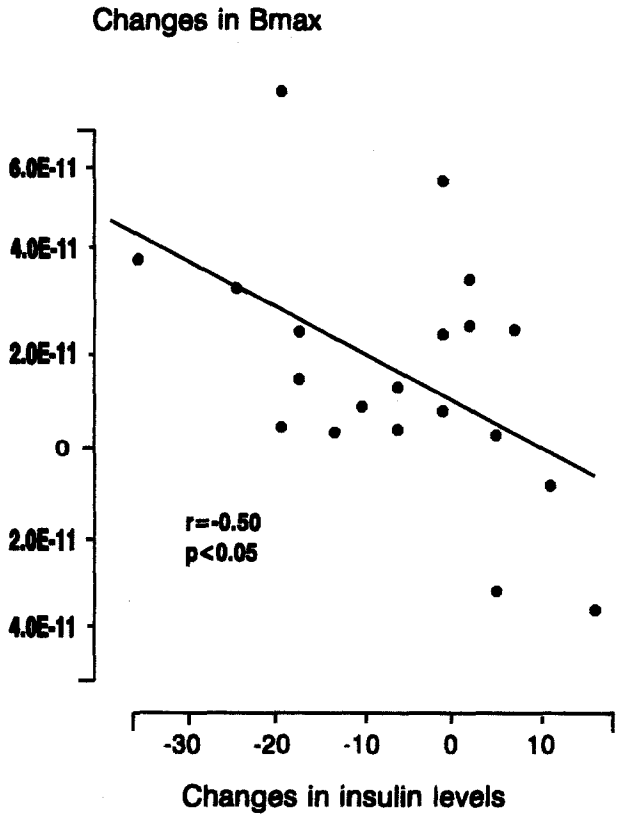

Fig 3. Relationship between changes in insulinemia and changes in B_{max} of specific binding activity. After diabetic control, a significant decrease in insulin was observed and an inverse and significant correlation with the increase in B_{max} from Scatchard analysis of specific binding activity was obtained ($r = -.50$, $P < .001$).

glucose and insulin, fasting plasma total triglycerides, and VLDL triglyceride and phospholipid. The mean change in HbA_{1c} from the high levels before treatment to normal levels after treatment was particularly suggestive of adequate diabetic control. In addition, fasting plasma concentrations of HDL_3 cholesterol and apo AI were significantly increased. These changes were associated with an apparent increase in high-affinity binding sites for HDL_3 on leukocytes isolated from patients after versus before the intervention.

After subtraction of the nonspecific binding, the maximum HDL_3 specific binding of leukocytes was higher after treatment. Analysis of the equilibrium binding data by the method described by Scatchard yielded important observations. First, the linearity of Scatchard plots for HDL_3 binding assays ($r = .84$, $r = .89$, $P < .01$) is indicative of only one class of binding sites and suggests a lack of cooperation among the receptors. Second, the change in binding was due to an increase in the number of receptors as suggested by the increase in maximum binding (B_{max}). Finally, no significant changes were observed in the dissociation constant (K_d).

In addition, we observed that the change in insulin levels after treatment correlated with the change in B_{max}. Several different lines of evidence in vitro indicate that insulin and insulin-like growth factor-I downregulate HDL_3 binding in human fibroblasts, and the activity of the HDL_3 receptor has been related to cell growth,[20,21] which in turn requires insulin

action. Furthermore, in healthy men and subjects with type 2 diabetes, higher plasma insulin levels are associated with lower HDL cholesterol,[22] and this clustering has been demonstrated to occur in populations with a high prevalence of coronary heart disease.[8,23] In our study, the intervention in type 2 diabetic patients following weight loss and improved glycemic control was associated with a decrease in fasting serum insulin. Afterward, we observed that these changes in insulin levels had a significant inverse correlation with the changes in maximum HDL_3 binding activity to freshly isolated granulocytes from type 2 diabetic patients. Thus, in this study, these findings suggest that the hyperinsulinemia observed in most subjects with type 2 diabetes may be associated with a decrease in HDL_3 binding activity.

We consider that the increase in HDL_3 binding observed after diabetic treatment is probably the result of an increase in high-affinity HDL_3 specific binding at its cellular receptors. The existence of specific structural domains of apo AI has been demonstrated that may be recognized by putative HDL receptor,[24] and it is implicated in the interaction between HDL and several cell lines.[25,26]

HDL is a heterogeneous lipoprotein fraction, and HDL_3 particles are a major HDL subpopulation that can promote cholesterol efflux from peripheral tissues. This reaction is the first step in the transport of cholesterol from extrahepatic cells to the liver for excretion. The class B scavenger receptor (SR-BI) has been demonstrated as the first HDL receptor to be well defined at a molecular level,[27] and it may be important in the delivery of cholesterol esters via a selective-uptake pathway to the liver (reverse cholesterol transport) and steroidogenic tissues, in determining plasma HDL concentrations, and in controlling cholesterol concentrations in bile.[28,29]

HDL_3 from normolipidemic subjects was used in our experiments, and although in humans HDL_3 with apo E normally represents only a minor component,[30] this apolipoprotein can also bind to apo B,E receptors. Results from the current study on competition experiments demonstrate that unlabeled HDL_3 can almost completely block the specific binding of ^{125}I-HDL_3, while native LDL exerted only minimal competitive effects with ^{125}I-HDL_3 and had only a slight overlap with the LDL binding sites. Furthermore, the apo E content of the native HDL_3 used in the experiments was only about 5% and no apo B was detected. However, the endogenous expression of apo E by granulocytes from diabetic patients was not studied, and it has recently been shown that endogenous apo E expression can modulate HDL_3 binding to macrophages.[31] The effects of variations in apo E content in HDL_3 and different expression levels of apo E in granulocytes on cellular binding in this study are unknown.

On the other hand, the effect of the 3-month interval on differences in the binding of HDL_3 to leukocytes may be uncertain. Binding values are known to vary from one bath of iodinated lipoprotein preparation to another and with lipoprotein storage. In our study, we calculated the precision (intraassay variance) and reproducibility (interassay variance) from replicate determinations on 5 healthy subjects, and the mean variance was 8% ($r = .891$) and 12% ($r = .878$), respectively. However, since all subjects in the study underwent the intervention, we cannot exclude the possibility that the binding may have increased independently of the intervention.

After diabetic control, changes in the lipid profile were observed in our patients that could also be attributed to an improvement in metabolic control. The greatest changes in serum lipid concentrations were a decrease in plasma triglycerides associated with an increase in apo AI. It has been suggested that a small reduction in body weight associated with restricted total energy intake is perhaps the only effective measure to reduce hypertriglyceridemia and resistance to the peripheral action of insulin. In subjects with type 2 diabetes, insulin resistance has been shown to be possibly reversible, and the major component may be attributed to the metabolic disorder.[32,33] In addition, the decrease in triglycerides may be at least partially explained by an increase in lipolysis associated with a decrease in insulin resistance,[34,35] and a direct correlation has been observed between triglyceride levels and apo AI fractional catabolic rates.[36,37] In this study, we observed that the fasting concentration of apo AI was significantly increased after treatment of diabetic patients. The HDL cholesterol level is related directly to the apo AI concentration, and of the 2 major density subclasses of plasma HDL, variations in apo AI have a major effect on the more dense HDL_3.[38,39] Finally, we found that the mean serum HDL_3 cholesterol concentration was significantly increased after intervention in type 2 diabetic patients, while serum HDL_2 cholesterol was not affected. Together, these findings could support the hypothesis that reversible defects in insulin action are acquired during the course and development of NIDDM and implicate the metabolic pathways of glucose and lipids. However, the biochemical causes and precise functional significance of these defects are not within the scope of this study.

In summary, we have demonstrated that metabolic control in type 2 diabetes is associated with increased HDL_3 binding activity in leukocytes due to an apparent increase in high-affinity binding sites. This finding was associated with a significant decrease in fasting insulin and a significant increase in plasma HDL_3 cholesterol. These results suggest that weight loss and improved metabolic control may have beneficial effects on HDL metabolism in patients with type 2 diabetes.

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