Hyperinsulinemia and insulin resistance are associated with multiple abnormalities of lipoprotein subclasses in glucose-tolerant relatives of NIDDM patients

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Abstract We studied the subclasses of plasma lipoproteins in normolipidemic, glucose-tolerant male relatives of noninsulin dependent diabetic patients (NIDDM), who represented either the lowest (n = 14) or the highest (n = 18) quintiles of fasting plasma insulin. The higher plasma triglyceride level in the high insulin group (1.61 mmol/l vs. 0.87 mmol/l, $P < 0.001$) was due to multiple differences in triglyceride-rich lipoproteins. The concentrations of larger $VLDL_1$, smaller $VLDL_2$ particles, and IDL particles were 3.8-fold, 2.5-fold, and 1.5-fold higher, respectively, in the high insulin group than in the low insulin group ($P < 0.01$ or less). In addition, hyperinsulinemic subjects had $VLDL_1$, $VLDL_2$, and IDL particles enriched in lipids and poor in protein. The lower plasma HDL cholesterol level in the high insulin group (1.20 mmol/l vs. 1.44 mmol/l, $P < 0.01$) compared to the low insulin group was a consequence of a 27% reduction of HDL_{2a} concentration ($P < 0.05$) and a significantly reduced percentage of cholesterol in HDL_{3a}, HDL_{3b}, and HDL_{3c} subclasses. On the other hand, the percentages of triglycerides in HDL_{2b}, HDL_{2a}, HDL_{3a}, and HDL_{3b} subclasses were 76%, 79%, 61%, and 50% higher, respectively, in the high insulin group than in the low insulin group ($P < 0.01$ or less). In the combined group, the concentration of $VLDL_1$ and $VLDL_2$ correlated positively with the concentrations of LDL_2 and LDL_3 and negatively with HDL_{2b} and HDL_{2a} subclasses ($P < 0.05$ or less). In conclusion, normolipidemic, glucose-tolerant but hyperinsulinemic relatives of NIDDM patients have qualitatively similar lipoprotein abnormalities as NIDDM patients. These abnormalities are not observed in insulin-sensitive relatives, suggesting that they develop in concert with insulin resistance.—**Tilly-Kiesi, M., P. Knudsen, L. Groop, M-R. Taskinen, and the Botnia Study Group.** Hyperinsulinemia and insulin resistance are associated with multiple abnormalities of lipoprotein subclasses in glucose-tolerant relatives of NIDDM patients. *J. Lipid Res.* 1996. **37:** 1569–1578.

Supplementary key words cholesterol • triglycerides • VLDL • IDL • HDL • insulin

Elevated serum triglyceride and reduced HDL cholesterol concentrations are key features of dyslipidemia in the insulin resistance syndrome (1). Elevation of serum triglycerides is mainly due to enhanced input of very low density lipoprotein (VLDL) particles into circulation (1–3). In particular, the number of large, triglyceride-rich VLDL particles is increased in NIDDM (4). This abnormality is associated with metabolic consequences for the lipoproteins. Recently, insulin resistance has been associated with the preponderance of atherogenic small, dense LDL particles (5–7) and postprandial lipemia (8, 9).

Insulin sensitivity is an important determinant of both triglyceride and HDL cholesterol metabolism (1, 10, 11). In non-diabetic subjects, in patients with impaired glucose tolerance (IGT), and in NIDDM patients, the rate of glucose disposal during an euglycemic clamp correlates inversely to fasting serum triglyceride concentration and positively to HDL cholesterol concentration (12–14). In nondiabetic populations, fasting insulin concentration correlates with insulin sensitivity and therefore an increased insulin level may be used as a marker of insulin resistance and concomitant changes in lipid and lipoprotein levels (15). The relationship between insulin level and concentrations of serum triglycerides

Abbreviations: NIDDM, noninsulin dependent diabetes mellitus; VLDL, very low density lipoproteins; IDL, intermediate density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; CETP, cholesteryl ester transfer protein; BMI, body mass index.

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and HDL cholesterol has been established in population studies (10, 11, 16–19).

First degree relatives of NIDDM patients are frequently characterized by insulin resistance and also by a higher prevalence of dyslipidemia than the general population (20) and show also the full profile of metabolic abnormalities associated with insulin resistance (21, 22). The specific purpose of the present study was to investigate whether hyperinsulinemia/insulin resistance is associated with coordinated alterations of the concentration, density distribution, and composition of various lipoprotein subclasses in normoglycemic first degree relatives of NIDDM patients. To address this question we studied subjects representing either the lowest or the highest quintiles of fasting plasma insulin. This choice of study groups allowed us to examine insulin-sensitive versus insulin-resistant individuals of the cohort.

SUBJECTS AND METHODS

Subjects

Thirty-two healthy first degree male relatives of NIDDM patients were included in this study. The subjects were recruited among 120,000 inhabitants of the west coast of Finland, where an extensive family study was initiated in 1990 in order to explore the genetic defect(s) implicated in the development of NIDDM. Altogether, 505 men with normal glucose tolerance have been studied in four primary health care centers (23). They were classified according to the quintiles of fasting serum insulin. The cutoff points of the lowest and highest quintiles were 4.6 and 10.0 mU/l. Sixty subjects among the lowest and the highest quintiles were invited to the community health center for a repeat study. At the second examination a total of 28 subjects had fasting insulin levels between 4.6 mU/l and 10.0 mU/l and were excluded from this study. A detailed analysis of all subfractions of the major lipoprotein classes was performed in 32 subjects. All subjects had an oral glucose tolerance test (75 g glucose) performed in the morning after a 12-h fast. Glucose and insulin were determined at 0, 30, 60, and 120 min.

To be eligible for the study, the participants had to have a normal oral glucose tolerance test according to the criteria of the World Health Organization (24) having both fasting and 2-h blood glucose levels less than 6.7 mml/l. Other inclusion criteria were age, between 25 and 65 years, and body mass index (BMI), between 23 and 29. All subjects with a known or discovered serious illness, or receiving lipid lowering medication or hormone therapy, were excluded. Two subjects included in the study were on medication for high blood pressure. Their fasting insulin levels were 11.0 and 19.2 mU/l.

Preparative separation of lipoproteins

Blood samples were obtained in the morning after a 12-h fast and serum was separated by a low-speed centrifugation (at 2000 rpm, 30 min, at 4°C). Lipoproteins were isolated by ultracentrifugation in a Beckman Optima TL Ultracentrifuge using Beckman 1/2 × 2 polycarbonate centrifuge tubes and a TLA 100.3 rotor. For isolation of VLDL and IDL, tubes containing 2.0 ml of serum were overlayered with 1.0 ml of NaBr solution of density 1.0190 g/ml and ultracentrifuged at 100.000 rpm, for 3 h, at 20°C using slow acceleration and deceleration (9/9). After ultracentrifugation a thin, fairly yellowish layer containing VLDL and IDL was observed at the top of the tubes followed by a colorless section of 2 cm before a strongly yellowish, distinct layer containing LDL. The VLDL and IDL were removed from the top of the tubes by careful aspiration in a volume of 1 ml. The density of the infranatant was increased to 1.0900 g/ml by adding 0.3 ml of NaBr solution d 1.535 g/ml and was further used for LDL density gradient ultracentrifugation. To obtain a sample for the HDL density gradient ultracentrifugation, the apoB-containing lipoproteins (VLDL, IDL, and LDL) were first removed by preparative ultracentrifugation. Using Beckman 1/2 × 2 polycarbonate centrifuge tubes, 2 ml of serum in 0.3 ml NaBr solution d 1.535 g/ml was overlayered with 0.7 ml NaBr solution d 1.0600 g/ml. After ultracentrifugation in a Beckman Optima TL ultracentrifuge with a Beckman TLA 100.3 rotor at 100,000 rpm for 5 h at 20°C the supernatant layer containing VLDL, IDL, and LDL was removed by aspiration and the infranatant was used for HDL density gradient ultracentrifugation. All density gradient ultracentrifugation studies were performed from fresh samples.

VLDL and IDL density gradient ultracentrifugation

Density gradient ultracentrifugation was performed in a Beckman L8-70 ultracentrifuge with an SW 40TI swinging-bucket rotor using Beckman Ultraclear 9/16 × 33/4 13 ml centrifuge tubes. The discontinuous gradient was prepared using NaBr solutions of distinct densities in the following order from bottom of the tube to the top: 1 ml d 1.0280 g/ml; 3 ml sample solution (1 ml VLDL + IDL in 2 ml NaBr solution d 1.0100 g/ml); 3 ml d 1.0060 g/ml; 3 ml d 1.0030 g/ml; and 2.5 ml of distilled water. All salt solutions contained 0.05% EDTA and their densities were measured with a DMA 46 Digital Density Meter (Anton Paar, Graz, Austria).

After ultracentrifugation at 40,000 rpm for 55 min at 20°C, the centrifuge was allowed to stop with the brake off. The bottoms of the tubes were punched and three fractions of volumes 5 ml, 5 ml, and 2.5 ml were collected

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TABLE 1. Clinical characteristics and serum lipid and lipoprotein concentrations of the study groups

	Low Insulin Group (n = 14)	High Insulin Group (n = 18)
Age (years)	45.8 ± 6.8	44.2 ± 6.9
Body mass index (kg/m^2)	24.8 ± 1.4	26.5 ± 1.8[a]
Waist circumference (cm)	88.8 ± 6.4	96.8 ± 6.1[a]
Fasting plasma insulin (mU/l)	3.3 ± 0.5	13.5 ± 3.1[c]
Total cholesterol (mmol/l)	5.53 ± 1.30	5.96 ± 1.07
Triglycerides (mmol/l)	0.87 ± 0.26	1.61 ± 0.59[c]
HDL cholesterol (mmol/l)	1.44 ± 0.22	1.20 ± 0.26[b]
LDL cholesterol (mmol/l)	3.67 ± 1.19	4.02 ± 1.01

Values are given as mean ± SD. HDL, high density lipoproteins; LDL, low density lipoproteins.
[a] $P < 0.05$, [b] $P < 0.01$, and [c] $P < 0.001$ for significance of differences between groups.

using a peristaltic pump operating at a flow rate 2.5 ml/min. The protein absorbance profiles in the tubes were monitored with an absorbance meter (Pharmacia, Uppsala, Sweden) and the density gradient of the tubes was controlled with a DMA 46 density meter placed before the fraction collector. The first fraction (d 1.0185–1.006 g/ml) contained IDL, the second 5-ml fraction contained the dense $VLDL_2$ (d 1.006–1.003 g/ml), and the 2.5-ml fraction (d 1.003–0.988 g/ml) contained the light $VLDL_1$.

LDL density gradient ultracentrifugation

The LDL subfractions LDL_1, LDL_2, and LDL_3 were separated by density gradient ultracentrifugation in a Beckman SW40 TI swinging-bucket rotor and Beckman Ultraclear 9/16 × 33/4 tubes from the sample obtained from preparative ultracentrifugation in Beckman Optima TL centrifuge. The method used is similar to the method described by Griffin et al. (25) for plasma samples. The discontinuous NaBr solution gradient was prepared by layering from bottom to top: 0.5 ml d 1.1900 g/ml; 2.3 ml sample (the infranatant of a volume of 2.0 ml from VLDL + IDL separation in 0.3 ml NaBr d 1.5350 g/ml); 1.5 ml d 1.0630 g/ml; 1.5 ml d 1.056 g/ml; 1.5 ml d 1.0450 g/ml; 2.0 ml d 1.0340 g/ml; 2.0 ml d 1.0240 g/ml; and 0.7 ml d 1.0190 g/ml. The tubes were centrifuged in a Beckman L8-70 ultracentrifuge at 40,000 rpm for 24 h at 23°C and the rotor was allowed to stop without using the brake.

After ultracentrifugation the tubes were discharged from the top using Beckman Recovery System and by infusing Maxidens solvent (Nyegaard & C. A/S, Oslo, Norway) to the tubes. The protein absorbance profile and the density gradient of the tubes were monitored as described for VLDL + IDL. Three LDL subfractions LDL1 (d 1.024–1.031 g/ml), LDL2 (d 1.031–1.040 g/ml), and LDL3 (d 1.040–1.054 g/ml) were collected in a volume of 1.5 ml each.

HDL density gradient ultracentrifugation

The HDL density gradient ultracentrifugation method was based on the method previously described by Groot et al. (26), except that the apoB-containing lipoproteins were first removed from serum samples by a centrifugation in Beckman Optima TL centrifuge as described above. The density of the infranatant was increased by adding 1.0 g dry NaBr. A 2-ml volume of this sample solvent was pipetted to the bottom of Beckman Ultraclear 9/16 × 33/4 tubes and the discontinuous gradient was prepared by layering NaBr solutions d

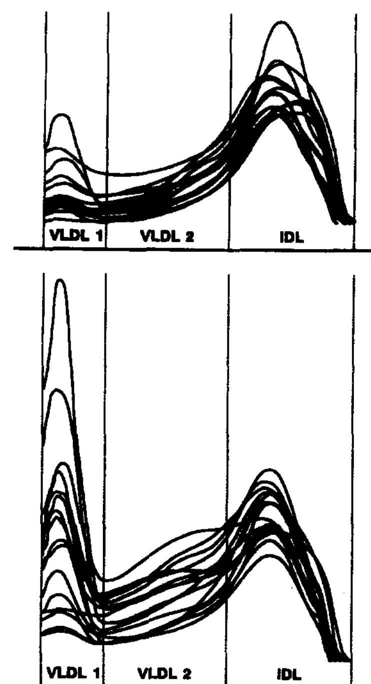

Fig. 1. Protein absorbance profiles of $VLDL_1$, $VLDL_2$, and IDL subclasses obtained by density gradient ultracentrifugation. The individual curves of subjects representing the lowest quintile of fasting serum insulin level are presented in the upper panel and the curves of the subjects in the highest quintile in the lower panel.

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TABLE 2. Mean concentrations and composition of lighter ($VLDL_1$) and denser ($VLDL_2$) VLDL subclasses and of IDL in subjects with low (Group I, n = 14)) and high (Group II, n = 18) plasma insulin levels

	Total Concentration	Chol	Tg	Pl	Prot
	mg %		%		
$VLDL_1$					
Group I	6.2 ± 2.8	11.4 ± 2.5	64.7 ± 5.4	12.9 ± 1.6	11.0 ± 3.7
Group II	23.7 ± 12.1[c]	11.7 ± 1.6	66.8 ± 3.3	14.0 ± 1.3[a]	7.5 ± 2.0[b]
$VLDL_2$					
Group I	28.6 ± 11.8	15.4 ± 2.7	52.8 ± 4.2	17.4 ± 1.1	14.4 ± 1.9
Group II	71.3 ± 34.0[c]	15.5 ± 1.6	55.3 ± 2.5[a]	18.3 ± 1.0[a]	11.0 ± 1.5[c]
IDL					
Group I	50.3 ± 19.5	22.0 ± 4.0	36.0 ± 5.3	20.2 ± 1.5	21.8 ± 4.9
Group II	74.2 ± 23.7[b]	21.6 ± 3.3	40.1 ± 5.2[a]	20.8 ± 1.3	17.6 ± 3.6[b]

Values given as mean ± SD. Abbreviations: Chol, cholesterol; Tg, triglycerides; Pl, phospholipids; Prot, proteins.
[a]$P < 0.05$, [b]$P < 0.01$, and [c]$P < 0.001$ for significance of differences between groups.

1.2500 g/ml and d 1.2200 g/ml, 1.5 ml and 6.7 ml above the sample, respectively, and 2.0 ml distilled water above the salt solutions.

After ultracentrifugation in Beckman L8-70 ultracentrifuge with a SW40 TI rotor (40,000 rpm, 18 h, 20°C) the tubes were discharged from the top as described for separation of LDL subfractions. Five 1.3-ml fractions corresponding to HDL_{2b}, HDL_{2a}, HDL_{3a}, HDL_{3b}, and HDL_{3c} were collected.

Analytical methods

Cholesterol, triglycerides, and phospholipids were measured by an enzymatic method with a Cobas Mira analyser (Hoffman-La Roche, Basel, Switzerland) using reagent kits (Nos. 0715166 and 0722138, Hoffman-La Roche, Basel, Switzerland) for cholesterol and triglycerides and reagent kit No. 990-54009 (Wako Chemicals GmBH, Germany) for phospholipids. Total serum HDL cholesterol concentration was determined with the Cobas Mira analyser by precipitation assay using reagent kit (No. 0720674, Hoffman-La Roche, Basel, Switzerland). Total serum LDL cholesterol was calculated using the formula of Friedewald, Levy, and Fredrickson (27). Blood glucose concentration was measured by a glucose oxidase method (Auto-Analyzer, Technicon, Tarrytown, NY). Fasting insulin levels were measured by a radioimmunoassay after precipitation with polyethylene glycol using the Phadeseph insulin radioimmunoassay kit (Pharmacia, Uppsala, Sweden) normal range being 2–20 mU/l.

Statistical analyses

Statistical analyses were performed with the BMDP statistical software (University of California Press, 1993). Values are expressed as mean ± SD. Comparison of data between groups was calculated by analysis of variance (ANOVA). The analysis of covariance (ANCOVA) was used to evaluate the influence of BMI on between-group comparisons. Relationships between variables were estimated with Pearson's correlation coefficients.

RESULTS

Serum lipids

The mean serum triglyceride concentration was 85.1% higher and the mean HDL cholesterol concentration 16.7% lower in the high-insulin compared with the low-insulin group, $P < 0.001$ and $P < 0.01$, respectively (**Table 1**). Total cholesterol and LDL cholesterol levels were similar in the two groups.

Concentrations and composition of triglyceride-rich lipoproteins

The individual protein absorbance profiles of $VLDL_1$, $VLDL_2$, and IDL of the subjects in the low and high insulin groups are presented in **Fig. 1.** The mean concentrations of $VLDL_1$ and $VLDL_2$ were 3.8-fold and 2.8-fold higher, respectively, in the high insulin compared with the low insulin group ($P < 0.01$ or less, **Table 2**). The differences remained significant after adjusting for BMI. The mean percentage content of protein in $VLDL_1$ and $VLDL_2$ was 3.5% and 3.0% less, respectively, in the high insulin compared with the low insulin group ($P < 0.01$ or less) indicating that subjects in the high insulin group had lipid-enriched and protein-poor $VLDL_1$ and $VLDL_2$ particles. The proportion of triglycerides in $VLDL_2$ was somewhat higher in the high insulin than in the low insulin group. Otherwise the lipid content of $VLDL_1$ and $VLDL_2$ particles were similar in both groups.

The mean IDL concentration was increased by 48% in the high insulin group compared with the mean IDL concentration of the low insulin group ($P < 0.001$, Table

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2). Similar to VLDL subclasses, the content of protein in IDL was significantly reduced and the content of triglycerides increased in subjects with higher insulin levels (Table 2).

LDL subclasses

The LDL hydrated density distribution and the LDL peak density did not correlate with fasting insulin in either group, whereas the LDL peak density correlated strongly with the serum triglyceride concentration (r = 0.660, $P < 0.001$). The protein absorbance profiles of LDL density distribution of individuals in the low and high insulin groups are shown in **Fig. 2.** The mean LDL peak density 1.0321 ± 0.0029 g/ml of the low insulin group differed significantly ($P < 0.01$) from the mean LDL peak density 1.0365 ± 0.0046 g/ml of the high insulin group, reflecting a change in LDL particles towards smaller and more dense particles. The mean concentration of LDL_1 subclass was similar in both groups. However, the mean concentration of LDL found in the major subclass LDL_2 was 43% higher in the high compared with the low insulin group ($P < 0.01$, **Table 3**). This difference remained significant after adjusting for BMI. A similar trend was noted in the concentration of LDL_3, but this difference reached statistical significancy only after adjusting for BMI. No major differences were observed in the composition of LDL_1, LDL_2, and LDL_3 subclasses between the two groups (Table 3). These findings indicate that the slight difference in serum LDL cholesterol concentration between the two groups is primarily due to the increase in the number of intermediate dense particles, while the composition of LDL particles is not affected by the plasma insulin level.

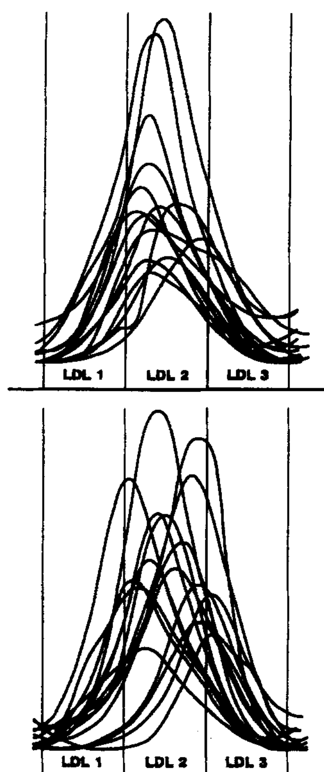

Fig. 2. Protein absorbance profiles of LDL obtained by density gradient ultracentrifugation. The individual curves of the subjects in the lowest quintile are shown in the upper panel and in the lower panel are the curves of the subjects in the highest quintile.

Characteristics of HDL subclasses

The individual hydrated density distribution curves of HDL demonstrated diminished HDL_{2b} and HDL_{2a} subclasses in the majority of subjects in the high insulin group in comparison to the subjects in the low insulin group (**Fig. 3**). The mean concentration of HDL_{2a} subclass was reduced by 27% in the high compared with the low insulin group ($P < 0.05$, **Table 4**). Similarly, the concentration of HDL_{2b} tended to be lower in the high insulin group. The groups did not differ from each other with respect to the concentrations of HDL_{3a}, HDL_{3b}, and HDL_{3c} particles (Table 4). These findings remained the same after adjusting for BMI.

The percentage of triglycerides in HDL_{2b}, HDL_{2a}, HDL_{3a}, and HDL_{3b} subclasses were 76%, 79%, 61%, and 50% higher, respectively, in the high insulin group than the corresponding percentages in the low insulin group ($P < 0.01$ or less, Table 4). In addition, the cholesterol content in HDL_{3a}, HDL_{3b}, and HDL_{3c} subclasses was reduced by 10.3%, 9.6%, and 9.7%, respectively, in the high compared to the low insulin group.

Correlations between lipoprotein subclasses

In all subjects the concentration of $VLDL_1$ subclass correlated positively with the concentrations of LDL_2 and LDL_3 subclasses ($P < 0.01$ in both) and negatively with the concentrations of HDL_{2b} and HDL_{2a} subclasses ($P < 0.05$ and $P < 0.001$, **Table 5**). The concentration of $VLDL_2$ correlated positively with concentrations of LDL_2 and LDL_3 subclasses ($P < 0.01$ and $P < 0.05$) and negatively with the concentrations of HDL_{2b}, HDL_{2a}, and HDL_{3a} ($P < 0.01$, $P < 0.001$ and $P < 0.05$, respectively, Table 5). The concentration of LDL_1 correlated positively with the concentration of HDL_{3b}, but no relationship was found between LDL_2 and LDL_3 subclasses and HDL_{2b}, HDL_{2a}, HDL_{3a}, HDL_{3b}, and HDL_{3c} (data not shown).

DISCUSSION

Hypertriglyceridemia and reduced HDL cholesterol concentration are common in NIDDM patients and are also frequently present in subjects with insulin resis-

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TABLE 3. Mean concentrations and compositions of different LDL subfractions in normoglycemic relatives of NIDDM patients having low normal (Group I, n = 14) or high normal (Group II, n = 18) plasma insulin level

	Total Concentration	Chol	Tg	Pl	Prot
	mg %		%		
LDL_1					
Group I	92.7 ± 39.5	41.7 ± 2.5	6.7 ± 2.5	26.8 ± 0.6	24.8 ± 1.3
Group II	86.7 ± 27.2	40.1 ± 2.0	8.2 ± 2.9	26.2 ± 0.8[a]	25.5 ± 1.6
LDL_2					
Group I	91.1 ± 37.1	40.7 ± 2.6	6.0 ± 2.4	25.9 ± 0.7	27.4 ± 1.1
Group II	130.5 ± 41.3[b]	40.4 ± 1.7	5.8 ± 1.5	25.7 ± 0.9	28.1 ± 1.7
LDL_3					
Group I	36.5 ± 13.1	37.9 ± 3.3	7.7 ± 2.3	25.0 ± 1.3	29.4 ± 2.1
Group II	48.7 ± 20.5	36.9 ± 8.1	7.9 ± 2.1	25.6 ± 3.1	29.7 ± 4.3

Values given as mean ± SD. Abbreviations: Chol, cholesterol; Tg, triglycerides; Pl, phospholipids; Prot, protein.
[a]$P < 0.05$ and [b]$P < 0.01$ for significance of differences between groups.

tance (1, 12, 28, 29). Previous data have revealed that an elevation of insulin concentration precedes the development of a number of metabolic disorders associated with the insulin resistance syndrome (22, 30, 31). Likewise, in the present study, men with high insulin levels who did not have frank hypertriglyceridemia had mean serum triglyceride concentration significantly higher compared to men with low insulin levels. Multiple alterations in triglyceride-rich lipoproteins contributed to the observed increase of serum triglycerides in these subjects. The concentrations of both larger and smaller VLDL particles and of IDL particles were increased in these hyperinsulinemic men. Notably, hyperinsulinemic men had a 4-fold elevation of large $VLDL_1$ particles as compared to normoinsulinemic men. In addition, in the high insulin group several abnormalities were observed in the composition of $VLDL_1$, $VLDL_2$, and IDL. The lipid/protein ratio and the content of triglycerides were significantly increased in $VLDL_1$, $VLDL_2$, and IDL indicating larger particle size in each of these lipoprotein subclasses. Similar compositional abnormalities in VLDL and IDL of NIDDM patients have been reported previously (4, 32, 33).

Metabolic studies have indicated that hypertriglyceridemia in NIDDM patients is a consequence of an overproduction of triglyceride-rich VLDL particles as well as of an impaired clearance of these particles (2, 28, 34). In the present study, fasting plasma insulin levels correlated positively with $VLDL_1$, $VLDL_2$, IDL, LDL_2, and LDL_3 concentrations and a significant negative correlation existed between the concentrations of $VLDL_1$ and $VLDL_2$ and HDL_2 subfractions HDL_{2b} and HDL_{2a}. These data suggest that the major abnormality behind dyslipidemia associated with insulin resistance is the increased concentration of large triglyceride-rich particles ($VLDL_1$) in the circulation. The observed interrelations indicate that the preponderance of smaller LDL particles, the reduced concentrations of HDL_2 subfractions, and increased content of triglycerides in HDL_2 particles result from abnormalities in the VLDL-IDL-LDL cascade. Our findings are consistent with the results previously reported in normolipidemic and hy-

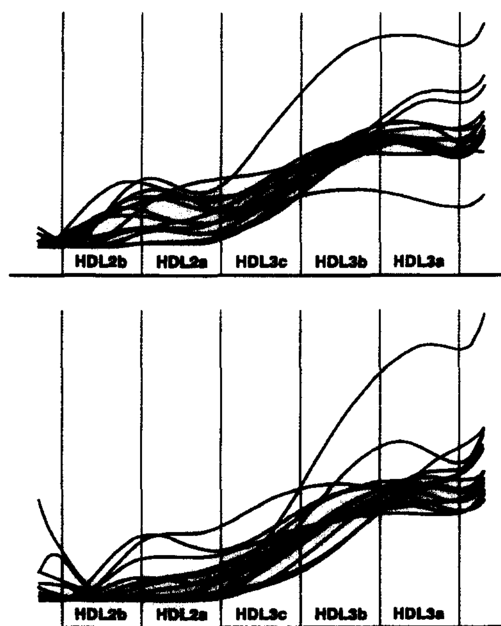

Fig. 3. Protein absorbance profiles of HDL obtained by density gradient ultracentrifugation. The individual curves of the subjects among the lowest quintile of fasting serum insulin level are presented in the upper panel and the curves of the subjects representing the highest quintile are shown in the lower panel.

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TABLE 4. Concentration and composition of HDL subclasses separated by density gradient ultracentrifugation in normoglycemic relatives of NIDDM patients with low (Group I, n = 14) and high fasting plasma insulin level (Group II, n = 18) plasma insulin level

	Total Concentration	Chol	Tg	Pl	Prot
	mg %		%		
HDL_{2b}					
Group I	30.3 ± 10.4	21.9 ± 1.8	6.3 ± 2.5	30.4 ± 4.0	41.5 ± 5.8
Group II	24.3 ± 12.3	21.0 ± 3.7	11.1 ± 5.3[b]	26.5 ± 3.9[b]	41.4 ± 9.3
HDL_{2a}					
Group I	41.8 ± 13.0	19.9 ± 1.1	4.7 ± 1.9	31.2 ± 2.8	44.2 ± 3.9
Group II	30.5 ± 16.2[a]	18.6 ± 4.4	8.4 ± 3.4[c]	29.4 ± 5.2	43.6 ± 4.7
HDL_{3a}					
Group I	52.2 ± 11.2	17.3 ± 1.2	3.8 ± 1.3	28.9 ± 2.6	49.9 ± 2.8
Group II	48.7 ± 18.1	15.5 ± 1.3[c]	6.1 ± 2.1[b]	27.4 ± 2.0	51.0 ± 3.3
HDL_{3b}					
Group I	72.7 ± 6.7	15.7 ± 1.0	3.0 ± 1.1	25.8 ± 2.2	55.4 ± 2.0
Group II	70.8 ± 13.1	14.2 ± 0.9[c]	4.5 ± 1.1[c]	24.8 ± 1.8	56.5 ± 2.0
HDL_{3c}					
Group I	78.4 ± 8.9	13.4 ± 1.1	2.7 ± 0.9	21.6 ± 2.2	62.3 ± 2.5
Group II	79.5 ± 12.0	12.0 ± 1.2[b]	5.7 ± 6.6	20.8 ± 2.8	61.5 ± 6.4

Values are given as mean ± SD. Abbreviations: Chol, cholesterol; Tg, triglycerides; Pl, phospholipids; Prot, proteins.
[a]$P < 0.05$, [b]$P < 0.01$, and [c]$P < 0.001$ for significance of differences between groups.

pertriglyceridemic subjects by Packard et al. (35), Griffin et al. (36), and Watson et al. (37).

Plasma postheparin lipoprotein lipase activity (LPL) has been shown to be subnormal or reduced in NIDDM patients (38–40). Recently, we (41) and Chen et al. (42) have reported that the LPL activity is reduced in subjects with insulin resistance compared to insulin-sensitive subjects. Interestingly, Ahn et al. (43) reported an association between a variation in the LPL gene and the insulin resistance syndrome. Thus, besides overproduction of VLDL, reduced or subnormal LPL activity may contribute to the accumulation of triglyceride-rich lipoproteins in men with high insulin levels.

LDL density distribution and LDL peak density were different in the two groups. In the high insulin group the LDL peak density was increased and LDL_2 concentration was significantly and LDL_3 moderately elevated compared to the low insulin group. Stewart et al. (44) have reported a significant shift in the hydrated density distribution of LDL and increased LDL peak density in NIDDM patients. In addition, in NIDDM patients and in subjects with insulin resistance, the LDL particle size is reduced (6, 7, 44). Plasma triglyceride concentration has been shown to be one major determinant of LDL density distribution, composition, and particle size in nondiabetic subjects (45–47). Accordingly, the concentration of plasma triglycerides predicts LDL size in diabetic populations (44, 48). We did not observe any differences in LDL density distribution, composition, or particle size between NIDDM patients and healthy subjects with similar plasma triglyceride concentrations (49). In the present study the lipid composition of different LDL subclasses did not differ between the two groups, suggesting that hyperinsulinemia does not directly affect LDL composition and that the reduced lipid/protein ratio of LDL associated with insulin resistance is obviously related to the relative abundance of small LDL particles. As small, dense LDL more readily undergoes oxidative modification, the accumulation of these LDL particles probably increases the risk for atherosclerotic cardiovascular disease in insulin-resistant subjects (50–52)

Several differences were observed in concentrations and composition of HDL subclasses between the two groups. First, the concentrations of larger and more buoyant HDL_{2b} and HDL_{2a} were lower in the high insulin group. Second, the content of cholesterol in all HDL_3 subfractions (HDL_{3a}, HDL_{3b}, and HDL_{3c}) was significantly reduced in the high insulin group. Taken together, these findings may explain the lower total serum HDL cholesterol concentration in the high insulin group. In addition, substantial reciprocal increases in the triglyceride content of HDL subfractions were noted in the high insulin group. The metabolism of VLDL and HDL is closely interrelated and regulated by several key enzymes. HDL_2 particles are formed as a

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TABLE 5. Pearson correlation coefficients for the relationship between triglyceride-rich lipoprotein subclasses and LDL and HDL subclasses

	VLDL1	VLDL1	IDL
LDL_1	-0.14	-0.11	0.04
LDL_2	0.54[b]	0.51[b]	0.55b
LDL_3	0.46[b]	0.42[a]	0.37a
HDL_{2b}	-0.44[a]	-0.49[b]	-0.33
HDL_{2a}	-0.56[c]	-0.62[c]	-0.50b
HDL_{3a}	-0.34	-0.41[a]	-0.24
HDL_{3b}	-0.22	-0.27	-0.04
HDL_{3c}	-0.04	-0.02	0.26

[a]$P < 0.05$, [b]$P < 0.01$, and [c]$P < 0.001$ for significance of correlations between variables.

result of lipoprotein lipase-induced lipolysis of VLDL and chylomicrons. In this process phospholipids, free cholesterol, and apolipoproteins are released and transferred to HDL_3 particles. The increase in cholesteryl ester content of HDL_3 particles by the action of lecithin:cholesterol acyltransferase (LCAT) results in the conversion of HDL_3 to HDL_2 particles. The concentration of HDL_2 has been shown to correlate positively with LPL activity (53, 54). Hepatic lipase (HL) acts in the opposite direction, promoting conversion of HDL_2 to HDL_3 (55, 56). In addition, cholesteryl ester transfer protein (CETP) regulates the cholesterol-triglyceride transfer between HDL and VLDL (57). Our previous results from this same cohort demonstrated decreased LPL activity and LPL/HL ratio in insulin-resistant subjects (40).

Dullaart et al. (58) have shown that CETP activity correlates positively with both body mass index and C-peptide levels. The increased content of Tg and reduced content of cholesterol in the HDL subfraction of insulin-resistant subjects may be related to higher serum triglyceride concentration (45). However, men with high insulin level had slightly higher BMI. Therefore, enhanced CETP activity and accelerated cholesteryl ester transfer may contribute to the compositional differences of HDL subfractions between the groups (58).

Our results show that hyperinsulinemic, glucose-tolerant first degree offspring of NIDDM patients have qualitatively and quantitatively similar lipoprotein abnormalities as NIDDM patients despite having plasma insulin and lipid levels within the normal range. We propose that increased concentration of large VLDL particles results in a relative abundance of smaller LDL particles and reduced concentration of larger HDL_2 particles with concomitant increase of triglycerides in their composition. As insulin resistance and family history of NIDDM are major predictors of subsequent NIDDM, our results suggest that atherogenic lipoprotein abnormalities may exist for years before clinical diagnosis of frank NIDDM. This could partly explain the manifestation of coronary heart disease in these patients already at the time of clinical diagnosis of NIDDM.

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