Postprandial Changes in High-Density Lipoprotein Composition and Subfraction Distribution Are Not Altered in Patients With Insulin-Dependent Diabetes Mellitus

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A detailed analysis of postprandial changes in the size, density, composition, and relative proportion of the major high-density lipoprotein (HDL) subfractions, HDL_2 and HDL_3, was performed in seven normolipidemic patients with insulin-dependent diabetes mellitus (IDDM) in moderate glycemic control and seven age-, sex-, and weight-matched healthy nondiabetic controls. IDDM subjects received an overnight insulin infusion to maintain euglycemia, with an incremental increase in the insulin infusion rate at the time of the test meal (containing 60 g fat/m^2). Samples for detailed analysis of HDL by gradient density ultracentrifugation and nondenaturing gradient gel electrophoresis (GGE) were collected at 0, 4, 8, and 12 hours after the test meal. The composition of HDL, HDL_2, and HDL_3 was significantly altered in the postprandial state in IDDM subjects and controls with an increase in triglyceride content at 4 to 8 hours and a reciprocal decrease in cholesteryl ester, reflecting exchange of lipid constituents of HDL with triglyceride (TG)-rich lipoproteins. In addition, the phospholipid content of the particles increased at 8 hours after the meal. Peak density of HDL_2 and HDL_3 decreased slightly at 4 to 8 hours, reaching significance only in controls at 8 hours ($P < .05$), whereas the mean radius size of these subfractions did not change significantly. In controls and IDDM subjects, the ratio of HDL_3 to HDL_2 at 8 to 12 hours increased significantly ($P < .005$). Significant differences in the composition, size, density, or subfraction distribution of HDL between subjects with IDDM and controls were not observed following ingestion of the lipid-rich meal. We conclude from these data that in patients with IDDM in moderate glycemic control, there do not appear to be any significant gross abnormalities in postprandial HDL metabolism with respect to the size, density, or compositional changes of HDL particles.

PATIENTS WITH insulin-dependent diabetes mellitus (IDDM) have a high incidence of premature atherosclerosis[1,2]; however, high-density lipoprotein (HDL) cholesterol concentration, which is inversely related to the incidence of coronary artery disease,[3,4] is not decreased and may even be elevated in IDDM.[5-8] The mechanism by which low HDL cholesterol is believed to contribute to the atherosclerotic process is not fully understood, but HDL plays a central role in the complex regulation of lipoprotein catabolism and is involved in the exchange and transfer of lipid and protein constituents with lipoproteins and cells.[9] Levels of HDL, particularly the HDL_2 subfraction, are strongly related to the activity of the lipoprotein lipase system,[10-12] which in turn is regulated to a large extent by insulin.[13]

To the best of our knowledge, this is the first study to investigate whether a more subtle alteration of HDL metabolism exists in treated IDDM patients in the postprandial state. We examined the change in HDL composition, size, and density following ingestion of a lipid-rich mixed meal and compared these responses with those in age-, weight-, and gender-matched nondiabetic controls. In addition, using the combined techniques of gradient density ultracentrifugation and nondenaturing gradient gel electrophoresis (GGE), we were able to examine relative postprandial changes in distribution of the main subfractions of HDL, HDL_2 and HDL_3.

A detailed analysis of postprandial changes in triglyceride (TG)-rich particles and nonesterified fatty acids under varying conditions of insulin replacement in these subjects has been reported previously.[14] In the present study, IDDM subjects were maintained in moderate to good prior glycemic control and received an overnight insulin infusion to maintain euglycemia, with a postprandial increase in the insulin infusion rate designed to mimic the plasma concentration of endogenously released insulin in the controls.

SUBJECTS AND METHODS

Description of Subjects

Seven patients with IDDM who were free of complications and otherwise in good health and seven nondiabetic controls participated in the study. Diabetic and control subjects were well matched for age (30.3 ± 2.0 *v* 30.0 ± 2.2 years, respectively, mean ± SEM; range, 21 to 40), gender (six women and one man in each group), and body mass index (23.9 ± 0.8 *v* 23.8 ± 0.9 kg/m^2; range, 20.5 to 27.1). All subjects were normolipidemic as defined by fasting plasma TG less than 2.0 mmol/L and total cholesterol less than 90th percentile and HDL cholesterol greater than 10th percentile for the US population for age and sex.[15] No subject was taking any medication known to affect lipid metabolism other than insulin, and all had stable weight for at least 1 month before entry into the study. The duration of diabetes in seven subjects with IDDM was 16.3 ± 0.8 years (range, 13 to 19), and all had an absolute deficiency of endogenous insulin production as evidenced by an absent C-peptide response to the test meal. Five subjects with IDDM were treated with a conventional insulin regimen consisting of intermediate-acting and regular insulin, and two were treated with continuous subcutaneous insulin infusion. Mean hemoglobin A_{1C} concentration in subjects with IDDM was 9.9% ± 0.4% (range, 8.8% to 11.7%).

Subjects with IDDM had previously been instructed in and adhered to a diet recommended by the American Diabetes

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Association.[16] Individualized weight-maintaining diabetic diets were designed for each control subject, and dietary patterns were followed for 2 weeks before receiving the high-fat test meal. Subjects provided written informed consent, and the protocol was approved by the Institutional Review Board of the University of Chicago.

Study Design

Subjects with IDDM were admitted to the Clinical Research Center of The University of Chicago Hospital the evening before the test meal. An intravenous infusion of regular human insulin (Eli Lilly & Co, Indianapolis, IN) was started in a forearm vein, and blood glucose was held constant in the 5- to 6-mmol/L range overnight by adjusting the insulin infusion rate according to the method described by White et al.[17] Blood sampling was performed through a second intravenous-sampling catheter inserted into the opposite forearm. The arm was maintained in a heating blanket to ensure arterialization of the venous sample. The subjects were given a high-fat mixed meal containing 60 g fat/m^2 body surface area and consisting of 66% calories as fat, 18% as carbohydrate, and 16% as protein. The meal, ingested in 20 minutes, provided 1,363 cal/100 g fat, 239 mg cholesterol/100 g fat, and a polyunsaturated to saturated fatty acid ratio of 0.28, and consisted of whole wheat bread with margarine and peanut butter, peach halves with pasteurized whipping cream, whole milk with heavy cream, and an omelette consisting of egg substitute with margarine, heavy cream, and cheddar cheese. After completing the meal, subjects did not eat again for 24 hours but were allowed free access to water after 8 hours. Blood samples for glucose, insulin, C-peptide, and lipid parameters were drawn before the meal and every hour after the meal until 6 hours, then every 2 hours until 14 hours, and at 24 hours. In addition, blood for detailed analysis of HDL was drawn at 0, 4, 8, and 12 hours after the meal. At the start of the test meal, the insulin infusion was increased incrementally so that the peak postprandial glucose level did not increase above 10.0 mmol/L. The rate was then slowly decreased, and after 6 to 8 hours the glucose level was held constant between 5 and 6 mmol/L by a variable-rate insulin infusion.

Controls were admitted to the Center on the morning of the test meal following a 14-hour overnight fast. They did not receive an insulin infusion, and a single sampling catheter was inserted into a forearm vein. The test meal and sampling was identical to that described for subjects with IDDM.

Laboratory Measurements

Serum insulin[18] and plasma C-peptide[19] levels were measured as previously described. Plasma glucose level was measured by a glucose analyzer (model 23A; YSI Yellow Springs, OH).

Blood samples for lipid analysis were collected in tubes containing a final concentration of 0.1% EDTA. Plasma was immediately separated by centrifugation (3,000 rpm) for 10 minutes at 4°C. Enzymatic kits were used to determine TG (Boehringer Mannheim, Indianapolis, IN), cholesterol, and cholesteryl esters (Lancer, St Louis, MO). Phospholipids were determined by the Bartlett inorganic phosphorus method.[20] All measurements were performed in the chemistry core laboratory for the Specialized Centre of Research (SCOR) in Atherosclerosis at the University of Chicago according to standardization criteria established by the Centers for Disease Control–National Heart, Lung, and Blood Institute Standardization Program. In addition, protein content was measured by a modified Lowry procedure[21] with sodium dodecyl sulfate to disrupt the lipid micelles.

All blood samples for detailed HDL determination were adjusted to a final concentration of 1.2 g/L sodium EDTA, 1 mmol/L phenylmethylsulfonyl fluoride, 0.1 g/L sodium azide, 1 mmol/L BHT, 80 mg/L chloramphenicol, 80 ng/L gentamicin sulfate, and 10,000 U/L kallikrein inhibitor. Lipoprotein fractions were isolated by density gradient ultracentrifugal flotation using a simplified gradient procedure (2 mL plasma, discontinuous 3% to 20% sodium bromide gradient, equilibrium centrifugation for 66 hours at 38,000 rpm and 15°C in a Beckman SW 41Ti rotor). With this gradient, the lipoproteins showed bands between density 1.006 and 1.25 g/mL (Fig 1). The density of the fractions was determined from the refractive index of the solution based on the density of a reference solution of sodium bromide. Fractions of 0.4 mL were collected using an ISCO gradient collector with UV monitor at 280 nm (Instrument Specialties, Lincoln, NE). Fractions corresponding to the relevant lipoprotein peaks were pooled (Fig 1), dialyzed in Tris-buffered saline (10 mmol/L Tris, 150 mmol/L NaCl, 0.01% EDTA, and 20 mmol/L NaN_3, pH 7.4), and used for lipid and lipoprotein analysis.

The size of lipoprotein particles was determined in a nondenaturing gel system using commercially prepared 4% to 30% polyacrylamide gels (Pharmacia, Piscataway, NJ) following procedures recommended by the manufacturer. Thirty micrograms of protein was applied as a mixture by volume with three parts sample and one part solution of 40% sucrose with 0.01% bromphenol blue. A mixture of standard molecular weight proteins (HMW Calibration Kit; Pharmacia) consisting of thyroglobulin (radius, 8.50 nm), ferritin (6.10 nm), catalase (5.20 nm), lactate dehydrogenase (4.08 nm), and bovine serum albumin (3.55 nm) was included on a separate lane in each gel. The gels were stained with Coomassie G250 in perchloric acid (0.1% stain and 5% perchloric acid), destained, and stored in 7% acetic acid (Fig 2). The radius of the particles was assessed after densitometric scanning (Instruments Specialties) based on the relative distance of migration of the standard. The total area under the HDL peak and its main subfractions (HDL_2 and HDL_3) was analyzed from the output of the scanner by a computerized curve-fitting digitizer program (Sigma Scan; Jandel Scientific, Corte Madera, CA). The respective

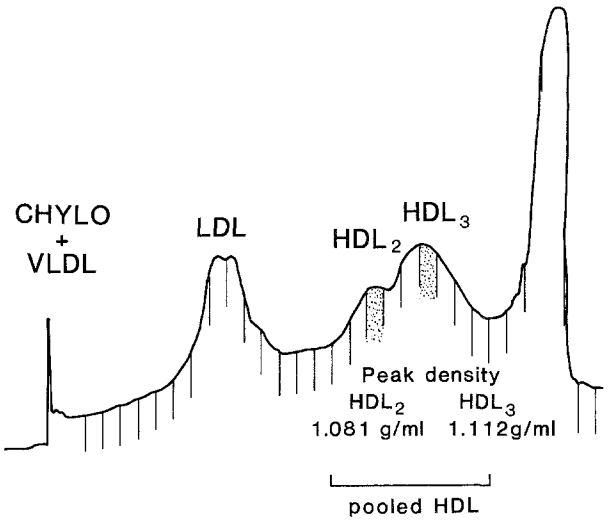

Fig 1. Representative gradient density ultracentrifugation lipoprotein profile at time 0 in a diabetic subject. HDL_2 and HDL_3 peaks can be clearly distinguished using this discontinuous 3% to 20% sodium bromide gradient, permitting detailed chemical analysis of HDL_2 and HDL_3 peak fractions and the pooled total HDL fraction. (▨) 0.4-mL fractions used for protein and lipid analysis. Densities of these fasting HDL_2 and HDL_3 peaks are shown. CHYLO, chylomicron; VLDL, very–low-density lipoprotein; LDL, low-density lipoprotein.

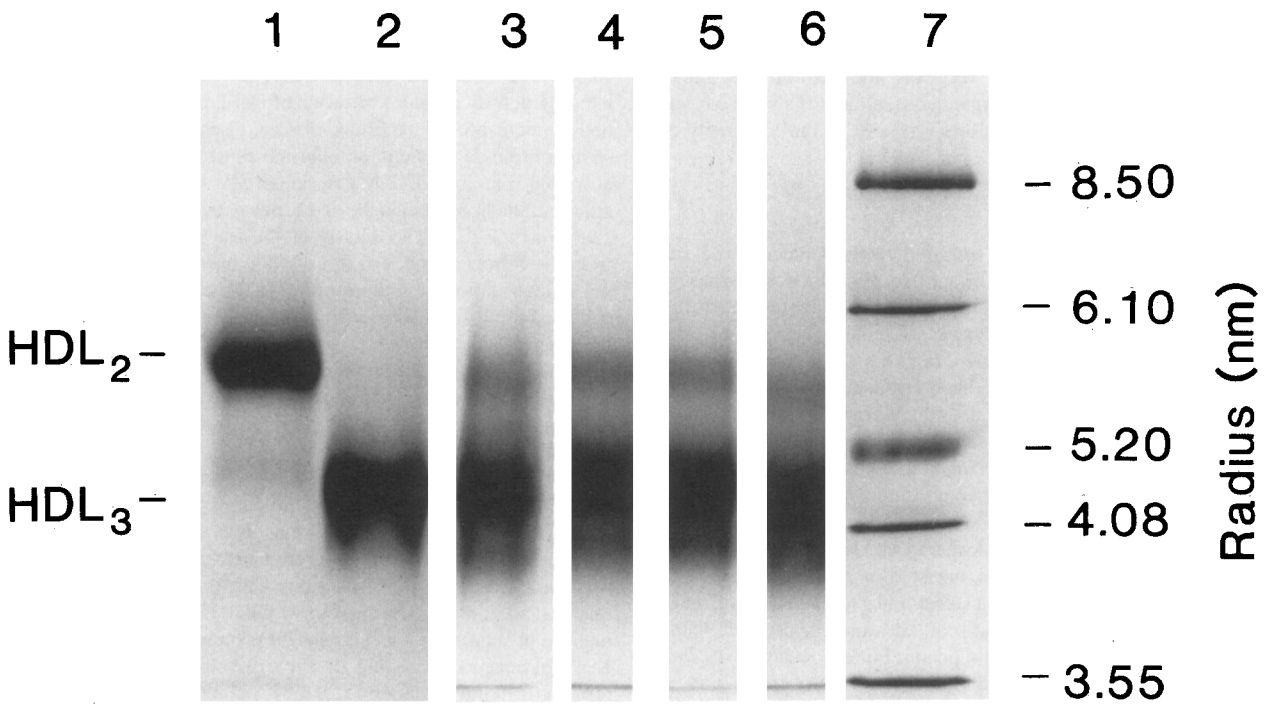

Fig 2. Representative 4% to 30% nondenaturing polyacrylamide GGE pattern from a normal control. Lanes 1 and 2, isolated HDL_2 and HDL_3, respectively, corresponding to peak fractions indicated in Fig 1. Lanes 3 to 6, pooled total HDL at 0, 4, 8, and 12 hours, respectively. Lane 7, are particle size standards.

areas of HDL_2 and HDL_3 were resolved by a perpendicular line at the nadir between the respective peaks.

Statistical Methods

All results are expressed as the mean ± SEM. Baseline differences between controls and subjects with IDDM were tested by the two-sample *t* test. Areas under glucose and insulin concentration curves were calculated by the trapezoid rule and used as summary measures for comparison. Postprandial responses of lipids and lipoproteins were tested by repeated-measures ANOVA with a Tukey allowance for multiple comparisons ($P < .05$ was regarded as statistically significant). Within-group changes over time were tested for significance using the paired *t* test.

Data analysis was performed using the Statistical Analysis System (SAS Version 6 for Personal Computers; SAS Institute, Cary, NC).

RESULTS

Fasting and Postprandial Glucose and Insulin Concentrations

After the overnight insulin infusion maintaining euglycemia, fasting glucose level in IDDM subjects was 5.7 ± 0.2 mmol/L, versus 5.0 ± 0.2 mmol/L in controls ($P < .05$). Following ingestion of the test meal, glucose concentration increased to a peak of 8.8 ± 0.6 mmol/L at 1 hour in IDDM subjects and then stabilized between 5 and 6 mmol/L from 6 hours onward. Glucose concentration increased minimally after the meal in control subjects, and the area under the glucose concentration curve was greater in subjects with IDDM (142.3 ± 3.5 *v* 120.2 ± 3.3 mmol/L/h, $P < .001$) (Fig 3).

Fasting insulin concentrations were higher in subjects with IDDM (78.0 ± 6.8 *v* 37.7 ± 5.2 pmol/L, $P < .001$). Peak postprandial insulin level was 360.0 ± 69.6 pmol/L at 3 hours in subjects with IDDM and 166.2 ± 43.1 pmol/L at 1 hour in controls. Although the absolute level was significantly greater in subjects with IDDM ($P < .01$), this represented a 3.9-fold increase over baseline, versus a comparable 4.4-fold increase in controls (Fig 3).

Fasting and Postprandial Lipid Concentrations

There were no significant differences between subjects with IDDM and controls for fasting TG (0.75 ± 0.04 *v* 0.82 ± 0.07 mmol/L, respectively), HDL cholesterol (1.15 ± 0.07 *v* 1.13 ± 0.05 mmol/L), or total cholesterol (3.93 ± 0.32 *v* 4.10 ± 0.32 mmol/L). Plasma TG concentrations increased to a maximum at 4 to 5 hours in both groups (peak TG, 1.82 ± 0.36 mmol/L in IDDM and 1.78 ± 0.33 in controls). There were no significant differences in the TG increment, peak TG response, or timing of the peak response between the groups. Total cholesterol tended to decrease overall with time after the test meal, but there was extreme interindividual variability. Overall, no clearly significant trends were detected.

Fasting HDL Composition

Total HDL, HDL_2, and HDL_3 composition did not differ significantly between subjects with IDDM and controls. HDL_3 composition most closely approximated total HDL composition, whereas HDL_2 had proportionately greater

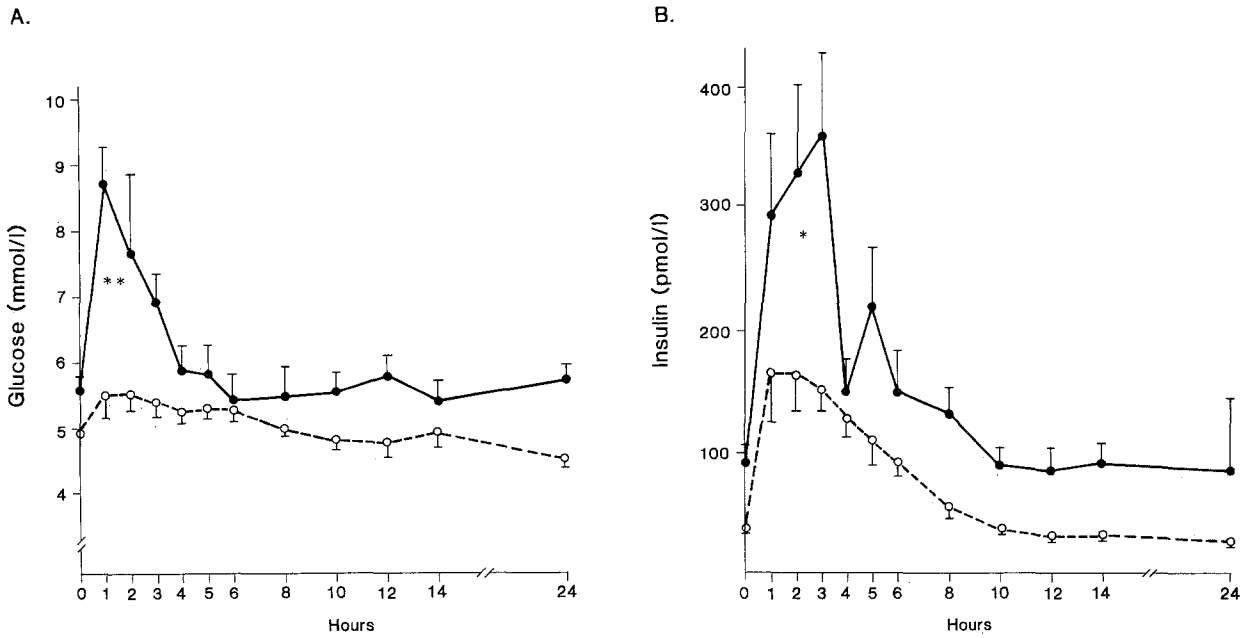

Fig 3. Fasting and postprandial (A) glucose and (B) insulin concentrations for subjects with IDDM (●) and controls (○) at baseline and for 24 hours following ingestion of the test meal (ingested at time 0). Fasting and postprandial glucose and insulin levels were greater in subjects with IDDM. Statistics are for differences between areas under the response curves. *$P < .01$, **$P < .001$. Bars denote the mean ± SEM.

TG, cholesterol, and phospholipids and less protein (Table 1).

Postprandial Changes in HDL Composition

Significant changes in particle composition occurred in total HDL, HDL_2, and HDL_3 following ingestion of the meal (Fig 4). TG increased at 4 to 8 hours with a corresponding decrease in cholesteryl ester at 4 to 8 hours in all fractions, and phospholipids increased significantly at 8 hours in total HDL and HDL_3. Changes in composition after the meal were similar in subjects with IDDM and controls. To more clearly delineate particle core compositional changes over time, core TG content was also calculated as (TG/TG + CE) × 100, where CE is cholesteryl-ester.

When phospholipid, free cholesterol, and protein were analyzed as percentage surface of the particle, there were no significant differences between IDDM and control subjects at zero hours, but at 12 hours after the meal, the percentage surface phospholipid (phospholipid/[phospholipid + protein + free cholesterol] × 100) was greater in subjects with IDDM than in controls (61.0% ± 3.8% *v* 45.7% ± 4.2%, respectively, $P < .02$) and the percentage protein was less (34.5% ± 4.1% *v* 49.4% ± 4.5%, respectively, $P < .05$).

Postprandial Particle Density Changes

Fasting HDL_2 and HDL_3 peak densities obtained from peak fractions by gradient density ultracentrifugation did not differ between subjects with IDDM and controls (HDL_2 = 1.081 ± 0.003 g/mL and HDL_3 = 1.112 ± 0.0032

Table 1. Fasting Total HDL, HDL_2, and HDL_3 Percentage Composition for Subjects With IDDM and Controls

	TG	Protein	FC	CE	PL	Core TG
IDDM subjects						
Total HDL	4.0 ± 0.5	41.8 ± 1.0	1.8 ± 0.2	17.0 ± 1.1	35.4 ± 1.3	19.1 ± 2.3
HDL_2	5.4 ± 0.9*	29.8 ± 2.8†	2.9 ± 0.3†	17.7 ± 1.6	44.2 ± 3.9*	23.7 ± 4.1*
HDL_3	3.6 ± 0.3	42.9 ± 1.5	1.4 ± 0.2	19.1 ± 0.6	32.9 ± 1.4	15.9 ± 1.3
Controls						
Total HDL	4.3 ± 0.4	43.0 ± 1.2	2.1 ± 0.1	16.8 ± 0.7	33.0 ± 1.4	20.5 ± 1.9
HDL_2	5.9 ± 0.8*	33.3 ± 1.4†	3.5 ± 0.4†	19.9 ± 1.0†	37.5 ± 0.9†	22.8 ± 0.3†
HDL_3	3.5 ± 0.5	45.2 ± 1.5	1.5 ± 0.1	18.3 ± 0.7†	31.4 ± 1.3	16.2 ± 0.2†

NOTE. Values are expressed as percentage composition by weight (mean ± SEM). Statistics are for differences from total HDL composition. There were no statistically significant differences between IDDM subjects and controls.

Abbreviations: FC, free cholesterol; CE, cholesteryl ester; PL, phospholipid.

*$P < .005$.

†$P < .0005$.

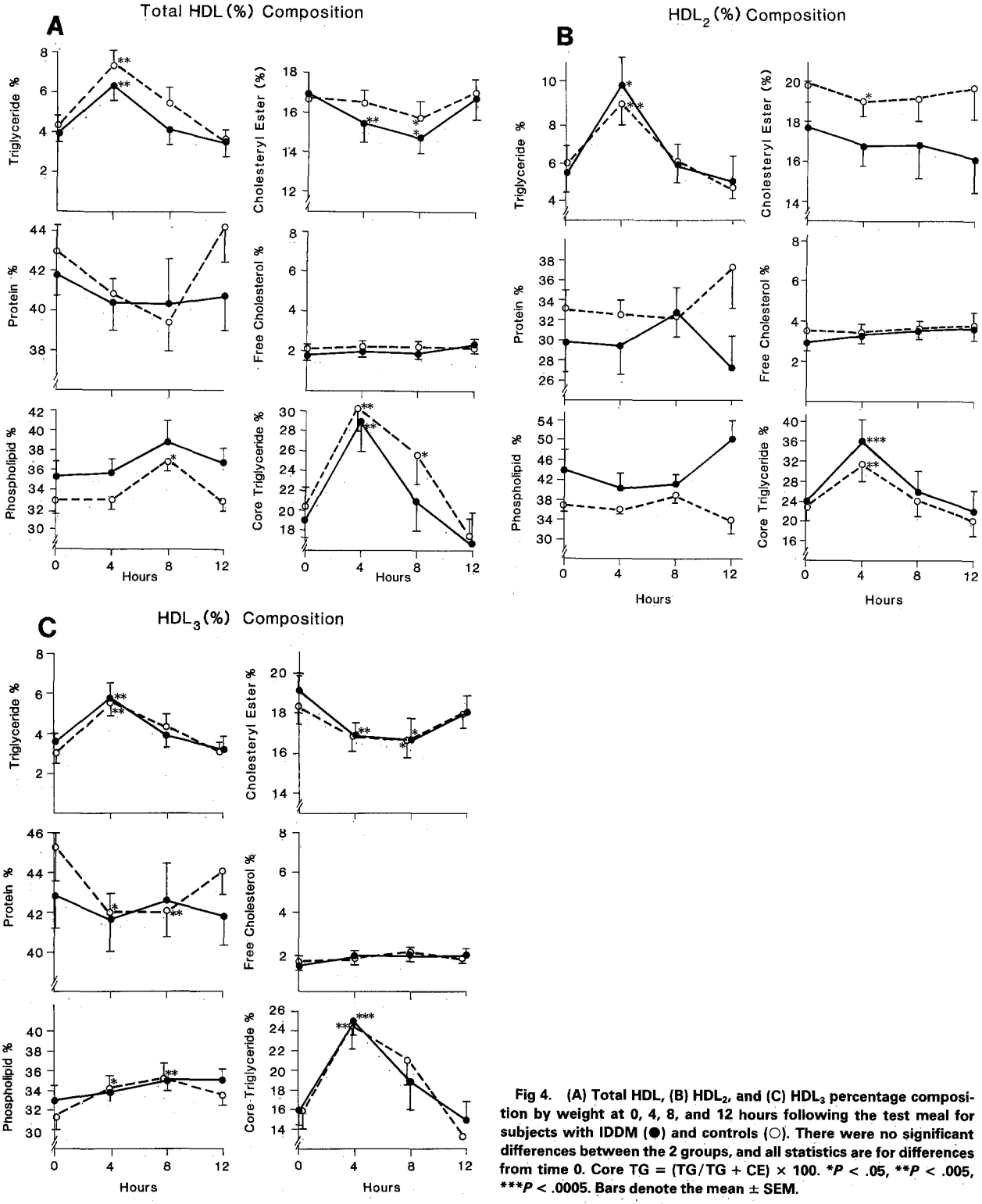

Fig 4. (A) Total HDL, (B) HDL_2, and (C) HDL_3 percentage composition by weight at 0, 4, 8, and 12 hours following the test meal for subjects with IDDM (●) and controls (○). There were no significant differences between the 2 groups, and all statistics are for differences from time 0. Core TG = (TG/TG + CE) × 100. *$P < .05$, **$P < .005$, ***$P < .0005$. Bars denote the mean ± SEM.

g/mL in both groups). The density of both HDL_2 and HDL_3 tended to decrease slightly at 4 and 8 hours in subjects with IDDM (HDL_2 = 1.079 ± 0.003 g/mL and HDL_3 = 1.110 ± 0.003 g/mL at 8 hours) and controls (HDL_2 = 1.075 ± 0.002 g/mL and HDL_3 = 1.104 ± 0.003 g/mL at 8 hours), reaching significance only in controls ($P < .05$ for HDL_2 change and $P < .002$ for HDL_3 at 8 hours). Although the shift to a lower density appeared to be greater in controls than in subjects with IDDM, the differences between the groups were not significant.

Postprandial Changes in HDL Size

The radius of the HDL_2 band at baseline was 5.65 ± 0.07 nm in subjects with IDDM versus 5.61 ± 0.07 nm in controls (P = NS), and the HDL_3 radius was 4.64 ± 0.05 nm in subjects with IDDM versus 4.67 ± 0.07 nm in controls (P = NS). Despite marked postprandial changes in the relative distribution of particles within these discrete subpopulations of HDL (discussed later), there were no significant shifts in mean particle size after the meal (Fig 5).

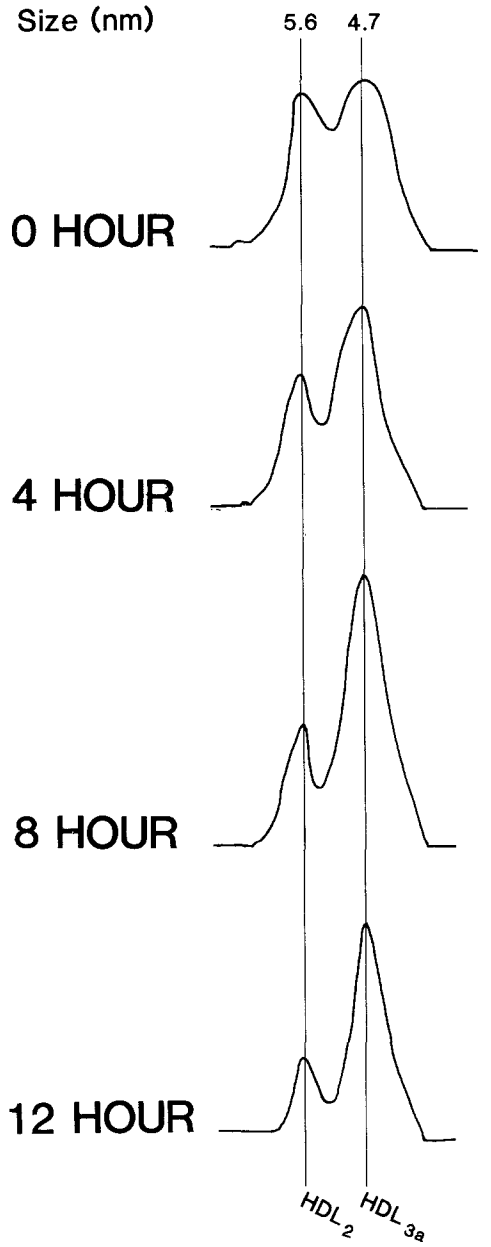

Fig 5. Densitometry scan profile of the gradient gel for total HDL of a representative diabetic subject at 0, 4, 8, and 12 hours. HDL subfraction peaks are well separated by size, with HDL_2 being the larger but less abundant subfraction. The relative proportion of HDL_3 to HDL_2 increased substantially after the meal, but the mean size of the subfractions remained constant.

Table 2. Postprandial Changes in the Relative Concentration of HDL_3 and HDL_2

Time	HDL_3/HDL_2 by Density Gradient Ultracentrifugation	HDL_3 Area/HDL_2 Area by GGE
IDDM subjects		
0 h	1.27 ± 0.07	1.47 ± 0.24
4 h	1.24 ± 0.06	1.60 ± 0.25
8 h	1.39 ± 0.07†	1.92 ± 0.28
12 h	1.44 ± 0.09‡	2.22 ± 0.34*
Controls		
0 h	1.38 ± 0.09	1.69 ± 0.67
4 h	1.34 ± 0.06	1.97 ± 0.79
8 h	1.48 ± 0.06*	2.13 ± 0.76
12 h	1.57 ± 0.06*	2.37 ± 0.80

NOTE. Statistics are for differences from 0 h.
*P < .05.
†P < .005.
‡P < .0005.

Postprandial Changes in the Concentration of HDL_3 Relative to HDL_2

The ratio of HDL_3 to HDL_2 obtained from the peak optical density by gradient density ultracentrifugation (Fig 1) increased significantly in subjects with IDDM and controls at 8 to 12 hours (Table 2).

The ratio of HDL_3 to HDL_2 obtained by GGE was determined from the area under the HDL subfraction curves. There was a significant increase in the ratio in subjects with IDDM at 8 and 12 hours. Changes in the ratio of the HDL_3/HDL_2 area over time in controls did not reach statistical significance, although the trends were similar to those in subjects with IDDM. There were no significant differences between controls and IDDM subjects. With the data from both groups combined, both the increase in the ratio of HDL_3/HDL_2 optical density by gradient density ultracentrifugation and the increase in the ratio of HDL_3/HDL_2 areas by GGE were highly significant (P = .0005) at 8 and 12 hours.

DISCUSSION

In the present study, we were unable to demonstrate any significant abnormality in postprandial HDL composition, density, size, or subfraction distribution in IDDM patients. These results, together with those of previous studies demonstrating normal or increased HDL levels in IDDM,[5-8] suggest that abnormalities of postprandial HDL metabolism are unlikely to play a significant role in the process of premature atherosclerosis thought to occur in normolipidemic IDDM patients.[1,2]

Our patients were in moderate glycemic control and received an overnight euglycemic insulin infusion, and the present study design therefore does not rule out possible abnormalities of HDL metabolism that might occur in poorly controlled IDDM patients. HDL levels have been inversely correlated with glycemic control, but most of the studies do not differentiate between insulin-treated type I or type II subjects with IDDM.[22-24]

Since HDL can be viewed as the modified product of redundant surface lipids and proteins generated during the

process of TG transport,[9] the postprandial state provides an excellent model for the study of this process in vivo. The changes occurring in HDL and its subfractions in subjects with IDDM and controls in the present study reflect the net balance of the process of transfer and exchange of lipids and protein with TG-rich lipoproteins, as well as the interconversion of HDL_2 and HDL_3. The changes noted during alimentary lipemia represent the product of these various processes occurring simultaneously, and are probably better understood by reference to in vitro studies as discussed below.

The postprandial TG enrichment of HDL at the expense of cholesteryl ester in IDDM subjects and controls in the present study has been demonstrated previously in different population groups by us[25-27] and others.[28,29] Although we did not study the transfer process directly, the temporal relationship with postprandial lipemia strongly suggests that this compositional change reflects the exchange of core lipids between chylomicrons and HDL under the influence of cholesteryl ester transfer protein.[30] We have extended previous observations of total HDL compositional change after a meal by analyzing the relatively pure fractions of HDL_2 and HDL_3 isolated by gradient density ultracentrifugation, and found similar compositional changes in both subfractions. In addition, HDL became enriched with phospholipid, which almost certainly reflects the transfer of surface products en masse from chylomicrons to HDL.[31] Although we were unable to determine whether HDL_2 or HDL_3 is the major recipient of surface components from chylomicrons, others have demonstrated that HDL_3 is the major acceptor.[32] Since HDL_3 is the dominant subfraction of HDL,[33] the compositional characteristics of HDL_3 might be expected to most closely approximate those of total HDL, as was evident both in IDDM subjects and in controls in the present study. HDL_2, on the other hand, had a greater proportion of TG, cholesterol, and phospholipids and less protein than HDL_3.

The only significant difference in HDL composition between the subjects with IDDM and controls relates to the relative proportions of surface constituents of the HDL_2 subfraction. Fasting HDL_2 samples from subjects with IDDM tended to have a greater proportion of phospholipid and less protein, and this difference between the groups was accentuated after the meal, reaching significance at 12 hours. The significance of the greater postprandial enrichment of HDL_2 with phospholipid in patients with IDDM is not known, and its relationship, if any, to atherogenesis remains to be determined.

Our observation of a relative increase in HDL_3 versus HDL_2 at 8 to 12 hours after the high-fat meal might be explained, at least in part, by the preferential enrichment of HDL_3 with surface components of chylomicron particles undergoing hydrolysis by lipoprotein lipase. Since our methodology was qualitative and not quantitative and since HDL_2 and HDL_3 demonstrate overlapping density and size ranges and cannot be quantitatively separated using this methodology, we were unable to determine whether HDL_2 also increased in mass, albeit to a lesser extent. The demonstration of similar compositional changes in the HDL_2 subfraction after the meal suggests that it is also an active participant in the process of exchange and transfer with chylomicrons. Groot and Scheek[34] have previously demonstrated that both HDL_2 and HDL_3 can accept released surface components from TG-rich particles during lipolysis. Taskinen and Kuusi[35] found a postprandial increase in HDL_2 mass in normal women using the traditional ultracentrifugal density definition of HDL_2 ($1.063 < d < 1.125$ g/mL). They also analyzed postprandial changes in HDL using rate zonal techniques, and inspection of their data suggests an increase in HDL_3 as well. Of note is the fact that the HDL_3 density peak in the present study (1.112 g/mL) is within the traditional HDL_2 density range (specifically HDL_{2a}). Previous investigators have found that a significant part of the HDL_3 peak extends into the 1.100- to 1.125-g/mL region.[36,37] Our results are similar to those of Tall et al,[32] who found that both the HDL_2 peak ($d = 1.09$ to 1.11 g/mL) and the HDL_3 peak ($d = 1.11$ to 1.17 g/mL) increased after fat ingestion, but that the major increase was in the HDL_3 fraction ($d = 1.12$ g/mL).

A second possible explanation for the relative increase in HDL_3 after the meal is the net effect of the interconversion of HDL_2 and HDL_3. HDL_3 has been shown to be converted to HDL_2 when incubated with TG-rich particles in the presence of lipoprotein lipase,[38,39] and, reciprocally, HDL_2 can be converted to smaller, denser HDL_3 particles by hepatic TG lipase.[40,41] Patsch et al[28] have shown that it is the TG enrichment of HDL_2 particles that determines their rate of conversion to HDL_3, and in the postprandial state this is related to the magnitude of triglyceridemia. The net direction of these two opposing reactions will depend on the balance of influencing factors such as the activity of lipoprotein lipase and hepatic lipase, degree of enrichment of HDL_2 with TG, magnitude of transfer of surface components to HDL_2 and HDL_3, and activity of the cholesterol esterification (lecithin cholesterol acyltransferase) system.[9]

The test meal in this study was not typical of a North American breakfast. The rationale for using a high-fat stimulus was to detect subtle abnormalities in HDL metabolism that could not normally be detected in the fasting state. The fact that no abnormalities in postprandial HDL metabolism were found in individuals with IDDM in response to this high-fat meal makes it extremely unlikely that significant abnormalities exist in response to meals containing less fat.

The dynamic postprandial changes in HDL density, composition, and subfraction distribution demonstrated in this study did not differ substantially between IDDM subjects and controls, despite subtle differences in HDL surface composition. This is most likely a reflection of the normal postprandial metabolism of TG-rich particles that occurred in these subjects.[14] If premature atherosclerosis in patients with IDDM is indeed related to lipid or lipoprotein abnormalities, it would appear unlikely that perturbations in the HDL system during the postprandial period play a major role, particularly if glycemia is moderately well controlled. However, the present study does not rule out the possibility of abnormalities in composition, density, or size of postprandial HDL in diabetic patients who are in poor glycemic control.

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