Abnormal metabolism of postprandial lipoproteins in patients with non-insulin-dependent diabetes mellitus is not related to coronary artery disease

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Abstract To investigate whether abnormalities in alimentary lipemia explain the increased risk of coronary artery disease (CAD) in subjects with non-insulin-dependent diabetes mellitus (NIDDM), we performed an oral vitamin A fat-load test in four groups of men (each n = 15): *1)* NIDDM and angiographically verified CAD (DM+CAD+): *2)* CAD but no diabetes (DM−CAD+); *3)* NIDDM but no CAD, excluded by an exercise thallium scan (DM+CAD−); and *4)* healthy control subjects (DM−CAD−). The groups were matched for age and body mass index. Plasma obtained after an overnight fast and 2, 3, 4, 6, 9, 12, and 24 h after a fatty meal (78 g fat, 345,000 IU retinyl palmitate [RP]) was separated by density gradient ultracentrifugation into six fractions of triglyceride (TG)-rich lipoproteins: Svedberg flotation units (S_f) > 3200, S_f 1100–3200, S_f 400–1100, S_f 60–400, S_f 20–60, and S_f 12–20. TG, RP, and cholesterol concentrations were measured in plasma and in each lipoprotein fraction. Postprandial plasma TG responses were significantly larger in both NIDDM groups than in the healthy control group. The most marked differences were observed in the S_f 60–400 lipoproteins, whether measured as TG or RP responses. However, there were no differences between the DM+CAD+and DM+CAD− groups. The between-group differences in alimentary lipemia were only partially explained by fasting TG levels. In contrast to the healthy subjects, no significant negative correlation was observed in the NIDDM patients between alimentary lipemia and lipoprotein lipase activity, implying an abnormality of the lipolysis of TG-rich particles in NIDDM. Levels of atherogenic postprandial remnant lipoproteins are increased in NIDDM. However, in this study the magnitude of alimentary lipemia did not distinguish NIDDM patients with CAD from those without CAD symptoms and normal exercise thallium scans.—**Syvänne, M., H. Hilden, and M-R. Taskinen.** Abnormal metabolism of postprandial lipoproteins in patients with non-insulin-dependent diabetes mellitus is not related to coronary artery disease. *J. Lipid Res.* 1994. **35:** 15–26.

Supplementary key words atherogenesis • triglyceride-rich lipoproteins • chylomicron remnants • remnant lipoproteins • lipoprotein lipase • high density lipoproteins • plasminogen activator inhibitor-1

Hypertriglyceridemia, caused mainly by overproduction of very low density lipoprotein (VLDL) triglyceride (TG), is one of the characteristic lipoprotein abnormalities in non-insulin-dependent diabetes mellitus (NIDDM) (1). NIDDM patients are at several-fold higher risk of coronary artery disease (CAD) than nondiabetic subjects. The underlying mechanisms of this increased risk are not completely understood (2, 3), but a high serum TG level has been found to qualify as an independent predictor of CAD among NIDDM patients in cross-sectional (4) as well as in prospective (5) studies.

Based on experimental work (6–9) and cross-sectional studies (10–14), TG-rich lipoproteins and especially chylomicron remnants (CMRs) have been implicated as risk factors for atherosclerosis and the progression of CAD (15). There is emerging evidence that these lipoproteins may be particularly atherogenic in NIDDM (16, 17). In addition to this putative direct atherogenic effect, Grundy and Vega (18) have recently drawn attention to the various metabolic consequences of hypertriglyceridemia, including postprandial lipemia, low levels of high density lipoprotein (HDL) cholesterol, and abnormalities in thrombogenic factors. So far these associations have not been addressed in NIDDM patients with CAD.

Although several studies have demonstrated enhanced postprandial lipemia in hypertriglyceridemic subjects, very few data exist on alimentary lipemia in NIDDM (19, 20). The potential significance of CMRs in the genesis of CAD in NIDDM patients has recently been recognized (20), but as yet no studies have directly compared post-

Abbreviations: VLDL, very low density lipoprotein; TG, triglyceride; NIDDM, non-insulin-dependent diabetes mellitus; CAD, coronary artery disease; CMR, chylomicron remnant; HDL, high density lipoprotein; BMI, body mass index; RP, retinyl palmitate; S_f, Svedberg flotation unit; IDL, intermediate density lipoprotein; LPL, lipoprotein lipase; HL, hepatic lipase; HbA_{1c}, glycosylated hemoglobin A_{1c}; apo, apolipoprotein; PAI-1, plasminogen activator inhibitor-1; AUC, area under curve; AUIC, area under incremental curve; LDL, low density lipoprotein; NTG, normotriglyceridemic; ANCOVA, analysis of covariance; ANOVA, analysis of variance.

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prandial lipemia in NIDDM patients with or without CAD, or in CAD patients with or without NIDDM. In the present study, we performed the oral vitamin A fat-load test in four well-defined groups of men with one of these diseases, both, or neither. Our main purpose was to investigate whether patients with NIDDM and angiographically verified CAD differ from healthy individuals, subjects with NIDDM but no evidence of CAD, and from nondiabetic CAD patients with respect to postprandial lipoprotein metabolism.

SUBJECTS AND METHODS

Subjects

Between September 1989 and February 1992, all patients with a history of NIDDM undergoing coronary angiography at the Helsinki University Central Hospital were screened for this study. Subjects aged 65 years or younger who had at least 50% stenosis in one or more major coronary branches were eligible. Exclusion criteria were hepatic, thyroid, or renal dysfunction, and the use of lipid-lowering medications or sex steroids. From a total of 50 male NIDDM patients with CAD, 15 men were randomly selected for the present study (group 1, DM+ CAD+). Groups 2, 3, and 4 were matched with group 1 for sex, age, and body mass index (BMI). Group 2 (DM−CAD+) consisted of 15 patients who also had angiographically verified CAD, but no history of diabetes and normal fasting blood glucose and glycosylated hemoglobin levels. The 15 subjects in group 3 (DM+CAD−) were recruited among the NIDDM patients of outpatient diabetes clinics. They were required to have no history or symptoms of CAD. To exclude silent ischemic heart disease, they underwent a maximal exercise test with thallium-201 imaging (21). Group 4 (DM−CAD−) also comprised 15 men. They had no known diseases, were not taking any medications, and had normal cardiovascular physical examinations, resting ECGs, symptom-limited exercise ECG tests, and laboratory screens including fasting blood glucose and glycosylated hemoglobin.

Of the patients with CAD, 11 in each group later underwent a coronary artery bypass operation. Coronary angioplasty was performed in three group 1 patients and in four group 2 patients. The cardiac medications consisted of β-blockers (group 1, 12; group 2, 14 patients), calcium channel blockers (10 and 8), and long-acting nitrates (13 and 15, respectively). Only one patient in group 1 was taking a diuretic. None of the patients had clinically or radiologically evident congestive heart failure, and none had suffered a myocardial infarction within several months before the metabolic studies. All had stable angina pectoris. Thirteen patients in group 1 and 10 in group 2 had New York Heart Association grade 3 symptoms, and the remaining had grade 2 angina.

All NIDDM patients fulfilled the World Health Organization criteria for diabetes (22). In group 1, five patients were treated with diet only, eight patients were on sulfonylurea therapy, one was taking guar gum, and one had a combination of a sulfonylurea and insulin. In group 3, four patients were on diet, five on sulfonylureas, five on a combination of a sulfonylurea and metformin, and one on insulin therapy. Only one patient in this group was taking a β-blocker for hypertension.

All subjects gave their informed consent to participate in the study. The study protocol was approved by the Ethical Committee of the First Department of Medicine, Helsinki University Central Hospital.

Methods

Oral fat-load test. The oral fat-load test procedure has been previously described in detail (23). After an overnight fast, blood samples were drawn between 7:30 and 8 AM. The patients then received a standardized fatty meal consisting of 200 ml cream and an egg yolk, containing 78 g fat, 490 mg cholesterol, and 760 kcal energy. Vitamin A (345,000 IU) was added to the meal to label chylomicrons and their remnants with retinyl palmitate (RP). The postprandial blood samples were taken into tubes containing EDTA 2, 3, 4, 6, 9, 12, and 24 h after the meal. The patients remained fasting, with only water allowed, until the 9-h sample had been drawn. At 5 PM they received an ordinary hospital meal, and at 7:30 PM, a light snack.

Density gradient ultracentrifugation. Plasma obtained during the fat-load test was fractionated as described in an earlier publication (23). Briefly, three fractions in the chylomicron size range were first separated by sequential density gradient ultracentrifugation. These fractions will be referred to as chylo$_1$ (Svedberg flotation units [S_f] $>$ 3200), chylo$_2$ (S_f 1100–3200), and chylo$_3$ (S_f 400–1100). In a second ultracentrifugation chylomicron-free plasma was used, and VLDL$_1$ (S_f 60–400), VLDL$_2$ (S_f 20–60), and intermediate density lipoproteins (IDL, S_f 12–20) were obtained. The chylomicron fractions consist almost exclusively of intestinally derived particles, whereas the other three fractions contain lipoproteins of both hepatic and intestinal origin. As these two classes of particles cannot be separated according to their physical characteristics, we used RP to label the intestinal lipoproteins (20).

Lipoprotein ultracentrifugation. Fasting serum lipoprotein fractions were separated by sequential flotation in an ultracentrifuge (24). Briefly, after isolation of chylomicrons from fresh fasting serum, VLDL ($d < 1.006$ g/ml), IDL ($1.006 < d < 1.019$ g/ml), LDL ($1.019 < d < 1.063$ g/ml), HDL$_2$ ($1.063 < d < 1.125$ g/ml), and HDL$_3$ ($1.125 < d < 1.210$ g/ml) were separated as described (25).

Plasminogen activator inhibitor-1 activity. Venous blood for

the determination of plasminogen activator inhibitor-1 (PAI-1) activity was gently drawn into a 10-ml syringe without stasis from the indwelling catheter after all other fasting samples had been drawn, the patient having lain supine for at least 10 min. The blood was mixed with 0.129 M sodium citrate (9:1, vol/vol) and centrifugated to platelet-poor plasma at 1400 *g* for 30 min. The samples were stored at −70°C until used. PAI-1 activity was determined with the functional assay COATEST PAI (Chromogenix, Mölndal, Sweden).

Postheparin lipase activities. In the morning preceding the day of the oral fat-load test, an intravenous bolus injection of heparin (100 IU per kg of body weight) was given to the fasted subject. Blood was drawn 5 and 15 min after the injection into lithium–heparin tubes chilled on ice. Plasma was promptly separated at 4°C and stored at −20°C. Lipoprotein lipase (LPL) and hepatic lipase (HL) activities were measured by an immunochemical assay as previously described (26).

Laboratory measurements. TG and cholesterol concentrations were determined by automated enzymatic methods (23). RP was measured by high performance liquid chromatography (23, 27). Blood glucose, serum C-peptide, serum free insulin, and glycosylated hemoglobin (HbA_{1c}) were measured as described (23). Apolipoprotein (apo) E phenotyping was done from serum by isoelectric focusing (28).

Quantification of postprandial lipemic responses. Postprandial TG, cholesterol, and RP responses were quantified in plasma and in the lipoprotein fractions as areas under the time-dependent concentration curves using the trapezoid rule (29). The total 24-h area under the curve (AUC, not normalized to fasting TG levels), i.e., between the concentration curve and zero, was calculated for plasma and all fractions. In addition, we determined the postprandial TG responses normalized to fasting levels for the first 9 h of the fat-load test. These 9-h areas under the incremental curve (AUIC) were calculated after subtracting the fasting value from each postprandial measurement. Because fasting RP concentrations were virtually always zero, only AUCs were used to characterize postprandial RP responses.

Statistical analyses. Data are expressed as mean ± SEM. TG, RP, plasma insulin, and PAI-1 values were logarithmically transformed before statistical comparisons and correlation analyses. Group means were compared by the analysis of variance, followed by five pairwise comparisons: groups 1, 2, and 3 versus group 4; group 1 versus group 2; and group 1 versus group 3 (30). Because all these pairwise tests have a rational theoretical basis, no corrections for multiple comparisons were made (31). Analysis of covariance was used to adjust the measures of postprandial lipemia for fasting TG concentrations and other selected metabolic variables. Pearson's coefficients were calculated to study correlations.

RESULTS

The groups were well matched for age and BMI (**Table 1**). The known duration of diabetes and the degree of glycemic control were similar in the diabetic groups.

TABLE 1. Clinical and metabolic characteristics of the patient groups

Variable	Group 1 DM + CAD + (n = 15)	Group 2 DM − CAD + (n = 15)	Group 3 DM + CAD − (n = 15)	Group 4 DM − CAD − (n = 15)
Age, yr	54.3 ± 1.6	55.2 ± 1.9	54.3 ± 2.2	56.3 ± 1.0
Body mass index, kg/m²	28.0 ± 0.7	27.7 ± 0.7	28.1 ± 0.9	28.0 ± 0.7
Duration of diabetes, yr	8.3 ± 1.5		6.4 ± 1.7	
Fasting blood glucose, mmol/l	8.2 ± 0.8[e]	4.8 ± 0.09	8.4 ± 0.4[e]	4.7 ± 0.1
HbA_{1c}, %[a]	7.2 ± 0.4[e]	5.2 ± 0.07	6.9 ± 0.3[e]	5.0 ± 0.1
Fasting C-peptide, nmol/l[b]	0.78 ± 0.07	0.83 ± 0.05	0.94 ± 0.10	0.78 ± 0.10
Fasting free insulin, mU/l	10.6 ± 1.6[e]	8.3 ± 1.0[e]	9.9 ± 1.5[e]	5.3 ± 0.7
Fasting plasma cholesterol, mmol/l	5.4 ± 0.2	5.3 ± 0.2	5.5 ± 0.2	5.2 ± 0.3
Fasting LDL cholesterol, mmol/l[c]	3.4 ± 0.2	3.3 ± 0.2	3.3 ± 0.2	3.3 ± 0.2
Fasting HDL cholesterol, mmol/l[c]	1.08 ± 0.05	1.22 ± 0.07	1.24 ± 0.09	1.24 ± 0.07
ApoE phenotype, n				
3/2	0	0	0	2
3/3	8	11	10	6
4/3	7	4	5	6
4/4	0	0	0	1
Lipoprotein lipase[d]	20.8 ± 1.2[e]	22.7 ± 1.7	23.1 ± 1.7	26.6 ± 2.0
Hepatic lipase[d]	42.3 ± 3.7[f]	30.6 ± 3.0	35.1 ± 3.9	34.1 ± 3.8

The data are mean ± SEM (except apoE phenotypes).
[a] Glycosylated hemoglobin; normal 4.0 to 6.0%.
[b] One insulin-treated patient from each diabetic group excluded.
[c] Data not available for one patient in group 1 and one patient in group 2.
[d] Postheparin plasma activities, expressed as μmol free fatty acids (FFA) • h^{-1} • ml^{-1}.
[e] Significantly different from group 4.
[f] Significantly different from group 2.

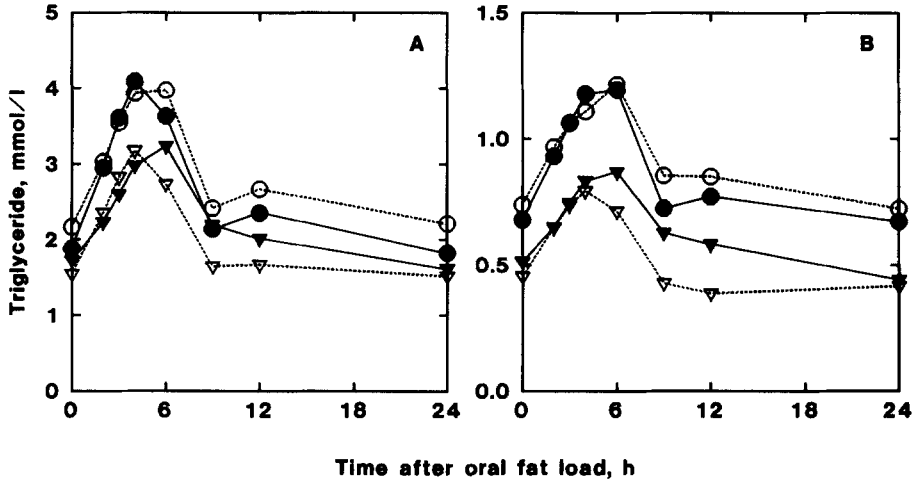

Fig. 1. Line graphs showing the postprandial triglyceride responses in plasma (panel A) and in the $VLDL_1$ (S_f 60–400) fraction (panel B). Group 1, DM+CAD+ (●); Group 2, DM−CAD+ (▼); Group 3, DM+CAD− (○); Group 4, DM−CAD− (▽).

Plasma insulin levels were significantly lower in the group of healthy subjects than in groups 1, 2, or 3. Plasma and low density lipoprotein (LDL) cholesterol concentrations were similar in all groups. HDL cholesterol was lowest in group 1, but the differences were not significant. The distribution of apoE phenotypes did not significantly differ among the groups. The postheparin plasma LPL activity was lowest and HL was highest in group 1.

Postprandial lipemic responses

Plasma triglycerides. Postprandial plasma TG responses are shown in **Fig. 1A** and **Table 2.** Overall, the fasting TG level varied between 0.84 and 3.80 mmol/l, and the mean values of the four groups were: 1.88 ± 0.12 (group 1); 1.72 ± 0.21 (group 2); 2.17 ± 0.23 (group 3); 1.54 ± 0.15 mmol/l (group 4). Only the difference between groups 3 and 4 was significant ($P < 0.02$). The postprandial TG values of both diabetic groups (1 and 3) were significantly higher than those of the healthy controls (group 4) at virtually all time points after the fatty meal. These differences resulted in significantly larger overall (AUCs) and incremental (AUICs) postprandial TG responses in the diabetic patients in comparison with the healthy subjects. Fig. 1A shows that the postprandial TG curves of the two diabetic groups were almost identical. The diabetic patients tended to have a secondary rise in TG levels at 12 h as a result of the ordinary meal they received at 5 PM, but this rise was not observed in the

TABLE 2. Triglyceride responses in the oral fat-load test

Fraction	Group 1 DM+CAD+	Group 2 DM−CAD+	Group 3 DM+CAD−	Group 4 DM−CAD−	P (ANOVA)[a]	P (ANCOVA)[b]
Plasma, AUC	60 ± 5[c]	52 ± 6	67 ± 6[d]	46 ± 5	0.034	0.489
$Chylo_1$, AUC	1.9 ± 0.3	1.6 ± 0.3	2.2 ± 0.2[d]	1.3 ± 0.3	0.031	0.273
$Chylo_1$, AUC	1.7 ± 0.3	1.7 ± 0.3	1.7 ± 0.2	1.2 ± 0.2	0.208	0.802
$Chylo_3$, AUC	2.9 ± 0.6	2.6 ± 0.2	3.5 ± 0.5[c]	2.0 ± 0.5	0.066	0.742
$VLDL_1$, AUC	20 ± 2[d,e]	15 ± 2	21 ± 3[d]	12 ± 2	0.008	0.671
$VLDL_2$, AUC	6.2 ± 0.5	5.5 ± 0.6	7.7 ± 1.1	5.7 ± 0.6	0.205	0.459
IDL, AUC	2.3 ± 0.2	2.0 ± 0.2	2.1 ± 0.2	2.6 ± 0.3	0.177	0.025
Plasma, AUIC	11 ± 1[c,e]	8.0 ± 1.2	10 ± 1	8.1 ± 1.7	0.039	0.221
$Chylo_1$, AUIC	1.6 ± 0.2	1.6 ± 0.3	1.8 ± 0.2[c]	1.3 ± 0.3	0.039	0.316
$Chylo_2$, AUIC	1.3 ± 0.2	1.1 ± 0.2	1.2 ± 0.1	0.9 ± 0.2	0.209	0.694
$Chylo_3$, AUIC	1.5 ± 0.2	1.1 ± 0.2	1.4 ± 0.2	1.2 ± 0.4	0.107	0.487
$VLDL_1$, AUIC	2.9 ± 0.4[d]	2.0 ± 0.3	2.6 ± 0.3[c]	1.7 ± 0.4	0.025	0.158

The data are areas under concentration curve (AUC), or under incremental concentration curve (AUIC), mmol/l • h, mean ± SEM (see Methods for definitions).

[a] Analysis of variance of logarithmically transformed data.

[b] Analysis of covariance of logarithmically transformed data, using log fasting plasma triglyceride as covariate.

[c] $P < 0.05$; [d] $P < 0.01$, compared with group 4.

[e] $P < 0.05$, compared with group 2.

nondiabetic subjects. Comparing the two groups with CAD, those with diabetes (group 1) had significantly higher TG levels than the nondiabetic CAD patients (group 2) at 2, 3, and 4 h, but not later during the test.

The nondiabetic patients with CAD (group 2) did not differ significantly from the healthy subjects (group 4) with regard to the TG concentrations at individual time points or to the overall magnitude of the TG response. However, the clearance of TG was clearly slower in group 2 (Fig. 1A). Eleven individuals in group 2 had the peak TG concentration at 6 h or later, compared with only one subject in group 4 ($P < 0.001$, Fisher's exact test).

Triglycerides in fractions $S_f > 400$. The overall postprandial TG responses in the three chylomicron fractions tended to be larger in all patient groups than in group 4, but statistical significance was reached only between groups 3 and 4 in $chylo_1$ and $chylo_3$ (Table 2). The upstroke of the TG curve was more rapid in diabetic than in nondiabetic subjects, reflected by significantly higher levels at 2, 3, and 4 h, but the differences leveled off at later time points (data not shown). No differences were observed between the two groups of diabetic patients.

Triglycerides in fractions $S_f < 400$. As shown in Fig. 1B, the most marked differences between the groups were observed in the $VLDL_1$ fraction (S_f 60–400). The diabetic groups 1 and 3 had almost twice as large overall TG responses in this fraction than group 4. The difference between groups 1 and 2 was significant as well. Also the incremental responses (AUICs) were elevated in both diabetic groups in comparison with the healthy subjects. The patients with CAD but no diabetes (group 2) tended to have somewhat higher TG levels than group 4 at 6 h after the fatty meal and later, but these differences did not reach statistical significance. Again, groups 1 and 3 showed similar postprandial profiles. The TG responses in $VLDL_2$ (S_f 20–60) and IDL (S_f 12–20) showed no major differences among any of the four groups.

Postprandial triglyceride responses adjusted for fasting TG. Because the fasting TG concentration is closely related to postprandial lipemia and because the groups differed in this respect, an analysis of covariance was performed to adjust for the differences in fasting TG levels. None of the differences in postprandial TG values remained significant after the adjustment (Table 2). By contrast, the adjusted postprandial IDL (S_f 12–20) response was significantly higher in the healthy control group than in groups 1 ($P = 0.074$), 2 ($P < 0.01$), or 3 ($P < 0.01$).

To further evaluate the interrelations of postprandial lipemia, NIDDM, and fasting TG concentrations, we selected all normotriglyceridemic (NTG, fasting TG below 1.75 mmol/l, median of the entire study population) diabetic patients from groups 1 (n = 6) and 3 (n = 6, total n = 12) and compared them with the NTG healthy individuals from group 4 (n = 9) (**Table 3**). NTG diabetic patients had significantly higher total and incremental TG responses in plasma and in the lipoprotein fractions with $S_f > 60$. As the NIDDM patients again had slightly higher mean fasting TG values than the nondiabetic NTG group, we adjusted for this difference using an analysis of covariance. As shown in Table 3, the differences between the NTG diabetic and nondiabetic subjects remained highly significant even when adjusted for fasting TG levels.

Retinyl palmitate patterns in plasma and $S_f > 400$ fractions. Postprandial lipoproteins of intestinal origin, chylomicrons and their remnants, were quantified by measuring RP concentrations in plasma and in the lipoprotein fractions during the fat-load test (**Table 4**). The overall responses in plasma or in fractions larger than S_f 400 were of similar magnitude in all groups. However, in agreement with the TG measurements, RP concentrations rose rapidly in group 1 (DM+CAD+), resulting in significantly higher levels at 2, 3, and 4 h as compared with groups 2 (DM−CAD+) or 4 (DM−CAD−).

Retinyl palmitate responses in $VLDL_1$ (S_f 60–400). In this

TABLE 3. Postprandial triglyceride (TG) responses in normotriglyceridemic (fasting serum TG < 1.75 mmol/l) diabetic patients (DM + CAD +, n = 6; DM + CAD −, n = 6) and healthy control subjects

Fraction	NIDDM Patients (n = 12)	Controls (n = 9)	P (ANOVA)[a]	P (ANCOVA)[b]
Fasting plasma TG, mmol/l	1.42 ± 0.08	1.12 ± 0.07	0.015	
Plasma, AUC	42 ± 2	32 ± 2	0.001	0.039
$Chylo_1$, AUC	1.7 ± 0.2	0.8 ± 0.1	0.007	0.024
$Chylo_2$, AUC	1.1 ± 0.08	0.8 ± 0.1	0.027	0.140
$Chylo_3$, AUC	1.7 ± 0.2	0.9 ± 0.2	0.008	0.050
$VLDL_1$, AUC	12 ± 0.9	6.8 ± 0.6	<0.001	0.001
Plasma, AUIC	7.8 ± 0.8	4.7 ± 0.6	0.008	0.065
$Chylo_3$, AUIC	0.9 ± 0.1	0.5 ± 0.09	0.023	0.176
$VLDL_1$, AUIC	2.1 ± 0.2	0.8 ± 0.2	<0.001	0.001

The data (except fasting TG) are areas under concentration curve (AUC), or under incremental concentration curve (AUIC), mmol/l • h, mean ± SEM (see Methods for definitions).

[a] Analysis of variance of logarithmically transformed data.

[b] Analysis of covariance of logarithmically transformed data, using log fasting plasma triglyceride as covariate.

TABLE 4. Retinyl palmitate responses in the oral fat-load test

Fraction	Group 1 DM + CAD +	Group 2 DM − CAD +	Group 3 DM + CAD −	Group 4 DM − CAD −	P (ANOVA)[a]	P (ANCOVA)[b]
Plasma, AUC	197 ± 19	179 ± 27	177 ± 21	181 ± 37	0.663	0.614
$Chylo_1$, AUC	6.1 ± 1.0	4.2 ± 0.8	4.9 ± 0.8	4.8 ± 1.1	0.368	0.676
$Chylo_2$, AUC	15 ± 2	14 ± 3	13 ± 2	14 ± 3.7	0.647	0.764
$Chylo_3$, AUC	41 ± 3	37 ± 10	29 ± 5	36 ± 12	0.357	0.191
$VLDL_1$, AUC	36 ± 5[c]	32 ± 7[c]	31 ± 4[d]	17 ± 4	0.008	0.017
$VLDL_2$, AUC	7.7 ± 1.0	6.1 ± 0.8	6.7 ± 0.6	9.6 ± 1.7	0.396	0.049
IDL, AUC	2.5 ± 0.4[c]	2.7 ± 0.5[d]	2.0 ± 0.2[d]	4.2 ± 0.4	0.002	0.002

The data are areas under concentration curve (AUC), μmol/l • h, mean ± SEM (see Methods for definition).
[a] Analysis of variance of logarithmically transformed data.
[b] Analysis of covariance of logarithmically transformed data, using log fasting plasma triglyceride as covariate.
[c] $P < 0.01$, [d] $P < 0.05$, compared with group 4.

fraction, the postprandial RP response was markedly larger in groups 1, 2, and 3, compared with the healthy control group (**Fig. 2A,** Table 4). These differences also persisted after adjustment for fasting TG concentrations. In addition, there were interesting differences between the diabetic groups with or without CAD on the one hand and the group with CAD but no diabetes on the other. The diabetic patients demonstrated a rapid upstroke in the RP curve and therefore had markedly elevated levels of RP from 2 to 6 h after the fatty meal. In contrast, group 2 (DM−CAD+) had a similar early response as the healthy controls, but delayed clearance of the particles, reflected by significantly elevated RP concentrations at 6, 9, and 12 h (Fig. 2A).

Retinyl palmitate in $VLDL_2$ (S_f 20–60) and IDL (S_f 12–20). Relatively low concentrations of RP were recovered from the smallest TG-rich lipoprotein fractions, suggesting that a substantial proportion of the CMRs had been removed from the circulation before being processed into this size range. Interestingly, however, the postprandial RP concentrations in $VLDL_2$ and IDL, in contrast to all other fractions, were highest in the healthy control subjects, compared with the other three groups (Table 4). The difference was especially marked in the IDL fraction, and persisted after adjustment for fasting TG levels (Fig. 2B, Table 4).

Cholesterol. The groups did not differ with respect to fasting plasma cholesterol or the postprandial AUCs in plasma (ANOVA $P = 0.760$). In the lipoprotein fractions, the responses of cholesterol paralleled closely those of TG. Both diabetic groups had significantly larger responses than group 4 in the $chylo_2$, $chylo_3$, and $VLDL_1$ fractions (data not shown). The ratio between TG and cholesterol concentrations was significantly higher in the diabetic groups than in group 4 at 2, 3, and 4 h, implying enrichment of the postprandial lipoproteins with triglyceride in the diabetic patients.

Determinants of postprandial lipemia

Fasting plasma triglyceride. The total postprandial plasma TG response (AUC) was closely correlated with the fasting TG concentration ($r = 0.925$, $P < 0.001$, log-

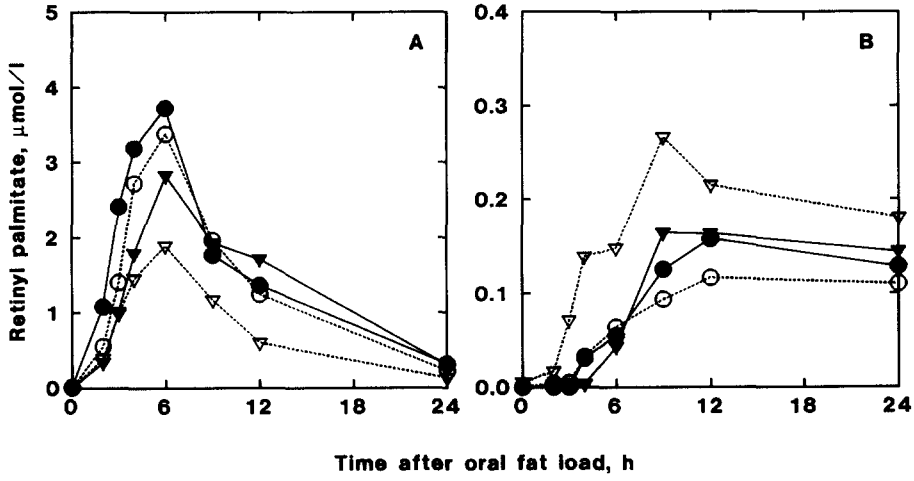

Fig. 2. Line graphs showing the postprandial retinyl palmitate responses in the $VLDL_1$ (S_f 60–400) fraction (panel A) and in the IDL (S_f 12–20) fraction (panel B). Group 1, DM+CAD+ (●); Group 2, DM−CAD+ (▼); Group 3, DM+CAD− (○); Group 4, DM−CAD− (▽).

transformed) in the entire study population, as well as within each of the four groups. The 9-h incremental response (AUIC) was less accurately predicted by the fasting TG level ($r = 0.581$, $P < 0.001$) than the total response. The correlation between the fasting TG concentration and the postprandial plasma RP AUC ($r = 0.641$, $P < 0.001$) was similar to that of TG AUIC. The total TG responses in all lipoprotein fractions were also related to fasting TG: $chylo_1$, $r = 0.520$; $chylo_2$, $r = 0.605$; $chylo_3$, $r = 0.786$; $VLDL_1$, $r = 0.900$; $VLDL_2$, $r = 0.800$ (all $P < 0.001$); IDL, $r = 0.368$, $P = 0.004$.

Lipoprotein lipase activity. There was a moderate negative correlation in group 4 between the postheparin plasma LPL activity and the plasma TG AUC ($r = -0.409$, not significant) and TG AUIC ($r = -0.571$, $P < 0.05$) (**Fig. 3B**). The relations with incremental responses in $chylo_3$ ($r = -0.453$) and $VLDL_1$ ($r = -0.415$) were comparable. Overall, the correlations were weaker and nonsignificant in groups 2 and 3. The group with diabetes and CAD (group 1) showed no negative relationships between LPL and postprandial lipemia (Fig. 3A).

Hepatic lipase activity. In the entire study population there were weak positive correlations between postheparin plasma HL activity and the plasma TG responses (AUC, $r = 0.286$, AUIC, $r = 0.318$, both $P < 0.05$). This relationship was strongest in the $VLDL_1$ fraction ($r = 0.408$, $P < 0.01$).

Glycemic control. To determine whether glycemic control influences postprandial lipemia, we calculated correlations between HbA_{1c} and the postprandial responses. In these analyses the two groups of diabetic patients were combined. Weak positive relations were found between HbA_{1c} and plasma TG AUC ($r = 0.284$, not significant), TG AUIC ($r = 0.338$, $P = 0.067$), and RP AUC ($r = 0.379$, $P < 0.05$). Correlations for TG responses in the lipoprotein fractions were similar in particles $S_f >$ 60, the most significant being $VLDL_1$ AUIC ($r = 0.483$, $P < 0.01$). $VLDL_2$ or IDL responses were unrelated to glycemic control. We further divided the diabetic patients into tertiles according to HbA_{1c} levels, and compared the lipemic responses between the top and the bottom tertile. Postprandial lipemia was larger in the highest compared with the lowest tertile measured as plasma TG AUC ($P < 0.03$), TG AUIC ($P < 0.03$), and similarly in all chylo fractions and $VLDL_1$. Likewise, the total postprandial amount of intestinally derived lipoproteins (plasma RP AUC) was larger in patients with the poorest glycemic control ($P < 0.01$), but for RP in the lipoprotein fractions, only $VLDL_1$ showed a significant difference ($P < 0.03$). We also tested by analysis of covariance whether the differences in postprandial lipemia between the four study groups were independent of glycemic control. All differences in TG responses lost their significance when adjusted for HbA_{1c}. In contrast, the adjusted postprandial RP $VLDL_1$ AUC was different between the groups (ANCOVA $P = 0.034$) as well as the unadjusted (ANOVA $P = 0.008$).

Fasting plasma insulin. Across the entire study population, there was a positive correlation between the plasma insulin level and TG AUC ($r = 0.360$, $P < 0.01$), and TG AUIC ($r = 0.329$, $P < 0.05$). Similar weakly positive relations were seen in the lipoprotein fractions larger than S_f 60. Among the diabetic patients with CAD (group 1), no consistent correlations were observed between insulin levels and postprandial lipemia. In all other groups, there was a trend to moderate positive correlations (data not shown).

ApoE phenotype. Because there were differences, albeit nonsignificant, in the apoE phenotype distributions be-

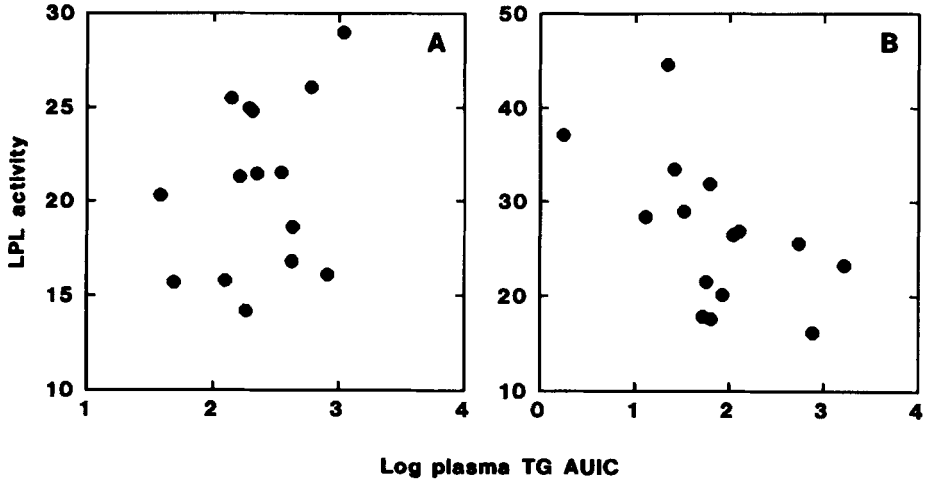

Fig. 3. Scatterplots showing correlations between incremental plasma triglyceride responses (AUIC, mmol/l • h, log-transformed) and postheparin plasma lipoprotein lipase activities (μmol free fatty acids [FFA] • h^{-1} • ml^{-1}). Panel A: Group 1 (DM+CAD+), $r = 0.300$, not significant. Panel B: Group 4 (DM−CAD−), $r = -0.571$, $P < 0.05$.

tween the study groups, we compared the lipemic responses in patients with or without the $\epsilon 4$ allele. In general, no consistent pattern emerged, and there were no significant differences across the whole study population. Only within group 2 were there larger responses for plasma TG ($P < 0.02$), TG $VLDL_1$ ($P < 0.05$), TG $VLDL_2$ ($P < 0.03$), and RP $VLDL_2$ ($P < 0.03$) in patients with phenotype 4/4 or 4/3, compared with phenotypes without the $\epsilon 4$ allele.

β-blocker therapy. We pooled the two diabetic groups and divided them into those using (n = 13) and not using (n = 17) adrenergic β-blocking drugs. No significant differences between users and nonusers were found. Plasma TG AUCs were similar in users (64.1 ± 4.6) and nonusers (62.8 ± 6.7, $P = 0.611$).

Postprandial lipemia and HDL

The HDL cholesterol concentration was inversely related to the magnitude of postprandial lipemia, whether quantified as the total TG response (AUC, $r = -0.360$, $P < 0.01$) or the incremental TG response (AUIC, $r = -0.319$, $P < 0.05$) when data from all study groups were combined. As shown in **Table 5,** these relations were slightly stronger when HDL_2 cholesterol was used instead of total HDL cholesterol. The TG responses in $chylo_3$, $VLDL_1$, and $VLDL_2$ were responsible for the observed negative relationship. Although the power of the correlation varied among the four groups, this was not consistently related to either NIDDM or CAD. A significant inverse correlation was also found between HDL_2 cholesterol and the RP responses in plasma ($r = -0.309$, $P < 0.02$), $chylo_3$ ($r = -0.328$, $P < 0.02$), $VLDL_1$ ($r = -0.396$, $P < 0.005$), and $VLDL_2$ ($r = -0.311$, $P < 0.02$). There were no significant correlations between postprandial lipemia and HDL_3 cholesterol or HDL_2 TG. HDL_3 TG was positively correlated with fasting plasma TG ($r = 0.572$, $P < 0.001$) and postprandial plasma TG AUC ($r = 0.468$, $P < 0.001$).

To investigate whether the magnitude of the inverse relationship between postprandial lipemia and HDL_2 cholesterol was dependent on the fasting TG concentration, we divided the study population into those below and those above the TG median (1.75 mmol/l). The correlation between the total plasma TG response (AUC) and HDL_2 cholesterol was similar in the NTG ($r = -0.325$) and hypertriglyceridemic ($r = -0.264$) subgroups.

Postprandial lipemia and PAI-1

The activity of PAI-1 was higher in the NIDDM than in the nondiabetic groups: 14.8 ± 3.2 (group 1); 12.7 ± 1.6 (group 2); 17.1 ± 4.4 (group 3); 11.9 ± 1.8 (group 4) arbitrary units ($P < 0.01$ between groups 3 and 4). Across the entire study population, PAI-1 activity was positively related to the fasting TG concentration ($r = 0.390$, $P < 0.005$), the total postprandial TG response ($r = 0.389$, $P < 0.005$), the incremental TG response ($r = 0.278$, $P < 0.05$), and the responses in $chylo_3$ ($r = 0.350$, $P < 0.01$), $VLDL_1$ ($r = 0.389$, $P < 0.005$), and $VLDL_2$ ($r = 0.310$, $P < 0.02$) fractions. As illustrated in **Fig. 4,** these relationships were especially strong within both groups of CAD patients (groups 1 and 2). Overall, no significant correlations existed between the postprandial RP responses and the PAI-1 activity.

DISCUSSION

The present study had three major findings. First, in agreement with Lewis et al. (19), we showed that NIDDM patients have higher plasma levels of postprandial remnant lipoproteins than nondiabetic subjects. Second, our results confirm the data of several previous investigations (10–14) that indicated that nondiabetic patients with CAD have enhanced postprandial lipemia. We extended these findings by demonstrating that most of these excess remnants reside in the size range S_f 60–400. Third, the present study suggests that the excessive alimentary lipe-

TABLE 5. Pearson's correlation coefficients between log-transformed measures of postprandial lipemia and HDL_2 cholesterol concentration

Fraction	All subjects (n = 58)	Group 1 DM + CAD + (n = 14)	Group 2 DM − CAD + (n = 14)	Group 3 DM + CAD − (n = 15)	Group 4 DM − CAD − (n = 15)
Fasting plasma TG	−0.493[a]	−0.175	−0.541[c]	−0.742[b]	−0.239
Plasma TG AUC	−0.475[a]	−0.210	−0.628[b]	−0.634[c]	−0.278
Plasma TG AUIC	−0.406[b]	−0.300	−0.475	−0.471	−0.299
$Chylo_1$ AUC	−0.205	−0.485	−0.113	−0.011	−0.246
$Chylo_2$ AUC	−0.272[c]	−0.276	−0.179	−0.420	−0.210
$Chylo_3$ AUC	−0.354[b]	−0.303	−0.357	−0.588[c]	−0.178
$VLDL_1$ AUC	−0.507[a]	−0.382	−0.580[c]	−0.717[b]	−0.271
$VLDL_2$ AUC	−0.451[a]	+0.065	−0.704[b]	−0.536[c]	−0.364
IDL AUC	−0.233	+0.292	−0.513	−0.175	−0.340

TG, triglyceride; AUC, area under curve; AUIC, area under incremental curve (see Methods for definitions).
[a] $P < 0.001$; [b] $P < 0.01$; [c] $P < 0.05$.

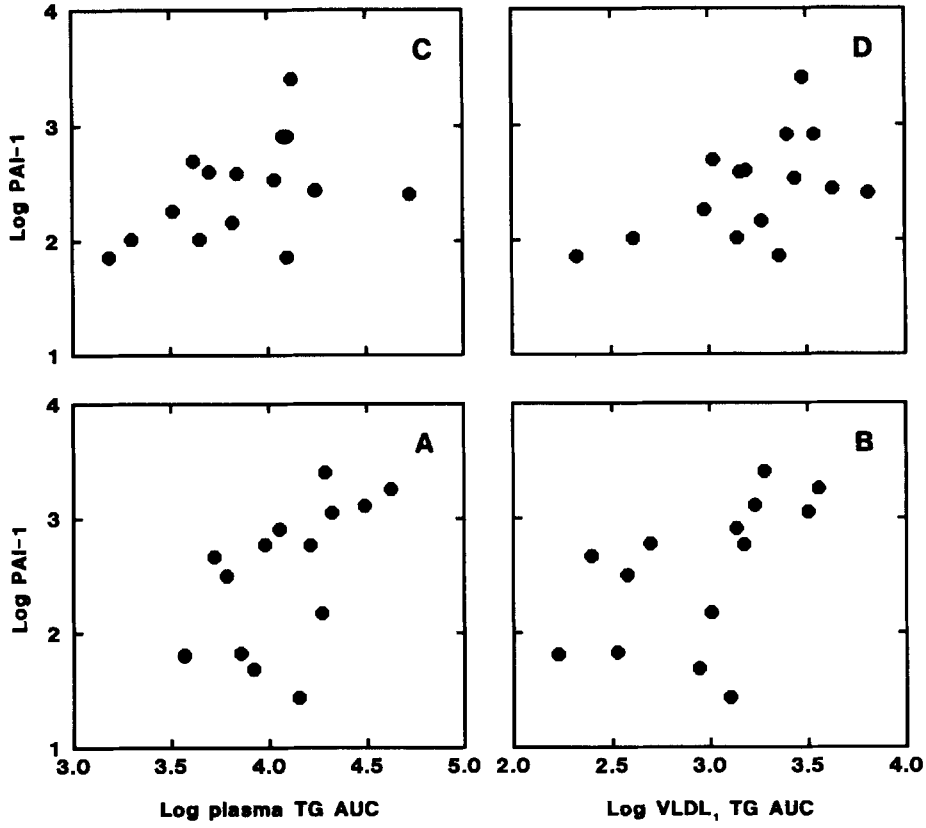

Fig. 4. Scatterplots showing correlations between postprandial lipemia and plasminogen activator inhibitor-1 (PAI-1) activity. Panel A: Plasma triglyceride (TG) area under curve (AUC, see Methods for definition) versus PAI-1 activity in group 1 (DM+CAD+), $r = 0.566$, $P < 0.05$. Panel B: $VLDL_1$ (S_f 60-400) TG AUC versus PAI-1 in group 1 (DM+CAD+), $r = 0.535$, $P < 0.05$. Panel C: Plasma TG AUC versus PAI-1 in group 2 (DM−CAD+), $r = 0.426$, not significant. Panel D: $VLDL_1$ (S_f 60-400) TG AUC versus PAI-1 in group 2 (DM−CAD+), $r = 0.506$, $P < 0.05$. The TG AUCs are mmol/l • h, log-transformed. PAI-1 activities are expressed in arbitrary units, log-transformed.

mia in NIDDM patients is equally present in individuals without evidence of CAD as in those with documented CAD. This finding was unexpected, and it challenges the view that alimentary lipemia is an important predictor of atherosclerosis among NIDDM patients.

As our sample size was relatively small, one must consider the possibility of a type II error in statistical comparisons between the two diabetic groups. We have calculated that our sample had an 80% power to detect a difference of 0.41 log-transformed units in the incremental postprandial plasma TG response (AUIC) at a two-sided 5% alpha level (32). This figure closely matches the actual difference of 0.52 units between groups 1 and 4 which was statistically significant. In non-transformed units, this corresponds to a difference of 26%. Thus, we conclude that even if a true difference in postprandial lipemia between the diabetic groups was concealed by an inadequate sample size in our study, this speculative difference should not be larger than approximately 25%.

Could the putative association between alimentary lipemia and CAD in NIDDM have been concealed by patient selection or confounding factors? Our group 1 patients undoubtedly had severe CAD, verified by coronary angiography. The NIDDM patients without CAD (group 3) did not undergo angiography and may thus have had asymptomatic coronary atherosclerosis. However, a negative exercise thallium scan effectively rules out significant ischemic heart disease (33). The two diabetic groups were well matched for age, BMI, and fasting TG levels. The treatment of diabetes was also similar in both groups. Some differences between the diabetic groups might even have exaggerated postprandial lipemia in the CAD patients. More NIDDM patients with CAD took β-blocking drugs, and they may have been physically less active than the non-CAD diabetics. However, the effect of β-blockers on postprandial lipemia seems to be small (34), although they tend to raise fasting TG levels (35). Likewise, physical inactivity could increase postprandial lipemia (36). On the other hand, one must also consider that the CAD patients might have changed their diets and lifestyles because of their illness. Indeed, the fasting plasma TG and cholesterol levels in our NIDDM patients with CAD were

clearly lower than those reported for a large cohort of Finnish NIDDM patients (37), although their degree of obesity was similar. Therefore, the role of postprandial lipemia in the atherogenesis in NIDDM could be missed in a cross-sectional study, and the present results should be confirmed or refuted in a prospective setting.

An interesting finding was that the largest differences in postprandial RP concentrations between the NIDDM patients and the nondiabetic subjects occurred during the early hours after the fatty meal. One possible explanation could be that chylomicron formation and secretion are enhanced in the diabetic gut, as suggested by some animal models of diabetes (38, 39). Although the relevance of these findings to humans is unclear, it has been proposed that hypertriglyceridemic individuals may package newly absorbed fat more efficiently than those with normal fasting TG concentrations (40). Secondly, one must consider the initial step in chylomicron catabolism, hydrolysis of their core TG by LPL. If this step is abnormal, then the net effect of continuing chylomicron secretion by the gut and inefficient lipolysis would be reflected in the early part of the postprandial TG concentration curve. As we have suggested earlier (23), the inverse relation between postprandial lipemia and LPL activity (41, 42) appears to be disrupted in NIDDM (Fig. 3). Thirdly, an impaired hepatocellular uptake of CMRs in NIDDM might also cause accumulation of intestinally derived particles during the early hours of alimentary lipemia, as CMR removal appears to be the rate-limiting step of postprandial fat clearance in healthy humans (43).

The abnormality in alimentary lipemia in NIDDM was not confined to the early part of the response, but there was an excess of CMRs in the late postprandial hours as well. Also, the nondiabetic group with CAD (group 2), whose rising part of the TG and RP concentration curves was identical with that of the healthy control subjects, had increased levels of TG-rich particles during the elimination phase. This finding agrees well with previous work by Simpson et al. (11), Groot et al. (12), and Patsch et al. (13), and suggests an ineffective removal of remnants via hepatic receptors. As pointed out by Chen and Reaven (20), the intestinally derived lipoproteins share catabolic sites with endogenous VLDL particles, and competition for removal could explain retarded clearance of alimentary lipoproteins in subjects with fasting hypertriglyceridemia. Although it has been suggested that CMRs are removed via receptors distinct from the LDL (apoB,E) receptor (44, 45), the issue is still controversial, and the LDL receptor may well be important for CMR catabolism also (46). The fact that adjusting for fasting TG levels eliminated most differences in postprandial lipoproteins between the groups in the present study is compatible with the competition hypothesis. However, other mechanisms may also operate, as suggested by the persistent differences for $VLDL_1$ RP responses despite adjustment for fasting TG. In addition, the subset of normotriglyceridemic NIDDM patients had increased postprandial lipemia in comparison with the normotriglyceridemic controls (Table 3), further indicating that some unknown factors retard the handling of alimentary lipoproteins in NIDDM. In this respect, our findings differ from those of Lewis et al. (19) who found that postprandial lipemia was increased only in hypertriglyceridemic NIDDM patients, whereas normotriglyceridemic NIDDM subjects had lipemic responses similar to controls. However, their patients were extremely obese (mean BMI > 35 kg/m^2), and the same investigators have demonstrated that normolipidemic obese subjects have greater postprandial lipemia than lean control subjects (47). Thus, their data may not be representative of all NIDDM patients.

The most striking differences in postprandial lipid concentrations between the NIDDM patients and the healthy controls were seen in the $VLDL_1$ fraction (S_f 60–400). This was true for TG, RP, and cholesterol levels. Particles in this size range are capable of transporting cholesteryl esters into the arterial intima, and are therefore potentially atherogenic (9). Furthermore, the postprandial responses in this fraction were positively related to the activity of PAI-1. This finding is in good agreement with the in vitro data of Stiko-Rahm et al. (48) who found that VLDL particles with S_f 100–400 obtained from hypertriglyceridemic individuals effectively stimulated PAI-1 production in cultured endothelial cells. Thus, high levels of postprandial lipoproteins may impair fibrinolysis and increase the risk of acute thrombotic events.

An unexpected finding was the high level of RP in the postprandial IDL (S_f 12–20) fraction in the healthy subjects (Fig. 2B). This may seem paradoxical, as intermediate density lipoproteins are atherogenic (49, 50). However, the absolute concentration of CMRs in this size range was very low. We suggest that the relative excess of these particles reflects a more efficient lipolysis in the healthy individuals compared with the other groups, and further supports the role of impaired LPL action in the postprandial lipemia of NIDDM.

As we have reported earlier (23), the negative relationship between alimentary lipemia and HDL cholesterol appears to be weaker in NIDDM patients than in the healthy normolipidemic subjects investigated by Patsch et al. (36, 42). In the present study, no significant correlation was found in the healthy control group. A lack of correlation between postprandial lipemia and HDL cholesterol has also been reported in endurance-trained men (51) and in subjects with hypoalphalipoproteinemia and mild hypertriglyceridemia (52). Although the differences in these relations between the groups of the present study may be due partly to chance, the results do suggest that

characteristics such as age, obesity, and fasting lipid concentrations may distort the metabolic interactions between TG-rich lipoproteins and HDL.

In conclusion, alimentary lipemia is enhanced in NIDDM patients in comparison with healthy individuals matched for sex, age, and BMI. The increased levels of postprandial remnant lipoproteins are not entirely explained by differences in endogenous TG-rich lipoprotein concentrations. Impaired lipolytic processing of chylomicrons and ineffective removal of the remnant particles by hepatic receptors both appear to contribute to these abnormalities. Although CMRs are potentially atherogenic, the magnitude of postprandial lipemia apparently does not distinguish NIDDM patients with symptomatic, angiographically verified CAD from those without cardiac symptoms and with normal myocardial perfusion scans. This finding should be interpreted with caution, given the sample size and cross-sectional design of the present study. Until data from prospective studies become available, the reduction of remnant lipoproteins remains a reasonable goal in the management of NIDDM. ■

We thank Dr. Kari S. Virtanen, M.D., for interpreting the exercise thallium scans. The skillful technical assistance of Leena Lehikoinen, Ritva Marjanen, Sirpa Rannikko, Sirkka-Liisa Runeberg, and Kirsi Usenius is greatly appreciated. This study was supported by grants from the Finnish Cardiac Research Foundation, the Aarne Koskelo Foundation, and the Sigrid Juselius Foundation.

Manuscript received 13 March 1993 and in revised form 1 July 1993.

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