Postprandial Lipoprotein Metabolism in Normotriglyceridemic Non–Insulin-Dependent Diabetic Patients: Influence of Apolipoprotein E Polymorphism

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Non–insulin-dependent diabetes mellitus (NIDDM) is associated with postprandial lipoprotein clearance defects that are correlated with the fasting hypertriglyceridemia widely observed in NIDDM patients. The aim of this study was to determine if such postprandial disturbances are found in NIDDM patients strictly normotriglyceridemic in the fasting state, and if the apolipoprotein E (apo E) polymorphism influences postprandial metabolism of intestinally derived lipoproteins. The vitamin A–fat loading test was used in 18 normotriglyceridemic NIDDM patients and seven normotriglyceridemic obese controls, and postprandial triglyceride (TG) and retinyl palmitate (RP) concentrations were evaluated in total plasma, and in the chylomicron (Sf > 1,000) and nonchylomicron (Sf < 1,000) fractions isolated by ultracentrifugation. NIDDM patients exhibited an amplified response of both TG and RP as compared with obese controls in the three fractions. Incremental TG response to the oral fat load was strongly correlated with fasting TG level ($r = .80$, $P < .0001$) in the whole study population. Postprandial lipoprotein profiles were distinguished in NIDDM patients according to apo E phenotype: despite normal fasting TG levels in E3/3 (n = 6), E2/3 (n = 6), and E3/4 (n = 6), postprandial RP response was twofold to threefold higher in E2/3 and E3/4 patients than in the common E3/3 phenotype. Contrasting lower postprandial TG increment and lower fasting and postprandial high-density lipoprotein (HDL) and HDL_3 cholesterol levels were observed in E3/4 versus E3/3 patients, possibly reflecting modifications in lipid content of the postprandial lipoproteins driven by a differential lipid transfer activity depending on apo E isoform. These data indicate an enhanced postprandial lipemia in normotriglyceridemic NIDDM patients, and demonstrate the influence of apo E polymorphism on their lipoprotein clearance. Postprandial alterations of lipoprotein remnants may thus accelerate atherogenesis even in normotriglyceridemic NIDDM patients.

SUBJECTS WITH glucose intolerance and non–insulin-dependent diabetes mellitus (NIDDM) are at higher risk for development of atherosclerosis,[1] and hypertriglyceridemia, which is the most common lipid disturbance in NIDDM, is identified in this population as a risk factor for development of coronary heart disease in univariate analysis.[2,3] Nevertheless, epidemiological studies have failed to demonstrate its impact as an independent risk factor in multivariate analysis.

Elevated fasting triglyceride (TG) level is linked to low serum high-density lipoprotein (HDL) and HDL_2 cholesterol in subjects with normal glucose tolerance, as in NIDDM, and these low HDL cholesterol levels are associated with an increased risk of coronary heart disease in NIDDM subjects.[4] In addition to this association of high TG/low HDL cholesterol, changes in lipoprotein composition such as high very–low-density lipoprotein (VLDL) TG and VLDL cholesterol are involved as powerful markers of coronary heart disease in NIDDM.[5] Abnormalities in low-density lipoprotein (LDL) composition are also observed in association with moderate elevation of fasting TG levels in type II diabetics,[6] and the pattern of small dense LDL closely related to TG levels is clearly associated with the risk of coronary heart disease.[7] Fasting TG concentrations thus poorly reflect the complex link between hypertriglyceridemia and the quantitative and qualitative associated changes in VLDL, LDL, and HDL that lead to its atherogenic influence.[8]

In 1979, Zilversmit[9] hypothesized that chylomicrons, which are synthesized and secreted after fat ingestion, and their remnants may be taken up by the arterial cells, and that accumulation of cholesterol-loaded remnants in the arterial wall may be critical in the development of the atherogenic process. In recent years, postprandial TG-rich lipoprotein (TRL) metabolism was extensively studied and was thought to play a role in the development of premature coronary heart disease.[10-13] The influence of several conditions such as aging, gender,[14] weight,[15] fasting TG and HDL cholesterol,[14-16] and apolipoprotein E (apo E) polymorphism[17-20] on postprandial lipemia was ascertained in further studies. Genetic variation in the apo E gene results in three common apo E isoforms: a normal allele, E3, and two variants, E4 and E2, which differ from E3 by a cysteine to arginine substitution at position 112 and an arginine to cysteine substitution at position 158, respectively.[21] These apo E alleles are functionally distinguished due to their differential catabolic rate in plasma: a faster catabolic rate of E4,[22] which behaves normally in receptor-binding assays,[23] and a delayed catabolic rate of E2, partly explained by the lower receptor-binding capacity of this isoform.[23] The development in type III hyperlipoproteinemia of a remnant chylomicron catabolism defect in patients with the E2 phenotype is dependent on environmental factors such as diet or coexistent disease such as diabetes.[24] Phenotypic distribution of apo E alleles is not different in NIDDM patients versus a nondiabetic population,[25] but recent studies suggest that apo E polymorphism should be a contributory factor for coronary heart disease in NIDDM.[26] Evaluation of postprandial lipemia was designed in diabetic patients to determine if alterations in postprandial lipopro-

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tein clearance could explain the high prevalence of coronary heart disease observed in this population.[1,27] Abnormalities in TRL clearance were found in NIDDM patients compared with nondiabetic subjects, with a strong positive correlation between these postprandial lipoprotein disturbances and the level of fasting TG,[28-30] but the question remains as to whether these postprandial abnormalities are attributable to higher fasting TG in NIDDM subjects as compared with subjects with normal glucose tolerance, or are explained by the diabetic condition per se.

This study was designed for elucidating the role of diabetes in postprandial TRL metabolism in strictly normotriglyceridemic subjects, and for determining if apo E polymorphism allows definition of a subgroup of diabetic subjects particularly prone to potentially atherogenic postprandial lipid disturbances.

SUBJECTS AND METHODS

Subjects

Eighteen obese NIDDM patients and seven obese subjects of both sexes participated in the study. Their clinical characteristics are summarized in Table 1. All subjects were normotriglyceridemic (TG < 1.7 mmol/L), and baseline lipid characteristics of both groups were not different (Table 2). Patients from the diabetic group were selected to constitute three subgroups according to apo E genotype (Table 1). In the diabetic group, oral antidiabetic agents were withdrawn 10 days before the test meal in 11 of 18 subjects, and insulin was withdrawn 48 hours before the test meal in six of 18. One patient was on diet alone. Compounds known to affect carbohydrate or lipid metabolism were also withdrawn 10 days before the test. Subjects were placed on a weight-maintenance diet determined by the dietician on a previous visit. All patients gave informed consent to the protocol.

Study Protocol

Subjects were admitted on the evening preceding the oral fat load test and ate a standard meal at 7 PM. After an overnight fast, patients were given at 11 AM a standardized fatty meal containing 60 g fat/m^2 body surface area, consisting of 67% calories as fat, 14% as carbohydrate, and 19% as protein and providing 1,315 cal/100 g fat and 280 mg cholesterol/100 g fat. The meal was ingested in 20 to 30 minutes and consisted of sausage, ground beef, mashed potatoes, butter, heavy cream, and cheddar cheese. Vitamin A (Avibon 100,000 UI; Rhône Poulenc Rorer Laboratories, Vitry, France) was ingested with 125 mL water at the start of the meal. Vitamin A is incorporated into chylomicrons in its retinyl ester form and thus is a good marker of chylomicron catabolism. After ingestion of the test meal, patients did not eat again for 20 hours and were allowed to drink 250 mL the first 8 hours and ad libitum after 8 hours. Baseline blood samples were drawn at 11 AM for determination of glucose, insulin, C-peptide, and apolipoproteins. Blood samples for lipid parameters were drawn before and 2, 4, 6, 8, 10, 12, and 20 hours after the test meal in tubes containing 0.1% EDTA shielded from light with aluminum foil, and at the same time for glucose in tubes containing sodium fluoride. Tubes were centrifuged at 3,000 rpm for 15 minutes at 4°C, and plasma was separated.

Table 1. Clinical Characteristics of the Patients (mean ± SEM)

Characteristic	Obese Controls	NIDDM
No.	7	18
Age (yr)	45 ± 4	54 ± 2*
BMI (kg/m^2)	35 ± 2	34 ± 1
Sex ratio (M/F)	2/5	5/13
Apo E isoforms (n)		
3/3	4	6
4/3	1	6
2/3	2	6

*P < .05, NIDDM *v* obese controls.

Table 2. Fasting Glycemic, Lipid, Lipoprotein, Apoprotein, and Hormone Levels in NIDDM Subjects and Obese Controls

Parameter	Obese Controls	NIDDM Subjects	P
HbA_{1c} (%)	<5.5	9.2 ± 0.5	<.05
Glucose (mmol/L)	5.2 ± 0.23	12.5 ± 0.85	<.05
Insulin (mU/L)	13.3 ± 1.7	17.5 ± 1.6	NS
C-peptide (nmol/L)	1.06 ± 0.16	1.12 ± 0.10	NS
TG (mmol/L)	1.06 ± 0.11	1.21 ± 0.07	NS
Cholesterol (mmol/L)	5.46 ± 0.51	5.28 ± 0.25	NS
HDL cholesterol (mmol/L)	1.31 ± 0.14	1.28 ± 0.08	NS
LDL cholesterol (mmol/L)	3.73 ± 0.45	3.43 ± 0.24	NS
Apo A1 (mg/dL)	145 ± 10	145 ± 7	NS
Apo B (mg/dL)	129 ± 13	121 ± 7	NS
Apo B:A1 ratio	0.91 ± 0.11	0.88 ± 0.07	NS

Gradient-Density Ultracentrifugation

Plasma samples were submitted to ultracentrifugation at 25,000 rpm for 25 minutes (Beckman L8 70M Ultracentrifuge, rotor TI 70,1; Beckman Instruments, Fullerton, CA) to separate the chylomicron fraction from the nonchylomicron fraction.[15]

Lipid and Lipoprotein Determination

TG and cholesterol concentrations were determined in total plasma and in the nonchylomicron fraction with an automated Hitachi 717 analyzer (Meylan, France) by enzymatic methods (kits 1-0580550 and 1-040839, respectively; Boehringer Mannheim, Germany). HDL cholesterol was assayed in the supernatant after precipitation of the other lipoproteins with polyethylene glycol solutions (kits 8251015 and 8252015 for total HDL and HDL_3, respectively, Quantolip; Immunofrance, Orly, France). Apo A1 and apoB were determined by nephelometry using a Behring Nephelometer Analyser (Rueil, France) (antisera OUED AC4 90073 and OSAN AC4 91153, respectively).

Retinyl Palmitate Assay

Blood samples for retinyl palmitate (RP) assay were collected in aluminum-covered tubes, and aliquots of whole plasma and the nonchylomicron fraction were kept frozen at −80°C. The day of the assay, samples were submitted to hexane extraction, redissolved in ethanol, and then submitted to high-performance liquid chromatography with a mobile phase of methanol at a flow rate of 2 mL/min, and the RP peak was measured at 325 nm. An ethanol solution of retinyl acetate at known concentration served as the standard to determine extraction recovery, which was approximately 95%.

Apo E Phenotyping and Genotyping

Apo E phenotypes were determined by an isoelectric focusing technique, and apo E genotypes were assessed by restriction isotyping from genomic DNA extracted from frozen leukocytes amplified by polymerase chain reaction restricted with *Hha*I.[31] Phenotype and genotype determinations were concordant except for one patient.

Other Laboratory Measurements

Plasma glucose was analyzed using the glucose oxidase method (kit Boehringer Mannheim for BM/Hitachi 717), insulin by radioimmunoassay (Phadeseph kit; Kabi Pharmacia Diagnostics, St Quentin, France), and C-peptide by RIA-coat (Byk-Sangtec Diagnostica, Mallinckrodt Medical, Evry, France). Hemoglobin A_{1c} (HbA_{1c}) level was measured by high-performance liquid chromatography.

Quantitation of Postprandial Lipemic Responses

Postprandial TG, RP, and cholesterol levels were measured in total plasma and in the nonchylomicron fraction and plotted against time to determine peak time and amplitude and clearance time. Areas under concentration curves (AUCs) for TG and RP and areas under incremental concentration curves (AUIC) for TG were determined according to Tai's model,[32] derived from the trapezoid rule.

Statistical Analysis

All results are expressed as the mean ± SEM. Group means were compared by a one- or two-variable ANOVA, with adjustment on variables not strictly matched and thus likely to introduce a bias.

RESULTS

Postprandial Lipemia and Glycemic Status: Comparison of NIDDM Patients and Obese Nondiabetic Patients

Eighteen NIDDM patients and seven obese controls were recruited for the fatty meal test. The diabetic group and the obese control group did not differ according to body mass index (BMI) and sex ratio, but the mean age of the NIDDM group was significantly higher ($P < .05$) than that of obese controls. Apo E phenotype distribution was not similar in the two experimental groups because of recruitment difficulties (Table 1).

Baseline plasma glucose and HbA_{1c} concentration were higher in diabetic patients versus obese controls, but insulin and C-peptide concentrations were not different between the groups. Baseline fasting lipids and lipoproteins of the diabetic group and the obese control group are shown in Table 2. Mean fasting TG concentrations in obese controls (1.06 ± 0.11 mmol/L) and diabetics (1.21 ± 0.07 mmol/L) were not significantly different, but were nevertheless 14% higher in the latter compared with obese controls. We therefore adjusted between-group comparisons on age, fasting TG, and apo E distribution to avoid statistical bias from these variables.

Postprandial lipid concentrations. After the oral fat load, TG relative concentrations were higher in diabetics than in obese controls (Fig 1). TG peak times between 4 and 6 hours were significantly higher in diabetics than in obese controls in whole plasma ($P < .01$) and in both the chylomicron ($P < .03$) and the nonchylomicron ($P < .01$) fractions (data not shown). TG AUCs were elevated in diabetics in comparison to obese controls in whole plasma ($P < .02$) and in chylomicron ($P < .01$) and nonchylomicron ($P < .03$) fractions (Table 3). In multivariate analysis, these differences between diabetics and obese controls were indepen-

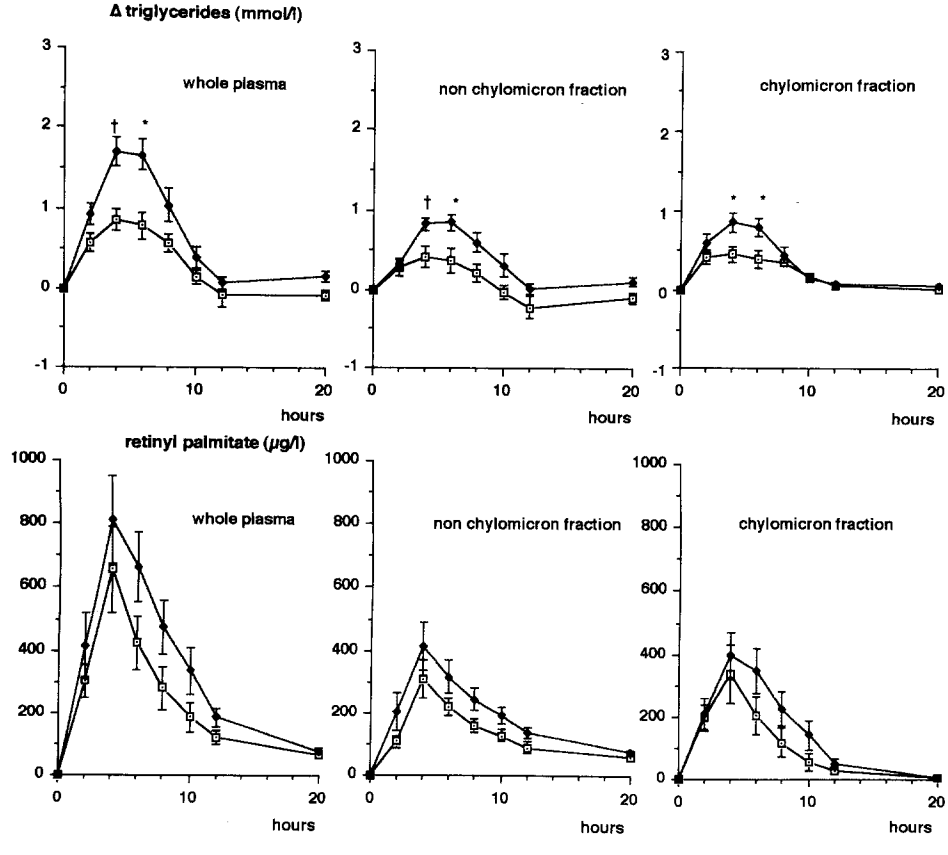

Fig 1. Incremental TG and RP concentrations after oral fat challenge in 18 NIDDM patients (◆) and 7 obese controls (□). *$P < .05$, †$P < .01$.

Table 3. TG and RP Responses to Oral Fat Load Test in NIDDM Subjects and Obese Controls

Response	Obese Controls (n = 7)	NIDDM (n = 18)
TG AUC		
Plasma	26 ± 2.8	36.7 ± 2.5†
Nonchylomicron	20.2 ± 2.1	27.5 ± 1.9*
Chylomicron	5.7 ± 0.8	9.2 ± 0.8‡
RP AUC		
Plasma	4,580 ± 518	6,650 ± 834
Nonchylomicron	2,572 ± 260	3,740 ± 496
Chylomicron	1,995 ± 306	2,930 ± 414

NOTE. Data are expressed as mmol/L · 20 h for TG and μg/L · 20 h for RP. Results are the mean ± SEM.

*$P < .03$.

†$P < .02$.

‡$P < .01$.

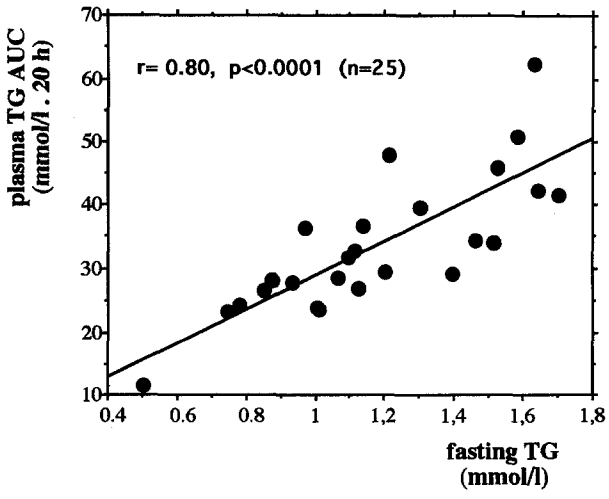

Fig 3. Correlation of TG AUC after oral fat challenge with fasting TG in 18 NIDDM patients and 7 obese controls.

dent of age and apo E distribution differences observed between the groups. These between-group differences were dependent on fasting TG, but remained significant after adjustment on this variable. When restricting the comparison to E3/3 NIDDM (n = 6) and E3/3 obese controls (n = 4), TG relative concentrations were still significantly higher at 4 hours ($P < .05$) and 6 hours ($P < .03$) in NIDDM than in obese controls (data not shown), and TG AUCs were larger in NIDDM than in obese controls (39 ± 5 *v* 25 ± 5 mmol/L · 20 h), with the difference tending toward significance ($P < .07$). Postprandial changes in mean total cholesterol were weak between groups, and there were no differences between diabetics and obese controls (data not shown). A slight postprandial decrease in total HDL, HDL_2, and HDL_3 cholesterol was observed in both groups, with no significant between-group difference nevertheless (Fig 2).

Analysis of the combined data from NIDDM and obese patients (Fig 3) showed that TG AUCs were significantly correlated with fasting plasma TG levels ($r = .80, P = .0001$), but not with HbA_{1C} and fasting HDL cholesterol, glucose, insulin, and C-peptide levels.

Postprandial RP concentrations. Postprandial intestinal lipoproteins were quantified by RP concentrations after vitamin A ingestion concomitantly with the fatty meal. Mean RP concentrations tended to be higher in diabetic subjects than in obese controls, and RP peak amplitude tended toward significantly higher levels in NIDDM than in obese controls ($P = .06$), whereas the peak time was not different between the groups (Fig 1). RP AUCs in whole plasma and in chylomicron and nonchylomicron fractions were 1.5-fold greater in the diabetic group than in obese controls, although these differences were not significant (Table 3).

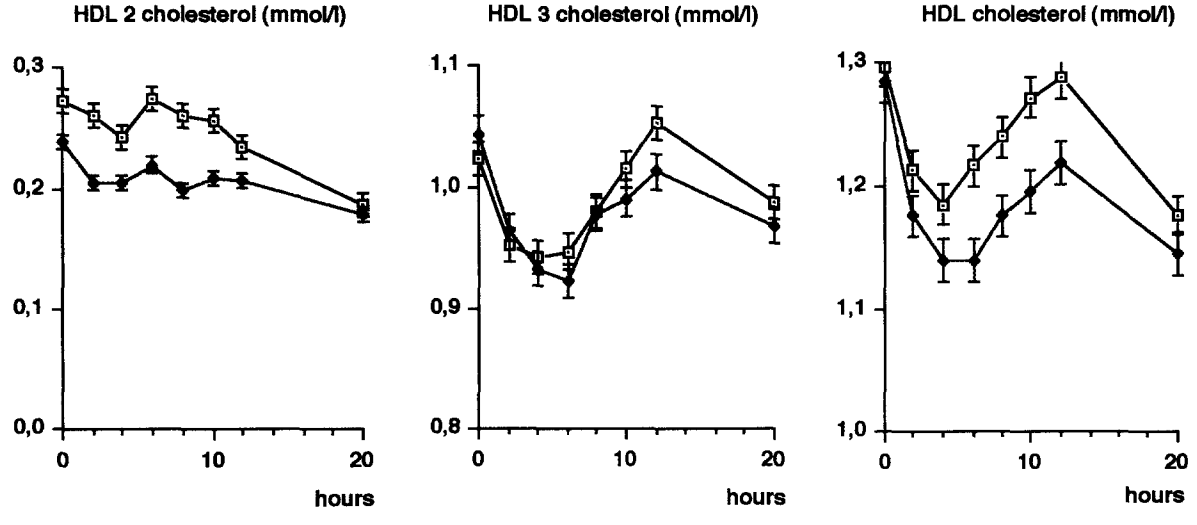

Fig 2. HDL_2, HDL_3, and total HDL cholesterol concentrations before and after oral fat challenge in 18 NIDDM patients (◆) and 7 obese controls (⊡).

Postprandial Lipemia and Apo E Phenotype in NIDDM Patients

We compared lipemic responses in diabetic patients according to apo E phenotype. The three phenotypic groups did not differ in mean age, but differed with respect to BMI, with E3/3 subjects exhibiting a lower BMI than E2/3 and E3/4 ($P < .05$). Biological characteristics of the patients are summarized in Table 4. Fasting TG was not significantly different among apo E phenotype groups, with E3/4 patients nevertheless exhibiting TG levels 16% higher than E2/3 and E3/3 patients (Table 4). Therefore, comparisons among the three apo E phenotype groups were adjusted on BMI, age, and fasting TG levels.

Postprandial lipid concentrations. After the oral fat load, TG relative concentrations were lower in E3/4 subjects than in E3/3 and E2/3 patients, with the difference reaching significance at 4 hours in whole plasma ($P < .05$). In the nonchylomicron fraction, TG relative concentrations were also lower in E3/4 subjects than in E3/3 and E2/3, but this difference did not reach significance (Fig 4). The incremental postprandial TG response (plasma TG AUIC) was lower in E3/4 subjects (7.7 ± 1.7 mmol/L · 20 h), than in E2/3 (13.9 ± 3.3 mmol/L · 20 h) and E3/3 (15.6 ± 2.4 mmol/L · 20 h), but this difference was not significant.

Cholesterol concentrations were not different according to apo E phenotype, and did not vary significantly after the oral fat load. Total HDL and HDL_3 cholesterol were lower in E3/4 than in E2/3 patients, with intermediate values in E3/3 patients. Both total HDL and HDL_3 cholesterol diminished early with the same magnitude in the three phenotype groups, with a nadir between 4 and 6 hours corresponding to the TG peak (Fig 5).

Postprandial RP concentrations. After the test meal, RP concentrations were higher in E2/3 and E3/4 subjects than in E3/3 subjects (Fig 4), with a RP peak amplitude in the whole-plasma fraction and in the nonchylomicron fraction significantly larger in E3/4 and E2/3 patients ($P < .02$) than in E3/3 patients (Table 5). Chylomicron RP peak amplitude was about twofold higher in E2/3 and E3/4 patients as compared with E3/3 patients, although these differences did not reach significance ($P = .06$). The RP peak time was similar in the three apo E phenotype groups (data not shown). RP AUCs in whole plasma and in chylomicron and nonchylomicron fractions were increased in E2/3 and E3/4 subjects (Table 5), but this difference had a trend toward significance only for the chylomicron fraction (E2/3 *v* E3/3, $P = .06$).

Table 4. Fasting Glycemic, Lipid, Lipoprotein, Apoprotein, and Hormone Levels in NIDDM According to Apo E Phenotype

Parameter	E3/3	E3/4	E2/3
HbA_{1c} (%)	8.6 ± 0.6	9.9 ± 1.6	9.0 ± 0.5
Glucose (mmol/L)	12.5 ± 1.9	13.2 ± 1.49	12.0 ± 1.17
Insulin (mU/L)	21 ± 3	19.4 ± 1.8	12.8 ± 2.5
C-peptide (nmol/L)	1.3 ± 0.2	1.3 ± 0.1	0.9 ± 0.1
TG (mmol/L)	1.16 ± 0.13	1.34 ± 0.09	1.15 ± 0.16
Cholesterol (mmol/L)	5.3 ± 0.6	5.5 ± 0.4	5.0 ± 0.4
HDL cholesterol (mmol/L)	1.26 ± 0.11	1.15 ± 0.17	1.44 ± 0.14
LDL cholesterol (mmol/L)	3.5 ± 0.5	3.7 ± 0.4	3.1 ± 0.3
Apo A1 (mg/dL)	142 ± 0.09	130 ± 0.10	165 ± 0.13
Apo B (mg/dL)	127 ± 0.13	132 ± 0.14	105 ± 0.13
Apo B:A1 ratio	0.91 ± 0.11	1.06 ± 0.13	0.67 ± 0.10

NOTE. No significant difference for any of the biological data was observed among apo E phenotypes.

Statistical analysis of apo E influence on postprandial lipemic responses. The different postprandial TG and RP responses observed in NIDDM patients among apo E phenotype groups were actually attributable to an apo E phenotype effect in multivariate analysis, because neither age, BMI, nor fasting TG level exerted an interaction, except for the nonchylomicron RP peak amplitude, where there was an interaction between apo E phenotype and age. Nevertheless, this interaction was ordinal and not transversal standard,[33,34] and therefore the difference between E2/3 and E3/4 phenotype on one hand and E3/3 phenotype on the other was effective whatever the age.

DISCUSSION

This study was designed foremost to evaluate the influence of diabetes on postprandial lipoprotein metabolism in a population of strictly normotriglyceridemic obese patients. Previous studies conducted in NIDDM patients clearly demonstrated that patients moderately hypertriglyceridemic in the fasting state had higher postprandial TRL plasma levels than nondiabetic control normotriglyceridemic subjects,[28-30] but fasting TG level, which reflects the size of the VLDL pool, is known to be a strong determinant of the postprandial TG increment in normal subjects,[14-16] obese patients,[15] type IV hyperlipoproteinemic patients,[35] and NIDDM patients.[28-29] In normotriglyceridemic patients, Chen et al[36] found postprandial TG plasma levels of the same magnitude in moderately obese nondiabetic patients and NIDDM patients, and the remnant chylomicron but not the chylomicron postprandial increment was significantly higher in NIDDM than in nondiabetic patients. Our data clearly demonstrate that postprandial TG concentrations in NIDDM patients with strictly normal fasting TG levels were higher than in obese nondiabetic subjects, independently of the age difference observed between groups. Total postprandial TG responses (AUCs) in chylomicron and nonchylomicron fractions were significantly higher in NIDDM patients, and postprandial RP AUCs were 45% higher in NIDDM patients than in obese patients. These data suggest that postprandial enhancement of TRL in NIDDM patients is partly attributable to TRL from the intestine, as observed in normal subjects submitted to a normal mixed meal[37] or a high-fat meal.[38-39] Our results do not exclude that TRLs from the liver contribute to the enhanced postprandial triglyceridemia observed in NIDDM patients.

Although our NIDDM and obese control groups were not strictly matched, we could demonstrate that the significantly higher postprandial lipemic response observed in NIDDM patients versus obese controls was independent of age and apo E phenotype distribution but dependent on fasting TG. By plotting fasting TG levels and TG AUCs of

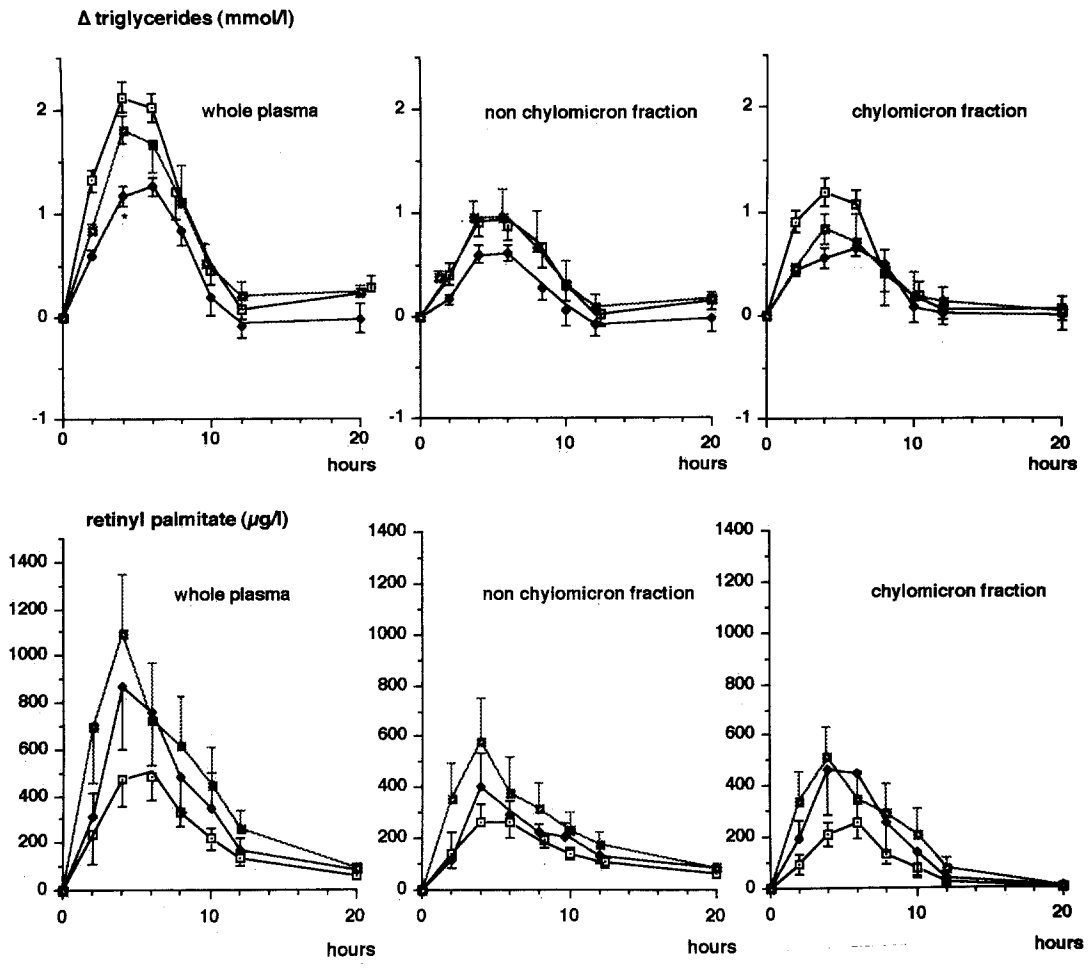

Fig 4. Incremental TG concentration and RP concentration after oral fat challenge in NIDDM patients separated in 3 subgroups according to apo E phenotype. (□) E3/3 (n = 6); (■) E2/3 (n = 6); (♦) E3/4 (n = 6). *$P < .05$.

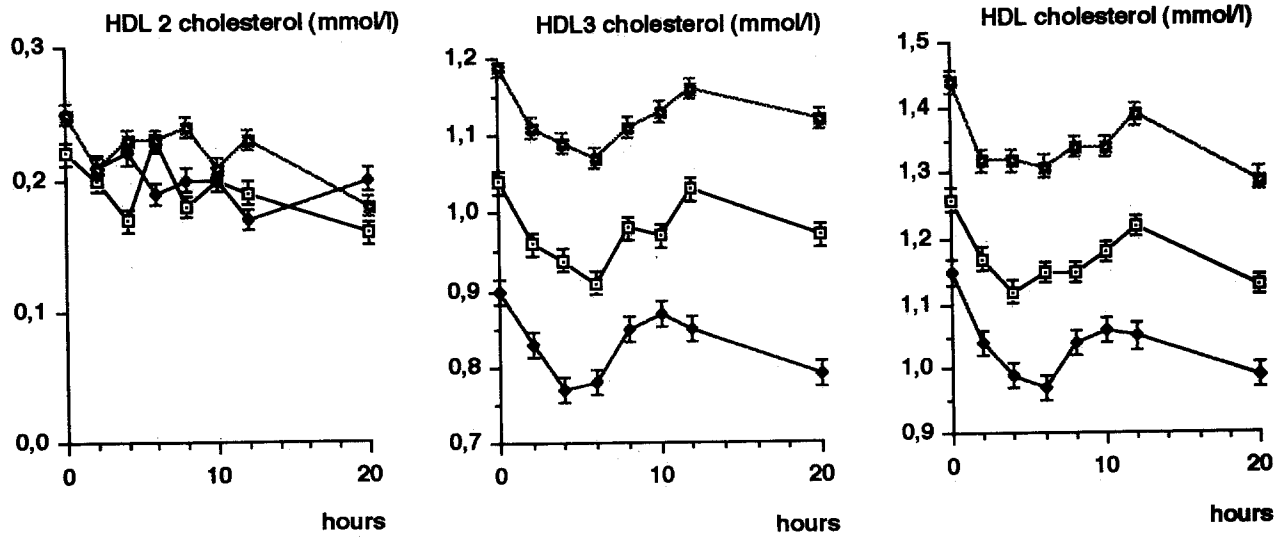

Fig 5. HDL_2, HDL_3, and total HDL cholesterol concentrations before and after oral fat challenge in NIDDM patients according to apo E phenotype. (□) (E3/3 (n = 6); (■) E2/3 (n = 6); (♦) (E3/4 (n = 6).

Table 5. RP Responses to an Oral Fat Load for Each of the Common Apo E Genotypes in 18 NIDDM Subjects

RP Response	E3/3	E3/4	E2/3
Plasma			
Peak amplitude	560 ± 116	1,330 ± 220*	1,470 ± 207†
AUC	4,430 ± 806	6,690 ± 914	8,840 ± 1,910
Nonchylomicron			
Peak amplitude	280 ± 70	610 ± 102*	753 ± 154†
AUC	2,760 ± 609	3,470 ± 288	4,990 ± 1,242
Chylomicron			
Peak amplitude	300 ± 53	720 ± 196‡	700 ± 83§
AUC	1,640 ± 315	3,220 ± 760	3,910 ± 730§

NOTE. Amplitude peaks are expressed as μg/L, time peaks as hours, and AUCs as μg/L · 20 h. Results are the mean ± SEM.

*$P < .02$, E3/4 *v* E3/3.

†$P < .02$, E2/3 *v* E3/3.

‡$P = .06$, E3/4 *v* E3/3.

§$P = .06$, E2/3 *v* E3/3.

both experimental groups, we actually observed a strong positive correlation between alimentary lipemia and fasting TG (Fig 3), as observed by others.[14,28,29] Nevertheless, the absence of interaction between both variables (eg, apo E phenotype and fasting TG) confirms that the different lipemic responses between diabetic and obese control groups were actually related to a group effect. No significant correlation was found between alimentary lipemia and HDL cholesterol, whereas Syvänne et al[29] found a weak inverse correlation in their study. In our study, postprandial TG and RP increments were unrelated to the level of glycemic control.

The second goal of this study was to investigate whether genetic variation in apo E affects postprandial fat metabolism in NIDDM patients. Apo E is a constituent of plasma VLDL, chylomicrons and their remnants, and HDL; in the early postprandial phase, a large redistribution of apo E occurs, with transfer from HDL to TRL.[40] The kinetics of this transfer process are partly dependent on apo E phenotype.[22] Apo E plays a crucial role in cellular uptake of apo E–enriched remnant lipoproteins by apo E–specific binding sites identified as the LDL receptor–related protein.[41] Lipoprotein binding to this receptor is tightly dependent on an apo E presence at the surface, is inhibited by apo C, which competes with apo E,[42-43] and is stimulated by insulin.[41] Other receptors involved in apo E–dependent TRL uptake are the LDL receptor and a lipolysis-stimulated receptor recently identified.[44] The respective contribution of these receptors in remnant chylomicron and intermediate-density lipoprotein clearance in vivo remains to be determined. The magnitude and duration of postprandial lipemia after a fat test meal in normal subjects is modulated by apo E polymorphism; studies designed in small-sample populations showed disagreement, with enhanced[17] or delayed[19] clearance of remnant chylomicrons in E4 individuals and delayed clearance in E2 heterozygote individuals,[17] whereas Brenninkmeijer et al[18] found such abnormalities only in E2/2 homozygote individuals. Boerwinkle et al,[20] in a large population of 474 individuals, observed a delayed clearance of retinyl esters in E2 heterozygote individuals compared with E4 heterozygote and E3 homozygote individuals, but no difference in postprandial TG responses according to apo E genotype. Postprandial metabolism of apo E has been poorly investigated in diabetes, but a recent study suggests an enrichment with apo E of the remnant lipoproteins in NIDDM patients.[45]

The influence of apo E polymorphism on postprandial lipemia in NIDDM patients has not been evaluated, and one might expect an influence of increased apo E glycosylation on the postprandial catabolism of apo E–rich lipoproteins.[46] In our study, E2/3 and E3/4 NIDDM patients had a delayed postprandial chylomicron clearance, as suggested by higher retinyl ester concentrations in the plasma and the chylomicron fraction. Moreover, postprandial remnant chylomicron clearance was delayed only in E2/3 patients. These different postprandial responses were actually attributable to the effect of apo E phenotype independently of age, weight, and fasting TG level. The lower TG peak amplitude and TG AUC contrasting with a higher RP peak observed in E3/4 patients as compared with E3/3 patients in our study, as in another study,[19] might reflect a faster hydrolysis of TG in E4 chylomicrons, possibly due to a modulation of lipoprotein lipase activity.[47] Alternatively, faster apo E4 transfer to TRL[22] could lead to enhanced TG exchange from TRL to HDL mediated by cholesteryl ester transfer protein,[48] resulting in a rapid turnover of TG-rich HDL more prone to hepatic lipase action[49] and consequently a lower HDL cholesterol level as observed in our study. Moreover, cholesterol enrichment of E4 TRL might favor rapid receptor-mediated uptake of their remnants by hepatocytes, leading in turn to downregulation of LDL receptors and to higher LDL levels.[50] These postprandial alterations in TRL catabolism might explain, in part, the contributory role of phenotype E4 to coronary heart disease in NIDDM,[26] even though the mechanism of such a process remains to be further evaluated.

In summary, the postprandial lipemic response to an oral fat challenge is amplified in NIDDM patients compared with obese nondiabetic patients, even in strictly normotriglyceridemic. This increased postprandial response partially reflects an impairment in the chylomicron catabolic pathway, whereas abnormalities in postprandial endogenous VLDL metabolism are also probably involved in this process. In NIDDM patients, intestinally derived TRL metabolism is modulated by apo E polymorphism, with a slower clearance in E2/3 and E3/4 than in E3/3 patients. Further studies need to be designed to evaluate whether these disturbances in TRL metabolism are involved in the higher incidence of coronary heart disease observed in the NIDDM population.

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