patient-oriented research

High postprandial triglyceridemia in patients with type 2 diabetes and microalbuminuria

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Abstract Microalbuminuria (MA) is an independent risk factor for atherosclerosis in patients with type 2 diabetes mellitus (T2DM). Postprandial lipemia is also associated with excess cardiovascular risk. However, the association between MA and postprandial lipemia in diabetes has not been investigated. A total of 64 patients with T2DM, 30 with and 34 without MA, were examined. Plasma total triglycerides (TGs), triglycerides contained in chylomicrons (CM-TG), and TGs in CM-deficient plasma were measured at baseline and every 2 h for 6 h after a mixed meal. Postheparin LPL and HL activities were also determined. Plasma levels of apolipoprotein A-V (apoA-V), apoC-II, and apoC-III were measured in the fasting state and 2 h postprandially. Patients with MA had higher postprandial total TG levels than those without MA ($P < 0.001$); this increase been attributed mainly to CM-TG. LPL activity and fasting concentrations of the measured apolipoproteins were not different between the studied groups, whereas HL activity was higher in the patients with MA. ApoC-II and apoC-III levels did not change postprandially in either study group, whereas apoA-V increased more in the patients with MA. These data demonstrate for the first time that MA is characterized by increased postprandial lipemia in patients with T2DM and may explain in part the excess cardiovascular risk in these patients.—Tentolouris, N., A. Stylianou, E. Lourida, D. Perrea, D. Kyriaki, E. C. Papavasiliou, A. D. Tselepis, and N. Katsilambros. **High postprandial triglyceridemia in patients with type 2 diabetes and microalbuminuria. *J. Lipid Res.* 2007.** 48: **218–225.**

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Macrovascular complications are the leading cause of morbidity and mortality in patients with type 2 diabetes mellitus (T2DM) (1). Microalbuminuria (MA) is common (prevalence rates of 10–48%) and is a well-established risk factor for macrovascular diseases in patients with T2DM (2, 3). A number of abnormalities have been described in diabetic patients with MA, including high blood pressure, dyslipidemia, insulin resistance, endothelial dysfunction, left ventricular hypertrophy, hypercoagulation, and high plasma homocysteine as well as C-reactive protein levels (4), all of which increase the cardiovascular risk in this group of patients.

Diabetic dyslipidemia is common in T2DM and is characterized by high levels of fasting triglycerides (TGs), low HDL cholesterol levels, and predominance of small, dense LDL cholesterol particles (5, 6). Moreover, the majority of patients with T2DM show high and prolonged postprandial lipemia after meals (7–9). Epidemiological data suggest that high plasma TG levels, both in the fasting state and postprandially, are associated with cardiovascular diseases in patients with diabetes (10, 11).

LPL is the key enzyme involved in the metabolism of TG-rich lipoproteins (12). However, literature data on the activity of LPL in patients with MA are not unanimous: some studies found decreased plasma LPL activity in microalbuminuric subjects (13), whereas others found no difference in LPL activity between normoalbuminuric and microalbuminuric subjects (14, 15). Reduced LPL activity in patients with T2DM and MA may result in enhancement in postprandial lipemia. However, the potential association between MA and postprandial lipemia has not been investigated to date.

Therefore, the main aim of this study was to examine the association between MA and postprandial lipemia in patients with T2DM. In addition, potential differences in LPL and HL activity, as well as in apolipoprotein A-V (apoA-V), apoC-II, and apoC-III involved in TG metabolism, between patients with and without MA were also investigated.

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Abbreviations: AER, albumin excretion rate; apoA-V, apolipoprotein A-V; AUC, area under the curve; BMI, body mass index; CM, chylomicron; CM-TG, triglycerides in chylomicron fraction; HOMA-IR, homeostasis model assessment equation insulin resistance; MA, microalbuminuria; MS, metabolic syndrome; T2DM, type 2 diabetes mellitus; TG, triglyceride; WHR, waist-to-hip ratio.

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METHODS

Subjects

A total of 64 patients with T2DM were examined. Patients were recruited from the outpatient diabetes clinic of our hospital. Subjects with diseases that may cause dyslipidemia (macroalbuminuria, abnormal liver or thyroid function) and those treated with medications affecting plasma lipids (statins, fibrates, ezetimide), urinary albumin excretion (angiotensin-converting enzyme inhibitors, angiotensin receptor blockers), and LPL activity (heparin in the previous 3 months, glitazones) were excluded (16, 17). To avoid the potential effect of smoking on plasma lipid levels, current smokers were also excluded. Equal numbers of patients in the two groups had been treated with diuretics and β-adrenergic blockers, drugs that may affect lipid metabolism. The protocol was approved by the ethics committee of our hospital, and written informed consent was obtained from all participants. Subjects were divided into two groups according to the presence or absence of MA.

Procedures and anthropometric measurements

Each subject attended the metabolic unit of our hospital twice in the morning after a 12–14 h fast. The antidiabetic medications were given at the end of the visits in the unit. In the first visit, a fasting blood sample was taken 15 min after contralateral intravenous administration of heparin (30 IU/kg body weight; Leo Pharmaceuticals, Weesp, The Netherlands) for the determination of LPL and HL activities. After ~1 week from the first visit, patients received a standard mixed meal consisting of four slices of toast bread, 225 g of a low-fat cheese, and 40 g of butter (total energy, 783 kcal: 52.5% as fat, 20% as protein, and 27.5% as carbohydrates, mainly as complex carbohydrates). Patients were permitted to consume only water during the study.

Blood was drawn in the fasting state and at 2, 4, and 6 h after the test meal for biochemical determinations. After proper preparation, plasma samples were stored in small aliquots at −80°C until assayed at the end of the study.

Weight and height were measured using standard methods. Body mass index (BMI) and waist-to-hip ratio (WHR) were measured and calculated. Absence of at least two of the four peripheral pulses in the feet, a history of intermittent claudication, ischemic rest pain, gangrene, any previous revascularization procedure at the aorta or the leg arteries, and ankle brachial index < 0.9 were all considered indications of the presence of peripheral vascular disease. Coronary artery disease was determined by resting 12 lead electrocardiography, from hospital records of myocardial infarction, and from history of angina or coronary revascularization procedure. Cerebrovascular disease was assessed by history, clinical examination, and hospital records of stroke. Arterial blood pressure was measured in the sitting position on three occasions with an interval of 1 min between determinations. The average of the last two of the three measurements was taken as the final blood pressure value.

Biochemical parameters

LPL and HL activities were measured in fasting, postheparin plasma samples by fluorometric assay using the Confluolip kit [Progen, Heidelberg, Germany; intra-assay coefficient of variation (CV) = 3.5%, interassay CV = 3.8%]. Briefly, 2 μl of plasma was used as the source of LPL or HL and was mixed with 200 μl of freshly reconstituted substrate. The assay was performed for 15 min at 37°C, and LPL and HL activities were determined by the increase in fluorescence intensity measured in a fluorescence spectrometer at an excitation wavelength of 342 nm and an emission wavelength of 400 nm (18).

Chylomicrons (CMs) were isolated from plasma by ultracentrifugation as described previously (19). CMs floating to the top of the tube were carefully aspirated with a Pasteur pipette. One milliliter of CM-deficient plasma was also carefully collected from the bottom of the tube.

Plasma apoA-V concentrations were measured at baseline and at the time of the peak concentration of plasma TGs (2 h after the test meal) by Linco Diagnostic Services (St. Charles, MO) using a dual-antibody apoA-V sandwich ELISA (20). ApoC-II and apoC-III were determined at the same time intervals by immunoturbidimetry with antisera from Kamiya Biomedical Co. (Seattle, WA) with commercially available kits and according to the manufacturer's instructions on an Olympus AU 640 automated analyzer (Diamond Diagnostics, Holliston, MA).

Plasma total cholesterol and creatinine levels were determined on a Technicon RA-XT analyzer (Technicon, Ltd., Dublin, Ireland). HDL cholesterol levels were measured by the same method in the supernatant of CM-deficient plasma after precipitation of apoB-containing lipoproteins by adding heparin and manganese chloride. LDL cholesterol levels were estimated using the equation of Friedewald, Levy, and Fredrickson (21). Triglycerides in the chylomicron fraction (CM-TG), in total plasma, and in CM-deficient plasma (non-CM-TG) were determined on a Technicon RA-XT analyzer. Plasma levels of FFAs were measured by enzymatic colorimetric assay (Bayer; Boehringer Mannheim). Serum glucose was measured by colorimetric method GOD-PERID (Bayer; Boehringer Mannheim). Glycosylated hemoglobin A_{1c} was measured by HPLC (Roche Diagnostics, Mannheim, Germany) with a nondiabetic reference range of 4.1–6.0%. MA was diagnosed when albumin excretion rate (AER), measured by RIA (Pharmacia and Upjohn Diagnostics AB, Uppsala, Sweden), was 30–300 mg/24 h in at least two of three 24 h urine collections over a 3 month period. Serum insulin levels were determined by RIA (Biosure, Brussels, Belgium). Glomerular filtration rate was calculated according to the equation of Cockcroft and Gault (22). The homeostasis model assessment equation was used to calculate insulin resistance (HOMA-IR) (23). Blood for the determination of fibrinogen was collected in tubes containing trisodium citrate (0.105 M) anticoagulant. The blood samples were immediately placed on melted ice and centrifuged at 1,700 *g* for 10 min at 4°C within 15 min after blood collection. Fibrinogen determination was performed using the Clauss method on a Sta Compact (Diagnostica Stago, Asnieres-Sur-Seine, France; intra-assay CV = 2.64%, interassay CV = 2.82%).

Statistical analysis

Statistical analysis was performed using programs available in the SPSS 10.0 statistical package. Student's *t*-test was used to compare parameters between patients with and without MA, and a Chi-square test was used for categorical variables. When data were skewed, logarithmic transformations were performed to improve normality for statistical testing and back-transformed for presentation in tables. One-way ANCOVA was used to assess differences in the tested variables, adjusting for the effect of confounding factors. ANOVA for repeated measurements was performed to test the timing effect of the studied parameters after the test meal. The Greenhouse-Geisser adjustment was used when the sphericity assumptions were not fulfilled. Postprandial responses over the 6 h period were calculated as incremental area under the curve (AUC) using the trapezoid rule. The AUCs adjusted for baseline values were calculated by subtracting the fasting value from each postprandial value before area calculation. A paired Student's *t*-test was used for comparison of the differences of the values of the studied parameters in the postprandial and the baseline state. Comparisons in the values of

the AUCs between subjects with and without MA were performed using the Mann-Whitney *U*-test. Correlations between the study parameters are expressed as Pearson's or Spearman's bivariate correlation coefficient (r). $P < 0.05$ (two-tailed) was considered statistically significant.

RESULTS

Baseline comparisons

Table 1 summarizes the characteristics of the studied patients with and without MA. The two groups were matched for sex and age. Duration of diabetes and diabetes control were similar in the two groups. Microalbuminuric patients had higher BMI, waist, and WHR than normoalbuminuric patients. More normoalbuminuric patients were treated with diet alone compared with microalbuminuric patients, but this difference was not statistically significant. Blood pressure was slightly higher in the microalbuminuric group. Concerning macroangiopathic complications, prevalence rates of coronary artery disease and peripheral vascular disease were similar in the two groups. Plasma insulin concentrations and HOMA-IR values were higher in the microalbuminuric group ($P < 0.01$).

At baseline, plasma concentrations of glucose, total cholesterol, HDL cholesterol, LDL cholesterol, FFAs, total TGs, CM-TG, and non-CM-TG were not different between patients with and without MA (**Table 2**, **Fig. 1**). However, patients with MA had higher plasma fibrinogen levels (Table 2).

Postheparin LPL activity values (mean ± SEM) were not different between patients with and without MA (180.1 ± 24.3 vs. 165.6 ± 25.4 pmol/ml/h; $P = 0.29$). Postheparin HL activity values were higher in microalbuminuric than in normoalbuminuric patients (161.7 ± 21.0 vs. 88.2 ± 17.2 pmol/ml/h; $P = 0.006$). The ratio of HL to LPL activity values was also higher in patients with MA (0.89 ± 0.05 vs. 0.53 ± 0.04; $P = 0.04$).

Fasting plasma apoA-V concentrations were not significantly different between patients with and without MA ($P = 0.84$). The same was valid for the plasma levels of apoC-II ($P = 0.41$) and apoC-III ($P = 0.80$) (Table 2) as well as the apoC-II/apoC-III ratio (0.39 ± 0.15 vs. 0.36 ± 0.12; $P = 0.32$).

Comparisons in the postprandial state

After the meal, plasma glucose, insulin, total TGs, CM-TG, and non-CM-TG increased significantly compared with baseline values in both normoalbuminuric and microalbuminuric subjects; the increase was significantly higher in the microalbuminuric than in the normoalbuminuric group in all of these variables, except for glucose and non-CM-TG (Table 2, Fig. 1). On the contrary, plasma levels of LDL cholesterol as well as of FFAs decreased after the meal compared with baseline values; however, this decrease was not significantly different between the studied groups. Plasma concentrations of total cholesterol, HDL cholesterol, and fibrinogen did not change significantly from baseline after the meal in either study group (Table 2).

Plasma apoA-V concentrations increased 2 h after the test meal compared with baseline values in the microalbuminuric patients ($P < 0.001$), whereas there was a trend towards an increase in the normoalbuminuric patients ($P = 0.06$) (Table 2). The percentage increase from the

TABLE 1. Demographic and clinical characteristics of the study subjects stratified according to MA status

Characteristics	Without MA (n = 34)	With MA (n = 30)	*P*
Male/female, n (%)	17 (50.0)/17 (50.0)	17 (56.7)/13 (43.3)	0.59
Age (years)	61.5 ± 7.6	63.0 ± 6.7	0.40
Body mass index (kg/m^2)	28.4 ± 5.7	30.8 ± 5.6	0.09
Waist (cm)	99.5 ± 12.0	108.8 ± 11.3	0.002
Waist-hip ratio	0.92 ± 0.06	0.96 ± 0.08	0.05
Systolic blood pressure (mmHg)	131.9 ± 16.8	135.7 ± 14.6	0.34
Diastolic blood pressure (mmHg)	77.9 ± 8.1	79.8 ± 8.9	0.38
Glomerular filtration rate (ml/min)	85.8 ± 24.5	96.4 ± 45.7	0.28
Duration of diabetes (years)	10.0 (1.0–18.3)	10.5 (3.0–17.0)	0.93
Glycosylated hemoglobin A_{1c} (%)	7.4 ± 1.2	7.8 ± 1.2	0.18
Treatment for diabetes, n (%)			
Diet alone	6 (17.6)	1 (3.3)	
Antidiabetic tablets	19 (55.9)	22 (73.3)	
Insulin	9 (26.5)	7 (23.3)	0.14
24 h urine albumin (mg/24 h)	7.6 (1.8–11.5)	68.8 (46.5–94.1)	<0.001
Insulin (pmol/l)	47.9 (22.1–73.6)	104.9 (79.8–114.6)	<0.001
Homeostasis model assessment equation insulin resistance index	2.5 (1.3–4.2)	6.1 (4.5–7.8)	0.001
Retinopathy, n (%)			
No	24 (70.6)	23 (76.7)	
Background	7 (20.6)	6 (20.0)	
Proliferative	3 (8.8)	1 (3.3)	0.65
Hypertension, n (%)	11 (32.4)	9 (30.0)	0.83
Coronary artery disease, n (%)	3 (8.8)	2 (6.7)	0.74
Peripheral vascular disease, n (%)	1 (2.9)	2 (6.7)	0.48
Use of β-adrenergic blockers, n (%)	6 (17.6)	3 (10.0)	0.38
Use of diuretics, n (%)	7 (20.6)	5 (16.7)	0.68

MA, microalbuminuria. Data are shown as means ± SD, n (%), or median (interquartile range).

TABLE 2. Fasting and postprandial profiles of the measured parameters in patients with (+) and without (−) MA

Parameter	Fasting	2 h	4 h	6 h	*P*	*P**
Glucose (mmol/l)						
MA−	8.82 ± 3.16	12.89 ± 5.25	11.63 ± 4.62	9.86 ± 3.99	<0.0001	0.30
MA+	9.28 ± 2.97	15.69 ± 4.98	12.37 ± 5.22	9.89 ± 4.68	<0.0001	
Insulin (pmol/l)						
MA−	57.5 ± 62.8	236.4 ± 138.8	159.46 ± 120.2	81.40 ± 62.2	<0.0001	0.03
MA+	105.7 ± 45.7	409.5 ± 380.8	256.48 ± 218.4	114.52 ± 73.4	0.005	
Total cholesterol (mmol/l)						
MA−	4.95 ± 0.84	4.89 ± 0.82	4.87 ± 0.80	4.92 ± 0.83	0.18	0.69
MA+	5.02 ± 0.84	5.01 ± 0.79	4.91 ± 0.86	5.02 ± 0.84	0.23	
Total TGs (mmol/l)						
MA−	1.25 ± 0.61	1.47 ± 0.61	1.46 ± 0.61	1.46 ± 0.61	<0.001	<0.001
MA+	1.27 ± 0.51	1.96 ± 0.59	1.88 ± 0.72	1.48 ± 0.84	<0.001	
CM-TG (mmol/l)						
MA−	0.44 ± 0.31	0.64 ± 0.31	0.63 ± 0.36	0.51 ± 0.16	<0.001	<0.001
MA+	0.44 ± 0.24	1.08 ± 0.46	1.01 ± 0.43	0.64 ± 0.19	<0.001	
Non-CM-TG (mmol/l)						
MA−	0.81 ± 0.21	0.83 ± 0.21	0.83 ± 0.19	0.79 ± 0.17	0.04	0.36
MA+	0.83 ± 0.19	0.89 ± 0.24	0.86 ± 0.21	0.83 ± 0.22	0.001	
HDL cholesterol (mmol/l)						
MA−	1.00 ± 0.17	1.00 ± 0.17	1.01 ± 0.18	1.00 ± 0.35	0.30	0.22
MA+	0.95 ± 0.22	0.94 ± 0.23	0.95 ± 0.23	0.95 ± 0.44	0.74	
LDL cholesterol (mmol/l)						
MA−	3.58 ± 0.89	3.32 ± 0.69	3.21 ± 0.77	3.31 ± 0.90	0.008	0.29
MA+	3.47 ± 0.66	3.00 ± 0.70	3.02 ± 0.84	3.18 ± 0.74	<0.0001	
ApoA-V (ng/ml)						
MA−	128.5 ± 49.8	145.8 ± 48.3	—	—	0.06	0.01
MA+	132.0 ± 78.6	168.1 ± 80.8			<0.001	
ApoC-II (mg/dl)						
MA−	3.41 ± 1.68	3.11 ± 1.64	—	—	0.18	0.58
MA+	3.77 ± 1.60	3.69 ± 1.56			0.56	
ApoC-III (mg/dl)						
MA−	9.43 ± 2.94	8.75 ± 2.54	—	—	0.08	0.14
MA+	9.63 ± 3.15	9.60 ± 2.86			0.91	
Fibrinogen (g/l)						
MA−	1.98 ± 0.56	1.97 ± 0.55	1.89 ± 0.52	1.96 ± 0.55	0.35	0.20
MA+	2.32 ± 0. 64	2.28 ± 0.71	2.26 ± 0.64	2.24 ± 0.71	0.36	
FFA (mmol/l)						
MA−	0.293 ± 0.150	0.188 ± 0.914	0.161 ± 0.099	0.186 ± 0.108	<0.0001	0.26
MA+	0.327 ± 0.138	0.208 ± 0.122	0.192 ± 0.113	0.215 ± 0.102	<0.0001	

ApoA-V, apolipoprotein A-V; CM, chylomicron; CM-TG, triglycerides in chylomicron fraction; TG, triglyceride. Data are means ± SD. *P* indicates the result of ANOVA for repeated measurements within each group (*P* value for the effect of time); *P** indicates the result of ANOVA for repeated measurements between the two groups (MA− vs. MA+) (time × group interaction).

baseline was significantly higher in patients with MA than in those without MA [median value (interquartile range), 36.5% (18.6, 72.1) vs. 6.0% (−10.2, 31.5), respectively; $P = 0.003$]. On the contrary, plasma levels of apoC-II and apoC-III did not change significantly 2 h after the test meal compared with the baseline values in either study group; moreover, no significant differences were observed between patients with and without MA (Table 2).

The incremental AUCs of total TGs and CM-TG were significantly higher in the patients with MA compared with those without MA (both $P < 0.001$) (Fig. 1). These differences remained significant ($P < 0.001$ for both AUCs of total TGs and CM-TG) even after adjustment for BMI, waist circumference, WHR, and HOMA-IR. The incremental AUC of non-CM-TG was higher in the microalbuminuric group, but this difference was not statistically significant (Fig. 1).

Additionally, we looked for differences in AERs and postprandial lipemia between study subjects stratified according to the presence (n = 50) or absence (n = 14) of metabolic syndrome (MS) using the ATP III criteria (24). Analysis showed that patients with MS had higher [median value (interquartile range)] AERs [49.1 (11.3, 82.5) vs. 27.0 (11.2, 50.0) mg/24 h; $P = 0.01$], AUC of total TGs [1.38 (0.72, 2.35) vs. 0.73 (0.27, 1.36) mmol/h/l; $P = 0.004$], and AUC of CM-TG [1.17 (0.60, 2.14) vs. 0.54 (0.17, 1.30) mmol/h/l; $P = 0.02$)]. The AUC of non-CM-TG was not different between the two groups [0.13 (−0.03, 0.24) vs. 0.04 (−0.09, 0.17) mmol/h/l; $P = 0.14$].

Bivariate correlations and multivariate analysis

In the total study population, AER correlated significantly with the incremental AUCs of total TGs ($r = 0.48$, $P = 0.002$) and CM-TG ($r = 0.43$, $P = 0.006$) but not with the incremental AUC of non-CM-TG ($r = 0.14$, $P = 0.38$). LPL activity values did not correlate significantly with the values of the fasting total TGs, CM-TG, and non-CM-TG or with the incremental AUCs after the test meal of these same parameters. HL activity values showed a significant negative association with the values of the fasting non-CM-TG ($r = -0.33$, $P = 0.03$) but not with those of the fasting total TGs and CM-TG or with the values of the incremental

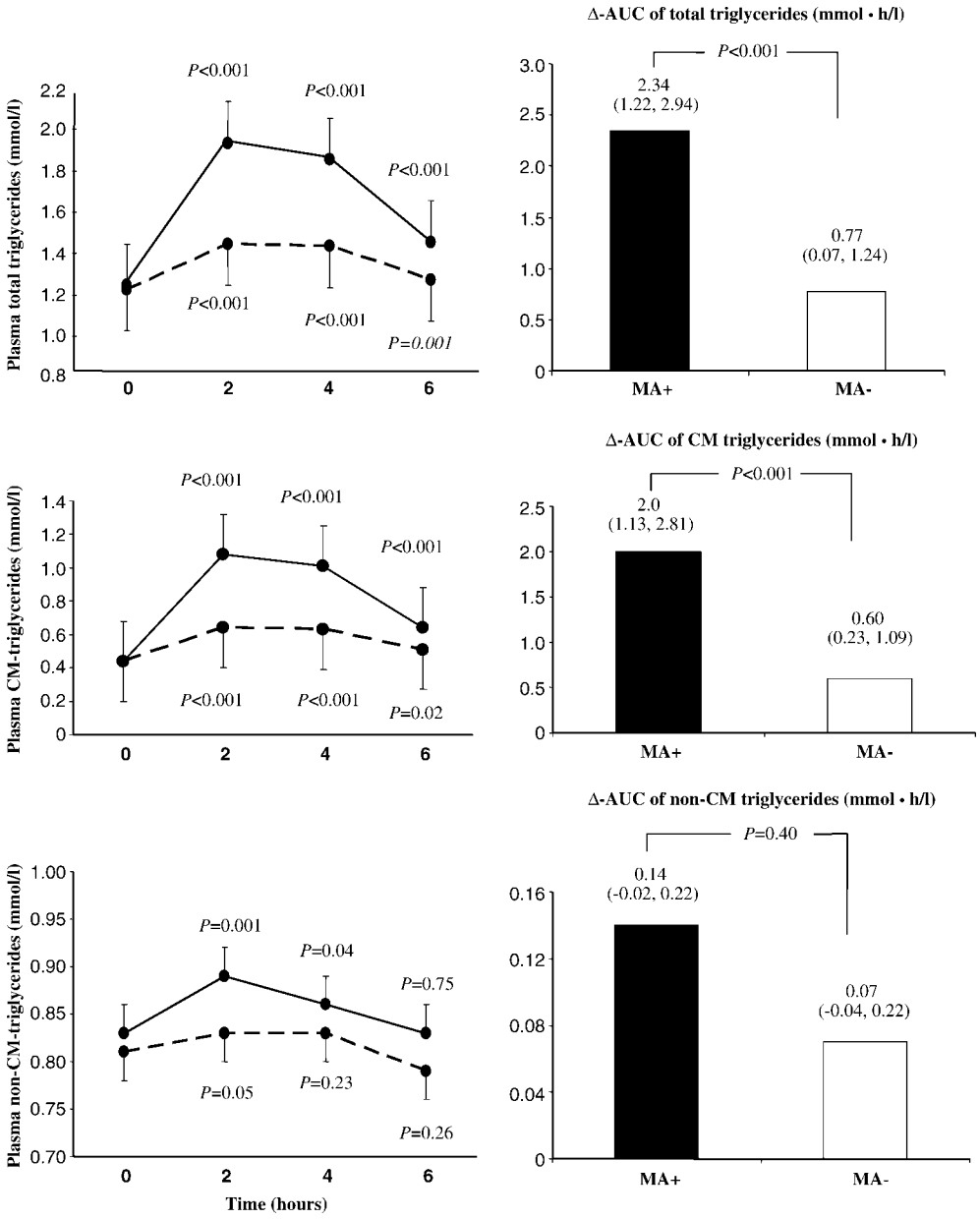

Fig. 1. Left panel: Plotted values are means ± SEM. Continuous lines, patients with microalbuminuria (MA); dashed lines, patients without MA. *P* values, which are for the comparison with baseline data, were determined with the use of the paired *t*-test. Right panel: Bars represent median values (interquartile range) of the area under the curve (AUC) of the increment of plasma triglyceride levels in patients with (black bars) and without (white bars) MA. *P* values, which are for the comparison between patients with (+) and without (−) MA, were determined with the use of the Mann-Whitney *U*-test. CM, chylomicron; non-CM, nonchylomicron.

AUCs of total TG, CM-TG, and non-CM-TG. In the fasting state, plasma apoC-II levels correlated significantly with fasting total TG ($r = 0.49$, $P < 0.001$), CM-TG ($r = 0.26$, $P = 0.04$), and non-CM-TG ($r = 0.31$, $P = 0.01$). Plasma apoC-III levels also correlated significantly with total TG ($r = 0.61$, $P < 0.001$), CM-TG ($r = 0.51$, $P < 0.001$) and non-CM-TG ($r = 0.50$, $P < 0.001$). However, fasting levels of apoC-II and apoC-III did not correlate significantly with the lipase activities or the postprandial AUCs of total TGs, CM-TG, and non-CM-TG. Furthermore, HOMA-IR correlated significantly with the incremental AUC of total TGs ($r = 0.32$, $P = 0.01$) and CM-TG ($r = 0.28$, $P = 0.03$); however, these relationships did not remain significant after controlling for the effect of MA. No significant associations were found between HOMA-IR and the incremental AUCs of non-CM-TG, LPL activity, and HL activity values.

Multivariate linear regression analysis in the total sample population, after adjustment for MA status or AER, waist circumference, HOMA-IR, and MS status, demon-

strated that the AUC of total TGs was independently and significantly associated only with the presence of MA [standardized regression coefficient (β) = 0.51, $P < 0.001$] and the degree of MA (β = 0.50, $P < 0.001$); the same was valid for the AUC of CM-TG (β = 0.51, $P < 0.001$ and β = 0.42, $P = 0.02$, respectively). The same analysis showed a suggestive association between the AUC of non-CM-TG and the degree of MA (β = 0.33, $P = 0.07$).

DISCUSSION

The novel finding of this study is that normotriglyceridemic patients with T2DM and MA have an almost 3-fold higher postprandial triglyceridemia than patients without MA after ingestion of a mixed test meal.

Exaggerated postprandial lipemia is related to proatherogenic conditions, and clinical studies provide evidence that exposure to postprandial lipoproteins is associated with cardiovascular diseases (10, 11). A variety of in vitro and clinical studies suggest that postprandial CM and VLDL are associated with adverse effects on vascular endothelium (8). In addition, CM and VLDL remnants as well as intermediate density lipoproteins and LDLs enter the arterial wall and are retained in the intima as well as in the atherosclerotic plaques in direct proportion to their plasma concentrations (25). In the postprandial state, similar to that of hypertriglyceridemic subjects, there is abundant formation of atherogenic small, dense LDL particles and less formation of antiatherogenic large HDL_2 particles (9, 10). Furthermore, high plasma TG levels are associated with changes in hemostatic factors that promote the risk for thrombotic events (26).

Although lipid metabolism in diabetes and overt nephropathy has been examined intensively, little is known about the effect of MA on postprandial lipemia. Previous studies have proved that patients with T2DM had higher and more prolonged increments in plasma TGs after a mixed meal compared with healthy individuals (6–9). Both hepatically and intestinally derived TGs contribute to the exaggerated postprandial lipemia in individuals with T2DM (8). The abundant offer of FFAs, glucose, and CM remnants to the liver in the presence of insulin resistance results in the overproduction of large VLDL particles, which compete with intestinally derived CM for clearance via the same lipolytic pathway (6–9).

Lipoprotein lipase is the key enzyme in the postprandial processing of TGs of both dietary origin and liver-derived VLDLs (12). At high plasma TG levels, LPL actions are saturated, leading to defects in the clearance of both hepatically and intestinally derived TG-rich lipoproteins (8, 12). LPL is attached to the luminal surface of the capillary endothelial cells via heparin sulfate proteoglycans (12). It has been suggested that MA in patients with diabetes might reflect widespread vascular damage resulting in low LPL activity (27). However, the results of this study showed that LPL activity was similar between patients with and without MA. Most of the previous studies reported similar plasma LPL and higher HL activity levels in patients with type 1 diabetes mellitus and MA compared with normoalbuminuric patients (14, 15). Moreover, one study showed that heparin-releasable LPL activity in muscle biopsies was not different between patients with and without MA (28), whereas other investigators have shown that LPL mass is reduced in microalbuminuric patients with T2DM (13). An explanation for the divergent results may be the fact that in the former studies the activity of LPL was measured, whereas in the latter study, the mass of the enzyme was determined.

The results of this study do not shed light on the mechanisms underlying the increase in postprandial lipemia in microalbuminuric patients. We tried to evaluate the potential role of the lipolytic enzymes on postprandial lipemia by measuring the activity of postheparin LPL and HL in the basal state. The postheparin levels of LPL were not different between the studied groups, whereas that of HL was higher in the microalbuminuric group. The nonsignificant association between lipase activity and postprandial triglyceridemia suggests that lipase activity may not influence postprandial lipemia in patients with MA. This finding is quiet conceivable. Lipase activity, determined in vitro after heparin injection, reflects the maximum available amount of enzymatically active lipase. However, the in situ clearance capacity is dependent on stimulatory and inhibitory factors (12, 29, 30). In vitro, insulin is a strong determinant of LPL activity (30). In clinical studies, however, conflicting data have been published on the association between plasma insulin levels and LPL activity (31, 32). Thus, some authors found a weak, but positive, association between insulin resistance and LPL as well as HL activity (32), whereas others found a negative one (31). In this study, we did not find significant associations between insulin resistance and postheparin lipase activity. In addition, results from a recent study showed that LPL activity was associated weakly with plasma TGs (33). Given that lipase activity is regulated by other factors, the usefulness of activity measurements made under optimized conditions in vitro as an indicator of the in vivo activity has been questioned (31).

ApoC-II activates but apoC-III is an effective inhibitor of LPL activity (12). ApoC-III synthesis is increased in hypertriglyceridemic patients with type 2 diabetes (34) and in overt nephropathy (17). Our results did not show differences in either apoC-II or apoC-III plasma concentrations between the participants with and without MA in both fasting and postprandial states. Other studies have also shown that plasma apoC-III levels do not change postprandially (35, 36). In addition, plasma levels of the recently described apoA-V, which is also involved in the metabolism of TG-rich lipoproteins (20), were not different between the studied groups. Additionally, the postprandial increase of apoA-V was higher in patients with MA who had higher lipemia, suggesting that the apoA-V mechanism in the regulation of TG metabolism is not impaired in patients with MA.

As the clearance of TG-rich lipoproteins may not be impaired in subjects with MA, the enhanced postprandial lipemia in these patients may be attributable to an altered metabolism of TG-rich lipoproteins in the intes-

tine. Emerging evidence suggests that animal models with insulin resistance have increased production of the highly atherogenic apoB-48 by the intestine, which may explain the profound postprandial lipemia observed in insulin-resistant states (9, 37). Our study subjects with MA had higher (by almost 2-fold) insulin resistance compared with subjects without MA, and the increase of postprandial lipemia was mainly attributable to the increase of intestinally derived TGs. Therefore, these findings support the notion that TG-rich lipoprotein assembly and secretion in the intestine may be different in patients with MA. However, it cannot be excluded that other, yet undetermined mechanisms may also be involved in the exaggeration of postprandial lipemia associated with MA, such as alterations in other apolipoproteins affecting lipid metabolism or decreased activity of lipoprotein receptors.

Interestingly, this study has shown that microalbuminuric patients had higher BMI, waist circumference, WHR, plasma insulin, HOMA-IR, and postprandial lipemia. However, multivariate analysis eliminated the role of these confounding factors and demonstrated that only the presence and degree of MA were associated independently with postprandial lipemia. It is noteworthy that MA was included in the diagnostic criteria for the MS proposed by the World Health Organization in 1998 (38). Therefore, the enhanced postprandial lipemia in patients with T2DM and MA may be a feature of the MS, if MA is included in its definition.

Poor glycemic control decreases (8, 12, 30), whereas treatment with glitazones increases, LPL activity (16). Glycemic control was similar in the studied groups, and none of the subjects was on treatment with glitazones. A similar percentage of patients was treated with β-adrenergic blockers and diuretics in the two groups. Therefore, the differential effects of MA on postprandial triglyceridemia cannot be attributed to differences in glycemia or to drug-mediated effects.

Recent data suggest that LPL gene variants associated with dyslipidemia may be important in the development of diabetic nephropathy, as they are associated with both the degree of AER and the progression of MA in T2DM (39). However, genetic studies have not been performed in this study. Therefore, interference between genetic variation of LPL activity and postprandial lipemia in patients with MA cannot be ruled out.

In agreement with previous reports, this study showed that fasting plasma fibrinogen levels were significantly higher in the MA compared with normoalbuminuric subjects (4). In addition, it was shown that the postprandial change of plasma fibrinogen levels was not different between the studied groups.

This study has enough power (>0.90 at the 0.001 significance level) to detect differences in postprandial triglyceridemia between subjects with and without MA. A limitation of the study is that the participants were a selective group; the results, therefore, cannot be extrapolated to the total diabetic population.

In conclusion, irrespective of the underlying mechanisms, the results of this study demonstrate for the first time that MA is associated with enhanced postprandial lipemia in normotriglyceridemic patients with T2DM. Because substantial evidence suggests that postprandial lipemia is atherogenic, the findings of this study are noteworthy and may explain in part the excess cardiovascular disease risk in patients with T2DM and MA. Clearly, further studies are needed to examine whether reduction of MA restores the exaggerated postprandial lipemia in patients with T2DM and MA and to elucidate the underlying mechanisms responsible for the abnormalities in lipid metabolism in the postprandial state in this group of patients.

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