# Increased plasma apoA-IV level is a marker of abnormal postprandial lipemia: a study in normoponderal and obese subjects

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**Abstract** **Plasma apolipoprotein A-IV (apoA-IV) levels are found elevated in hypertriglyceridemic patients. However, the relationship between plasma apoA-IV level and postprandial lipemia is not well known and remains to be elucidated. Thus, our objective was to study the relationship between plasma apoA-IV and postprandial TG after an oral fat load test (OFLT). Plasma apoA-IV was measured at fast and during an OFLT in 16 normotriglyceridemic, normoglucose-tolerant android obese subjects (BMI = 34.6 ± 2.9 kg/m$^2$) and 30 normal weight controls (BMI = 22.2 ± 2.3 kg/m$^2$). In spite of not statistically different fasting plasma TG levels in controls and obese patients, the former group showed an altered TG response after OFLT, featuring increased nonchylomicron TG area under the curve (AUC) compared with controls (516 ± 138 vs. 426 ± 119 mmol/l·min, $P < 0.05$). As compared to controls, obese patients showed increased apoA-IV levels both at fast (138.5 ± 22.4 vs. 124.0 ± 22.8 mg/l, $P < 0.05$) and during the OFLT (apoA-IV AUC: 79,833 ± 14,281 vs. 68,176 ± 17,463 mg/l·min, $P < 0.05$). Among the whole population studied, as among the control and obese subgroups, fasting plasma apoA-IV correlated significantly with AUC of plasma TG ($r = 0.60$, $P < 0.001$), AUC of chymomicron TG ($r = 0.45$, $P < 0.01$), and AUC of nonchylomicron TG ($r = 0.62$, $P < 0.001$). In the multivariate analysis, fasting apoA-IV level constituted an independent and highly significant determinant of AUC of plasma TG, AUC of chymomicron TG, AUC of nonchylomicron TG, and incremental AUC of plasma TG. In conclusion, we show a strong link between fasting apoA-IV and postprandial TG metabolism. Plasma fasting apoA-IV is shown to be a good marker of TG response after an OFLT, providing additional information on post-load TG response in conjunction with other known factors such as fasting TGs.**—Vergès, B., B. Guerci, V. Durlach, C. Galland-Jos, J. L. Paul, L. Lagrost, and P. Gambert. **Increased plasma apoA-IV level is a marker of abnormal postprandial lipemia: a study in normoponderal and obese subjects.** ***J. Lipid Res.*** **2001.** 42: **2021–2029.**

**Supplementary key words** triglycerides • oral fat load • syndrome X • kinetics

Human apolipoprotein A-IV (apoA-IV) is a 46-kDa plasma apolipoprotein that is synthesized predominantly in the small intestine (1–4). ApoA-IV is found to be associated in plasma with TG-rich lipoproteins and HDL particles (5). Although its precise function remains unclear, apoA-IV has been proposed to play a role in the metabolism of both TG-rich lipoproteins and HDL. ApoA-IV has been shown to modulate the activation of lipoprotein lipase in the presence of apoC-II (6). ApoA-IV is thought to play a potentially important role in reverse cholesterol transport because it has been shown to stimulate lecithin: cholesterol acyl transferase (LCAT) activity (7, 8), to bind to bovine aortic endothelial cells (9) and to hepatic tissue (10, 11), to stimulate cholesterol efflux from adipose cells (12, 13), and to participate in HDL particle conversion by cholesteryl ester transfer protein (CETP) (14, 15). ApoA-IV can modulate CETP-mediated transfer of cholesteryl esters between HDL and LDL fractions (16). Moreover, Weinberg, Ibdah, and Phillips (17) demonstrated that apoA-IV exhibits labile reversible binding to HDL3 and proposed that apoA-IV helps to maintain optimal surface pressure for CETP activity. Furthermore, apoA-IV could be a signal for satiety in the central nervous system (18, 19).

For several years, postprandial lipid metabolism has received considerable attention because it has been shown that postprandial TG-rich lipoproteins are involved in the

Abbreviations: apoA-IV, apolipoprotein A-IV; ALT, alanine amino-transferase; AST, aspartate amino-transferase; AUC, area under the curve; AUCi, incremental AUC; BMI, body mass index; γGT, gamma-glutamyl transferase; HOMA, homeostasis model assessment; LCAT, lecithin:cholesterol acyl transferase; OFLT, oral fat load test.

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development of atherosclerosis (20, 21). Many studies comparing patients with coronary heart disease and controls have demonstrated differences in postprandial TG after an oral fat load test (OFLT) (22, 23) and shown that postprandial TG level was an independent predictor of coronary artery disease in multivariate analysis (24, 25). Others and we have reported altered postprandial lipemia in obese subjects (26–28), which could explain, at least partly, the greater development of cardiovascular disease in the obese population.

Several studies have pointed out a link between apoA-IV and TG metabolism. Indeed, plasma apoA-IV levels are found elevated in hypertriglyceridemic patients (29–31). We have previously shown that increased plasma apoA-IV levels associated with hypertriglyceridemia are due to delayed catabolism of apoA-IV (31). However, very little is known about the relationship between plasma apoA-IV level and postprandial lipemia, and so far, only few studies performed in a limited number of normal subjects have been reported (3, 32, 33). Thus, to gain further insight into the relationship between apo-IV and postprandial lipid metabolism, we aimed to study the possible link between plasma apoA-IV and postprandial TG after an OFLT in normolipidemic obese subjects with altered post-fat load TG responses and in normal weight healthy controls.

## MATERIALS AND METHODS

### Patients and controls

A group of 30 normal weight controls (17 men, 13 women) and 16 obese patients (5 men, 11 women) were recruited. All had a normal glucose tolerance (fasting plasma glucose $<6.1$ mmol/l and 2-h plasma glucose $<7.8$ mmol/l after an oral glucose tolerance test) and normal fasting lipid levels (TG $\leq 1.50$ mmol/l, HDL cholesterol $>0.90$ mmol/l for men and 1.03 mmol/l for women, and LDL cholesterol $<4.13$ mmol/l).

Android obese patients were selected according to the following criteria: 1) body mass index (BMI) $>30$ kg/m$^2$; 2) abdominal (android) fat distribution, defined by a waist-to-hip ratio $>0.85$ for women and $>0.95$ for men; 3) none of the patients was morbidly obese. Healthy subjects were selected according to the following criteria: BMI 18–25 kg/m$^2$ and stable body weight ($<2\%$ change in the last 3 months).

All the subjects (obese and control) had no symptoms of illness, no family history of premature coronary disease (before 60 years), and normal values for blood creatinine, sodium, potassium, chloride, total protein, total and direct bilirubin, activities of aspartate (AST) or alanine (ALT) amino-transferase, and gamma-glutamyl transferase ($\gamma$GT). None had any endocrine or gastrointestinal disease, hypertension (systolic blood pressure $<140$ mmHg, diastolic blood pressure $<90$ mmHg), or were regular smokers (cigarette smoking was defined as the consumption of $>10$ cigarettes daily for at least 5 years). None of the participants (obese or controls) were on any medication known to affect carbohydrate or lipoprotein metabolism, and their alcohol intake was limited ($<20$ g/day). All the women were premenopausal (in follicular cycle) and none was taking oral contraceptive or hormone replacement therapy. This project was approved by the local Ethics committee of the Nancy University Hospital (France), and all subjects gave their written informed consent.

### Dietary assessments

A weight maintenance diet was prescribed for all subjects [50% carbohydrate, 33% fat (polyunsaturated/saturated ratio 80%) and 17% protein] for the 7 days before the study to ensure uniformity.

Three days before the OFLT, subjects were instructed to maintain their usual level of activity and to refrain from any strenuous exercise to limit the influence of acute exercise, which alters lipid metabolism (34, 35). For the same reason, alcohol was not allowed during the 3 days immediately before OFLT (36, 37). Subjects remained fasted during the 12 h before the oral fat load was given (at 8:00 AM).

### OFLT

The OFLT was performed as previously reported (28). In summary, the fat load consisted of 180 g of a blended emulsified meal containing 88 mg cholesterol, 35 g saturated fatty acid, and 30 g mono- and 15 g polyunsaturated fatty acid (Laboratoires Pierre Fabre Santé, Castres, France). It provided 890 calories (85% fat, 13% carbohydrates, and 2% protein). The fat load was ingested in 15 min with 200 ml water. No further food or drink was allowed during the study period. The participants were instructed to remain in bed in a supine position.

### Laboratory procedures

Plasma apoA-IV concentrations were measured using a competitive enzyme immunoassay standardized with purified apoA-IV, as previously described (29, 38). The coefficient of variability for this apoA-IV assay was 3.0% within runs and 3.9% between runs. ApoA-IV phenotyping was performed by isoelectric focusing of delipidated plasma samples and immunoblotting (39).

Total cholesterol and TG were measured enzymatically (bioMérieux, Marcy l'Etoile, France). HDL cholesterol was assessed by phosphotungstic acid precipitation, and LDL cholesterol was calculated according to the Friedewald formula (40). HDL2 and HDL3 cholesterol concentrations were determined by nondenaturating electrophoresis in discontinuous gradient gels (41). ApoA-I and apoB were determined by immunonephelometry with commercial kits (Beckman, Gagny, France). Plasma glucose was determined enzymatically (PAP 250; bioMérieux). Total plasma insulin concentration was measured by immunoenzymatic assay (Insulin IMX®; Abbott Laboratories, Tokyo, Japan). Cross reactivity with proinsulin was $<0.05\%$.

The apoE genotypes were determined using *Hha*I restriction enzyme and PCR (42).

Fasting plasma leptin concentrations were measured in triplicate by radioimmunoassay (LINCO Research Inc., Saint-Louis, MO). The intra- and inter-assay coefficients of variation were 4.5 and 8%, respectively.

Insulin sensitivity was assessed from blood samples collected 30, 20, and 10 minutes before the ingestion of the oral fat load using the homeostasis model assessment (HOMA) system described by Matthews et al. (43) with the formula (insulin mU/l $\times$ plasma glucose mmol/l)/22.5.

### Collection of sequential blood samples

Blood samples were monitored as previously reported (28). In summary, blood samples were taken 30, 20, and 10 minutes before the fat load, at the time of the fat load, and 2, 3, 4, 5, 6, and 8 hours later (T0, T2, T3, T4, T5, T6, and T8). Plasma apoA-IV levels were measured at T0, T4, and T8. The apoE genotype, apoA-I and apoB, plasma total LDL and HDL cholesterol, and HDL2 and HDL3 cholesterol concentrations were determined at T0. The lipoprotein fraction (supernatant) containing chylomicrons was isolated by ultracentrifugation for 30 min at 25,000 rpm in a Beckman (Palo Alto, CA) XL-80 ultracentrifuge, rotor Ti-SW 41. The infranatant was collected and named the nonchylo-

micron fraction, which contained TG-rich lipoproteins (chylomicron remnants, VLDL, and VLDL remnants). Mean recovery (±SD) was 98 ± 3% for TG. TG in the plasma and fractions were assayed within the day.

### Statistical analysis

Data are means ± SD. The areas under the time concentration curves [area under the curves (AUCs)] were calculated by the trapezoidal method (44). Incremental AUC (AUCi) was evaluated after subtracting the initial individual value (T0) from all respective postprandial measurements, yielding the net postprandial change.

Means were compared between two groups by Student's unpaired *t*-test and between several groups by ANOVA. For paired data, means between two groups were compared with the paired *t*-test. Data were compared by the nonparametric Mann-Whitney U-test when men and women were analyzed separately. The $\chi^2$ test was used to compare frequencies between controls and obese subjects.

When the distribution of a variable was not normal, as assessed by the Kolmogorov-Smirnov test, data were log-transformed for univariate and multivariate regression analyses. The correlation coefficients ($r$) were determined by linear regression analysis. Statistical significance of the correlation coefficients was determined by the method of Fisher and Yates (44a). Multiple linear regression analyses were performed to identify significant independent predictors of postprandial lipemia parameters and significant independent predictors of fasting plasma apoA-IV level. Significance was implied at $P < 0.05$. Statistical analyses were performed using the SPSS software (SPSS, Inc., Chicago, IL).

## RESULTS

### Characteristics and lipid concentrations of obese patients and controls

The clinical and biological characteristics of the obese subjects and controls are shown in **Table 1**. Obese subjects had significantly higher BMI, waist-to hip ratio, HOMA, plasma glucose, insulin, and leptin levels than controls. For all characteristics listed in Table 1, there were no significant differences between men and women except for waist-to-hip ratio (higher in men than in women in both controls and obese subjects) and leptin levels (higher in women than in men in both controls and obese subjects) (data not shown).

TABLE 1. Clinical and biological characteristics of the control and obese subjects

| | Controls (n = 30) | Obese Subjects (n = 16) |
|---|---|---|
| Sex ratio (male/female) | 17/13 | 5/11[a] |
| Age (years) | 30.9 ± 8.9 | 41.3 ± 9.2[b] |
| BMI (kg/m$^2$) | 22.2 ± 2.3 | 34.6 ± 2.9[b] |
| Waist/hip ratio | 0.82 ± 0.07 | 0.94 ± 0.06[b] |
| Fasting plasma glucose (mmol/l) | 4.76 ± 0.52 | 5.54 ± 0.64[b] |
| Fasting plasma insulin (pmol/l) | 31.3 ± 11.5 | 63.5 ± 26.2[b] |
| HOMA | 1.13 ± 0.49 | 2.65 ± 1.25[b] |
| Leptin (ng/ml) | 6.1 ± 5.1 | 27.5 ± 16.2[b] |

Data are means ± SD. BMI, body mass index. HOMA, homeostasis model assessment.
[a] Not significant.
[b] $P < 0.001$.

TABLE 2. Fasting lipid parameters of control and obese subjects

| | Controls (n = 30) | Obese Subjects (n = 16) |
|---|---|---|
| Total cholesterol (mmol/l) | 4.33 ± 0.77 | 4.66 ± 0.74[a] |
| Fasting TG (mmol/l) | 0.77 ± 0.32 | 0.93 ± 0.23[a] |
| HDL cholesterol (mmol/l) | 1.24 ± 0.22 | 1.08 ± 0.27[b] |
| HDL2 cholesterol (mmol/l) | 0.48 ± 0.18 | 0.33 ± 0.17[c] |
| HDL3 cholesterol (mmol/l) | 0.76 ± 0.14 | 0.76 ± 0.13[a] |
| LDL cholesterol (mmol/l) | 2.76 ± 0.67 | 3.15 ± 0.61[a] |
| ApoA-I (g/l) | 1.29 ± 0.20 | 1.19 ± 0.26[a] |
| ApoB (g/l) | 0.70 ± 0.15 | 0.79 ± 0.16[a] |
| Lp(a) (g/l) | 0.17 ± 0.25 | 0.18 ± 0.27[a] |
| Apo E genotype (n) | | |
| ε2/ε3 | 5 | 2[a] |
| ε3/ε3 | 19 | 10[a] |
| ε2/ε4 | 1 | 0[a] |
| ε3/ε4 | 5 | 4[a] |
| ApoA-IV phenotype (n) | | |
| A-IV 0/1 | 0 | 1[a] |
| A-IV 1/1 | 27 | 12[a] |
| A-IV 1/2 | 3 | 3[a] |

Data are means ± SD. Apo, apolipoprotein; Lp(a), lipoprotein (a).
[a] Not significant.
[b] $P < 0.05$.
[c] $P < 0.01$.

Fasting lipid parameters in control and obese subjects are shown in **Table 2**. HDL cholesterol concentrations were lower in obese subjects compared with controls but remained within the normal range. HDL2 cholesterol levels was significantly lower in obese patients than in controls ($P < 0.01$). For all lipid values listed in Table 2, there were no significant differences between men and women except for HDL2 cholesterol levels (higher in women than in men in both controls and obese subjects) (data not shown).

The distribution of apoE genotypes and apoA-IV phenotypes were not statistically different between controls and obese subjects (Table 2).

The three apoE genotypes had comparable clinical characteristics and lipid values. Furthermore, clinical characteristics and lipid parameters were not different between apoA-IV 1/1 and 1/2 phenotypes.

### ApoA-IV concentrations and postprandial TG responses in obese subjects and controls

TG responses after the OLFT and plasma apoA-IV levels at different time points (T0, T4, and T8) are shown in **Table 3**. Plasma levels of apoA-IV levels at T0 (fasting), at T4, and at T8 were significantly higher in obese subjects than in controls. The area under the plasma apoA-IV curve (AUC apoA-IV) was significantly greater in obese subjects. Plasma TG at T8 and nonchylomicron TG at T8 were significantly higher in obese subjects than in controls ($P < 0.05$). The AUC of plasma TG and the AUC of chylomicron TG were not significantly different between controls and obese subjects. The incremental AUC for plasma TG was not different between controls and obese subjects. The AUC of nonchylomicron TG and the incremental AUC of nonchylomicron TG were significantly greater in obese subjects than in controls ($P < 0.05$).

TABLE 3. Triglyceride and apoA-IV responses after oral fat load test (OFLT) in control and obese subjects

| | Controls (n = 30) | Obese Subjects (n = 16) |
|---|---|---|
| Plasma TG at T0 (mmol/l) | 0.77 ± 0.32 | 0.93 ± 0.23[a] |
| Plasma TG at T2 (mmol/l) | 1.47 ± 0.66 | 1.43 ± 0.43[a] |
| Plasma TG at T3 (mmol/l) | 1.67 ± 0.80 | 1.70 ± 0.55[a] |
| Plasma TG at T4 (mmol/l) | 1.75 ± 0.79 | 1.74 ± 0.48[a] |
| Plasma TG at T5 (mmol/l) | 1.79 ± 0.77 | 1.67 ± 0.60[a] |
| Plasma TG at T6 (mmol/l) | 1.46 ± 0.58 | 1.53 ± 0.51[a] |
| Plasma TG at T8 (mmol/l) | 0.93 ± 0.44 | 1.24 ± 0.46[b] |
| CM TG at T0 (mmol/l) | 0 | 0[a] |
| CM TG at T2 (mmol/l) | 0.52 ± 0.38 | 0.35 ± 0.22[a] |
| CM TG at T3 (mmol/l) | 0.62 ± 0.46 | 0.48 ± 0.34[a] |
| CM TG at T4 (mmol/l) | 0.71 ± 0.42 | 0.50 ± 0.22[a] |
| CM TG at T5 (mmol/l) | 0.74 ± 0.48 | 0.50 ± 0.26[a] |
| CM TG at T6 (mmol/l) | 0.53 ± 0.28 | 0.47 ± 0.25[a] |
| CM TG at T8 (mmol/l) | 0.25 ± 0.21 | 0.31 ± 0.18[a] |
| Non-CM TG at T0 (mmol/l) | 0.77 ± 0.32 | 0.93 ± 0.23[a] |
| Non-CM TG at T2 (mmol/l) | 0.96 ± 0.36 | 1.09 ± 0.29[a] |
| Non-CM TG at T3 (mmol/l) | 1.05 ± 0.39 | 1.22 ± 0.31[a] |
| Non-CM TG at T4 (mmol/l) | 1.04 ± 0.41 | 1.24 ± 0.34[a] |
| Non-CM TG at T5 (mmol/l) | 1.03 ± 0.39 | 1.18 ± 0.43[a] |
| Non-CM TG at T6 (mmol/l) | 0.93 ± 0.36 | 1.06 ± 0.35[a] |
| Non-CM TG at T8 (mmol/l) | 0.68 ± 0.27 | 0.93 ± 0.33[c] |
| AUC plasma TG (mmol/l·min) | 659 ± 205 | 705 ±182[a] |
| AUCi plasma TG (mmol/l·min) | 321 ± 160 | 259 ± 106 [a] |
| AUC CM TG (mmol/l·min) | 176 ± 70 | 136 ± 57[a] |
| AUC non-CM TG (mmol/l·min) | 426 ± 119 | 516 ± 138[b] |
| AUCi non-CM TG (mmol/l·min) | 91 ± 58 | 127 ± 57[b] |
| Fasting apoA-IV at T0 (mg/l) | 124.0 ± 22.8 | 138.5 ± 22.4[b] |
| ApoA-IV at T4 (mg/l) | 151.5 ± 46.1 | 182.7 ± 38.9[b] |
| ApoA-IV at T8 (mg/l) | 141.0 ± 34.6 | 161 ± 31.1[b] |
| AUC apoA-IV (mg/l·min) | 68,176 ± 17,463 | 79,833 ± 14,281[b] |

AUC, area under the curve; AUCi, incremental AUC; CM, chylomicron. Because CM TG = 0 at T0, AUC CM TG is equal to AUCi CM TG.

[a] Not significant.

[b] $P < 0.05$.

[c] $P < 0.01$.

The three apoE genotypes had comparable triglyceride and apoA-IV responses after OFLT. Furthermore, triglyceride and apoA-IV responses after OFLT were not different between apoA-IV 1/1 and 1/2 phenotypes (data not shown).

### Kinetic of apoA-IV after the oral fat load

**Figure 1** shows means of plasma TG and apoA-IV levels after the oral fat load in all subjects. As expected, plasma apoA-IV increased after the oral fat load, with significantly higher level at T4 [164 ± 47 vs. 129 ± 27 (at T0) mg/l, $P < 0.0001$] and at T8 [149 ± 34 vs. 129 ± 27 (at T0) mg/l, $P < 0.0001$]. As shown in Fig. 1, plasma apoA-IV at T8 was still significantly higher than basal values ($P < 0.0001$), whereas plasma TG had returned to nearly basal values, indicating a more prolonged plasma residence time postprandially for apoA-IV than for TG. Indeed, plasma TG levels at T8 had returned to basal values (TG level at T8 ⩽ TG level at T0) in 27 subjects (58.7%), when apoA-IV levels at T8 had returned to basal values in only 11 subjects (23.9%) ($\chi^2 = 12.8$, $P = 0.0007$).

### Correlation coefficients

**Table 4** shows the correlation coefficients between plasma fasting apoA-IV level and lipid parameters during the OFLT in all subjects and in control and obese subjects separately. A strong correlation was found in all subjects and in controls and obese subjects between fasting apoA-IV levels on the one hand and the AUC of plasma TG, the AUC of chylomicron TG, and the AUC of nonchylomicron TG on the other hand. The correlation between fasting apoA-IV level and the AUC of plasma TG is shown in **Fig. 2**. Fasting apoA-IV was also positively correlated with the incremental AUC for plasma TG in all subjects and in both controls and obese subjects. As shown in Table 4, apoA-IV was significantly correlated with plasma TG at all time points of the OFLT, from the beginning up to 6 h.

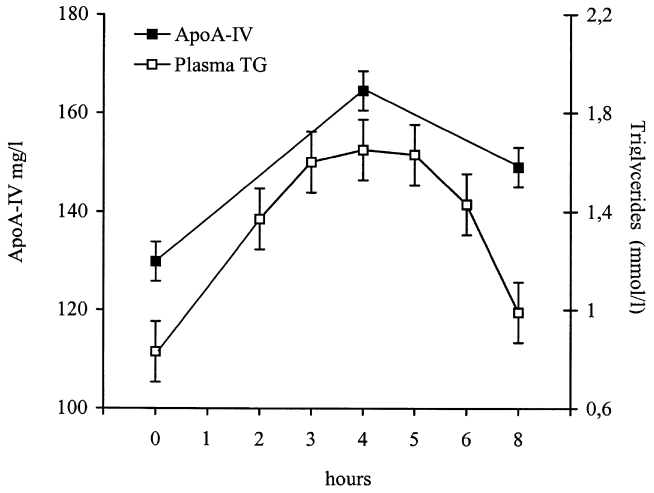


**Fig. 1.** Means of plasma TG and plasma apoA-IV levels during the OFLT in the whole population studied (control and obese subjects).

### Multivariate analysis

Stepwise multiple regression analyses were performed for all subjects to evaluate the independent effects of different factors [age, sex, type (obese or control), BMI,

TABLE 4. Univariate analysis. Correlation coefficients between plasma fasting apoA-IV level (at T0) and lipid parameters during the OFLT in all subjects and in each group (controls, obese subjects)

| | All Subjects (n = 46) | Controls (n = 30) | Obese Subjects (n = 16) |
|---|---|---|---|
| AUC plasma TG | $r = 0.60$[a] | $r = 0.56$[a] | $r = 0.66$[a] |
| AUC CM TG | $r = 0.45$[b] | $r = 0.57$[a] | $r = 0.54$[c] |
| AUC non-CM TG (log) | $r = 0.62$[a] | $r = 0.60$[a] | $r = 0.59$[c] |
| AUCi plasma TG | $r = 0.35$[c] | $r = 0.37$[c] | $r = 0.55$[c] |
| Plasma TG at T0 | $r = 0.65$[a] | $r = 0.63$[a] | $r = 0.56$[c] |
| Plasma TG at T2 | $r = 0.62$[a] | $r = 0.71$[a] | $r = 0.48$[d] ($P = 0.06$) |
| Plasma TG at T3 (log) | $r = 0.49$[a] | $r = 0.49$[b] | $r = 0.50$[c] |
| Plasma TG at T4 | $r = 0.63$[a] | $r = 0.61$[a] | $r = 0.64$[a] |
| Plasma TG at T5 | $r = 0.38$[b] | $r = 0.37$[c] | $r = 0.65$[a] |
| Plasma TG at T6 (log) | $r = 0.38$[b] | $r = 0.36$[c] | $r = 0.65$[a] |
| Plasma TG at T8 | $r = 0.17$[d] | $r = 0.07$[d] | $r = 0.34$[d] |

[a] $P < 0.001$.

[b] $P < 0.01$.

[c] $P < 0.05$.

[d] Not significant.

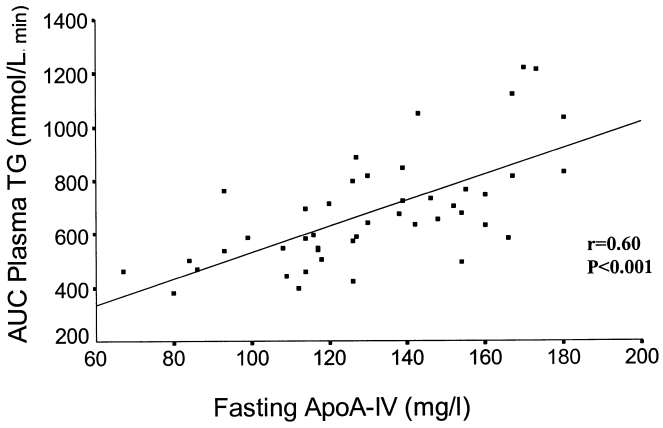


**Fig. 2.** Correlation between fasting apoA-IV level and the area under the curve (AUC) of plasma TG in the whole population studied (control and obese subjects).

waist-to-hip ratio, fasting TG, HDL2 cholesterol, fasting apoA-IV] on the AUC of plasma TG, the AUCi for plasma TG, the AUC of chylomicron TG, or the AUC of nonchylomicron TG (**Tables 5–8**).

Table 5 shows the results of the multiple linear regression with the AUC plasma TG as dependent variable. Both fasting TG ($P = 0.0065$) and fasting apoA-IV levels ($P = 0.0178$) were shown to influence independently and significantly the AUC of plasma TG and could explain 49% of its variance. When fasting apoA-IV was not introduced into the model, only 39% of the AUC of plasma TG could be predicted.

The results of the multiple linear regression with the AUCi of plasma TG as dependent variable are shown in Table 6. The type of subject (obese or control) ($P = 0.0001$), fasting apoA-IV ($P = 0.0011$), age ($P = 0.0024$), and HDL2 cholesterol ($P = 0.0031$) were shown to be independently associated with the AUCi of plasma TG, explaining 42% of its variance. When fasting apoA-IV was not introduced into the model, only 29% of the AUCi of plasma TG could be predicted.

Table 7 shows the results of the multiple linear regression with AUC of chylomicron TG as dependent variable. Fasting apoA-IV ($P < 0.0001$), the type of subject (obese or control) ($P < 0.0001$), HDL2 cholesterol ($P = 0.0049$), and age ($P = 0.0245$) were shown to influence independently the AUC of chylomicron TG, explaining 52% of its variance. When fasting apoA-IV was not introduced into the model, only 36% of the AUC of chylomicron TG could be predicted.

The results of the multiple linear regression with the log of AUC nonchylomicron TG are shown in Table 8. Fasting TG ($P = 0.0005$), age ($P = 0.0015$), fasting apoA-IV ($P = 0.0041$), and HDL2 cholesterol ($P = 0.0413$) were shown to be independently associated with the log of AUC nonchylomicron TG, explaining 69% of its variance. When fasting apoA-IV was not introduced into the model, only 62% of the log of AUC of nonchylomicron TG could be predicted.

To study the influence of different factors including fasting and postprandial lipids on fasting apoA-IV level, we performed a stepwise multiple linear regression analysis with fasting apoA-IV as dependent variable and age, sex, type (obese or control), BMI, waist/hip ratio, fasting TG, HDL2 cholesterol, and AUC of plasma TG as independent variables. As shown in **Table 9**, fasting plasma TG ($P = 0.0039$) and the AUC of plasma TG ($P = 0.017$) were independently associated with fasting plasma apoA-IV level, explaining 50% of its variance.

TABLE 6. Multivariate analysis. Multiple linear regression with AUCi TG as dependent variable

| | Coefficient | SD | *t* | *P* | $R^2$ of the Model |
|---|---|---|---|---|---|
| Significant and independent variables | | | | | 0.42 |
| Type (control/obese) | −214 | 47 | 4.50 | 0.0001 | |
| Fasting apoA-IV at T0 | 2.27 | 0.64 | 3.51 | 0.0011 | |
| Age | 6.54 | 2.02 | 3.23 | 0.0024 | |
| HDL2 cholesterol | −315 | 100 | 3.14 | 0.0031 | |
| Nonsignificant variables | | | | | |
| Plasma TG at T0 | | | | | |
| BMI | | | | | |
| Waist/hip ratio | | | | | |
| Sex | | | | | |

TABLE 5. Multivariate analysis. Multiple linear regression with AUC plasma TG as dependent variable

| | Coefficient | SD | *t* | *P* | $R^2$ of the Model |
|---|---|---|---|---|---|
| Significant and independent variables | | | | | 0.49 |
| Plasma TG at T0 | 290 | 101 | 2.86 | 0.0065 | |
| Fasting apoA-IV at T0 | 2.66 | 1.08 | 2.46 | 0.0178 | |
| Nonsignificant variables | | | | | |
| Age | | | | | |
| BMI | | | | | |
| HDL2 cholesterol | | | | | |
| Waist/hip ratio | | | | | |
| Sex | | | | | |
| Type (control/obese) | | | | | |

TABLE 7. Multivariate analysis. Multiple linear regression with AUC CM TG as dependent variable

| | Coefficient | SD | *t* | *P* | $R^2$ of the Model |
|---|---|---|---|---|---|
| Significant and independent variables | | | | | 0.52 |
| Fasting apoA-IV at T0 | 1.46 | 0.27 | 5.23 | <0.0001 | |
| Type (control/obese) | −101 | 20 | 5.06 | <0.0001 | |
| HDL2 cholesterol | −125 | 42 | 2.97 | 0.0049 | |
| Age | 1.98 | 0.85 | 2.33 | 0.0245 | |
| Nonsignificant variables | | | | | |
| Plasma TG at T0 | | | | | |
| BMI | | | | | |
| Sex | | | | | |
| Waist/hip ratio | | | | | |

TABLE 8. Multivariate analysis. Multiple linear regression with Log AUC non-CM TG as dependent variable

| | Coefficient | SD | *t* | *P* | $R^2$ of the Model |
|---|---|---|---|---|---|
| Significant and independent variables | | | | | 0.69 |
| Plasma TG at T0 | 0.42 | 0.11 | 3.80 | 0.0005 | |
| Age | 0.008 | 0.002 | 3.38 | 0.0015 | |
| Fasting apoA-IV at T0 | 0.003 | 0.001 | 3.03 | 0.0041 | |
| HDL2 cholesterol | −0.27 | 0.12 | 2.10 | 0.0413 | |
| Nonsignificant variables | | | | | |
| BMI | | | | | |
| Waist/hip ratio | | | | | |
| Sex | | | | | |
| Type (control/obese) | | | | | |

## DISCUSSION

In this study, we show that plasma fasting apoA-IV level is strongly correlated with the TG response after an OFLT, indicating a link between apoA-IV and postprandial TG metabolism. Plasma fasting apoA-IV level is shown to be a good marker of TG response after OFLT, giving additional information on post-load TG response complementary to other known factors such as fasting TG.

For a few years, special interest has been focused on apoA-IV because of its potentially important role in lipoprotein metabolism. A part of plasma apoA-IV is bound to TG-rich lipoproteins, and a link between plasma apoA-IV and TG metabolism has been found in clinical studies, showing a positive correlation between fasting plasma apoA-IV levels and fasting TG (29, 30, 45). However, so far, only few studies on apoA-IV in the postprandial state, performed in a limited number of normal subjects, have been reported (3, 32, 33), and the relationship between fasting plasma apoA-IV level and postprandial lipemia remains to be elucidated. Thus, we decided to study the possible association between apoA-IV and TG concentrations after an oral fat load in normal weight controls and in obese subjects who were normotriglyceridemic in the fasted state. Such obese patients with an increased risk for cardiovascular disease (46) are good candidates for an oral fat load test to detect atherogenic postprandial lipid abnormalities because they display no significant degree of hyperlipemia in the fasted state. This combined population of control and obese subjects gave us a broad range of lipid responses after the OFLT, giving us optimal conditions to analyze the relationship between apoA-IV and postprandial lipemia.

As expected, plasma apoA-IV levels rose after the oral fat load. Plasma apoA-IV, which is synthesized predominantly in the small intestine, is known to increase postprandially (3, 32, 47, 48). As previously reported, normolipidemic obese subjects had altered postprandial lipemia indicated by a significantly greater AUC of the TG in the nonchylomicron fraction than in controls (28). Fasting plasma apoA-IV was significantly higher in obese subjects than in controls when fasting TG were not significantly different between the two groups. apoA-IV at T4 and T8 and apoA-IV AUC were also significantly increased in obese subjects.

Several studies have pointed out structural and functional differences between the two major isoproteins, apoA-IV-1 and apoA-IV-2 (49–51). It has been reported that the hypercholesterolemic response to a high-cholesterol diet was attenuated in subjects with the apoA-IV 1-2 phenotype (52). However, in the present study, the TG response after the OFLT was not different between the two main apoA-IV phenotypes (1/1 and 1/2). Our results are in accordance with previous report of De Knijff et al. (53), who did not show any differences of fasting cholesterol and TG levels between the different apoA-IV phenotypes.

One of the most interesting results is that fasting plasma apoA-IV is highly correlated with AUC of plasma TG in the entire studied population as well as in each group, controls and obese subjects. Because this strong positive correlation is found in both groups, it is likely to represent an important link between fasting apoA-IV and postprandial TG metabolism. Furthermore, the association between fasting apoA-IV and AUC of plasma TG is not confounded by other factors, as demonstrated in the multivariate analysis. Interestingly, fasting apoA-IV is associated not only with the AUC of plasma TG but also with the AUCi of plasma TG, as shown in univariate and multivariate analyses. This reinforces the link between fasting plasma apoA-IV and the TG response after the oral fat load.

In a previous report, Dallongeville et al. (33) did not find any correlation between fasting plasma apoA-IV and postprandial changes in plasma TG. However, these authors used an apoA-IV assay that may be less precise than ours, as shown by the wide range of fasting apoA-IV values obtained in their healthy subjects. Thus, the different assay used by Dalongeville et al. to measure plasma apoA-IV may explain the discrepancies between their results and ours Moreover, surprisingly in their study, Dalongeville et al. did not find any correlation between fasting apoA-IV and fasting TG, although such a correlation has been found in many studies (29–31, 45).

Plasma fasting apoA-IV is found to be strongly correlated with both the AUC of chylomicron TG and the AUC

TABLE 9. Multivariate analysis. Multiple linear regression with fasting apoA-IV (at T0) as dependent variable

| | Coefficient | SD | *t* | *P* | $R^2$ of the Model |
|---|---|---|---|---|---|
| Significant and independent variables | | | | | 0.50 |
| Plasma TG at T0 | 40.1 | 13.2 | 3.04 | 0.0039 | |
| AUC plasma TG | 0.046 | 0.018 | 2.46 | 0.017 | |
| Nonsignificant variables | | | | | |
| Age | | | | | |
| BMI | | | | | |
| HDL2 cholesterol | | | | | |
| Waist/hip ratio | | | | | |
| Sex | | | | | |
| Type (control/obese) | | | | | |

of nonchylomicron TG. These results indicate that, although synthesized in the intestine, apoA-IV is likely to be a marker of the overall TG response after the OFLT rather than a marker of the lipid response of intestinal origin (chylomicrons) alone. Indeed, apoA-IV, after being synthesized in the intestinal cell and incorporated in the chylomicrons, dissociates very rapidly from chylomicrons to bind to other TG-rich lipoproteins (such as VLDL) or to HDL particles (2, 3). For this reason, it is not so surprising to find that apoA-IV is associated with the postprandial increase of all TG-rich lipoproteins derived from intestine or liver (chylomicrons and nonchylomicrons).

Although fasting TG are known to be good markers of postprandial lipid response, it has been shown that they could predict only a part of postprandial lipemia (54, 55). Interestingly, the results of the multivariate analysis indicate that fasting apoA-IV gives additional information on post-load TG response complementary to that of other known factors such as fasting TG.

On the other hand, plasma TG response after the oral fat load (AUC of the plasma TG) is shown to be, in association with fasting TGs, an independent predictor of fasting apoA-IV level. This confirms the link between fasting apoA-IV and TG metabolism, both fasting and postprandial.

ApoA-IV is found to be associated in plasma mainly with HDL particles but also with TG-rich lipoproteins (5, 56). ApoA-IV, after being synthesized in the intestinal cell and incorporated in the chylomicrons, dissociates very rapidly from chylomicrons to bind to other TG-rich lipoproteins (such as VLDL) or to HDL particles (2, 3, 57). The association between fasting apoA-IV and postprandial lipid metabolism is likely due to the fact that apoA-IV binds easily to TG-rich lipoproteins. Indeed, a significant increase of apoA-IV in the chylomicron fraction, after lipid ingestion has been reported in animals (18) and in humans (3), indicates that some apoA-IV remains associated with chylomicrons during chylomicron metabolism. Moreover, Seishima et al. (48) have shown a significant increase in apoA-IV in the TG-rich lipoprotein fraction after an oral fat load in normal subjects. We have previously shown that hypertriglyceridemic patients have a significant increase in apoA-IV within the TG-rich lipoprotein fraction (31). In patients with type 2 diabetes, it has been shown that the apoA-IV level in the apoB-containing lipoproteins was positively correlated with plasma TG (45). Thus, we may think that, during the postprandial state and in hypertriglyceridemia, a part of apoA-IV is associated with TG-rich lipoproteins. Furthermore, an increased level of TG-rich lipoproteins may promote the exchange of apoA-IV to HDL particles, explaining why, in hypertriglyceridemia, increased apoA-IV is observed not only in the TG-rich fraction but also in the HDL fraction (31).

Although apoA-IV catabolism is rapid (mean fractional catabolic rate of 0.096 pool/h) (31, 51), it is slower than TG catabolism (mean VLDL apoB fractional catabolic rate of 0.44 pool/h) (58). This could explain why the postprandial kinetics of apoA-IV is somewhat slower than the TG kinetics, as we observed in our study. Indeed, plasma apoA-IV levels did not return to basal values at T8 in the great majority of subjects whereas plasma TG returned to basal values in 59% of them. As previously shown in a kinetics study (31), increased fasting apoA-IV levels observed in hypertriglyceridemic patients are due to delayed apoA-IV catabolism. We hypothesize that when TG response is increased postprandially, the association of apoA-IV with the TG-rich lipoproteins may partly explain the slower clearance rate of apoA-IV; we also may hypothesize that an increased level of TG-rich lipoproteins promotes the exchange of apoA-IV to HDL particles and therefore may be responsible for its slower catabolism, because HDL catabolism is much slower than TG-rich lipoprotein catabolism. Thus, plasma apoA-IV level appears to be a "remanent marker" of increased TG-rich lipoproteins. Because the catabolism of apoA-IV is slower than that of TG, it is more likely to result, in the fasting state (12 h after the previous meal) in increased plasma apoA-IV levels than increased TG concentrations.

In conclusion, in this study we show a strong correlation between plasma apoA-IV and postprandial TG metabolism in both normoponderal and obese subjects. Plasma fasting apoA-IV is shown to be a good marker of TG response after an OFLT, providing additional information on post-load TG response complementary to that of other known factors such as fasting TG.

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