Effects of atorvastatin on fasting and postprandial complement component 3 response in familial combined hyperlipidemia

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Abstract VLDL overproduction by enhanced hepatic FFA flux is a major characteristic of familial combined hyperlipidemia (FCHL). The postprandial complement component 3 (C3) response has been associated with impaired postprandial FFA metabolism in FCHL. We investigated the effects of 16 weeks of treatment with atorvastatin on postprandial C3 and lipid changes in 12 FCHL patients. Atorvastatin significantly lowered fasting plasma C3 and triglyceride (TG) in FCHL. Fasting TG and insulin sensitivity were the best predictors of fasting and postprandial C3. Postprandial triglyceridemia and C3 response, estimated as area under the curve (AUC), were significantly lowered by atorvastatin by 19% and 12%, respectively, albeit still elevated, compared with 10 matched controls. Postprandial FFA-AUC and postheparin plasma lipolytic activities remained unchanged after atorvastatin, suggesting no major effect on lipolysis. After atorvastatin, postprandial hydroxybutyric acid-AUC, which was elevated in untreated FCHL patients, was decreased, reaching values similar to those in controls. The present data show reduction of postprandial hepatic FFA flux in FCHL by atorvastatin, providing an additional mechanistic explanation for the reduction of VLDL secretion reported previously for atorvastatin. This was accompanied by a decrease in fasting plasma C3 concentrations and a blunted postprandial C3 response to an acute oral fat load.—Verseyden, C., S. Meijssen, H. van Dijk, H. Jansen, and M. Castro Cabezas. **Effects of atorvastatin on fasting and postprandial complement component 3 response in familial combined hyperlipidemia. *J. Lipid Res.* 2003.** 44: **2100–2108.**

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Familial combined hyperlipidemia (FCHL) is the most frequent, dominantly inherited disorder of lipid metabolism leading to increased risk for atherosclerosis (1–8). The diagnosis is based on clinical criteria such as the presence of "multiple-type hyperlipidemia," increased plasma apolipoprotein B (apoB), and a positive family history of premature coronary heart disease (CHD) (1–10). Abdominal obesity and increased body mass index (BMI) have been identified as independent factors for the development of hyperlipidemia and CHD in FCHL (4, 8, 10–12). Associations with the complement system have also been reported (13, 14). The genetic basis of FCHL has not been elucidated, although several groups have provided evidence suggesting that different genes are involved in the pathogenesis of this disorder (15–23).

Impaired FFA metabolism in the postprandial as well as in the postabsorptive period is closely related to the expression of the FCHL phenotype (24–28). FCHL patients have increased postprandial FFA concentrations, compared with healthy controls (24, 26, 27). Increased postprandial FFA concentrations result in an increased postprandial hepatic FFA flux, which could explain in part the well-known VLDL overproduction in FCHL (26). More recently, an impaired postprandial complement component 3 (C3) response has been associated with the disturbed postprandial FFA handling (27).

In vitro and in vivo experiments have demonstrated that the uptake of FFA by peripheral cells is stimulated by acylation-stimulating protein (ASP) (29, 30), which is one of the immunologically inactive cleavage products of C3 (30). Different studies in FCHL and non-FCHL subjects have shown a strong correlation between fasting C3 concentrations and fasting lipid parameters, especially plasma triglycerides (TGs) (13, 14, 27, 31, 32). Furthermore, C3 is a powerful indicator for the risk of myocardial infarction in men (32), and recently, it has been shown that C3 depositions are found predominantly in ruptured atherosclerotic plaques in humans (33), suggesting a pathogenetic involvement in the process of atherosclerosis and acute coronary syndromes. Moreover, both fasting and post-

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prandial C3 concentrations can be decreased by treatment with statins, and this has been associated with an improvement in postprandial triglyceridemia (31).

The present study was carried out to evaluate the effects of atorvastatin in FCHL on postprandial lipemia and C3 changes. In addition, changes in postprandial FFA and hydroxybutyric acid (HBA) levels, reflecting hepatic FFA delivery, were also evaluated.

MATERIALS AND METHODS

Subjects

The study protocol was approved by the Human Investigations Review Committee of the University Medical Center Utrecht. All participants gave written informed consent. Twelve unrelated FCHL patients were recruited from the Lipid Clinic of the University Medical Center Utrecht. All untreated FCHL subjects met the criteria described previously (24, 26, 27) and used by different groups (8–23). In addition, all patients fulfilled the following inclusion criteria: absence of xanthomas; absence of secondary factors associated with hyperlipidemia, as demonstrated by normal thyroid, renal, and liver function tests; absence of diabetes mellitus; BMI <30 kg/m^2; absence of apoE2/E2 genotype; no use of drugs affecting lipid metabolism; and consumption of less than 3 units of alcohol per day. Ten normolipidemic, healthy controls without a family history of cardiovascular disease or type 2 diabetes, with the absence of apoE2/E2 genotype, not using drugs known to affect lipid metabolism, and not using more than 2 alcoholic beverages per day were recruited by advertisement. Controls were matched to FCHL subjects by age, BMI, and waist-to-hip ratio.

Oral fat-loading test

Cream was used as fat source. Added to the cream were 60 g/l dextrose and vitamin A (26). After an overnight fast of 12 h, the subjects ingested the fresh cream in a dose of 50 g fat/m^2 body surface and 7.5 g dextrose/m^2 body surface. After ingestion of the fat load, the participants were allowed to consume only water and sugar-free tea during the following 24 h. Peripheral blood samples were obtained in sodium EDTA (2 mg/ml) before the fat load (t = 0 h), at hourly intervals up to 10 h after the fat load, and then at 12 h and 24 h. For measurement of HBA, blood samples were obtained every 2 h in heparin tubes. Blood was placed on ice and centrifuged immediately for 15 min at 800 *g* at 4°C. Plasma samples were stored at −20°C immediately after centrifugation.

Study protocol

All patients stopped lipid-lowering drugs 4 weeks before the oral fat load, but they continued their usual diet. After the oral fat-loading test, the FCHL patients started with 10 mg atorvastatin once a day. Every 4 weeks, the patients visited our outpatient clinic, where fasting TG and cholesterol were measured. When plasma TG concentrations were >2.0 mmol/l and/or cholesterol >6.5 mmol/l, the dosage of atorvastatin was doubled, up to 80 mg/day after 12 weeks, followed by a second oral fat-loading test 4 weeks after the last dose adjustment.

The healthy volunteers received no treatment and underwent an oral fat-loading test only once. Those data were used as normal reference. All participants underwent a postheparin test to measure lipoprotein lipase (LPL) and hepatic lipase (HL) concentrations at the end of the oral fat-loading test, i.e., 24 h after fat intake.

Analytical methods

TG and cholesterol were measured in duplicate by commercial colorimetric assay (GPO-PAP and CHOD-PAP, Boehringer Mannheim, respectively). FFA was measured in plasma samples by the enzymatic colorimetric method (Wako Chemicals GmbH, Neuss, Germany). HBA was measured spectrophotometrically by the principle of converting NADH to NAD^+ after adding 3-hydroxybutyrate dehydrogenase. For this purpose, 0.5 ml heparin blood was denutriated by adding 1 ml 0.7 M $HClO_4$ (26). The detection limit of HBA is 0.02 mmol/l. C3 was measured by nephelometry (Dade Behring Nephelometry type II) (27, 31). HDL-cholesterol was determined using the phosphotungstic acid/$MgCl_2$ method. Plasma apoB was measured by nephelometry (27, 31). Glucose was measured by glucose oxidase by dry chemistry (Vitros GLU slides) and colorimetry, and insulin was measured by commercial ELISA (Mercodia, Uppsala, Sweden). ApoB48 and apoB100 concentrations in chylomicron fractions [Svedberg flotation (Sf >400)] were determined according to the method used by Karpe and Hamsten (34) and described in detail previously (35). Postheparin plasma LPL and HL activities were determined by the release of FFAs from ^{14}C-labeled trioleoyl emulsion. ASP was measured using the ELISA kindly provided by Dr. K. Cianflone, as described previously (27). For estimation of insulin sensitivity, the homeostasis model assessment (HOMA) (HOMA = glucose × insulin/22.5) was calculated.

Statistics

All values are expressed as mean ± standard error of the mean (SEM). Comparisons are made between FCHL patients and controls (unpaired *t*-test) and between FCHL patients before and after therapy (paired *t*-test). In addition, a subgroup analysis was performed between subjects on the highest dose of atorvastatin (80 mg/day) and those on the lowest doses. The area under the curve (AUC) and incremental AUC were calculated by the trapezoidal rule and after correction for fasting values, respectively. The first 8 h after ingestion of the fat load were used as representing the postprandial period (26, 27). Mean fasting differences between controls and FCHL subjects were calculated by unpaired *t*-test, and fasting difference before and after treatment in FCHL patients was calculated by paired *t*-test. Mean differences in apoB48-AUC and apoB100-AUC were calculated by nonparametric test due to the skewed distribution of these variables. Mean differences of fasting plasma TG were calculated by parametric tests after log transformation. For statistical analysis of changes in C3, TG, FFA and HBA concentrations, repeated measures ANOVA was used, with the least significant difference test as post hoc test. For the calculation of correlations in untreated patients and controls, Spearman's correlation coefficient was determined. All significantly correlated variables were used as independent variables in stepwise multiple regression analysis, with fasting C3 levels and postprandial C3-AUC as dependent variables. Calculations were performed using SPSS/PC 10.0 (SPSS, Inc., Chicago, IL). Calculations of AUCs were performed with GraphPad Prism, version 3.0 (GraphPad Software, Inc., San Diego, CA). Statistical significance was reached at $P < 0.05$ (2-tailed).

RESULTS

General characteristics of untreated FCHL patients and controls

FCHL patients and controls did not differ in anthropometric characteristics (**Table 1**). Fasting plasma TG, cholesterol, apoB, C3, and insulin were significantly higher in

TABLE 1. Anthropometric characteristics of 12 FCHL patients and 10 matched control subjects

	FCHL	Controls
Gender (F/M)	6/6	6/4
Age (year)	42.8 ± 5.5	44.8 ± 3.1
BMI (kg/m^2)	26.1 ± 0.5	25.2 ± 0.9
BP diastolic (mmHg)	85 ± 3	81 ± 4
BP systolic (mmHg)	133 ± 4	124 ± 5
WHR	0.89 ± 0.02	0.87 ± 0.02
apoE genotype	E2/3 n = 2	E2/3 n = 2
	E3/3 n = 7	E3/3 n = 6
	E3/4 n = 2	E3/4 n = 2
	E4/4 n = 1	

apoE, apolipoprotein E; BP, blood pressure; BMI, body mass index; FCHL, familial combined hyperlipidemia; WHR, waist-to-hip ratio. Data are mean ± SEM.

untreated FCHL patients, compared with controls. Fasting ASP concentrations were not different in untreated FCHL patients, compared with controls. FCHL patients were more insulin resistant than controls, according to the elevated HOMA index (**Table 2**).

Treatment effects of atorvastatin

After 16 weeks, two patients were using atorvastatin at a dose of 10 mg/day, four patients at 20 mg/day, one patient at 40 mg/day, and five patients at 80 mg/day. Except for age (36 ± 4 and 52 ± 2 years, respectively; $P = 0.01$) the anthropometric characteristics were not different between the group using the lower doses (up to 40 mg/day) and the five subjects on 80 mg/day. There were no significant differences in baseline (off-treatment) characteristics in the seven subjects at low doses, compared with the five at high doses.

After the final dose adjustment, the patients at 80 mg/day had significantly higher fasting plasma TG concentrations (2.92 ± 0.80 mmol/l), compared with the group at lower doses (1.90 ± 0.22 mmol/l; $P = 0.01$), but similar fasting plasma cholesterol (4.01 ± 0.17 mmol/l and 4.27 ± 0.36 mmol/l, respectively) and apoB concentrations (0.82 ± 0.07 g/l and 0.80 ± 0.07 g/l). Similar reductions of postprandial plasma TG concentrations were observed in the patients at low doses, compared with those using 80 mg/day of atorvastatin.

In the total FCHL group, atorvastatin significantly lowered plasma TG, cholesterol, apoB, and C3 concentrations (Table 2). Plasma apoB concentrations reached normal levels, as compared with controls, and plasma cholesterol concentrations were reduced below the mean levels in these control subjects. These effects were due mainly to a reduction of LDL-apoB (data not shown). LPL and HL concentrations were not different in FCHL patients and controls and were not effected by atorvastatin. HDL-cholesterol, apoAI, ASP, and anthropometric characteristics did not change after treatment.

Postprandial C3 changes

Fasting plasma C3 concentrations decreased after atorvastatin, but were still elevated, compared with controls (Table 2). Before treatment, the first significant increase in C3 concentration was seen 6 h after fat intake in FCHL patients (up to 1.49 ± 0.16 g/l). In controls, however, C3 increased significantly after only 2 h (up to 1.02 ± 0.04 g/l). After treatment, no significant increase was seen over time by repeated measures ANOVA, but there was a tendency to increase 4 h after fat intake (1.27 ± 0.06 g/l; $P = 0.09$).

The postprandial C3-AUC was reduced by atorvastatin in FCHL patients from 11.25 ± 0.69 g·h·l^{-1} to 9.70 ± 0.35 g·h·l^{-1} (in controls: 8.02 ± 0.29 g·h·l^{-1}) (**Fig. 1**, right panel), with a maximal relative postprandial increase of 9.8 ± 8.6% before and 6.2 ± 3.0% after atorvastatin, compared with a 21.8 ± 3.8% increase in controls. C3-AUC was still higher after treatment than in controls. When the group using up to 40 mg atorvastatin/day was compared with the group using 80 mg/day, fasting C3 (1.27 ± 0.07 g/l and 1.21 ± 0.08 g/l, respectively) and postprandial C3-AUC (9.49 ± 0.50 g/l and 9.36 ± 0.49 g/l, respectively) were not different.

Plasma TG, FFA, and HBA changes

In FCHL patients before and after treatment as well as in controls, postprandial plasma TG increased signifi-

TABLE 2. Fasting laboratory values at the time of the oral fat loading test of 12 FCHL patients before and after atorvastatin treatment and 10 matched control subjects

	1: FCHL Untreated	2: FCHL Treated	3: Controls	*P* 1 vs. 3	*P* 1 vs. 2	*P* 2 vs. 3
Cholesterol (mmol/l)	6.54 ± 0.36	4.02 ± 0.22	5.07 ± 0.25	<0.01	<0.01	0.02
TG (mmol/l)	3.19 ± 0.43	2.27 ± 0.23	1.15 ± 0.13	<0.01	0.03	<0.01
HDL-cholesterol (mmol/l)	0.93 ± 0.07	0.99 ± 0.08	1.12 ± 0.09	0.11	0.22	0.31
apoB (g/l)	1.27 ± 0.09	0.85 ± 0.05	0.83 ± 0.06	<0.01	<0.01	0.77
apoAI (g/l)	1.22 ± 0.08	1.23 ± 0.06	1.40 ± 0.05	0.08	0.98	0.04
LPL (mU/ml)	108 ± 14	101 ± 11	123 ± 9	0.39	0.63	0.15
HL (mU/ml)	328 ± 56	299 ± 39	382 ± 48	0.47	0.35	0.21
FFA (mmol/l)	0.39 ± 0.03	0.35 ± 0.03	0.31 ± 0.03	0.08	0.38	0.32
Complement 3 (g/l)	1.41 ± 0.04	1.24 ± 0.05	0.91 ± 0.03	<0.01	0.02	<0.01
ASP (nmol/l)	46.71 ± 6.08	36.04 ± 4.27	42.90 ± 10.74	0.38	0.15	0.13
Insulin (IU/l)	14.8 ± 1.9	14.2 ± 2.3	9.4 ± 1.1	0.02	0.80	0.08
Glucose (mmol/l)	5.2 ± 0.4	5.2 ± 0.1	4.9 ± 0.1	0.14	0.42	0.12
HOMA	3.26 ± 0.37	3.24 ± 0.61	2.04 ± 0.22	<0.01	0.82	0.12

ASP, acylation-stimulating protein; HOMA, homeostasis model assessment; LPL, lipoprotein lipase; TG, triglyceride. Data are mean ± SEM.

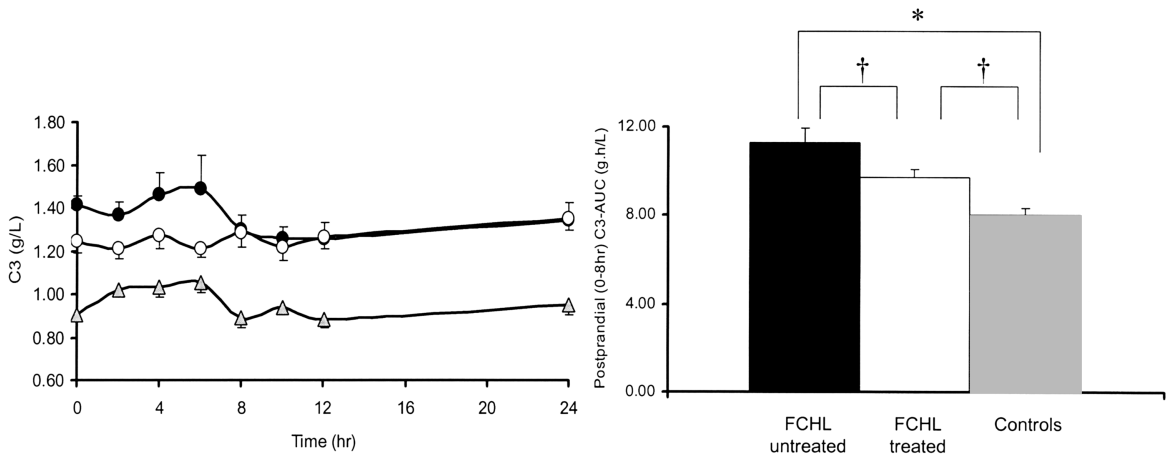

Fig. 1. Mean changes of plasma complement component 3 (C3) concentrations (left panel) and postprandial (0–8 h) C3 area under the curve (AUC) (right panel). Note: the y axis starts at 0.6 g/L. * $P < 0.05$; † $P < 0.01$.

cantly to maximal concentrations at 5 h from 3.19 ± 0.43 mmol/l to 5.31 ± 0.64 mmol/l in untreated FCHL patients ($P < 0.01$), from 2.27 ± 0.23 mmol/l to 3.88 ± 0.88 mmol/l in treated FCHL patients ($P < 0.01$), and from 1.15 ± 0.13 mmol/l to 2.36 ± 0.50 mmol/l in controls ($P < 0.01$). The maximal TG increase was not changed by treatment (160% and 173%, respectively). The postprandial TG-AUC in FCHL patients decreased after atorvastatin by 23% (from 33.77 ± 4.38 mmol·h·l^{-1} to 25.85 ± 2.81 mmol·h·l^{-1}), which was still elevated, compared with controls (14.30 ± 1.97 mmol·h·l^{-1}) (**Fig. 2A**). The group using up to 40 mg/day and the group on 80 mg/day atorvastatin had a similar reduction in fasting TG (19% and 21% reduction, respectively) and in postprandial (0–8 h) TG-AUC (16% and 23% reduction, respectively).

Postprandial FFA increased in untreated FCHL patients from 0.39 ± 0.03 mmol/l to a maximum of 0.80 ± 0.05 mmol/l at t = 5 h ($P < 0.01$), in treated FCHL patients from 0.35 ± 0.03 mmol/l to a maximum of 0.89 ± 0.07 mmol/l at t = 6 h ($P < 0.01$), and in controls from 0.31 ± 0.03 mmol/l to 0.68 ± 0.06 mmol/l ($P < 0.01$), reaching a peak at t = 7 h ($P < 0.01$). The postprandial FFA-AUC was significantly higher in FCHL patients before and after atorvastatin (4.96 ± 0.23 mmol·h·l^{-1} and 5.30 ± 0.29 mmol·h·l^{-1}, respectively), compared with controls (4.07 ± 0.18 mmol·h·l^{-1}). The postprandial FFA-AUC was not statistically different between the untreated and treated patients (Fig. 2B, right panel).

HBA concentrations increased in untreated FCHL patients from 0.02 ± 0.001 mmol/l (fasting) to a maximum of 0.30 ± 0.04 mmol/l (at t = 6 h) ($P < 0.01$) and in treated FCHL patients from 0.02 ± 0.001 mmol/l (fasting) to a maximum of 0.18 ± 0.03 mmol/l (at t = 8 h) ($P < 0.01$). In controls, a postprandial HBA response similar to that in treated FCHL patients was seen up to 10 h. The postprandial (0–8 h) HBA-AUC was higher in untreated patients (1.39 ± 0.16 mmol·h·l^{-1}) than in both treated patients (0.81 ± 0.11 mmol·h·l^{-1}) and controls (0.95 ± 0.16 mmol·h·l^{-1}) (Fig. 2C, right panel).

apoB48, apoB100, and TG changes in the chylomicron fraction

Fasting concentrations of apoB48 in the chylomicron fraction (Sf >400) were similar in untreated (0.31 ± 0.14 mg/l) and treated FCHL patients (0.21 ± 0.09 mg/l), but significantly higher, compared with controls (0.02 ± 0.01 mg/l; $P = 0.05$) (**Fig 3A**). Fasting chylomicron-B48 increased postprandially, reaching maximal concentrations after 4 h (0.91 ± 0.27 mg/l; $P < 0.05$, compared with fasting concentrations) in untreated FCHL patients. In treated patients and in controls, a delayed peak was found at t = 6 h, reaching lower concentrations (0.70 ± 0.26 mg/l and 0.16 ± 0.02 mg/l, respectively; $P < 0.05$ for each), than in FCHL patients before treatment. The postprandial apoB48-AUC did not improve significantly with atorvastatin (5.07 ± 1.35 mg·h·l^{-1} before vs. 4.31 ± 1.31 mg·h·l^{-1} after atorvastatin; $P = 0.52$). Control subjects had a significantly lower apoB48-AUC (0.92 ± 0.20 mg·h·l^{-1}; $P < 0.05$).

A significant increase of apoB100 from fasting to a peak at 6 h postprandially was found in untreated FCHL patients before (from 4.27 ± 1.46 mg/l to 9.13 ± 2.62 mg/l; $P < 0.05$) as well as after atorvastatin (from 2.71 ± 0.99 mg/l to 6.34 ± 2.90 mg/l; $P < 0.05$) and in controls (from 0.07 ± 0.04 mg/l to 0.55 ± 0.25 mg/l; $P < 0.05$) (Fig. 3B). The postprandial apoB100-AUC was not significantly decreased by atorvastatin (55.5 ± 14.9 mg·h·l^{-1} and 38.3 ± 14.5 mg·h·l^{-1}, respectively; $P = 0.26$). Controls showed a significantly lower apoB100 response (3.2 ± 1.0 mg·h·l^{-1}; $P < 0.01$).

Fasting TG in the Sf >400 fraction tended to decrease after atorvastatin (0.25 ± 0.07 mmol/l to 0.13 ± 0.03 mmol/l; $P = 0.11$), but the TG concentrations in this fraction were still elevated, compared with controls (0.03 ± 0.01 mmol/l; $P < 0.01$) (Fig. 3C). All subjects reached their peak value 4 h after fat intake. The percentage increase of plasma TG concentration was not different in untreated and treated FCHL patients (595% and 634%, respectively). The postprandial TG-AUC of the Sf >400 fraction was not significantly different in untreated or

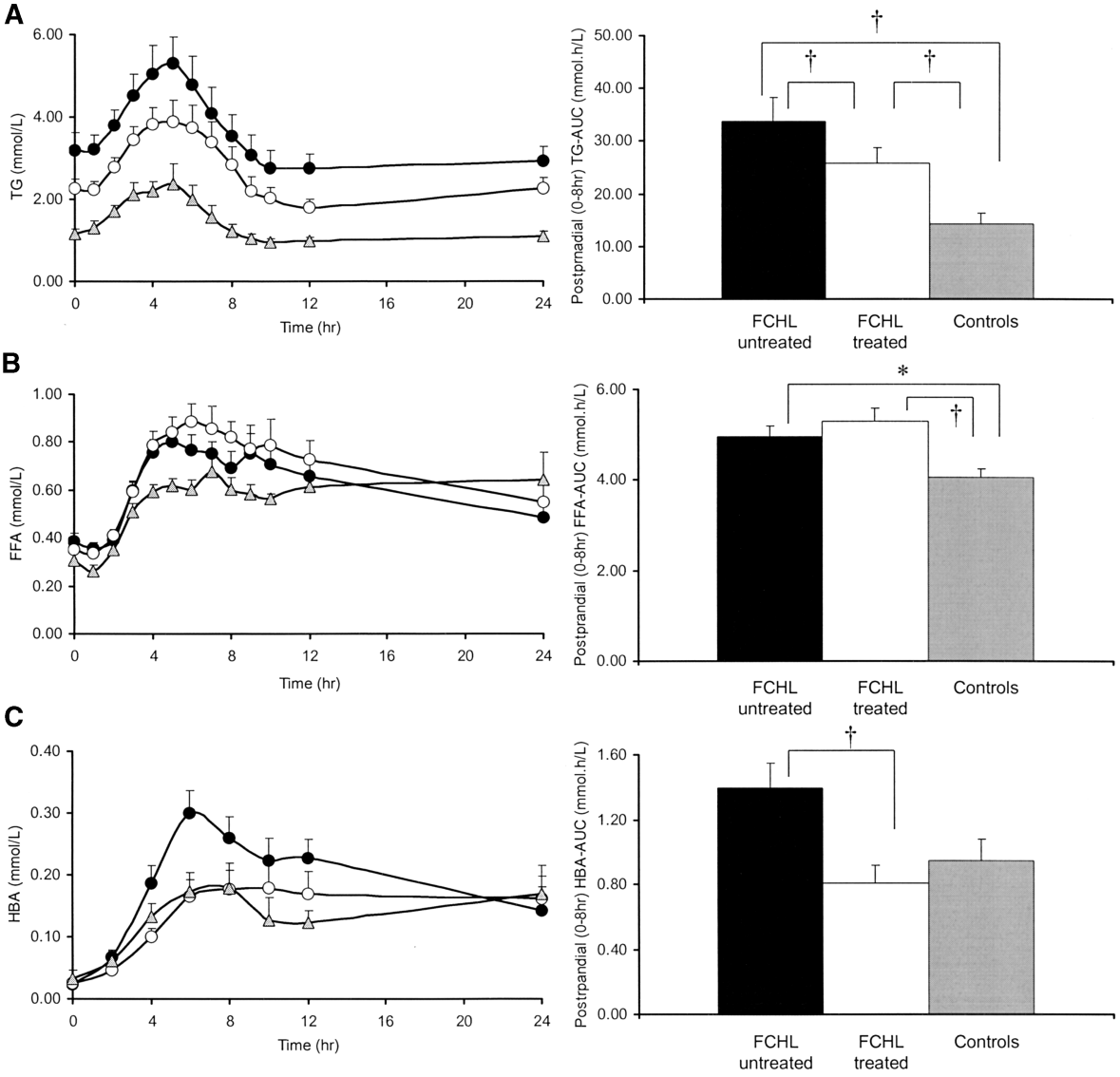

Fig. 2. A: Mean changes of triglyceride (TG) concentrations (left panel) and postprandial TG-AUC (right panel) in 12 untreated familial combined hyperlipidemia (FCHL) patients (closed circles) and 12 treated FCHL patients (open circles), compared with 10 matched controls (gray triangles). B: Mean changes of FFA concentrations (left panel) and postprandial (0–8 h) FFA-AUC (right panel). C: Mean changes of hydroxybutyric acid (HBA) concentrations (left panel) and postprandial HBA-AUC (right panel) in 12 untreated FCHL patients (closed circles) and 12 treated FCHL patients (open circles), compared with 10 matched controls (gray triangles). Data are mean ± SEM. * $P < 0.05$; † $P < 0.01$.

treated patients (5.00 ± 1.38 mg·h·l^{-1} and 3.70 ± 1.38 mg·h·l^{-1}), compared with controls (2.34 ± 0.43 mg·h·l^{-1}).

Determinants of fasting and postprandial C3 levels

All significant correlations between fasting C3 and postprandial C3 (represented as C3-AUC), apoB, postprandial plasma TG, FFA, apoB48, apoB100 and Sf >400-TG, as well as general characteristics, are listed in **Table 3**. Multiple regression analysis showed that the best determinant of fasting C3 in all subjects was the fasting plasma TG (adjusted $R^2 = 0.55$; $\beta = 0.75$; $P < 0.001$). Addition of HOMA and plasma apoB further improved the model significantly (adjusted $R^2 = 0.68$ and 0.72, respectively). Postprandial C3-AUC was best predicted by the HOMA (adjusted $R^2 = 0.60$; $\beta = 0.78$; $P < 0.001$). Addition of fasting plasma TG also improved this model (adjusted $R^2 = 0.73$; $P < 0.01$).

DISCUSSION

The present study shows an improvement of postprandial triglyceridemia, a blunted postprandial C3 response, and a suppressed hepatic FFA flux in FCHL subjects by monotherapy with atorvastatin. Atorvastatin, a 3-hydroxy-methylglutaryl CoA (HMG-CoA) reductase inhibitor, reduces VLDL secretion in vitro by impairing the translocation of apoB in the lumen of the endoplasmatic reticulum (36). This is supported by in vivo stable isotope studies in patients with combined hyperlipidemia (37). Atorvastatin improves the clear-

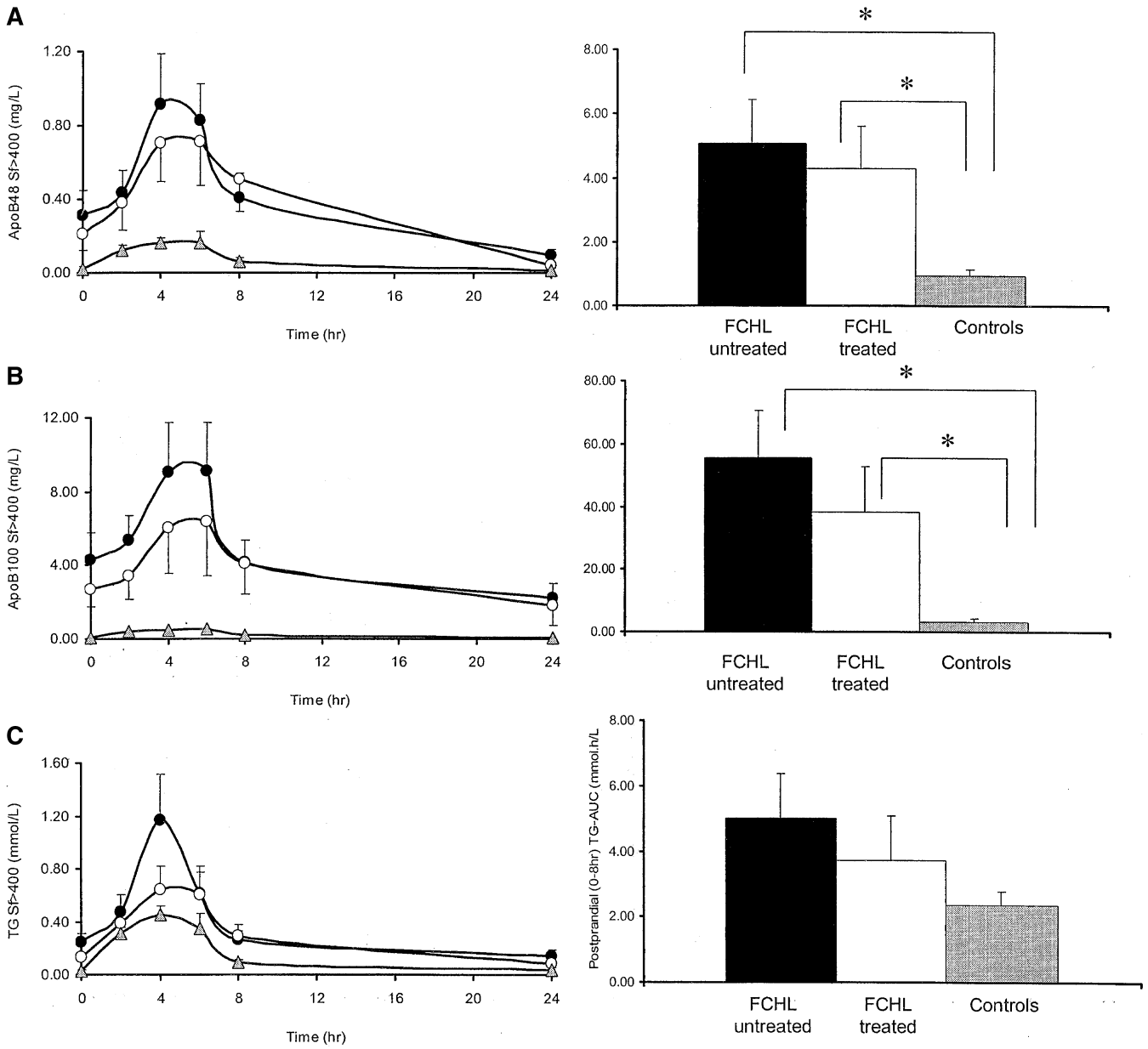

Fig. 3. A: Mean changes of apoB48 concentrations (left panel) and postprandial apoB48-AUC (right panel) in 12 untreated FCHL patients (closed circles) and 12 treated FCHL patients (open circles), compared with 10 matched controls (gray triangles). B: Mean changes of apoB100 concentrations (left panel) and postprandial (0–8 h) apoB100-AUC (right panel). C: Mean changes of TG [in Svedberg flotation (Sf) >400 fraction] concentrations (left panel) and postprandial TG-AUC (right panel) in 12 untreated FCHL patients (closed circles) and 12 treated FCHL patients (open circles), compared with 10 matched controls (gray triangles). Data are mean ± SEM. * $P < 0.05$.

ance of chylomicron remnants, but it does not have an effect on the production of intestinal lipoproteins, e.g., chylomicrons (38–40). In our FCHL patients, atorvastatin lowered fasting plasma TG, cholesterol, and apoB. Neither HDL-cholesterol nor apoAI improved after treatment.

Fasting C3 levels were correlated with the different lipid parameters, such as fasting plasma TG, cholesterol, and apoB concentrations, and also with insulin and HOMA, as a marker for insulin resistance, in accordance with different previous studies (13, 14, 27, 31, 32). C3 is the precursor of ASP and plays an important role in FFA uptake by adipocytes, following lipolysis of chylomicrons (29). In a previous study, we found that untreated FCHL patients had higher fasting C3 and an exaggerated and delayed postprandial C3 response (27). We concluded that this could be a consequence of C3 resistance at the level of adipocytes. The latter could result in decreased uptake of FFA by peripheral cells and consequently could lead to VLDL overproduction (26, 41). In this study, evidence is provided suggesting that atorvastatin improves peripheral FFA trapping, which potentially may reduce VLDL overproduction in FCHL patients. Studies directly investigating the effects of statins on FFA fluxes and VLDL secretion in FCHL patients are needed.

Maslowska et al. (42) demonstrated in vitro that the main stimulators of C3 and ASP production in human adipocytes are insulin and large TRL (Sf >400), which the

TABLE 3. Spearman's correlation coefficients between fasting and postprandial C3-AUC and biochemical variables in untreated FCHL patients and controls

	Fasting C3	C3-AUC
Fasting plasma TG	0.72[a]	0.76[a]
Total cholesterol	0.56[a]	0.56[a]
Fasting apoB	0.63[a]	0.66[a]
Fasting apoB48	0.46[b]	0.59[a]
Fasting apoB100	0.65[a]	0.72[a]
Fasting (Sf >400)-TG	0.65[a]	0.73[a]
apoB100-AUC	0.49[b]	0.53[b]
Insulin	0.63[a]	0.59[a]
HOMA	0.69[a]	0.66[a]
TG-AUC	0.66[a]	0.72[a]
Fasting C3	—	0.97[a]

AUC, area under the curve; C3, complement component 3; Sf, Svedberg flotation. Only significant correlations are given.

[a] $P < 0.01$.

[b] $P < 0.05$.

authors defined as "chylomicrons." However, this Sf >400 fraction also contains large apoB100 particles in addition to the apoB48-containing lipoproteins, as clearly shown here and by different groups (35, 43, 44). The positive correlations between fasting C3 and postprandial C3 with the postprandial apoB100 (and not with apoB48) suggest that chylomicrons may not be the main stimulators of C3 production in vivo, but rather large VLDLs. A reduction in VLDL secretion may be responsible for the decrease in C3 production seen after atorvastatin treatment. Previous work from our department has shown that postprandial C3 responses decreased in non-FCHL/coronary artery disease (CAD) patients by expanded-dose simvastatin (31), providing support for the view that C3 reduction may be one of the consequences of statin therapy.

Untreated FCHL had a delayed C3 peak, in accordance with previous work (27). Atorvastatin lowered fasting and postprandial C3 concentrations, but also changed the shape of the postprandial C3 curve. Although no normalization of the fasting C3 and the postprandial curve was reached, HBA concentrations returned to control values after atorvastatin. HBA was measured as a marker of hepatic FFA flux, because the largest amount of ketone bodies are formed in the liver from acetyl-CoA (45) derived from fatty acid oxidation. This could be interpreted in different ways. One explanation could be that the conversion of acetyl-CoA into ketone bodies in the liver is inhibited by atorvastatin, in a way similar to that in which atorvastatin inhibits the HMG-CoA reductase. However, to our knowledge, there is no evidence supporting such a theory. Another explanation is that there is a decreased postprandial hepatic FFA flux after atorvastatin treatment. It is not likely that net production of FFA released by hydrolysis of TRL decreased by atorvastatin, because the lipolytic activities were not changed after atorvastatin and the amount of TG hydrolyzed by LPL was not affected substantially, as indicated by the unchanged postprandial TG in plasma and Sf >400 fractions. Also, the postprandial FFA concentrations (from 0–8 h after fat intake) were not changed by atorvastatin. In our view, the data indicate improved peripheral fatty acid uptake by extrahepatic tissues. Because the FCHL patients did not gain weight after treatment, improved peripheral FFA trapping by adipocytes cannot be the only explanation for the observed postprandial decrease of ketone bodies. It remains to be determined which other tissues extract FFA at a higher rate and thereby decrease the hepatic FFA flux in FCHL by atorvastatin.

It has been suggested that chylomicrons compete efficiently with VLDL for the same lipolytic pathway (46), which leads to accumulation of postprandial VLDL (47). In the present study, we found a significant reduction of postprandial plasma TGs without improvement of large triglyceride-rich lipoprotein (TRL)-apoB48 or apoB100 clearance. This may have been due to the relatively small sample size of FCHL patients and to the fact that fasting plasma TGs (the best determinants of postprandial lipemia) were not normalized. However, in other postprandial studies investigating the effects of atorvastatin, including similar numbers of non-FCHL participants, statistically significant improvements have been reported (39, 40). This underlines once again how difficult it is to reduce postprandial lipemia in FCHL subjects (48, 49). The small nonsignificant changes obtained by atorvastatin in postprandial apoB100 and apoB48 clearance suggest that a more effective reduction of fasting plasma TG could improve postprandial lipemia in this disorder.

It should be noted that total plasma apoB was normalized in FCHL patients by atorvastatin, in contrast to the less-efficient reduction of fasting plasma TG. This may be explained by the fact that atorvastatin reduced LDL dramatically, with less-significant effects on large TRLs. In a recent paper by Parhofer, Laubach, and Barrett (50), atorvastatin significantly reduced the postprandial concentrations of large TRLs, with less effect on small TRLs in 10 hypertriglyceridemic patients. Detailed data on the effects of atorvastatin on lipoprotein composition in FCHL will be published separately.

Each patient was treated with the minimal dose of atorvastatin needed to reach a normolipidemic state, as is done in clinical practice, where titration to optimal doses is prescribed individually to patients. However, five of the 12 patients were treated with the maximal dose, 80 mg/day, and still had increased fasting and postprandial plasma TG levels. Although these patients had a higher triglyceridemia, the percentage decrease in fasting plasma TG, postprandial triglyceridemia, fasting and postprandial C3, as well as HBA-AUC was not different between the two groups, indicating a similar response of hepatic FFA flux after treatment. Why some patients need higher doses of atorvastatin than others to reach the fasting treatment targets is not clear at present and may depend on different genetic and environmental factors.

In conclusion, atorvastatin decreased the postprandial hepatic FFA flux and the total postprandial plasma triglyceridemia in FCHL patients. This effect was accompanied by reduced fasting and postprandial C3 concentrations. Further studies investigating FFA metabolism in vivo in different tissues will be necessary to estimate the FFA handling by different cells and to better delineate the role of C3 after lipid-lowering intervention.

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