In vivo evidence of defective postprandial and postabsorptive free fatty acid metabolism in familial combined hyperlipidemia

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Abstract **Overproduction of very low density lipoprotein (VLDL) is the major characteristic of subjects with familial combined hyperlipidemia (FCHL). As enhanced free fatty acid (FFA) flux to the liver may be one of the determinants of VLDL overproduction, we studied FFA changes and products of hepatic FFA metabolism in response to a 24-h oral fat loading test (50 g/m^2) in 7 FCHL subjects and 7 matched control subjects. The response to the meal was subdivided into a postprandial (up to 8 h after ingestion of the meal) and postabsorptive period (from 8 to 24 h). Although postheparin plasma lipolytic activities were not different between both groups, the postprandial FFA area under the curve (FFA-AUC) and FFA incremental area under the curve (FFA-dAUC) were higher in FCHL subjects than in control subjects (6.05 ± 0.45 vs. 3.43 ± 0.46 and 2.60 ± 0.49 vs. 0.96 ± 0.31 mmol·h/L, respectively; $P < 0.01$ for each). The postprandial increase in ketone bodies was almost four times higher in FCHL patients. As ketogenesis occurs predominantly in hepatocytes, these findings suggest that during the postprandial period in FCHL an increased flux of FFA to the liver occurs, possibly because of inadequate incorporation of FFA into triglycerides (TGs) in adipocytes. In the postabsorptive period, FFA and ketone bodies significantly decreased in FCHL subjects, in contrast to control subjects, in whom both increased. These results may represent a diminished release of FFA from adipocytes by hormone-sensitive lipase (HSL) in FCHL patients. The decrease in postabsorptive FFA and ketone bodies in FCHL patients could not be explained by insulin-mediated inhibition of HSL, as both FCHL subjects and control subjects had similar postabsorptive insulin concentrations, which were below fasting concentrations. This study provides in vivo evidence of impaired metabolism of postprandial FFA in FCHL, which may explain in part the hepatic VLDL overproduction characteristic of FCHL subjects.**—Meijssen, S., M. Castro Cabezas, T. B. Twickler, H. Jansen, and D. W. Erkelens. **In vivo evidence of defective postprandial and postabsorptive free fatty acid metabolism in familial combined hyperlipidemia.** ***J. Lipid Res.*** **2000.** 41: **1096–1102.**

Supplementary key words atherosclerosis • apoB overproduction • VLDL • triglycerides • ketone bodies • postprandial lipemia • acylation-stimulating protein • hormone-sensitive lipase

Familial combined hyperlipidemia (FCHL) is the most frequent, dominantly inherited disorder of lipid metabolism leading to increased risk of atherosclerosis (1–5). The diagnosis is based on clinical criteria such as the presence of "multiple-type hyperlipidemia" (1, 2, 4, 5), increased concentrations of plasma apolipoprotein B (apoB), reflecting very low density lipoprotein (VLDL) overproduction, and a positive family history of premature coronary heart disease (CHD). 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 (6–8).

Several metabolic characteristics have been described during the last 20 years. Patients with FCHL are characterized by a hepatic apoB overproduction (9–11), delayed clearance of chylomicron remnants (12, 13), insulin resistance (12, 14, 15), and a primary defect in the metabolism of triglycerides and plasma free fatty acids (12, 16). In addition, in a subset of patients decreased activity of postheparin plasma lipoprotein lipase has been described (17, 18). Recently, free fatty acid (FFA) metabolism has received considerable interest as a major determinant of the metabolic disturbances in FCHL (19, 20). Several investigators have linked an impaired FFA metabolism to the hepatic apoB overproduction and the insulin resistance as seen in FCHL (12, 16). Cianflone, Maslowska, and Sniderman (20) have shown in vitro that the fatty acid uptake by peripheral cells and incorporation into triglycerides in patients with hyperapobetalipoproteinemia (hyperapoB) is impaired, because of diminished activity and binding of

Abbreviations: ACA, aceto-acetate; ASP, acylation stimulating protein; AUC, area under the curve; dAUC, delta (incremental) area under the curve; CHD, coronary heart disease; ELISA, enzyme-linked immunosorbent assay; FCHL, familial combined hyperlipidemia; FFA, free fatty acid; HL, hepatic lipase; HDL-C, high density lipoprotein-cholesterol; HSL, hormone-sensitive lipase; HBA, hydroxybutyric acid; LPL, lipoprotein lipase; TG, triglyceride; VLDL, very low density lipoprotein.

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acylation-stimulating protein (ASP). In addition, decreased activity of hormone-sensitive lipase (HSL) in adipocytes of FCHL patients was demonstrated by in vitro studies (21). HSL is most active in the postabsorptive state, releasing FFA from the adipocytes. HSL exists in an active and inactive form and is activated by catecholamines through cAMP-dependent phosphorylation, whereas insulin prevents this phosphorylation by hydrolysis of cAMP (22, 23). The defects described in in vitro studies of FCHL have led to the hypothesis that there might be a decreased metabolism of FFA in the adipose tissue of FCHL patients, which may lead to enhanced flux of FFA to the liver, resulting in overproduction of VLDL-apoB (19). However, there are no in vivo data available to support this hypothesis. The present study was aimed to provide in vivo evidence of an increased delivery of FFA to the liver in FCHL patients. For this purpose we measured concentrations of ketone bodies as hepatic products of free fatty acid metabolism after an oral fat loading test in FCHL patients and matched control subjects.

MATERIALS AND METHODS

Index FCHL patients

The study protocol was approved by the Human Investigations Review Committee of the University Hospital Utrecht. All participants gave informed consent before the test. Seven unrelated FCHL patients were recruited from the Lipid Clinic of the Utrecht University Hospital. These subjects met the following criteria: a primary hyperlipidemia with varying phenotypic expression and at least one first degree relative with a different hyperlipidemic phenotype; elevated plasma apoB concentrations (>0.9 g/L), and a positive family history of premature coronary heart disease (CHD) defined as myocardial infarction or cerebrovascular disease before the age of 60 years in at least one blood-related subject of the index patient. In addition, the patients fulfilled the following inclusion criteria: absence of xanthomas, absence of secondary factors associated to hyperlipidemia as demonstrated by normal thyroid, renal, and liver function tests, BMI <30 kg/m^2, absence of apoE-2/E-2 genotype, no use of drugs affecting lipid metabolism, and consumption of less than 3 units of alcohol per day. All FCHL patients stopped lipid-lowering drugs 4 weeks before the test.

Seven normolipidemic, healthy control subjects without a family history of cardiovascular disease, without the apoE-2/E-2 genotype, and not using drugs known to affect lipid metabolism were recruited by advertisement. Control subjects were matched to FCHL patients by age, body mass index (BMI), and gender.

Anthropometric measurements

On the morning of inclusion blood pressure and waist-to-hip index were measured. Body fat was estimated with a body impedance analyzer (RJL Systems, Detroit, MI) according to instructions provided by the manufacturer (24, 25).

Oral fat loading test

Cream was used as fat source; this is a 40% (w/v) fat emulsion, with a ratio of polyunsaturated to saturated fat (P/S ratio) of 0.06, that contains 0.001% (w/v) cholesterol and 2.8% (w/v) carbohydrates. After the ingestion of the fat load, subjects were allowed to drink water and sugar-free tea during the following 24 h. Peripheral blood samples were obtained in sodium-EDTA (2 mg/mL) before (T = 0) and at hourly intervals up to 10 h, at 12 h, and 24 h after the fat load. Blood was placed on ice and centrifuged immediately for 15 min at 800 *g* at 4°C.

Analytical methods

Triglycerides (TGs) and cholesterol were measured in duplicate by commercial colorimetric assay (GPO-PAP and Monotest cholesterol kit, respectively; Boehringer Mannheim, Mannheim, Germany). FFA was measured in plasma samples by enzymatic colorimetric method (Wako Chemicals GmbH, Neuss, Germany). Plasma samples were stored at −20°C immediately after centrifugation. The ketone bodies, hydroxybutyric acid (HBA), and aceto-acetate (ACA) were measured spectrophotometrically by the principle of converting NADH to NAD$^+$ after adding 3-hydroxybutyrate dehydrogenase. For this purpose 0.5 ml of EDTA blood was denutriated by adding 1 ml of 0.7 M $HClO_4$. HDL-cholesterol (HDL-C) was determined according to Gidez et al. (26). The quantitative assays of apolipoprotein B have been described in detail (27). Postheparin plasma lipoprotein lipase (LPL) and hepatic lipase (HL) activities were determined by the release of free fatty acids from ^{14}C-labeled trioleoyl emulsion (28). Lipolytic activity is expressed as nanomoles of free fatty acids (FFAs) per minute (mU) per milliliter of plasma. ApoE genotypes were determined as described (29). Glucose was measured by glucose oxidase by dry chemistry (Vitros GLU slides, Rochester, NY) and colorimetry, and insulin was measured by commercial enzyme-linked immunosorbent assay (ELISA) (Mercodia, Uppsala, Sweden). For estimation of insulin sensitivity the homeostasis model assessment [HOMA index = (glucose × insulin)/22.5] was calculated (30).

Statistics

All values are expressed as means ± standard error of the mean (SEM). The area under the curve (AUC) and incremental area under the curve (dAUC) were, respectively, calculated by the trapezoidal rule and after correction for fasting values. The curves were subdivided into postprandial and postabsorptive periods. The transition point was arbitrarily set at 8 h after ingestion of the fat load, as plasma TG concentrations usually reach basal values at 8 h (31). The first 8 h was referred to as the postprandial period and the remainder was referred to as the postabsorptive period. The incremental postprandial and postabsorptive TG-AUC and FFA-AUC were calculated after correction for the fasting plasma TG and FFA concentrations and at 8 h, respectively. Mean differences between control subjects and FCHL subjects were calculated by unpaired *t* test. AUCs and dAUCs were calculated by Mann–Whitney U test, as well as the fasting TG concentrations and HOMA index. Statistical significance was reached when $P < 0.05$ (two-tailed). Calculations were performed with SPSS/PC+ 8.0 (SPSS, Chicago, IL).

RESULTS

General characteristics

FCHL patients and healthy control subjects did not differ in anthropometric characteristics (**Table 1**). Fasting concentrations of plasma TG, cholesterol, apoB, and glucose were significantly higher in FCHL patients (**Table 2**). Fasting plasma FFA and HBA concentrations were similar in both groups. HDL-C was higher in control subjects. The HOMA index was higher in FCHL patients than in control subjects. Postheparin plasma lipolytic enzymes were not different between FCHL and control subjects.

TABLE 1. Individual characteristics of 7 untreated FCHL patients and 7 matched control subjects

	Gender	Age	BMI (kg/m²)	WH Index	Absolute Fat Mass (kg)	Relative Fat Mass (%)
FCHL Patients						
1	M	47	26	0.99	16.0	19.5
2	F	52	27	0.84	26.7	37.5
3	M	40	24	0.94	31.2	41.1
4	F	56	30	0.85	25.5	34.9
5	M	45	28	0.93	22.2	22.4
6	M	55	28	0.95	36.0	36.3
7	F	50	21	0.85	18.7	30.1
Mean (SEM)		49.3 (2.5)	26.4 (1.1)	0.91 (0.04)	25.4 (2.6)	31.7 (3.1)
Controls						
1	F	42	25	0.68	23.3	30.2
2	M	47	26	0.86	13.5	15.6
3	M	49	24	0.88	14.8	18.5
4	M	55	27	0.87	21.8	27.2
5	M	42	28	0.91	23.6	21.8
6	F	50	24	0.85	17.6	23.4
7	F	34	27	0.84	24.4	32.5
Mean (SEM)		45.6 (2.6)	25.8 (1.6)	0.85 (0.04)	19.9 (1.7)	24.2 (2.3)

TG and FFA changes in response to an oral fat loading test

After ingestion of the cream, plasma TG rose from 3.58 ± 0.42 mmol/L to a peak value of 7.05 ± 0.83 mmol/L at 4.3 ± 0.2 h in FCHL patients. In control subjects, however, TG started with 0.78 ± 0.06 mmol/L and had a peak of 2.20 ± 0.31 mmol/L at 3.0 ± 0.4 h ($P < 0.01$ compared with FCHL patients for TG increase and time of peak) (**Fig. 1A**).

The absolute TG response to the oral fat loading, calculated as area under the 24-h TG curve, was higher in FCHL than in control subjects (90.4 ± 10.6 and 22.1 ± 1.7 mmol·h/L, respectively; $P < 0.01$) (Fig. 1B). The incremental TG-AUC was similar in FCHL and control subjects (4.52 ± 10.25 vs. 3.28 ± 1.35 mmol/L; NS). This difference between FCHL and control subjects was seen in the total postprandial (41.4 ± 4.0 vs. 11.0 ± 1.3 mmol·h/L; $P < 0.01$) and postabsorptive TG-AUC (48.9 ± 6.6 vs. 11.0 ± 0.5 mmol·h/L; $P < 0.01$) (Fig. 1C). The incremental TG-AUC of the postprandial period (12.8 ± 3.4 vs. 4.8 ± 1.0 mmol·h/L; $P < 0.05$) was higher in FCHL. The postabsorptive incremental TG-AUC (−17.3 ± 4.3 vs. −0.9 ± 1.0; $P < 0.01$) was higher in control subjects.

The FFA curve increased from a basal value of 0.43 ± 0.06 mmol/L to a maximum of 1.20 ± 0.11 mmol/L at 5.3 ± 0.3 h in FCHL and from 0.31 ± 0.05 to 0.57 ± 0.003 mmol/L at 4.9 ± 0.3 h in control subjects ($P < 0.01$; FCHL compared with control subjects for FFA increase) (**Fig. 2A**). The absolute and incremental AUCs of the FFA curve were not different between both groups (Fig. 2B), but the absolute and incremental postprandial FFA-AUCs

TABLE 2. Individual fasting laboratory values at the time of oral fat loading test of 7 untreated FCHL patients and 7 matched control subjects

	Chol (mmol/L)	TG (mmol/L)	HDL–C (mmol/L)	ApoB (g/L)	FFA mmol/L)	HL (mU/ml)	LPL (mU/ml)	Insulin (IU/L)	Glucose (mmol/L)	HOMA Index	ApoE Gen.
FCHL Patients											
1	7.8	4.92	0.85	1.43	0.45	630	211	9.09	6.1	2.46	2/3
2	7.6	3.33	0.72	1.60	0.69	324	194	8.85	6.5	2.56	3/4
3	6.2	3.56	0.91	1.37	0.59	423	98	5.87	5.6	1.46	3/4
4	10.1	2.19	0.85	2.62	0.37	NA	NA	9.87	6.1	2.67	3/3
5	7.0	3.53	0.73	1.61	0.36	248	111	9.98	6.3	2.79	3/3
6	3.7	5.10	0.44	0.88	0.30	552	68	24.08	6.0	6.42	3/4
7	6.0	2.40	0.60	1.25	0.26	344	79	10.10	6.3	2.83	3/3
Mean (SEM)	6.92 (0.74)	3.58 (0.42)	0.73 (0.06)	1.54 (0.19)	0.43 (0.06)	420.2 (55.1)	126.2 (22.9)	11.12 (2.23)	6.1 (0.1)	3.03 (0.59)	
Controls											
1	5.13	0.61	1.30	1.21	0.15	256	135	4.69	4.9	1.02	3/4
2	3.80	0.85	1.15	0.89	0.12	NA	NA	4.75	5.9	1.25	3/3
3	4.69	0.71	1.00	0.79	0.33	252	136	6.15	5.9	1.61	3/3
4	5.92	0.8	1.51	0.99	0.37	478	110	8.21	5.9	2.15	3/3
5	3.94	1.02	1.11	0.70	0.36	581	166	11.19	5.2	2.59	3/3
6	5.40	0.90	1.19	0.70	0.29	NA	NA	8.27	5.2	1.91	3/3
7	5.20	0.59	1.83	0.82	0.54	247	169	6.03	5.4	1.45	2/3
Mean (SEM)	4.78 (0.29)	0.78 (0.06)	1.30 (0.12)	0.87 (0.07)	0.31 (0.05)	262.8 (59.2)	143.2 (9.3)	7.04 (0.88)	5.5 (0.2)	1.71 (0.20)	
P	<0.05	<0.01	<0.01	<0.01	NS	NS	NS	NS	<0.01	<0.05	

NA, not available; Gen., genotype; NS, not significant.

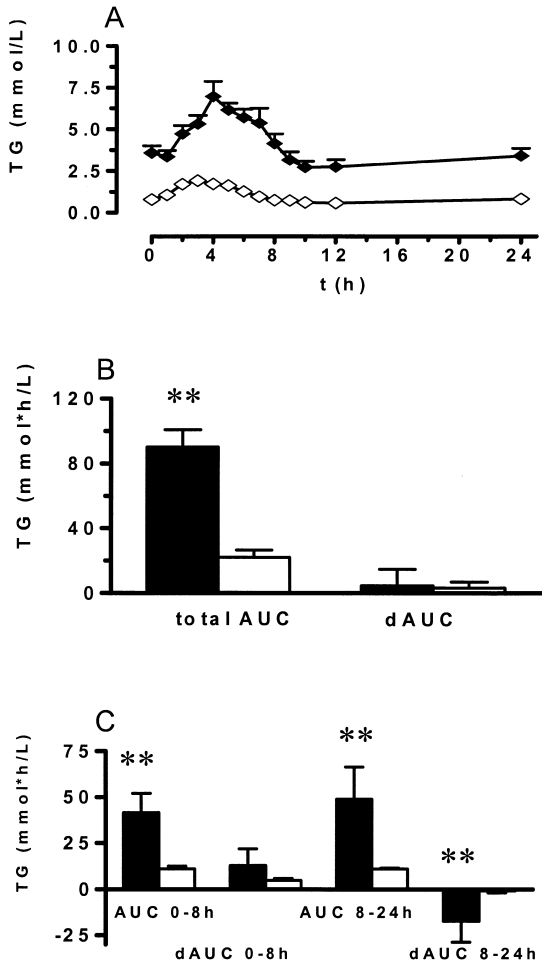

Fig. 1. A: Mean changes in plasma triglycerides (TGs) in 7 untreated FCHL patients (solid symbols) compared with 7 matched control subjects (open symbols). B: Areas under the curve (AUC) and incremental areas under the curve (dAUC) of total area. C: AUC and dAUC of postprandial and postabsorptive period. Data represent means ± SEM. ** $P < 0.01$, FCHL compared with control subjects. Note: Error bars for control subjects are so small that they are not visible at all times.

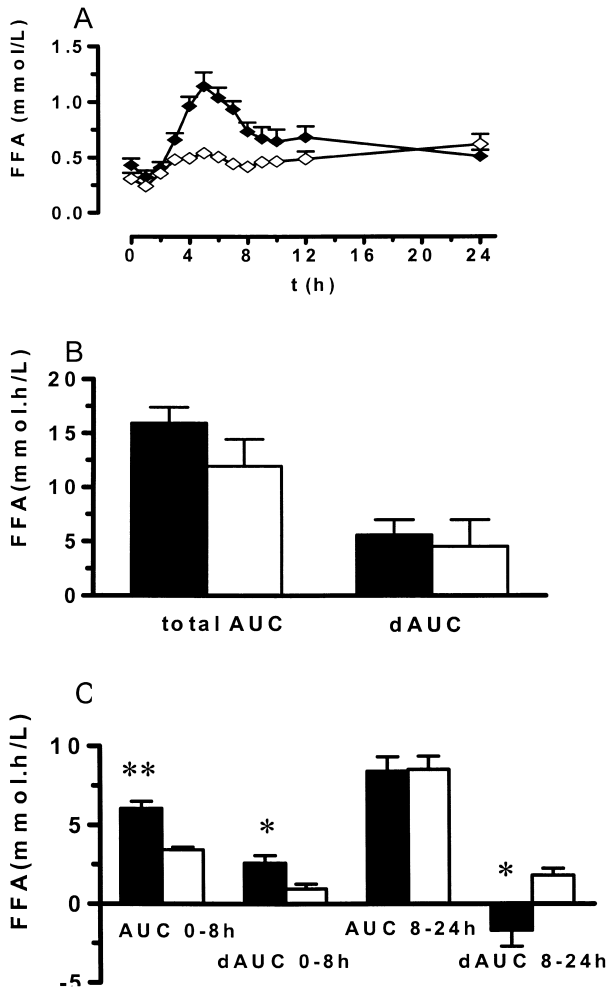

Fig. 2. A: Mean changes in plasma free fatty acids (FFAs) in 7 untreated FCHL patients (solid symbols) compared with 7 matched control subjects (open symbols). B: Areas under the curve (AUC) and incremental areas under the curve (dAUC) of total area. C: AUC and dAUC of postprandial and postabsorptive periods. Data represent means ± SEM. * $P < 0.05$, ** $P < 0.01$ FCHL compared with control subjects. Note: Error bars for control subjects are so small that they are not visible at all times.

were higher in FCHL than in control subjects (respectively, 6.05 ± 0.45 vs. 3.43 ± 0.46 mmol·h/L, $P < 0.01$; and 2.60 ± 0.49 vs. 0.96 ± 0.31 mmol·h/L, $P < 0.05$). In the postabsorptive period, the incremental FFA-AUC was lower in FCHL than in control subjects (−1.69 ± 0.90 vs. 1.81 ± 0.45 mmol·h/L; $P < 0.05$) (Fig. 2C). In this period an increase in FFA was seen in control subjects, from 0.42 ± 0.04 (t = 8 h) to 0.62 ± 0.09 mmol/L (t = 24 h) ($P < 0.05$). In FCHL, the FFA concentrations decreased (from 0.74 ± 0.08 at t = 8 h to 0.51 ± 0.05 mmol/L at t = 24 h; $P < 0.05$) (**Fig. 3A** and **B**).

HBA changes in response to an oral fat loading test

FCHL subjects started with a fasting level of 0.02 ± 0.0 mmol/L compared with control subjects, who started at 0.06 ± 0.02 mmol/L (NS). Both groups had an HBA peak at 6 h (FCHL: 0.43 ± 0.03 vs. control subjects: 0.20 ± 0.03 mmol/L; $P < 0.01$) (**Fig. 4**). The absolute increase in HBA concentration of FCHL and control subjects (0.41 ± 0.03 vs. 0.14 ± 0.04 mmol/L; $P < 0.01$) and the relative increase (1925 ± 162 vs. 538 ± 170%; $P < 0.01$) from baseline to peak values were higher in FCHL. Control subjects had a second HBA peak of 0.29 ± 0.08 mmol/L at 12 h in the postabsorptive period. In FCHL, the HBA concentration decreased after the peak value at 6 h during the rest of the test, up to 24 h. FCHL subjects showed a 59% decrease in postabsorptive HBA, in contrast to a mean 128% increase in control subjects. The absolute postabsorptive change in HBA concentrations (−0.18 ± 0.07 vs. 0.12 ± 0.07 mmol/L, respectively; $P < 0.01$) was lower in FCHL (Fig. 3C and D). The aceto-acetate curves were similar to the hydroxybutyric acid curves for FCHL and control subjects (data not shown).

Insulin changes in response to an oral fat loading test

Fasting insulin concentrations were not different in FCHL and control subjects (11.12 ± 0.20 vs. 7.04 ± 0.88

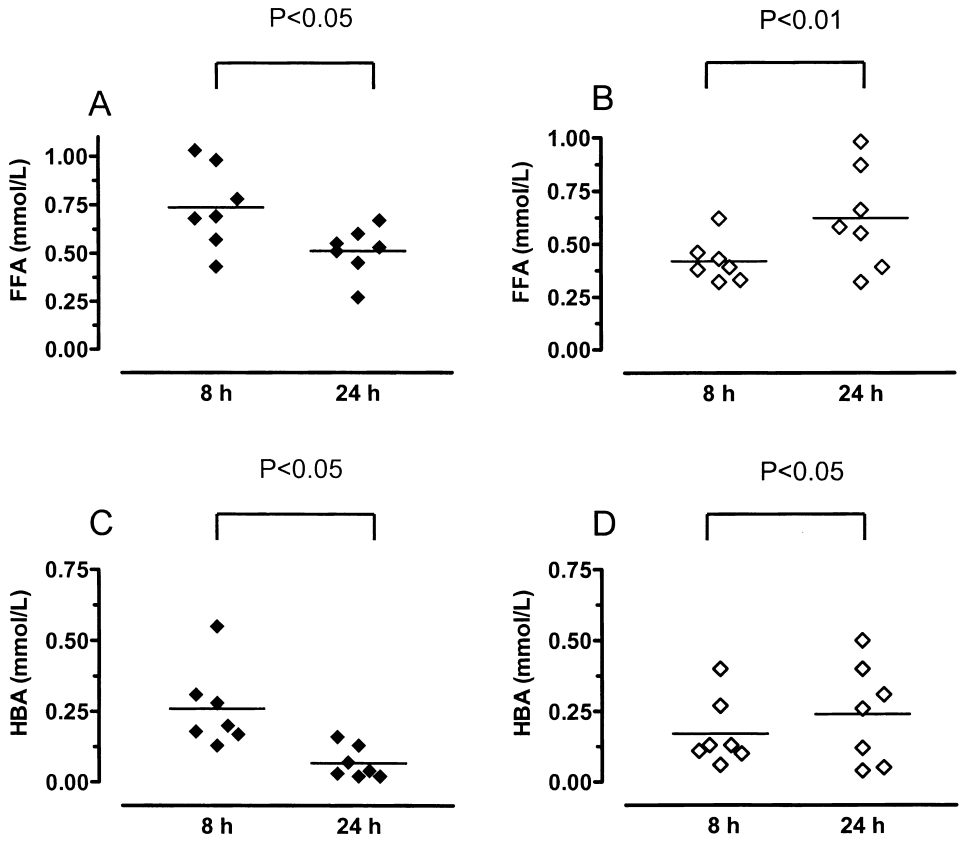

Fig. 3. A and B: Individual FFA concentrations at 8 and 24 h after fat load of 7 untreated FCHL patients (solid symbols) and 7 matched control subjects (open symbols). C and D: Individual HBA concentrations at 8 and 24 h. Bars represent mean values of the significant differences, between mean concentrations at t = 8 and t = 24 h, and were calculated by paired t test.

IU/L). From 8 to 12 h insulin concentrations decreased both in FCHL subjects (from 6.98 ± 1.73 to 4.89 ± 1.36 IU/L; $P < 0.01$) and in control subjects (from 4.14 ± 0.46 to 3.17 ± 0.36 IU/L; $P < 0.05$) (**Fig. 5**). During this entire period the insulin concentrations were below basal values in both groups. From 12 to 24 h insulin concentrations increased in both groups until basal values were reached.

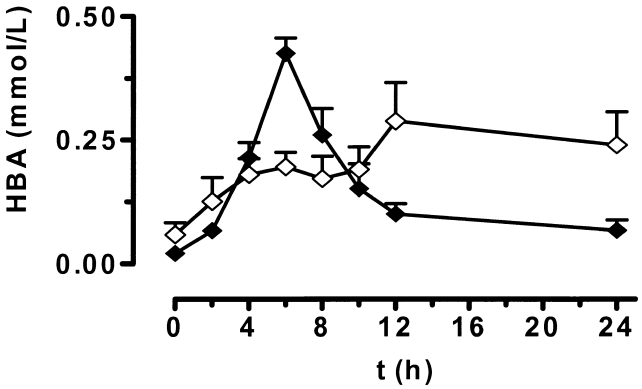

Fig. 4. Mean changes in hydroxybutyric acid (HBA) concentrations in 7 untreated FCHL patients (solid symbols) and 7 matched control subjects (open symbols) after an oral fat load. Data represent means ± SEM.

DISCUSSION

In the present study in vivo evidence of disturbed FFA metabolism in FCHL has been provided, which is in agreement with several in vitro studies. It has been suggested that in FCHL there is a defect in the adipose tissue metabolism of FFA and part of the features mentioned in FCHL could be explained by these defects (19). Separate analysis of postprandial and postabsorptive changes in TG, FFA, and ketone bodies clearly demonstrated a different FFA processing in FCHL than in control subjects. After a fat meal the intestines form a large amount of chylomicrons. Chylomicrons compete with VLDL for the action of LPL (32). As chylomicron-TG is preferred above VLDL-TG as a substrate for LPL, the clearance of the latter is reduced (33). After the same fat load as control subjects, FCHL patients tended to have a higher incremental TG-AUC. This is not surprising, as FCHL patients have a higher circulating VLDL and chylomicron (remnant) pool (31). However, the absolute and incremental postprandial FFA-AUCs were significantly higher in FCHL patients. This could be due either to an increase in FFA production or to a decrease in the clearance of FFA. As the incremental TG-AUC was slightly elevated, this could partly explain the differences seen in the FFA-AUC. It has been shown in vitro (34) and in vivo (35) that FFA released by LPL may be

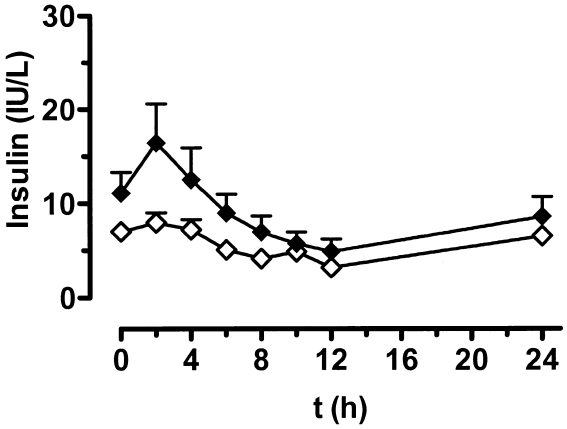

Fig. 5. Mean insulin concentration changes in 7 untreated FCH patients (solid symbols) and 7 matched control subjects (open symbols) after an oral fat load. Data represent means ± SEM.

taken up by the adipocyte for esterification and storage as TG, by the action of ASP. Not surprisingly, Maslowska et al. (36) found a positive correlation with the amount of ASP and FFA in healthy subjects. Chylomicrons increase the amount of ASP after an oral fat load (37). Although FCHL patients are known to have high postprandial levels of chylomicrons and chylomicron remnants (31), the storage of FFA in the adipocytes by ASP in FCHL patients may be diminished. In FCHL, an exaggerated increase in ketone bodies occurred compared with control subjects. As HBA is formed in liver mitochondrions solely from fatty acids (38), this exaggerated increase in HBA in FCHL may represent an enhanced hepatic FFA delivery. Our data are well in line with the in vitro studies of Sniderman and co-workers (19, 20), showing in FCHL a decreased ASP-mediated uptake of FFA by peripheral cells, theoretically, resulting in enhanced flux toward the liver. However, we cannot exclude the possibility that as a result of a larger pool of triglyceride-rich proteins (TRPs; i.e., VLDL and chylomicrons) and in the presence of well-functioning LPL, more FFAs are released, resulting in higher plasma concentrations. Ultimately, both situations will lead to increased delivery of FFA to the liver, most likely resulting in VLDL overproduction (19, 39). The differences in FFA and HBA between both groups in the present study do not seem to be a consequence of differences in fat mass, as described in obese subjects who have higher ASP levels (36), as no significant differences in weight, relative or absolute fat mass, were found. However, we cannot rule out that the small, not statistically significant difference in fat mass might have influenced some of our results.

In the postabsorptive period, the FFA concentrations increased in the control subjects. As adipose tissue is the main source of circulating FFA in this period (40), we may assume that the FFA increase is derived from the adipocytes. The rate-limiting step for mobilization of adipose tissue TG is hydrolysis by HSL (41). HSL is most active when the inhibitory effect of insulin is blunted (42). In the postabsorptive period, the insulin levels of control subjects as well as FCHL patients decreased, even below basal values. The FFA liberated from the adipose tissue might lead to an increase in HBA formed in the liver. In the FCHL group, no postabsorptive elevation of FFA followed by an increase in HBA concentration was seen. In contrast, both FFA and HBA declined during this period. Impaired ketogenesis is not likely, as postprandial HBA increase was seen in FCHL patients, suggesting high conversion of FFA in HBA. Therefore, the postabsorptive decline in FFA and HBA might represent decreased HSL activity in FCHL patients, in agreement with the in vitro findings of Reynisdottir et al. (43). Alternatively, as HSL is inhibited by insulin, the decreased insulin sensitivity in FCHL patients, as suggested by the higher HOMA index, or the slightly higher postabsorptive insulin concentrations compared with control subjects, might explain the impaired action of this lipase. However, in patients with insulin resistance syndrome, HSL activity has been shown to be normal (43). Furthermore, both FCHL patients and control subjects had insulin levels below baseline in the postabsorptive period. Thus, decreased insulin sensitivity in FCHL does not seem to explain the FFA and HBA changes in the postabsorptive period. Therefore, our data seem to be more in line with the primary decrease in HSL activity. In a report by Pihlajamäki et al. (44), the authors demonstrated decreased suppressibility of plasma FFA concentrations during hyperinsulinemic clamp in FCHL family members with and without hyperlipidemia. Similar results have been reported before in hyperlipidemic FCHL subjects (15, 16). In contrast to our study these two reports describe impaired inhibition of HSL by increased insulin levels in vivo. In the present article, impaired activation of HSL-mediated FFA release in the postabsorptive period is hypothesized, as plasma FFA concentrations decreased in FCHL (Fig. 3A), during lower insulin concentrations (Fig. 5).

In summary, in the postprandial period, diminished ASP-stimulated FFA incorporation into TG, in adipocytes, might be responsible for the elevated flux of FFAs to the liver in FCHL. In the postabsorptive period, however, less FFA seems to be released from the adipose tissue as a result of decreased action of HSL. These defects in the adipose tissue of FCHL patients could be the origin of several metabolic characteristics of FCHL, such as VLDL apoB overproduction and prolonged FFA concentrations. Further in vivo studies are necessary to elucidate the mechanisms behind the impaired action of ASP and HSL in FCHL patients.

Manuscript received 3 December 1999 and in revised form 16 March 2000.

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