Impaired Fatty Acid Metabolism in Familial Combined Hyperlipidemia

A Mechanism Associating Hepatic Apolipoprotein B Overproduction and Insulin Resistance

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Abstract

To establish whether insulin resistance and/or postprandial fatty acid metabolism might contribute to familial combined hyperlipidemia (FCH) we have examined parameters of insulin resistance and lipid metabolism in six FCH kindreds. Probands and relatives (n = 56) were divided into three tertiles on the basis of fasting plasma triglycerides (TG). Individuals in the highest tertile (TG > 2.5 mM; n = 14) were older and had increased body mass index, systolic blood pressure, and fasting plasma insulin concentrations compared with individuals in the lowest tertile (n = 24). The former also presented with decreased HDL cholesterol and increased total plasma cholesterol, HDL-TG, and apoprotein B, E, and CIII concentrations. Insulin concentrations were positively correlated with plasma apo B, apo CIII, apo E, and TG, and inversely with HDL cholesterol. Fasting nonesterified fatty acids (NEFA) were elevated in FCH subjects compared to six unrelated controls and five subjects with familial hypertriglyceridemia. Prolonged and exaggerated postprandial plasma NEFA concentrations were found in five hypertriglyceridemic FCH probands. In FCH the X2 minor allele of the AI-CIII-AIV gene cluster was associated with increased fasting plasma TG, apo CIII, apo AI, and NEFA concentrations and decreased postheparin lipolytic activities. The clustering of risk factors associated with insulin resistance in FCH indicates a common metabolic basis for the FCH phenotype and the syndrome of insulin resistance probably mediated by an impaired fatty acid metabolism. (*J. Clin. Invest.* 1993. 92:160–168.) Key words: apolipoprotein B • fatty acids • hyperinsulinemia • insulin resistance • premature atherosclerosis

Introduction

The majority of hyperlipidemic survivors of premature myocardial infarction and cerebral stroke have familial combined hyperlipidemia (FCH)[1] (1, 2). The incidence of FCH may be as high as 1% in Western societies (1, 3). Multiple-type hyperlipidemia in combination with elevated concentrations of apolipoprotein (apo) B and a history of premature coronary heart disease (CHD) in first degree relatives are characteristic features of FCH (1–5). Manifest hyperlipidemia in FCH usually does not occur before the age of 20 yr (3, 5), although a recent report demonstrated the presence of the FCH phenotype in 67% of children with familial hyperlipidemia (6). At present, a clinical marker specific for FCH is not available and family studies are necessary to establish the diagnosis (3–5). Several reports have documented hepatic overproduction of VLDL-apo B100 in FCH subjects (4, 7–9). The spectrum of FCH also comprises hyperapobetalipoproteinemia (hyperapoB) (10) and partial lipoprotein lipase (LPL) deficiency (11). Associations with the X2 allele of the apo AI-CIII-AIV gene cluster have been demonstrated in half of the FCH families (12). Increased concentrations of VLDL remnants (intermediate density lipoproteins) and increased synthesis of LDL, both metabolic products of hepatic VLDL, are likely to contribute to the atherosclerotic process (3, 5). We have recently demonstrated that chylomicron remnant clearance is delayed in FCH patients (13). Insulin resistance which has been associated to increased atherosclerotic risk (14), has also been associated with FCH (15). In subjects with the syndrome of insulin resistance, also known as "syndrome X" (16, 17), "familial dyslipidemic hypertension" (15, 18), or the "deadly quartet" (19), clustering of risk factors may be found. This atherosclerotic state has also been called the "atherogenic lipoprotein phenotype" (20) and a candidate gene cluster comprising the insulin receptor and LDL receptor genes has been found on the short arm of chromosome 19 (21).

The aim of the present study was to evaluate in FCH kindreds the relationship among lipids, apolipoproteins, and markers associated with the syndrome of insulin resistance.

Methods

Index FCH patients (Table I). The study protocol was approved by the Human Investigations Review Committee of the University Hospital Utrecht. Six unrelated male patients with FCH were recruited from the Lipid Clinic of the Utrecht University Hospital. These subjects met the following criteria: (*a*) a primary hyperlipidemia with varying phenotypic expression (fasting plasma cholesterol > 6.5 mM and/or fasting

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1. *Abbreviations used in this paper:* CHD, coronary heart disease; FCH, familial combined hyperlipidemia; FHTG, familial hypertriglyceridemia; HL, hepatic lipase; hyperapo B, hyperapobetalipoproteinemia; LPL, lipoprotein lipase; NEFA, nonesterified fatty acids; RP, retinyl palmitate.

Table I. General Characteristics of Index Patients and Blood Relatives in Six FCH Kindreds (n = 56)

Kindred (*n*)	I (11)	II (13)	III (15)	IV (6)	V (7)	VI (4)
X1X2 polymorphism of index patient	X1X2	X1X1	X1X1	X1X2	X1X2	X1X1
Age (*yr*)	39±6	37±4	38±5	40±6	50±8	33±6
(range)	(16–79)	(20–68)	(18–87)	(20–73)	(21–80)	(22–51)
M/F	3/8	4/9	10/5	3/9	5/2	2/2
Medication* (*n*)	4	1	7	1	2	2
Smokers (*n*)	5	8	6	2	3	0
CAD+‡ (*n*)	1	0	1	0	2	0
BMI (kg/m^2)	22.8±1.1	23.2±0.7	21.9±0.5	26.6±1.1	26.0±0.7	23.0±2.7

Data are given as mean±SEM. Abbreviations: BMI, body mass index; CAD, coronary artery disease; M/F, male/female.
* Number of subjects on lipid-lowering drug treatment during the study. ‡ Coronary artery disease characteristic includes the number of subjects with a positive history of angina pectoris or proven myocardial infarction.

plasma triglycerides [TG] > 2.0 mM), (*b*) at least one first-degree relative had a different hyperlipidemic phenotype (3–5), (*c*) elevated plasma apo B concentrations (> 0.9 g/liter), and (*d*) a positive family history of premature CHD defined as myocardial infarction or cerebrovascular diseases before the age of 60 yr in at least one blood-related subject of the index patient.

The six index FCH patients had normal fasting plasma glucose and thyroid-stimulating hormone concentrations. The apo E phenotype of the six index FCH patients was E4/E4 (kindreds I and II) and E3/E3 (kindreds III–VI).

Family studies. 50 direct FCH relatives from these six kindreds were studied. These relatives were parents, siblings, children, nephews, and nieces of the index patients. They attended our department for collection of fasting blood specimens and physical examination.

Familial hypertriglyceridemia controls (FHTG). Five hypertriglyceridemic male subjects (45.4±3.0 (SEM) yr) with normal apo B (0.83±0.06 g/liter) from families with primary hypertriglyceridemia were studied as a control group (4, 5). None of these subjects was obese (body mass index < 30 kg/m^2).

Analysis of the X1X2 apo AI-CIII-AIV polymorphism. The presence of the X2 minor allele which results from a polymorphic XmnI restriction site (X1 major allele) at the 5′ end of the apo AI gene on chromosome 11, was determined in DNA isolated from EDTA blood as described (12). These samples were sent by mail, at room temperature, and were always received 1 d later.

Oral fat-loading tests. In order to study the postprandial fatty acid metabolism in FCH and control subjects, nonesterified fatty acids (NEFA) were monitored after a standardized oral fat load in five unrelated hypertriglyceridemic FCH male probands (fasting TG 6.2±2.4 mM, cholesterol 8.4±2.4 mM, HDL-cholesterol 0.63±0.24 mM, insulin 13.8±9.8 mU/liter, age 49.0±10.8 yr, body mass index 26.0±2.3 kg/m^2; mean±SD) and six unrelated, normolipidemic male controls (TG 1.1±0.5 mM, cholesterol 5.0±0.8 mM, HDL-cholesterol 1.0±0.2 mM, insulin 5.0±0.2 mU/liter, age 42.5±6.8 yr, body mass index 23.1±3.2 kg/m^2). Cream was used as fat source; this is a 40% (wt/vol) fat emulsion with a P/S ratio of 0.06, which contains 0.001% (wt/vol) cholesterol and 2.8% (wt/vol) carbohydrates. After an overnight fast of 12 h, the subjects ingested the fresh cream, to which 120,000 U aqueous retinyl palmitate (RP) had been added 18 h before the test, in a dose of 50 g/m^2 body surface. After the ingestion of the fat load, subjects were only allowed to drink water during the following 24 h. Peripheral blood samples were obtained before ($t = 0$), at hourly intervals up to 10 h, and at 12, 14, and 24 h after the meal in sodium EDTA (2 mg/ml). The results of the oral fat loading tests have been published separately in detail (13).

Nonesterified fatty acids. NEFA were measured in plasma samples by an enzymatic colorimetric method (Wako Chemicals GmbH, Neuss, FRG). Plasma samples were stored at −20°C immediately after centrifugation and assayed within 1 wk after the fat load. Samples from normolipidemic controls and FCH patients were treated in the same fashion. Pilot experiments showed that generation of NEFA in stored plasma samples, presumably caused by LPL bound to lipoproteins, was quantitatively insignificant. NEFA concentrations in plasma samples from 20 different subjects (0.72±0.27 mU/liter; mean±SD) did not change significantly after storage for 9 mo at −20°C (0.87±0.44 mU/liter; paired *t*-test).

Analytical methods. Venous blood after an overnight fast of 12 h was drawn for biochemical analysis. Fasting plasma insulin was assayed by use of a commercial radioimmunoassay kit (Phadaseph Insulin RIA, Pharmacia, Uppsala, Sweden) and glucose was determined by the glucose oxidase method (22). TG and cholesterol (chol) were measured in duplicate by commercial colorimetric assay (GPO-PAP, Boehringer Mannheim no. 701912 and Monotest cholesterol kit, Boehringer Mannheim no. 237574, respectively) (22). HDL was prepared from whole plasma by precipitation with phosphotungstate-$MgCl_2$ as described (22). Plasma apo B and apo AI were determined by immunoturbidimetry (22, 23). Plasma apo E was measured by commercial immunoturbidimetric assays (Daiichi Pure Chemicals Ltd., Tokyo, Japan) according to manufacturer's instructions (24). Plasma apo CIII was measured by radial immunodiffusion using plates and apo CIII standards, according to manufacturer's instructions (Daiichi Pure Chemicals Ltd.). The diameter of the precipitation ring was measured by an investigator unaware of the specimens' identity. Postheparin plasma LPL and hepatic lipase (HL) activities were determined by the release of free fatty acids from ^{14}C-labeled trioleoyl emulsion, according to Huttunen et al. (25) as described (22). Lipolytic activity is expressed as nmol free fatty acids min^{-1} (mU) per ml plasma. Apo E phenotypes were determined by the single-dimension isoelectric focusing technique of VLDL isolated by ultracentrifugation in a model 40.3 rotor (40,000 rpm for 20 h at 4°C; Beckman Instruments, Inc., Fullerton, CA) and tube slicing. This method was validated in the laboratory of Dr. L. M. Havekes (Leiden, The Netherlands).

Statistical analysis. All values are expressed as mean±standard deviation (in all figures) or standard error of the mean (in all tables). The areas under the curves for TG and RP, were calculated by the trapezoidal rule (13, 22). Pearson's correlation coefficients were calculated by linear regression analysis after log-transformation in the case of TG, apo E, and apo CIII. Mean changes between more than two groups were calculated by ANOVA and Scheffé's test as post-hoc test. Significant postprandial changes of NEFA compared to baseline were calcu-

Table II. Fasting Plasma Lipids and Apoproteins of Blood Relatives and Index Patients in Six FCH Kindreds

Kindred (*n*)	I (11)	II (13)	III (15)	IV (6)	V (7)	VI (4)	Reference value*
TG (*mM*)	3.2±0.9	1.5±0.3	1.4±0.1	2.1±0.4	2.7±0.5	2.9±0.6	<2.0
(range)	(1.0–11.9)	(0.7–4.1)	(0.6–2.3)	(0.9–3.1)	(1.3–4.8)	(1.2–4.2)	
Cholesterol (*mM*)	6.3±0.5	5.7±0.3	6.0±0.4	6.3±0.4	7.0±0.6	7.6±0.8	<6.5
(range)	(4.7–10.2)	(4.4–7.8)	(3.5–8.4)	(5.0–7.2)	(5.1–10.0)	(6.1–9.7)	
HDL-C (*mM*)	1.2±0.1	1.2±0.1	1.0±0.1	1.2±0.1	1.2±0.1	0.8±0.1	>0.9
(range)	(0.70–1.61)	(0.81–1.50)	(0.60–1.87)	(0.98–1.69)	(0.80–1.38)	(0.60–1.00)	
Apo B (*g/liter*)	1.2±0.1	1.1±0.1	1.1±0.1	1.1±0.1	1.3±0.1	1.6±0.2	0.6–0.9
(range)	(0.7–1.7)	(0.5–2.0)	(0.5–1.6)	(0.8–1.3)	(0.9–1.5)	(1.1–2.2)	
Apo AI (*g/liter*)	2.0±0.1	1.7±0.1	1.6±0.1	1.9±0.1	1.9±0.1	1.7±0.2	1.4–1.6
(range)	(1.7–2.5)	(1.4–2.0)	(1.3–2.1)	(1.6–2.2)	(1.5–2.3)	(1.4–2.2)	
Apo E (*mg/liter*)	83.0±13.5	41.6±5.7	53.5±4.8	47.5±3.6	51.0±3.6	72.3±18.0	30±3
(range)	(33–176)	(18–99)	(28–78)	(37–61)	(37–63)	(37–122)	
Apo CIII (*mg/dl*)	11.6±1.7	7.6±1.0	9.4±0.9	12.2±1.5	15.7±3.3	13.2±4.4	8.1±0.9
(range)	(5.3–34.0)	(2.4–15.0)	(4.5–15.0)	(5.0–16.6)	(9.1–37.0)	(4.5–18.0)	

Data are given as mean±SEM.
* Reference values for nine normolipidemic male controls without FCH (13).

lated by repeated measures ANOVA and Fisher's least significant difference test. Mean differences between two groups were calculated by the unpaired *t* test. Statistical significance was reached when $P < 0.05$ (two-tailed).

Results

General characteristics, plasma lipids, and apoproteins of FCH relatives (Table II; Fig. 1). At the time of investigation, three relatives (5%) had had a nonfatal myocardial infarction; 17 subjects (30%) were using hypolipidemic drugs (HMG-CoA reductase inhibitors). None of the subjects used other drugs known to affect lipid metabolism such as beta-blockers or thiazides. One subject (26 yr) had previously undiagnosed type II diabetes (fasting plasma glucose 11.6 mM and insulin 102 mU/liter); 24 subjects (43%) were current smokers. 17 subjects (30%) had a body mass index > 25, but only one subject was obese with a body mass index of 30.6 kg/m^2. The mean plasma lipids and apoproteins of the FCH relatives are given in Table II. When the diabetic FCH relative was excluded, from the 56 relatives who visited our department, 5 subjects (9%) had isolated hypertriglyceridemia (TG > 2.5 mM), 15 (27%) had isolated hypercholesterolemia (cholesterol > 6.5 mM), 9 (16%) had combined hyperlipidemia, and 16 (29%) had increased insulin concentrations (> 10 mU/liter). Mean fasting HDL cholesterol concentrations were decreased (< 0.9 mM) in only one kindred (kindred VI). The mean plasma apo B concentrations were elevated (> 0.9 g/liter) in all kindreds, and mean plasma apo AI was elevated in five of the six kindreds. Plasma apo E was elevated in all kindreds and apo CIII in five of the six kindreds. Elevated plasma apo E and apo CIII indicated the presence of increased concentrations of VLDL and remnant particles in FCH subjects.

Effect of plasma TG on factors related to the syndrome of insulin resistance in FCH relatives (Tables III–V). Because elevated plasma TG concentrations are a common characteristic feature of FCH and insulin resistance, we have divided the relatives in the FCH kindreds into three tertiles of fasting plasma TG concentrations (Table III). This approach resulted in clustering of FCH phenotype-positive subjects in the highest tertile. The subjects in the highest tertile had decreased concentrations of HDL cholesterol and increased concentrations of plasma cholesterol, as well as plasma apoproteins B, E, and CIII. None of the subjects in the lowest tertile used lipid lowering medication or had elevated plasma cholesterol concentrations. The characteristic features of the syndrome of insulin resistance in the three tertiles of plasma TG are listed in Table IV. The subjects in the highest TG tertile were significantly older, had a higher body mass index, raised systolic blood pressure and increased fasting insulin concentrations. By ANOVA, a trend was found which suggested elevated NEFA plasma concentrations in hypertriglyceridemic FCH relatives compared to normolipidemic relatives in the lowest TG tertile ($P = 0.09$). No significant association was found between fasting NEFA plasma concentrations and fasting plasma triglycerides ($r = 0.20$; NS) in FCH relatives. When the nondiabetic FCH relatives were subdivided into lipoprotein phenotypes, e.g., normolipidemia (chol ≤ 6.5 mM and TG ≤ 2.5 mM; $n = 26$), hypercholesterolemia (chol > 6.5 mM and TG ≤ 2.5 mM; $n = 15$), hypertriglyceridemia (chol ≤ 6.5 mM and TG > 2.5 mM; $n = 5$), and combined hyperlipidemia ($n = 9$) fasting plasma NEFA were significantly increased in the subjects with combined hyperlipidemia compared to normolipidemic and hypercholesterolemic subjects (Table V). In addition, five subjects with primary hypertriglyceridemia and normal plasma apo B concentrations (FHTG), showed normal fasting NEFA plasma concentrations. This finding suggested that hypertriglyceridemia per se is not necessarily associated with increased plasma NEFA concentrations in non-FCH subjects. The data show that increased fasting plasma NEFA are elevated in FCH relatives with different phenotypes, but most pronounced in the group with combined hyperlipidemia and elevated plasma apo B concentrations.

Postprandial fatty acid metabolism in hypertriglyceridemic FCH subjects and normolipidemic controls (Figs. 2 and 3). The postprandial chylomicron and chylomicron remnant clear-

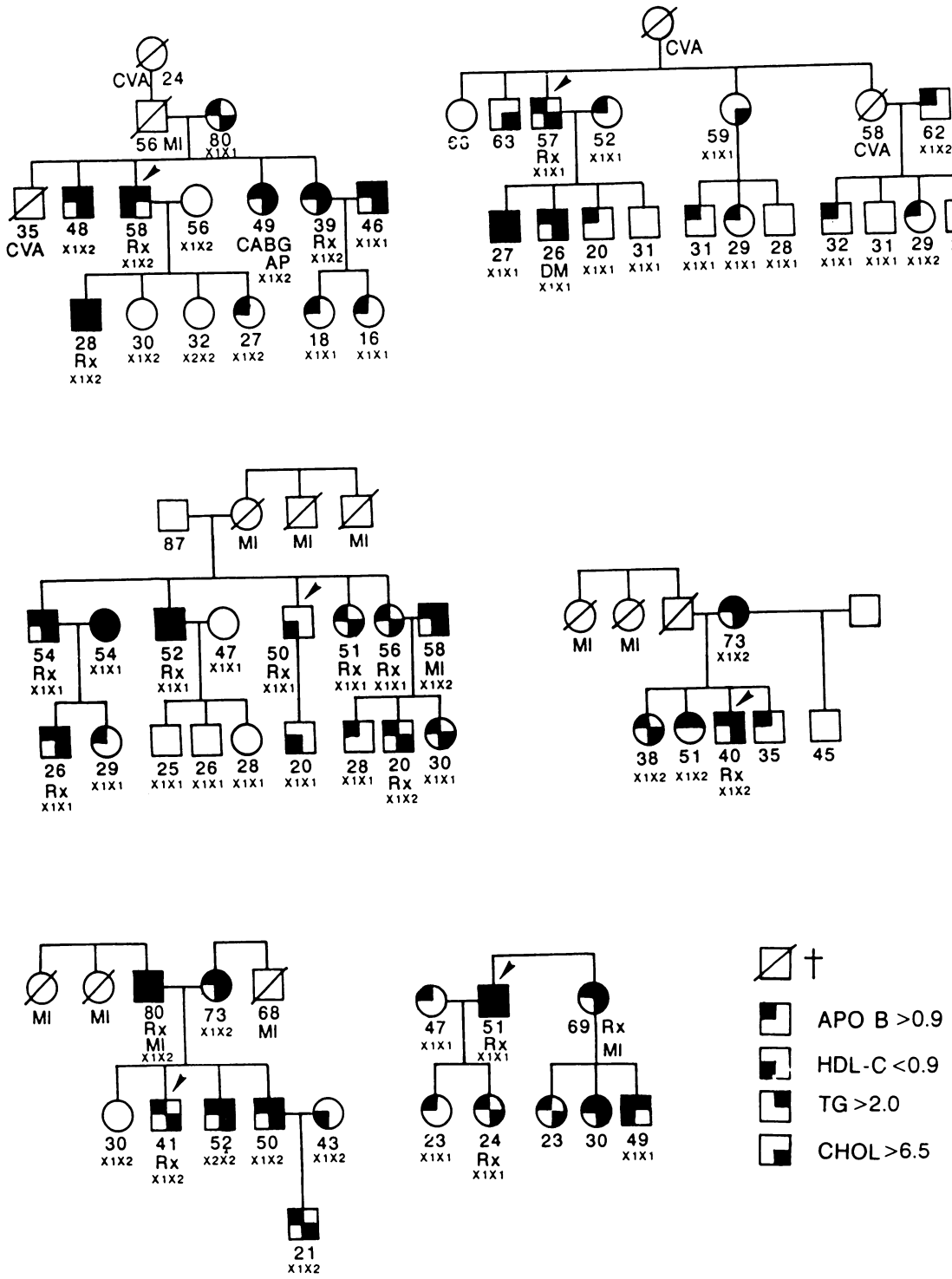


Figure 1. Examples of the pedigrees showing direct relatives of the six FCH kindreds participating in the study. The age of each subject at the time of the study is given. The squares represent males and the circles represent females. Abbreviations: MI, myocardial infarction; AP, angina pectoris; CABG, coronary artery bypass graft; Rx, using hypolipidemic medication; DM, diabetes mellitus; CVA, cerebrovascular accident. The data in this report are based on 56 relatives who visited our department. Data on five additional relatives shown in the figure were obtained by contacting their general practitioners with permission.

ance was delayed in the five FCH subjects studied, as indicated by the increased plasma RP area under the curve (RP-AUC) in FCH (104.6±39.2 h · mg/liter) compared to six normolipidemic controls (46.0±32.7 h · mg/liter; $P = 0.02$) (13). The postprandial triglyceridemia calculated as the area under the plasma TG curve (TG-AUC) was also increased in FCH (141.8±68.18 h · mM) compared to controls (23.25±12.30 h · mM; $P = 0.06$), also suggesting a delayed clearance of postprandial TG. Fasting NEFA concentrations were elevated in the five hypertriglyceridemic FCH subjects (0.94±0.38 mM)

Table III. Fasting Plasma Lipids and Apolipoproteins in FCH Blood Relatives Subdivided in Tertiles Based on Plasma TG

Tertile (*n*)	TG ≤ 1.5 (24)	1.5 < TH ≤ 2.5 (17)	TG > 2.5 (14)	*P* ANOVA
No. of smokers	9	9	6	NS
Medication*	4	5	8	0.03
TG (*mM*)	1.0±0.05	1.9±0.07	4.2±0.6‡§	0.0001
Cholesterol (*mM*)	5.4±0.2	6.7±0.3‡	7.2±0.4‡	0.0002
HDL-chol (*mM*)	1.2±0.05	1.2±0.07	0.96±0.06‡	0.02
HDL-TG (*mM*)	0.19±0.01	0.24±0.02	0.34±0.04‡§	0.0001
Apo B (*g/liter*)	0.94±0.06	1.22±0.07‡	1.40±0.09‡	0.0001
Apo AI (*g/liter*)	1.76±0.05	1.68±0.06	1.85±0.09	NS
Apo E (*mg/liter*)	44.96±4.29	53.56±4.04	79.50±9.97‡§	0.0007
Apo CIII (*mg/dl*)	6.80±0.51	10.89±0.74‡	17.04±2.27‡§	0.0001

Data are given as mean±SEM.
One diabetic FCH relative has been excluded. * Number of subjects on lipid lowering medication. ‡ $P < 0.05$ vs. lowest tertile; § $P < 0.05$ vs. middle tertile.

compared to controls (0.58±0.21 mM) (Fig. 2). NEFA remained significantly elevated from $t = 4$ to $t = 12$ in FCH subjects with a peak at 6 h, and the course of the NEFA was different from controls ($P = 0.02$ by ANOVA), indicating an abnormal postprandial metabolism of fatty acids in hypertriglyceridemic FCH. Fasting plasma NEFA correlated positively to plasma RP area under the curve ($r = 0.45$; $P = 0.02$) and plasma TG area under the curve ($r = 0.76$; $P = 0.007$) (Fig. 3), suggesting a relationship between postprandial clearance of TG-rich lipoproteins and fasting plasma NEFA.

Associations with fasting plasma insulin concentrations in FCH (Fig. 4). In FCH relatives, fasting plasma insulin correlated positively with body mass index ($r = 0.57$; $P < 0.0001$) and fasting plasma TG ($r = 0.44$, $P = 0.001$) as reported by others (15, 16, 19). In addition, we also found significant positive correlations with fasting plasma apo B ($r = 0.42$; $P = 0.002$), apo CIII ($r = 0.39$; $P = 0.005$), and apo E ($r = 0.37$; $P = 0.008$). This finding suggested a relationship between atherogenic lipoproteins which contain apo B as structural apoprotein (and apo E and CIII as exchangeable apoproteins) and insulin concentrations in FCH. In addition, insulin concentrations were also positively correlated with fasting plasma glucose ($r = 0.30$; $P = 0.04$) and inversely with plasma HDL cholesterol concentrations ($r = -0.29$; $P = 0.03$). The well-known relationship between fasting plasma TG and HDL-cholesterol concentrations was also demonstrated in FCH (Fig. 4). When the subjects on hypolipidemic treatment were excluded from the analysis identical significant correlations were found, except for the correlation of insulin with HDL cholesterol ($r = -0.22$; NS).

Associations of parameters of the syndrome of insulin resistance and polymorphisms of the apo AI-CIII-AIV gene cluster (Table VI). Three of the six FCH index patients studied had the X2 minor allele of the AI-CIII-AIV gene cluster of chromosome 11. The XmnI restriction length polymorphisms were also determined in a subset of FCH relatives ($n = 52$). 21 nondiabetic relatives had an X2 allele (19 were X1X2 and 2 X2X2) and 30 nondiabetic relatives were homozygous for the X1 allele (X2 negative). Compared to the X2-negative subjects, FCH relatives with the X2 allele had significantly increased fasting plasma TG, plasma apo AI, apo CIII, and NEFA concentrations. No significant differences were found in fasting plasma cholesterol, HDL cholesterol, or plasma apoproteins B and E. Postheparin plasma LPL and hepatic lipase activities were significantly decreased in the X2-positive ($n = 12$) subjects compared to the X2-negative subjects ($n = 13$).

Table IV. Variables Related to the Syndrome of Insulin Resistance in FCH Blood Relatives Subdivided into Tertiles Based on Plasma TG

Tertile (*n*)	TG ≤ 1.5 (24)	1.5 < TG ≤ 2.5 (17)	TG > 2.5 (14)	*P* ANOVA
Age (*yr*)	31.1±3.1	43.6±3.6	46.0±5.2*	0.01
BMI (kg/m^2)	22.13±0.40	23.27±0.72	27.0±0.92*‡	0.0001
BP (*mm Hg*)				
Systolic	118±2	128±6	135±4*	0.02
Diastolic	77±3	81±2	83±2	NS
Insulin (*mU/liter*)	7.48±0.64	7.82±0.93	16.62±2.46*‡	0.002
NEFA (*mM*)	0.65±0.06	0.62±0.08	0.90±0.14	0.09
Glucose (*mM*)	4.93±0.12	4.96±0.12	5.46±0.45	NS

Data are given as mean±SEM. Abbreviations = BMI, body mass index; BP, blood pressure.
* $P < 0.05$ vs. lowest tertile. ‡ $P < 0.05$ vs. middle tertile.

Table V. Fasting Plasma NEFA in FCH Relatives Who Were Divided into Different Lipoprotein Phenotypes

	FCH (normolipidemic) TC ≤ 6.5 TG ≤ 2.5	FCH (hypercholesterolemic) TC > 6.5 TG ≤ 2.5	FCH (hypertriglyceridemic) TC ≤ 6.5 TG > 2.5	FCH (combined) TC > 6.5 TG > 2.5	*P* ANOVA	FHTG
n	26	15	5	9		5
NEFA (*mM*)	0.67±0.07	0.58±0.06	0.69±0.19	1.02±0.19	0.05	0.42±0.04*‡§‖
TG (*mM*)	1.14±0.07	1.78±0.12*	3.59±0.36*‡	4.53±0.95*‡	<0.0001	7.15±0.83*‡§‖
Cholesterol (*mM*)	5.05±0.13	7.42±0.23*	5.73±0.35‡	8.01±0.39*§	<0.0001	5.17±0.28‡‖
Apo B (*g/liter*)	0.89±0.04	1.35±0.06*	1.17±0.07	1.53±0.13*	<0.0001	0.83±0.06‡§‖

The mean values of five unrelated male subjects with primary hypertriglyceridemia (FHTG) are included as reference. Data are given as mean±SEM.

* $P < 0.05$ vs. normolipidemic FCH relatives; ‡ $P < 0.05$ vs. hypercholesterolemic FCH relatives; § $P < 0.05$ vs. hypertriglyceridemic FCH relatives by Scheffé's test as post-hoc test; ‖ $P < 0.05$ vs. FCH combined. Note: Normolipidemic and hypercholesterolemic FCH relatives had significantly lower plasma NEFA than combined hyperlipidemic FCH subjects by Student's *t* test ($P = 0.03$ and $P = 0.02$, respectively).

Fasting plasma glucose and insulin concentrations were not different.

Discussion

Insulin resistance and FCH. In the present study we have demonstrated that clustering of risk factors is found in a subgroup of subjects with FCH who are characterized by elevated plasma TG and apo B concentrations. These risk factors are part of the syndrome of insulin resistance. Others have demonstrated a higher incidence of the syndrome of insulin resistance in FCH subjects but this was found to be associated with obesity (15, 18). In the present study, obesity was not a feature in FCH subjects since the mean body mass index in the tertiles was lower than in the study of Hunt et al. (15) and Selby et al. (18). Nonetheless, in the present study insulin concentrations correlated significantly with the body mass index in FCH relatives. Therefore, obesity does not appear to be an obligatory feature of the insulin resistance syndrome in FCH.

Insulin resistance has also been associated with upper-body fat distribution measured by the waist-hip ratio (26). An increased waist-hip ratio represents accumulation of intra-abdominal fat (27), which may provide an increased flux of fatty acids to the liver as well as insulin resistance by an unknown mechanism (16). In the present study we did not measure the waist-hip ratio of our patients. However, recently we compared in our laboratory 27 hypertriglyceridemic FCH subjects (age

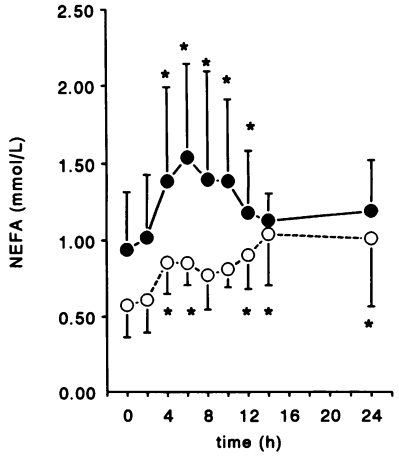
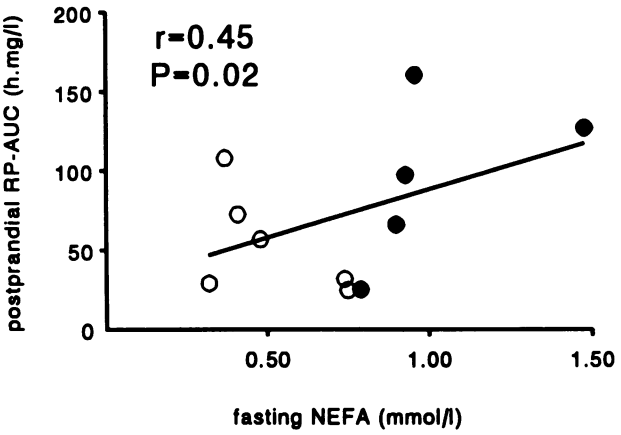


Figure 2. Postprandial changes in NEFA in (●) five hypertriglyceridemic FCH subjects (in the highest TG tertile), compared to (○) six unrelated normolipidemic controls. The asterisks represent significant changes by repeated measures ANOVA and Fisher's least significant difference test compared to fasting values. The course of the NEFA curves between FCH and controls was significantly different by ANOVA ($P = 0.02$). Data are mean±SD. L, liter.



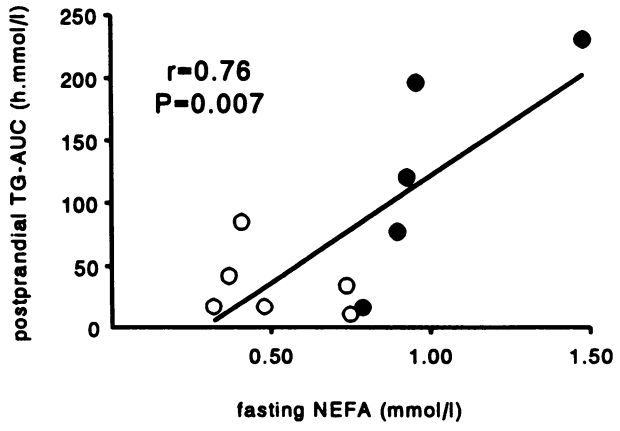


Figure 3. Correlations between fasting plasma NEFA and plasma RP area under the curve (RP-AUC) and plasma TG area under the curve (TG-AUC) in five hypertriglyceridemic FCH subjects (●) and six unrelated normolipidemic controls (○). Pearson's correlation coefficients (*r*) were calculated by linear regression analysis. l, liter.

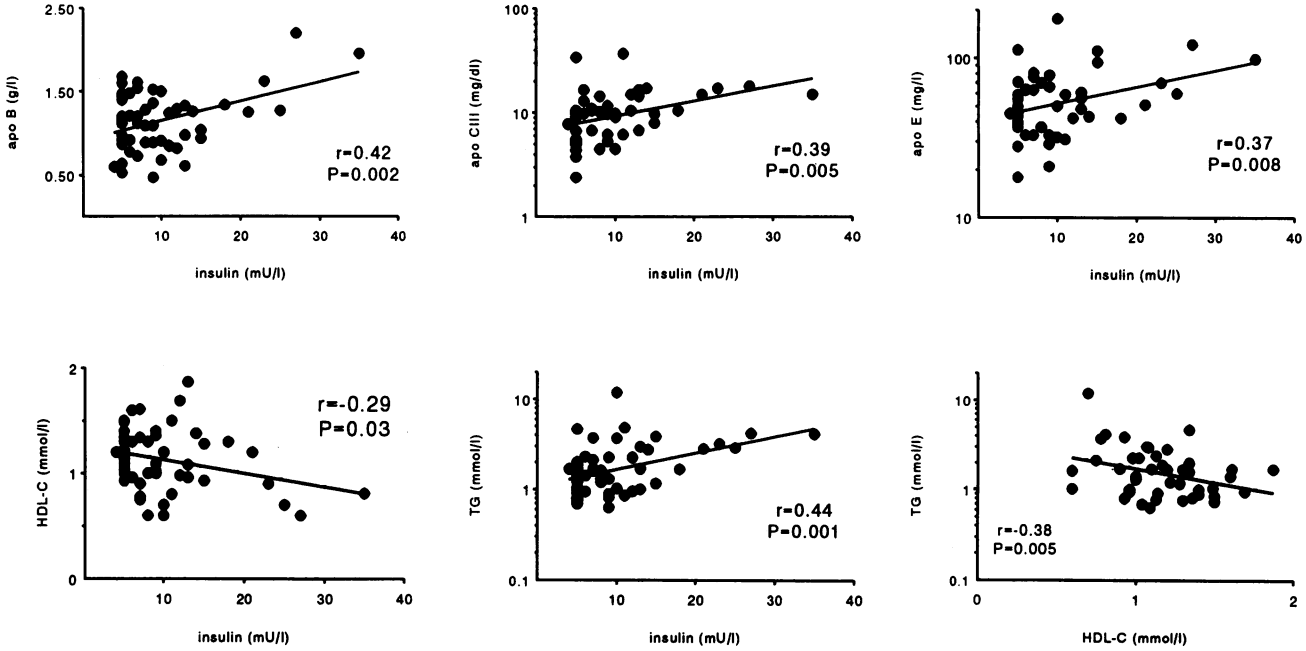


Figure 4. Correlations between fasting plasma insulin concentrations with plasma apo B (*upper left panel*), apo CIII (*upper middle panel*), apo E (*upper right panel*), HDL cholesterol concentrations (HDL-C, *lower left panel*), and plasma TG (TG, *lower middle panel*) concentrations in FCH relatives. The lower right panel shows the relationship between HDL cholesterol and fasting plasma TG concentrations. Pearson's correlation coefficients (r) were calculated by linear regression analysis. l, liter.

54.2±3.01 yr; body mass index 25.9±0.6 kg/m^2; TG 3.03±0.2 mM) to 18 normotriglyceridemic controls (TG 1.33±0.09 mM; $P < 0.0001$), who had been matched for age (60.6±2.1 yr) and body mass index (25.4±0.9 kg/m^2), and found no differences in waist-hip ratio (0.91±0.01 in FCH vs. 0.88±0.02 in controls). Therefore, the insulin resistance in FCH appears to be linked to other factors than body weight and fat distribution per se. It should be noted that the FCH subjects with the characteristics of insulin resistance (highest TG tertile) were significantly older than those without (in the lowest TG tertile). Insulin resistance increases with aging (28) and it is well known that the FCH phenotype does not generally develop before the age of 20 yr (1, 3, 5), suggesting an association between insulin resistance and the development of hyperlipidemia in FCH. Insulin resistance may be a common genetic mechanism in FCH subjects with hypertriglyceridemia. The hypertriglyceridemic FCH subjects in the present study were shown to cluster atherogenic factors like hyperlipidemia, relatively lower HDL-cholesterol concentrations, increased insulin concentrations and increased blood pressure. This phenotype in combination with the presence of small-dense LDL in plasma, is known as the atherogenic lipoprotein phenotype (ALP) (20), and has been linked recently to the insulin receptor locus on chromosome 19 (21). Furthermore, this phenotype may be associated with an insulin-resistant state (21) and is frequently found in FCH kindreds (29), in agreement with the findings described here. Thus, a frequently found gene abnormality may be assumed to underly the phenotype and also contribute to FCH.

Table VI. Plasma Lipids, Apoproteins, Postheparin Lipolytic Activities, and Parameters of Carbohydrate Metabolism in Relation to the XmnI Polymorphism of the AI-CIII-AIV Gene Cluster in Nondiabetic FCH Relatives

	FCH relatives		
(*n*)	X2 positive (21)	X2 negative (30)	*P*
BMI (*kg/m^2*)	24.4±0.8	23.7±0.6	NS
Age (*yr*)	42.4±3.8	36.2±3.0	NS
TG (*mM*)	3.04±0.53	1.67±0.18	0.003
Cholesterol (*mM*)	6.54±0.35	6.23±0.29	NS
HDL-C (*mM*)	1.17±0.05	1.09±0.06	NS
Apo B (*g/liter*)	1.20±0.05	1.17±0.08	NS
Apo AI (*g/liter*)	1.89±0.06	1.67±0.05	0.008
Apo E (*mg/liter*)	61.2±7.9	57.3±4.7	NS
Apo CIII (*mg/dl*)	13.4±1.9	9.34±0.8	0.04
Glucose (*mM*)	5.0±0.33	5.15±0.1	NS
Insulin (*mU/liter*)	9.3±1.2	10.8±1.4	NS
NEFA (*mM*)	0.86±0.1	0.59±0.05	0.04
LPL (*mU/ml*)*	91±10	133±15	0.03
HL (*mU/mL*)*	327±26	438±28	0.008

X2 positive denotes X2X2 (n = 2) and X1X (n = 19) subjects. Data are given as mean±SEM.

* LPL and HL activities were determined in 12 X2-positive and 13 X-negative subjects.

Metabolism of fatty acids and FCH. In this study the FCH subjects in the highest TG tertile had increased concentrations of plasma NEFA. An abnormal metabolism of fatty acids in the postprandial state in hypertriglyceridemic FCH subjects was found, resulting in prolonged and exaggerated concentrations of NEFA. In addition, significant correlations between fasting NEFA plasma concentrations and the area under the postprandial plasma RP and TG curves supported the association between fatty acids and postprandial lipoprotein catabolism. Re-

cently Lewis and co-workers (30) found similar results in subjects with non-insulin-dependent diabetes mellitus. Hypertriglyceridemic patients with diabetes had identical fasting NEFA plasma concentrations as those with normotriglyceridemia, which is in agreement with our findings in subjects with FHTG. More studies are necessary to investigate NEFA metabolism in subjects with insulin resistance and hypertriglyceridemia, but without FCH.

Our data are in agreement with the findings of Sniderman and co-workers (31), who demonstrated impaired metabolism of fatty acids in vitro by fibroblasts of patients with hyperapoB. Decreased binding, uptake and degradation of the acylation-stimulating protein (i.e., basic protein I) by hyperapoB cells was responsible for the impaired fatty acid metabolism (32–34). Increased delivery of fatty acids to hepatocytes in an vitro model has been shown to result in increased synthesis and secretion of VLDL (34, 35), resembling the VLDL overproduction in FCH. Moreover, increased NEFA in plasma inhibits LPL which could result in slower clearance of triglyceride rich particles from plasma (36, 37). It cannot be excluded that elevated levels of NEFA are responsible for the decreased lipolytic activities as found in FCH subjects (11, 13). In addition, free fatty acids promote the release of LPL from the endothelial cells resulting in increased uptake of LPL by the liver (38). Our data therefore provide an alternative explanation for the frequently found decreased concentrations of LPL mass and LPL activity in FCH (11, 13). The abnormal metabolism of fatty acids may be a characteristic feature of FCH and provide a pathophysiological link to the hepatic apo B overproduction and partial LPL deficiency that are characteristic in FCH (11, 39).

XmnI polymorphisms and FCH. The X2 allele has been proposed as a possible marker for FCH. Three of the six FCH index patients studied had the X2 allele which is in agreement with a previous report (12). Subjects with the X2 allele had increased concentrations of fasting plasma NEFA as well as decreased postheparin plasma LPL and HL activities. However, fasting plasma insulin concentrations were not significantly different in the X2 positive and negative subjects. In both groups the fasting plasma apo B, E, and CIII, characteristic features of FCH subjects (13), were elevated. Therefore, the X2 allele may discriminate between two different pathogenetic mechanisms for the FCH phenotype, one of those related to high NEFA. In our laboratory studies are underway to investigate in detail the effect of the X2 allele on lipoprotein variables in FCH and non-FCH subjects.

In agreement with other authors (1, 3), a high frequency of lipoprotein abnormalities was found in the spouses of affected FCH subjects. This suggests that lipoprotein parameters in these families might be largely influenced by nongenetic variables like age, diet, and smoking. This should be taken into consideration when studying genetic determinants in FCH. Extensive family studies including relatives of the spouses are underway in our department.

Hepatic apo B overproduction and hyperinsulinemia in FCH. Although apo B overproduction is a well established feature of FCH (3, 7–9), the underlying mechanism is not known. Apo B gene defects have been ruled out by linkage analysis in FCH kindreds (40). Several studies have demonstrated that insulin decreases apo B secretion in vitro by enhanced intracellular degradation (41–44). However, the hyperinsulinemia in FCH may be a consequence of the insulin resistance and this may be associated to impaired metabolism of fatty acids by peripheral cells. In vivo evidence has been provided suggesting a pivotal role of increased fatty acids in insulin resistance (45–47). Fatty acids activate gluconeogenesis by supplying acetyl-CoA which stimulates pyruvate carboxylase, one of the enzymes involved in hepatic gluconeogenesis (48, 49). Increased production of glucose is a trigger to the pancreas to secrete insulin in order to maintain normoglycemia and this can result in increased fasting plasma insulin concentrations (50). Therefore, we propose that the increased concentrations of NEFA in FCH subjects may be the primary metabolic factor causing hepatic apo B overproduction (31, 34).

In conclusion, in FCH increased fasting plasma insulin concentrations which are associated with the syndrome of insulin resistance may be secondary to impaired postprandial fatty acid metabolism. Elevated fatty acid plasma concentrations may provide an explanation for the hepatic overproduction of atherogenic apo B–containing lipoproteins, a characteristic feature of FCH.

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