Lipoprotein Phenotype and Insulin Resistance in Familial Combined Hyperlipidemia

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The study objective was to investigate the relationship of insulin resistance (IR) with the lipoprotein phenotype in familial combined hyperlipidemia (FCH). Thirty-seven FCH men diagnosed by clinical and biochemical criteria and classified as lipoprotein phenotype IIa (n = 9), IIb (n = 17), or IV (n = 11) were compared with a healthy control group of 30 men of similar age, body mass index (BMI), waist to hip ratio (WHR), and systolic and diastolic blood pressure. In all subjects, the plasma lipoprotein profile and baseline and post–oral glucose tolerance test (OGTT) glucose and insulin plasma values were measured. An intravenous glucose tolerance test was performed and IR was studied by the peripheral insulin sensitivity index (Si). After the OGTT, significantly higher values for insulinemia (at 0, 60, 90, and 120 minutes) and the area under the curve (AUC) of insulin secretion were observed in FCH. The AUC of insulin was greater in FCH subjects with the hypertriglyceridemic phenotype as compared with the controls and significantly lower Si levels, indicating greater IR, were found in the three FCH groups (control, 3.48 ± 1.87 mU/L/min; FCH IIa, 2.09 ± 1.08; FCH IIb, 1.54 ± 0.77; FCH IV, 1.47 ± 0.93; $P < .001$). The prevalence of IR ($Si < 2 \times 10^{-4}$ mU/L/min) was greater in FCH, independent of the lipoprotein phenotype, as compared with the controls ($P < .0001$). Higher plasma glucose and insulin levels at 120 minutes and lower Si values were found in the FCH IIa group compared with the controls ($P < .05$), indicating a state of IR in this subgroup of normotriglyceridemic subjects. In conclusion, IR was found in the three FCH lipoprotein phenotypes, being more severe in subjects with hypertriglyceridemia. Hence, the therapeutic goals in FCH should include measures to normalize plasma lipids and improve peripheral insulin sensitivity.

FAMILIAL COMBINED HYPERLIPIDEMIA (FCH), described in 1973 as a dyslipidemic syndrome and identified in family studies of young myocardial infarction survivors,[1-2] is one of the most common genetic hyperlipidemias. Transmission is autosomal dominant, although a polygenic inheritance cannot be ruled out.[3] The condition is characterized by an elevation in the plasma concentration of apolipoprotein B (apo B) and the presence of multiple lipoprotein phenotypes within the same individual (expressed over a period of time), as well as affected family members. The lipoproteins involved are low-density lipoprotein ([LDL] phenotype IIa), very–low-density lipoprotein ([VLDL] phenotype IV), or both (phenotype IIb).[4]

In addition to the increase in VLDL and LDL, there is a qualitative alteration in the composition of the lipoproteins (particularly an increase in the proportion of apo B), together with a frequent decrease in plasma high-density lipoproteins (HDLs).[5] The increased risk of ischemic heart disease observed in FCH families has been related, among other risk factors, to the plasma lipid alterations.[6]

Studies performed in heterogeneous populations have examined the relationship of insulin resistance (IR) and hyperinsulinemia to hypertriglyceridemia, hypertension, type 2 diabetes mellitus, and central trunk obesity.[7,8] In FCH, IR appears as a basic element implicated in the metabolic derangements observed in these patients; moreover, FCH patients with central obesity or hypertriglyceridemia show further deterioration of insulin sensitivity.[9] Thus, the presence of IR could explain, in part, the elevated cardiovascular risk observed in FCH.[10]

As part of a study of cardiovascular risk factors clustering in FCH, we have investigated the alterations in glucose metabolism and insulin secretion and the degree of IR in relation to the lipoprotein phenotype.

SUBJECTS AND METHODS

Subjects

We studied 37 unrelated FCH males selected on the basis of consecutive attendance at our Lipid Clinic. The diagnosis of FCH was based on the lipoprotein profile, the presence of a variable lipoprotein phenotype in first-degree family members, a positive family history of premature ischemic heart disease, and the absence of hyperlipidemia in first-degree relatives younger than 20 years of age.[9,10] The selected probands had an LDL cholesterol (LDL-C) level of 4.1 mmol/L or higher and/or plasma triglyceride (TG) at least 2.3 mmol/L, with apo B greater than 1.2 g/L and baseline plasma glucose less than 7 mmol/L. The study group included 9 subjects with phenotype IIa (LDL-C $\geq$ 4.1 mmol/L and plasma TG $<$ 2.3 mmol/L), 17 with phenotype IIb (LDL-C $\geq$ 4.1 mmol/L and plasma TG $\geq$ 2.3 mmol/L), and 11 with phenotype IV (LDL-C $<$ 4.1 mmol/L and plasma TG $\geq$ 2.3 mmol/L). In IIa subjects, familial hypercholesterolemia (FH) was ruled out by clinical and genetic criteria. The clinical criteria for a diagnosis of FH were as follows: the presence of xanthomas, hypercholesterolemia in first-degree relatives less than 20 years of age, and an autosomal inherited pattern of a lipid IIa phenotype.[11] Genetic diagnosis of FH was based on Southern blot analysis to detect major rearrangements or polymerase chain reaction–single-strand conformational polymorphism analysis to detect point and small mutations of the LDL receptor gene.[12]

Exclusion criteria were as follows: a diagnosis of secondary hyperlipidemia and other conditions that can modify the metabolic parameters under study (hepatic or renal disease, alcohol intake, and myocardial infarction less than 3 months previously) or the use of medications known to modify carbohydrate or lipid metabolism. All subjects were non-smokers or ex-smokers for at least 1 year. Each subject had a fasting plasma glucose less than 7.8 mmol/L and a glucose level at 120 minutes post–oral loading with 75 g glucose of less than 11.1 mmol/L.

A control group of 30 healthy subjects with similar age, gender, waist to hip ratio (WHR), body mass index (BMI), and blood pressure were also studied. They had no personal or family history of metabolic or cardiovascular disease and used no medications. They were recruited

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from plasma donors, health personnel, and researchers from our Hospital.

In all subjects, the WHR was calculated and blood pressure was measured in the sitting position after a 10-minute rest as the mean of 3 determinations. A trained investigator performed the clinical tests between 8 and 9 AM in the Metabolic Unit. All subjects were European Caucasians.

Study recruitment of the patients and control subjects followed fully informed consent, and the study was approved by the Ethics Committee of the Hospital.

Laboratory Methods

During the 4 weeks prior to the study, all medications that can affect lipid metabolism or influence insulin sensitivity were suspended. Following a 12- to 14-hour overnight fast, total cholesterol (TC) and TG levels were measured by enzymatic methods[13,14]; HDL-C was determined by precipitation of apo B–containing lipoproteins with polyanions,[15] and VLDL-C was determined after the separation of VLDL (density < 1.006 g/mL) by ultracentrifugation (18 hours at $105{,}000 \times g$ at 15°C) in a Ti 50.3 fixed-angle rotor in a Beckman L8-80 ultracentrifuge.[16] The value for LDL-C was calculated by subtraction of VLDL-C and HDL-C from TC. Plasma free fatty acid (FFA) levels were measured by enzymatic colorimetry,[17] and plasma apo B and apo A-I levels by immunoturbidimetry.[18]

Clinical Methods

Metabolic tests were performed in the Metabolic Unit near the laboratory under the supervision of a physician or nurse.

The oral glucose tolerance test (OGTT) with 75 g glucose was performed following World Health Organization recommendations,[19] with determinations of plasma glucose[20] by a multichannel autoanalyzer (Technicon RA-1000, Swords, Dublin, Ireland) and plasma insulin by radioimmunoassay.[21] The area under the curve (AUC) of glucose and insulin was calculated by the trapezoidal method.[22]

The intravenous glucose tolerance test, with multiple blood samples for the measurement of glucose and insulin and calculation of the minimal-model peripheral insulin sensitivity index (Si), was performed after a 12-hour fast and with the patient resting supine for at least 15 minutes before the test. Two baseline venous blood samples ($t = -10$ and $t = -5$ minutes) for glucose and insulin were obtained. At time 0 minutes, a bolus of glucose 300 mg/kg body weight in a 50% glucose-saline solution was administered over a period of approximately 60 seconds. At 20 minutes, a 0.03-U/kg body weight bolus of regular insulin (Actrapid; Novo Nordisk Pharma, Copenhagen, Denmark) was administered. Following the two baseline samples, 26 additional blood samples were taken for the determination of glucose and insulin at 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 22, 24, 25, 27, 30, 40, 50, 60, 70, 90, 100, 120, 140, 160, and 180 minutes. The Si was calculated using the MINMOD program.[23]

Statistical Analyses

All statistical analyses were performed using the SSPS program[24] and the results are expressed as the mean ± SD. Because of the sample size and the measurement of variables that do not fulfill the criteria of normality, nonparametric tests were used for the statistical analyses. For the comparison of mean values, we used the Mann-Whitney *U* test for 2 variables or the Kruskal-Wallis test for 3 or more variables, while Fisher's exact test was used for the comparison of proportions. The degree of relationship between two quantitative variables was determined by Spearman's correlation coefficient. *P* values less than .05 were considered statistically significant.

RESULTS

The general characteristics of the control and FCH subjects classified by lipoprotein phenotype are presented in Table 1. We found no significant differences for the age, BMI, WHR, and blood pressure between the groups. Plasma concentrations of lipids, apo B, and the different lipoprotein fractions were significantly different by selection criteria. Fasting FFA plasma levels were significantly higher in the FCH group compared with the controls.

The results of the OGTT are summarized in Table 2. We found significant differences in plasma glucose and insulin and Si values between groups (Kruskal-Wallis test). The AUC for insulin was greater in FCH phenotype IV versus phenotypes IIb or IIa and the control group ($P < .001$). The corresponding Si values were 3.48 ± 1.87 in the control group, 2.09 ± 1.08 in FCH IIa, 1.54 ± 0.77 in FCH IIb, and 1.47 ± 0.93 in FCH IV ($P < .001$). The FCH IIa group showed higher plasma glucose and insulin at 120 minutes and glucose AUC and lower Si values than control subjects ($P < .05$, Mann-Whitney).

We considered the presence of IR when the Si value was above the 10th percentile of the corresponding value in the

Table 1. General Characteristics of the Subjects

Characteristic	FCH IIa	FCH IIb	FCH IV	Control	*P*
No. of subjects	9	17	11	30	
Age (yr)	48.2 ± 12.9	47.4 ± 9.5	46.2 ± 8.3	44.5 ± 8.4	NS
BMI (kg/m²)	26.5 ± 3.1	27.5 ± 2.6	27.6 ± 2.3	26.7 ± 2.8	NS
WHR	0.96 ± 0.06	1.01 ± 0.05	0.98 ± 0.05	0.96 ± 0.07	NS
SBP (mm Hg)	138.3 ± 15.6	131.5 ± 11.6	137.0 ± 13.5	128.5 ± 9.2	NS
DBP (mm Hg)	80.0 ± 7.1	81.2 ± 9.7	86.0 ± 11.5	77.1 ± 6.5	NS
TC (mmol/L)	7.39 ± 1.30	7.94 ± 1.36	5.90 ± 0.66	5.26 ± 0.64	*
TG (mmol/L)	2.02 ± 0.46	4.70 ± 1.90	4.82 ± 1.61	1.77 ± 0.47	*
LDL-C (mmol/L)	5.60 ± 1.30	5.33 ± 1.10	3.43 ± 0.43	3.45 ± 0.59	*
VLDL-C (mmol/L)	0.78 ± 0.20	1.61 ± 0.51	1.68 ± 0.49	0.73 ± 0.28	*
HDL-C (mmol/L)	1.00 ± 0.09	0.95 ± 0.20	0.92 ± 0.16	1.06 ± 0.11	*
LDL-C/HDL-C	5.67 ± 1.69	5.78 ± 1.46	3.79 ± 0.59	3.27 ± 0.65	*
Apo B (g/L)	1.56 ± 0.20	1.71 ± 0.43	1.41 ± 0.25	1.05 ± 0.15	*
Apo A-I (g/L)	1.14 ± 0.13	1.09 ± 0.26	1.08 ± 0.19	1.02 ± 0.14	*
FFA (mg/L)	446.5 ± 192.2	563.2 ± 168.9	518.0 ± 122.7	351.7 ± 116.4	*

Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure; NS, non–statistically significant differences.

*Statistically significant differences due to selection criteria.

Table 2. Glucose and Insulin Response to an Oral Glucose Load and Si in FCH Subjects and Controls

Parameter	FCH IIa	FCH IIb	FCH IV	Control	*P*
No. of subjects	9	17	11	30	
Age (yr)	48.2 ± 12.9	47.4 ± 9.5	46.2 ± 8.3	44.5 ± 8.4	NS
BMI (kg/m²)	26.5 ± 3.1	27.5 ± 2.6	27.6 ± 2.3	26.7 ± 2.8	NS
Glucose (mmol/L)					
0 min	5.2 ± 0.5	5.2 ± 0.6	5.8 ± 0.9	4.7 ± 0.9	.02
30 min	9.0 ± 1.3	9.2 ± 1.6	10.7 ± 2.2	7.4 ± 2.1	.001
60 min	9.8 ± 1.8	9.7 ± 2.7	12.9 ± 3.5	6.9 ± 2.4	.001
90 min	8.3 ± 2.4	9.1 ± 3.2	10.8 ± 5.6	4.2 ± 3.2	.001
120 min	6.9 ± 2.0†	8.3 ± 3.2	8.9 ± 4.4	5.0 ± 1.5	.001
AUC (mmol/L/min)‡	998 ± 137†	982 ± 356	1,300 ± 368	812 ± 145	.001
Insulin (pmol/L)					
0 min	80.5 ± 29.8	121.0 ± 70.2	158.3 ± 72.4	79.5 ± 36.5	.001
30 min	503.1 ± 355.9	899.8 ± 101.3	766.4 ± 522.8	355.5 ± 335.6	.06
60 min	677.9 ± 370.3	1,347.7 ± 1,293	1,243 ± 1,128.6	462.9 ± 385.9	.001
90 min	643.1 ± 323.6	1,249.0 ± 1,058.3	1,485.2 ± 1,137.6	358.8 ± 323.8	.001
120 min	481.3 ± 168.4*	1,096.3 ± 787.6	1,185.8 ± 1,097.1	260.9 ± 272.6	.001
AUC (pmol/L/min)‡	63,150 ± 26,005	1,308,750 ± 104,065	137,340 ± 93,044	58,137 ± 24,220	.001
Si (10^{-4} mU/L/min)	2.09 ± 1.08†	1.54 ± 0.77	1.47 ± 0.93	3.48 ± 1.87	.001

*$P < .05$, IIa *v* control (Mann-Whitney).
†$P < .01$, IIa *v* control (Mann-Whitney).
‡During the oral glucose load.

control group. With this criterium (Fig 1), 13.3% of control subjects, 66.6% of FCH IIa, 76.5% of FCH IIb, and 81.8% of FCH IV subjects had IR (Si $< 2 \times 10^{-4}$ mU/L/min, $P < .001$). No significant differences in age, BMI, WHR, and blood pressure were found among the 4 groups.

DISCUSSION

The presence of IR has been demonstrated in FCH,[25] and in our experience,[26] it is aggravated by truncal obesity[27] and is related to plasma levels of TG and FFA.[9] In the present study in 37 FCH males, we observed fasting and post-OGTT hyperinsulinemia together with lower Si values, indicating a state of IR, irrespectively of the lipoprotein phenotype. In addition, in a previous study,[26] we observed a significant inverse correlation ($r = -.34$, $P < .02$) between Si values and plasma TG levels. However, in the present study, the FCH subgroup without hypertriglyceridemia (IIa phenotype) also showed significantly higher mean fasting and post-OGTT plasma insulin, lower Si values, and a higher prevalence of IR than the control group.

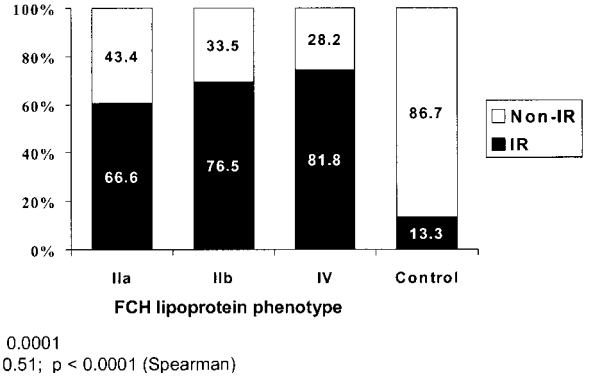

Fig 1. Prevalence of IR expressed as Si (peripheral Si by minimal model) $< 2 \times 10^{-4}$ mU/L/min in the study groups.

Our findings support the notion that IR is part of the metabolic alterations in FCH, independent of the lipoprotein phenotype. Thus, is IR the cause or the consequence of the dyslipidemia and high plasma FFA observed in FCH? As hypothesized by Frayn,[28] the observed lipid changes could stem from a disruption of the normal, precise coordination of postprandial lipid metabolism by insulin. One important consequence of IR is the loss of the suppressive effect of insulin on fat mobilization from adipose tissue; as a result, there is an increase in FFA flux to the liver. There is also a failure to suppress FFA release in the postprandial period that could explain the increased hepatic VLDL-TG secretion described in IR subjects.[29] Our results are in concordance with Frayn's hypothesis. We hypothesize that IR is the first metabolic alteration in subjects with FCH, indicating a state of IR and hyperinsulinism with elevated fasting plasma FFA levels. However, our cross-sectional study makes this causality only hypothetical. In support of this hypothesis, IR has been demonstrated in non-obese normotensive FCH subjects,[9,26-28] as well as FCH subjects with phenotype IIa, as our present results have shown. Moreover, other evidence has suggested that hypertriglyceridemia is a consequence of IR irrespective of the glucose tolerance status, and it was demonstrated in two prospective studies that fasting hyperinsulinemia precedes hypertriglyceridemia and low plasma HDL-C.[30,31] In contrast, Karjalainen et al[32] recently demonstrated that first-degree relatives from FCH families without lipid alterations showed elevated fasting FFA but normal glucose metabolism as compared with controls. These findings were interpreted as an indication that abnormalities in FFA metabolism precede IR in FCH kindreds. Moreover,

abnormal lipid and lipoprotein concentrations are known to impair insulin action. Steiner and Vranic[33] have shown that hypertriglyceridemia can lead to IR even without concomitant obesity or non–insulin-dependent diabetes, and that high plasma VLDL concentrations downregulate insulin receptors.

Another possibility as hypothesized by Cianflone et al[34] could be the failure in FCH subjects of FFA ''trapping'' by the adipocyte.[35-37] In this situation wherein FFAs of dietary origin are not taken up by the adipocyte, their flux to the liver is increased, leading to an increase in fasting and postprandial FFA levels. In addition, other metabolic alterations could appear: prolonged postprandial TG clearance, accumulation of chylomicron remnants, increased VLDL release, preponderance of small, dense LDL particles, and decreased HDL-C. All of these findings are well-known lipoprotein alterations in FCH. This mechanism could be complementary to our hypothesis.

In conclusion, IR is a common finding in FCH regardless of whether the affected subjects have hypertriglyceridemia. This frequent alteration could explain, in part, the elevated cardiovascular risk in FCH subjects.[10,38,39] Hence, IR should be considered as part of the therapeutic goals together with lipid control in this high–coronary risk group.

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