A Study of Insulin Resistance Using the Minimal Model in Nondiabetic Familial Combined Hyperlipidemic Patients

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The presence of insulin resistance in 20 male nondiabetic patients with familial combined hyperlipidemia (FCH) and 20 controls of similar age and body mass index (BMI) was investigated using the minimal model method modified by the administration of insulin and an oral glucose tolerance test. The peripheral sensitivity of insulin, expressed as the insulin sensitivity index (Si), was 1.91 ± 1.05 and 2.86 ± 1.19 × $10^{-4} \cdot min^{-1} \cdot mU/L$ in FCH patients and controls, respectively ($P < .01$), and the corresponding value for the peripheral utilization of glucose independently of insulin (Sg) was 1.70 ± 1.13 in FCH patients and 2.35 ± 0.60 × $10^{-2} \cdot min^{-1}$ in controls ($P < .02$). In the FCH group, the Si value correlated significantly ($P < .05$) with the waist to hip ratio (WHR), plasma triglycerides (TG), free fatty acids (FFA), and the area under the curve of glucose (AUCg) and insulin (AUCi). In the control group, the correlation also reached statistical significance ($P < .05$) with age, BMI, WHR, blood pressure, TG, AUCg, and AUCi. Subgrouping the subjects with respect to central obesity defined as a WHR of 0.95 or greater, we observed lower Si values in obese and non-obese FCH subjects relative to controls ($P < .02$). The mean Si value in obese subjects was significantly lower than in non-obese FCH subgroups (1.40 ± 0.79 *v* 2.68 ± 0.95 × $10^{-4} \cdot min^{-1} \cdot mU/L$, respectively, $P < .01$). In conclusion, a higher degree of insulin resistance relative to control values appears to be an integral part of the metabolic derangements observed in FCH, and central-trunk obesity exacerbates the insulin resistance syndrome.

FAMILIAL COMBINED HYPERLIPIDEMIA (FCH) is an alteration in lipid metabolism first described in family studies of subjects who survived a myocardial infarction (MI).[1,2] Its prevalence in the population is between 0.5% and 2%, and it is the cause of more than 10% of MIs observed in subjects less than 60 years of age.[3]

The etiology of FCH is not known, since the phenotypic expression of the condition is modified by genetic, metabolic, and environmental factors. Implicated among the pathophysiological factors are a defect in the activity of the enzyme lipoprotein lipase (LPL),[4] hyperproduction of apolipoprotein B (apo B),[5] and insulin resistance.[6]

The common finding in all FCH patients is an elevation of plasma low-density lipoprotein [LDL] (phenotype IIa), very–low-density lipoprotein ([VLDL] phenotype IV), or both (phenotype IIb) with an increase in apo B with or without a concomitant increase in the cholesterol moiety of the lipoprotein.[7] Other associated abnormalities are low levels of high-density lipoprotein cholesterol (HDL-C), alterations in the composition of the lipoproteins,[8] the presence of small, dense LDL particles,[9] and an increase of free fatty acids (FFA) in the plasma.[6]

The frequent observation of obesity, hypertension, glucose intolerance, and non–insulin-dependent diabetes mellitus (NIDDM)[6,10] associated with the disorder is indicative of the metabolic heterogeneity and its possible overlap with other metabolic derangements such as hyperapobetalipoproteinemia,[11] familial dyslipemic hypertension,[12] and syndrome X.[13]

An increase in basal insulinemia related to an increase in plasma concentrations of FFA and triglycerides (TG)[6,14] has been described in FCH subjects expressing the IIb and IV phenotypes.[5] Similarly, post–oral glucose load hyperinsulinemia together with a decrease in the peripheral sensitivity to insulin independently of obesity has been observed in subjects with phenotypes IIb and IV relative to control subjects and patients expressing the IIa phenotype.[15] Using the glucose clamp technique, Karhapää et al[16] demonstrated the presence of insulin resistance in a group of 12 obese normotensive subjects with hyperlipidemia type IIb and hyperapobetalipoproteinemia relative to a group of control subjects matched for age and BMI.

In the present study, we investigated the presence of insulin resistance in nondiabetic FCH patients using the minimal model approximation of the metabolism of glucose (MMAMG) as described by Bergman et al[17] and modified with the administration of insulin[18] and an oral glucose tolerance test. At the same time, we evaluated the relationship between insulin resistance and the BMI, waist to hip ratio (WHR), and FFA concentrations in plasma.

SUBJECTS AND METHODS

Patients

All patients were selected from among those attending the Lipid Clinic at the Hospital Clinico Universitario de Valencia. Men aged 30 to 60 years (N = 20) were diagnosed as FCH based on clinical and laboratory data of the index patient together with all first-degree family members. The focus on men within this age range excluded variations in the measurement of the peripheral sensitivity to insulin (Si) associated with the menstrual cycle and menopause and with extremes of age.[19,20] Inclusion into the study was based on consistent consecutive lipoprotein profiles between April 1994 and October 1995. All subjects were nonsmokers or ex-smokers for at least 1 year. All subjects had a fasting plasma glucose less than 7.8 mmol/L and a glucose level at 120 minutes post–oral load with 75 g glucose less than 11.1 mmol/L.

The diagnosis of FCH was based on the presence of hyperlipidemia in the index patient together with variable phenotypes IIa, IIb, or IV in first-degree relatives, a family history of arteriosclerosis, an elevated concentration of plasma apo B (>1.20 g/L), and the absence of xanthomas in the patient and first-degree family members.[21] The assignment of phenotype was made according to the North American National Cholesterol Education Program,[22] whereby phenotype IIa is defined as LDL-C at least 4.1 mmol/L and TG less than 2.3 mmol/L,

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type IIb as LDL-C at least 4.1 mmol/L and TG at least 2.3 mmol/L, and type IV as LDL-C less than 4.1 mmol/L and TG at least 2.3 mmol/L.

The WHR was measured according to standard methods.[23] Exclusion criteria were unstable angina or MI in the previous 3 months, smoking habit, consumption of greater than 30 g alcohol per day, pursuit of intense physical fitness or weight loss programs, body weight fluctuation greater than 10% in the previous 3 months, other chronic diseases, other secondary hyperlipidemias, renal or hepatic insufficiency, and hypothyroidism.

A group of healthy nonsmoking control subjects of similar age, gender, and BMI selected from among the clinical and laboratory staff volunteered to participate in the study. Excluded were subjects with plasma TG greater than 2.3 mmol/L and/or with antecedents of NIDDM, hyperlipidemia, or premature arteriosclerosis.

Recruitment into the study 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 before the study, all medications that affect lipid levels or that could produce alterations in Si were suspended. Following an overnight fast of greater than 12 hours, total cholesterol (TC) and TG levels were measured by enzymatic methods,[24,25] HDL-C levels were measured following precipitation of apo B–containing lipoproteins with polyanions,[26] and VLDL-C levels were measured after separation of VLDL (density <1.006 g/mL) by ultracentrifugation (18 hours at 105,000 × *g* at 15°C) in a Ti 50.3 fixed-angle rotor in a Beckman L8-80 ultracentrifuge (Irvine, CA).[27] The value for LDL-C was obtained by subtraction of VLDL-C and HDL-C from TC. FFA levels in plasma were measured by enzymatic colorimetry,[28] and apo B levels by immunoturbidometry.[29]

Clinical Methods

The tests were performed in the Metabolic Unit near the laboratory with a clinician or a nurse constantly in attendance.

The oral glucose-load test with 75 g glucose was performed as recommended by the World Health Organization,[30] with determinations of glucose[31] made by a multichannel autoanalyzer (Technicon RA-1000) and plasma insulin by radioimmunoassay.[32] Calculation of the area under the curve (AUC) was made with the trapezoidal method.[33]

The intravenous glucose tolerance test, with extraction of multiple blood samples for measurement of glucose and insulin levels and calculation of the MMAMG indices of Si and Sg, was conducted following a 12-hour fast and with the patient resting supine for at least 15 minutes before commencement of the test. Two baseline venous blood samples ($t = -15$ and -5 minutes) were taken for glucose and insulin. At time 0, 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 time 20 minutes, a bolus of insulin 0.03 U/kg body weight (Actrapid; Novo Nordisk Pharma, Copenhagen, Denmark) was administered. Following the two baseline samples, 26 more blood samples were taken for determination of glucose and insulin at times 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.[17,18] The indices of insulin sensitivity (Si) and utilization of glucose independently of insulin (Sg) were calculated following the method described by Pacini and Bergman[34] using the MINMOD program.

Statistical Analyses

All analyses were conducted using the Statgraphics Plus 1.4 program,[35] and 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, we used nonparametric tests for statistical analyses. For comparison of mean values, we used the Mann-Whitney *U* test for two variables or the Kruskal-Wallis test for three or more variables. For comparison of proportions, we used Fisher's exact test. The degree of relationship between two quantitative variables was determined by the correlation coefficient of Spearman. The line of regression was calculated using the criterion of least-squares. ANOVA and stepwise multivariate analysis were used. Values for *P* less than .05 were considered significant.

RESULTS

General characteristics of the FCH patients and control subjects are presented in Table 1. There were no significant differences in the age, BMI, and WHR between the groups, since these were the selection criteria; nor were there differences in blood pressure. The plasma concentrations and apo B levels of the different lipoprotein fractions were significantly higher and HDL-C concentrations were significantly lower in the FCH group relative to the controls ($P < .01$), as expected by our selection criteria.

The results of the response to the glucose load show a significant ($P < .001$) increase in the AUCg in subjects with FCH (881.7 ± 180.3 mmol/L/min) compared with the control group (740.0 ± 84.2 mmol/L/min), as well as the AUCi (104,676 ± 80,023 and 59,091 ± 24,306 pmol/L/min in FCH and control groups, respectively, $P < .01$) (Table 2 and Fig 1).

The peripheral sensitivity to insulin expressed as the Si index was 1.91 ± 1.05 and 2.86 ± 1.19 × $10^{-4} \cdot min^{-1} \cdot$ mU/L in the FCH and control groups, respectively ($P < .01$), and the corresponding values for Sg were 1.70 ± 1.13 in the FCH group and 2.35 ± 0.60 × $10^{-2} \cdot min^{-1}$ in the control group ($P < .02$).

In the FCH group, the values for Si correlated significantly ($P < .05$) with the WHR, TG, FFA, AUCi, and AUCg, whereas in the control group the significant correlations ($P < .05$) were with the age, BMI, WHR, blood pressure, TG, AUCi, and AUCg. In the multivariate analysis using Si as the independent variable while controlling for the WHR, TG, basal FFA, and AUCG, the correlation with the WHR and basal FFA remained significant ($P < .01$ and $P < .03$, respectively) (Table 3).

When subjects with FCH were grouped by tertiles of plasma TG levels (≤2.37, 2.38 to 3.91, and ≥3.92 mmol/L), Si values were significantly lower when TG levels were elevated ($P < .04$) (Table 4).

Table 1. Anthropometric and Lipid Parameters in FCH Patients and Control Subjects (mean ± SD)

Parameter	FCH Patients (n = 20)	Control Subjects (n = 20)	*P*
Age (yr)	45.9 ± 8.0	45.4 ± 7.21	NS
BMI (kg/m²)	26.59 ± 2.05	27.09 ± 2.72	NS
WHR	0.96 ± 0.05	0.96 ± 0.04	NS
BPs (mm Hg)	132.5 ± 13.1	128.4 ± 9.2	NS
BPd (mm Hg)	77.7 ± 8.0	77.1 ± 6.3	NS
TC (mmol/L)	6.85 ± 0.98	5.26 ± 0.64	.001*
TG (mmol/L)	3.39 ± 1.68	1.48 ± 0.39	.001*
HDL-C (mmol/L)	0.90 ± 0.15	1.06 ± 0.11	.001*
LDL-C (mmol/L)	4.53 ± 0.91	3.42 ± 0.57	.001*
Apo B (g/L)	1.49 ± 0.18	1.04 ± 0.15	.001*
Basal FFA (mg/L)	454.5 ± 129.2	353.7 ± 116.8	.01

Abbreviations: BPs, systolic blood pressure; BPd, diastolic blood pressure.

*Differences explained by selection criteria.

Table 2. Glucose Tolerance and MMAMG Indices in FCH Patients and Control Subjects of Similar Age and BMI (mean ± SD)

Parameter	FCH Patients (n = 20)	Control Subjects (n = 20)	P
Glucose 0 min (mmol/L)	5.11 ± 0.59	5.02 ± 0.36	NS
Glucose 120 min (mmol/L)	6.71 ± 1.42	5.57 ± 1.04	.01
AUCg (mmol/L/min)	1,122 ± 278	897 ± 158	.001
Insulin 0 min (pmol/L)	102.2 ± 40.1	86.5 ± 24.4	NS
Insulin 120 min (pmol/L)	856.0 ± 885.8	361.7 ± 257.6	.02
AUCi (pmol/L/min)	104,676 ± 80,023	59,091 ± 24,306	.01
Si ($\times 10^{-4} \cdot min^{-1} \cdot mU/L$)	1.91 ± 1.05	2.86 ± 1.19	.01
Sg ($\times 10^{-2} \cdot min^{-1}$)	1.70 ± 1.13	2.35 ± 0.60	.02

When subjects were subgrouped with respect to central-trunk obesity (WHR ≥0.95), significantly lower ($P < .01$) values for Si (indicating greater insulin resistance) were observed in obese versus non-obese subjects irrespective of inclusion in the FCH group or control group. In FCH subgroups (whether obese or non-obese), the differences in Si values were significantly less ($P < .02$) compared with the corresponding control subgroups (Table 5).

Table 3. Correlation (r) Between Si and Other Measured Parameters in FCH Patients and Control Subjects

Linear Regression of Measured Variables v Si as the Dependent Variable	FCH Patients (n = 20)	Control Subjects (n = 20)
Age (yr)	.12	−.77*
BMI (kg/m²)	−.37	−.67*
WHR	−.60*	−.77*
BPs (mm Hg)	.42	−.55*
BPD (mm Hg)	.12	−.68*
TC (mmol/L)	−.29	−.21
TG (mmol/L)	−.60*	−.51*
HDL-C (mmol/L)	.26	.33
Apo B (g/L)	−.27	−.37
Basal FFA (mg/L)	−.47†	−.50†
Glucose 0 min (mmol/L)	−.26	−.34
Glucose 120 min (mmol/L)	−.54*	−.25
AUCg (mmol/L/min)	−.48†	−.60*
Insulin 0 min (pmol/L)	−.59*	−.67*
Insulin 120 min (pmol/L)	−.57*	−.60*
AUCi (pmol/L/min)	−.64*	−.74*

*$P < .01$.
†$P < .03$.

DISCUSSION

Many quantitative and qualitative alterations of plasma lipoproteins have been described in FCH,[8] particularly a high frequency of LDL pattern B (smaller, denser lipoprotein particles)[36] and a reduction in the clearance of chylomicrons in the postprandial state.[37] Other metabolic abnormalities have been described, including an increase in plasma levels of FFA in the basal state, decreased levels of HDL-C, hyperinsulinemia at baseline or at 120 minutes of a glucose load, and increases in the AUCg and AUCi with respect to values in normal subjects.[21]

FCH is a heterogeneous dyslipidemic syndrome in which the interaction of various metabolic and environmental factors (central-trunk obesity, diet rich in saturated fat, and sedentary life-style) can modulate the phenotypic expression.[37,38] Many studies[6,10,14,15] suggest that insulin resistance could be another of these metabolic alterations implicated in the pathophysiology of the disease, with an increase in basal plasma insulin in FCH or in subjects with the IIb phenotype observed relative to the levels in control subjects.

In the present study using the MMAMG modified additionally by intravenous administration of insulin, we quantified in vivo the peripheral sensitivity to insulin in patients diagnosed as

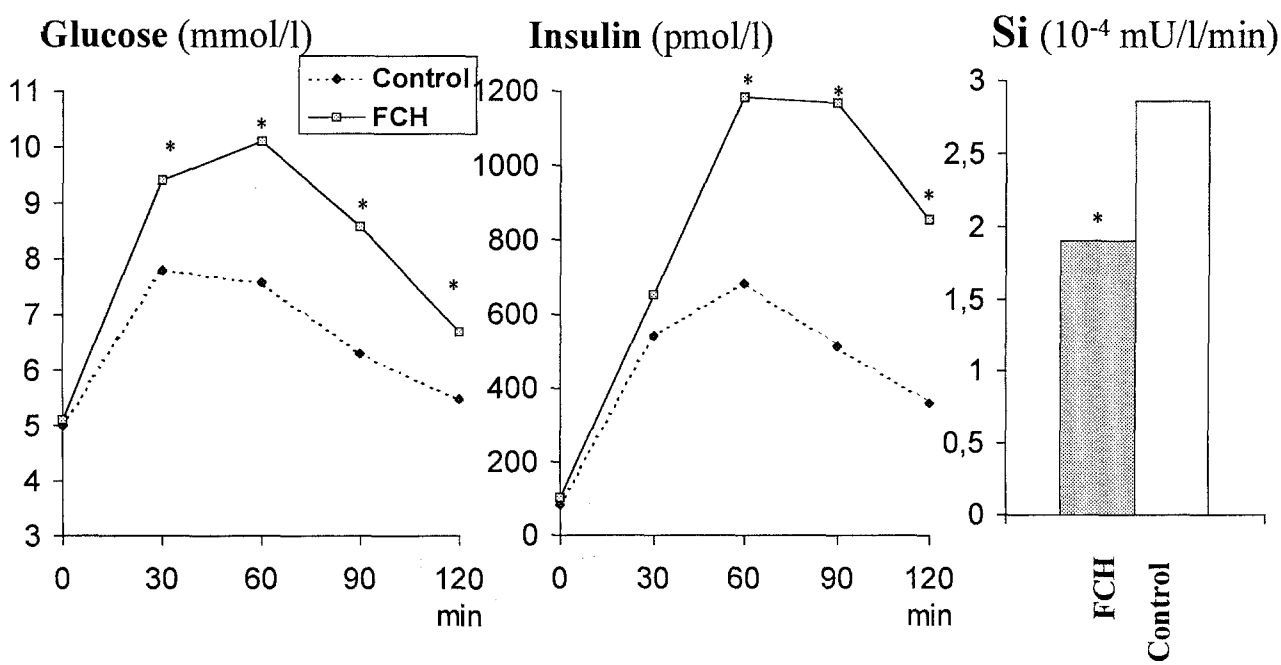

Fig. 1. Glucose and insulin responses during the oral glucose tolerance test and MINMOD in 20 FCH males and 20 controls of similar age and BMI. Glucose is the plasma glucose during a 75-g oral glucose load; insulin is the plasma insulin during a 75-g oral glucose load. *$P < .05$.

Table 4. Si Values (mean ± SD) in FCH Subjects Subgrouped With Respect to Plasma TG Tertile Assignment

Group	Si ($\times 10^{-4} \cdot min^{-1} \cdot mU/L$)
FCH patients (n = 20)	
Lower tertile (TG ≤ 2.37 mmol/L)	2.40 ± 1.18
Middle tertile (TG 2.38-3.91 mmol/L)	2.28 ± 0.89
Upper tertile (TG ≥ 3.92 mmol/L)	1.12 ± 0.65
	$P < .04$ (ANOVA)
Control subjects (n = 20)	
Lower tertile (TG ≤ 1.32 mmol/L)	3.45 ± 1.32
Middle tertile (TG 1.33-1.69 mmol/L)	2.62 ± 1.32
Upper tertile (TG ≥ 1.70 mmol/L)	2.50 ± 0.97
	NS

FCH and in a group of control normolipidemic subjects matched for age, gender, and BMI. The MMAMG modified with intravenous insulin administration is a technique that has been widely validated with respect to the hyperinsulinemic-euglycemic clamp. A good correlation between the indices calculated by both methods indicates that the MMAMG is an easier but still reliable method for calculation of Si and Sg indices in normal subjects and in patients with glucose intolerance, IDDM, and NIDDM.[39,40]

Our results demonstrate that compared with the controls, subjects with FCH have insulin resistance even when the BMI is less than 27 kg/m^2 and the WHR is less than 0.95. The Si index obtained with the MMAMG was $1.91 \pm 1.05 \times 10^{-4} \cdot min^{-1} \cdot \mu U/mL$ in the FCH group and $2.86 \pm 1.19 \times 10^{-4} \cdot min^{-1} \cdot \mu U/mL$ in the control group ($P < .01$).

In our control group, values for the Si index were comparable to those obtained by other investigators, with minor variations resulting from the age, BMI, life-style, and ethnic differences of the groups studied.[40,41] We observed significant negative correlations of the Si index with age, obesity, central-trunk fat distribution (measured as the WHR), systolic and diastolic blood pressure, TG, AUCi, AUCg, and insulinemia at baseline and at 120 minutes of the glucose load.

In the group of patients with FCH, we observed significant negative correlations of the Si index with the WHR, plasma TG, basal FFA, AUCi, AUCg, glycemia at 120 minutes of the glucose load, and insulinemia both at baseline and at 120 minutes of the glucose load.

One of the factors implicated in insulin resistance is the increase in plasma FFA, which provokes a decrease in the insulin-mediated utilization of glucose.[42] An elevation in circulating FFA can increase the risk of NIDDM by inhibiting the secretion of insulin.[43] On the other hand, insulin resistance implies the loss of the inhibitory effect of insulin on lipolysis, which in turn facilitates the flux of FFA from adipose tissue and its elevation in circulating plasma.[44] FFA stimulate hepatic production of apo B, TG, and glucose, as well as insulin liberation from the pancreas to maintain normoglycemia.[13] A reduced Si and an increase in plasma FFA in the basal state with a significant negative correlation between them ($r = -.47$, $P = .03$) confirms the association between insulin resistance and FFA in the group of patients with FCH.

An increase of plasma FFA tends to inhibit the activity of LPL with a concomitant reduction in the clearance of TG-rich particles,[45] and would result in the increase of TG in the circulation. Partial confirmation of this is the observation in the present study and another study[6] of a significant positive correlation between basal plasma FFA and TG ($r = .45$, $P = .04$).

The associations between hypertriglyceridemia, glucose intolerance, and hyperinsulinemia have been described for decades.[46,47] The results suggest that the hypertriglyceridemia is a consequence of the insulin resistance and is independent of the degree of glucose tolerance.[48] We observed a significant negative correlation between insulin resistance (as measured by the Si index) and plasma TG. When FCH subjects were grouped by tertiles based on TG levels, the lower tertile had a Si value significantly higher than the upper tertile (Table 4). This is in agreement with previous reports showing that insulin resistance increases with hypertriglyceridemia.[49] In the multivariate analysis, the TG level correlated with Si ($P < .01$) and not with AUCg ($P < .69$, NS), and this would tend to confirm the hypothesis that the triglyceridemia was a consequence of insulin resistance and independent of the grade of glucose tolerance.

Several studies have demonstrated a relationship between obesity and insulin resistance, especially in subjects with central-trunk obesity.[50,51] In obesity, whether central or peripheral, there is an increase in the portal flux of FFA through the liver that provokes the metabolic derangements discussed earlier. It would be of interest to determine whether the insulin resistance in FCH is confined to obese individuals or is present in the lean[16] as well. In the overall study groups with a similar BMI and WHR, the Si index was significantly higher in the control group compared with the FCH group, with a significant

Table 5. Variation in the Peripheral Sensitivity to Insulin in FCH Patients and Control Subjects Subgrouped With Respect to Central-Trunk Obesity (WHR)

	FCH Patients (n = 20)		Control Subjects (n = 20)	
Parameter	WHR < 0.95 (n = 6)	WHR ≥ 0.95 (n = 14)	WHR < 0.95 (n = 7)	WHR ≥ 0.95 (n = 13)
WHR	0.92 ± 0.01	1.00 ± 0.03†	0.92 ± 0.02	0.99 ± 0.03‡
BMI (kg/m^2)	25.6 ± 1.6	27.0 ± 1.6†	25.1 ± 1.7	28.1 ± 2.5‡
TG (mmol/L)	2.39 ± 0.96*	3.89 ± 1.67†	1.37 ± 0.46	1.57 ± 0.33
Basal FFA (mg/L)	439.6 ± 118.2*	464.4 ± 140.3	302.6 ± 79.5	387.7 ± 128.0
AUCg (mmol/L/min)	1,006 ± 221*	1,199 ± 294*	822 ± 64	947 ± 183
Si ($\times 10^{-4} \cdot min^{-1} \cdot mU/L$)	2.68 ± 0.95*	1.40 ± 0.79*†	3.87 ± 1.01	2.19 ± 0.75‡

*$P < .02$ *v* control subjects with similar WHR.
†$P < .001$ *v* FCH subjects with WHR < 0.95.
‡$P < .001$ *v* control subjects with WHR < 0.95.

negative correlation between the Si index and WHR. However, when we divided each group into two subgroups with respect to the presence or absence of central-trunk obesity (defined as a WHR cutoff point of 0.95),[52,53] we observed that the Si index was lower in non-obese and obese FCH subjects compared with the respective control subgroups. Also, obese subjects with FCH were more insulin-resistant than the non-obese subjects (Si, 1.40 ± 0.79 *v* $2.68 \pm 0.95 \times 10^{-4} \cdot \text{min}^{-1} \cdot \mu\text{U/mL}$, respectively, $P < .05$). Hence, central-trunk obesity appears to be an exacerbating factor in the insulin resistance present in FCH, although obesity per se did not appear to influence plasma lipoprotein distributions in the FCH group: there were no statistically significant differences between obese and non-obese subgroups with respect to lipoprotein concentrations.

In summary, the MMAMG modified with insulin administration is an effective and sensitive method for measurement of several parameters, including the index of peripheral sensitivity to insulin. In patients with FCH, the Si index was decreased relative to that of control subjects of similar age, gender, and BMI. Also, the Si index was significantly and negatively correlated with the WHR, plasma TG, FFA, AUCi, AUCg, basal insulinemia, and glycemia and insulinemia at 120 minutes post–glucose load.

We conclude that a higher degree of insulin resistance relative to the levels in control subjects appears to be an integral part of the metabolic derangements observed in FCH, and central-trunk obesity exacerbates the insulin resistance syndrome.

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