C-174G Polymorphism in the Promoter of the Interleukin-6 Gene Is Associated With Insulin Resistance

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OBJECTIVE — The C-174G promoter polymorphism of the interleukin (IL)-6 gene was found to influence transcriptional activity and plasma IL-6 levels in humans. We addressed the question of whether the C-174G IL-6 polymorphism contributes to variation of insulin sensitivity.

RESEARCH DESIGN AND METHODS — Two cohorts of subjects were genotyped. Cohort 1 includes 275 nondiabetic subjects who underwent a euglycemic-hyperinsulinemic clamp. Cohort 2 includes 77 patients with morbid obesity who underwent laparoscopic adjustable gastric banding (LAGB).

RESULTS — The genotypes were consistent with Hardy-Weinberg equilibrium proportions. In cohort 1, insulin sensitivity was reduced in carriers of the *−174G/G* genotype as compared with subjects carrying the *C* allele ($P = 0.004$). Carriers of *−174G/G* displayed significantly higher plasma IL-6 levels in comparison with carriers of the *C* allele. In a stepwise linear regression analysis, the C-174G polymorphism was independently associated with insulin sensitivity; however, after inclusion of plasma IL-6 concentrations, the polymorphism was excluded from the model explaining insulin sensitivity variability, thus suggesting that the polymorphism was affecting insulin sensitivity by regulating IL-6 plasma levels. IL-6 mRNA levels were measured by real-time RT-PCR in subcutaneous fat obtained from obese patients of cohort 2 during LAGB. Carriers of *−174G/G* showed increased IL-6 expression compared with subjects carrying the *C* allele ($P = 0.04$). There was a significant correlation between adipose IL-6 mRNA expression and insulin resistance assessed by homeostasis model assessment ($\rho = 0.28$, $P = 0.014$).

CONCLUSIONS — These results indicate that the *−174G/G* genotype of the IL-6 gene may contribute to variations in insulin sensitivity.

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Abbreviations: IL, interleukin; LAGB, laparoscopic adjustable gastric banding.

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A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

There is increasing evidence suggesting the concept that chronic low-grade activation of the immune system may play a role in the pathogenesis of insulin resistance and type 2 diabetes (1–3). Cross-sectional and prospective studies have shown that increased levels of systemic inflammatory markers such as interleukin (IL)-6, a major proinflammatory cytokine expressed in several tissues including leukocytes, adipocytes, and endothelial cells, are associated with type 2 diabetes and glucose disorders (1–3). Both circulating levels of IL-6 and adipose tissue IL-6 content have been correlated with insulin resistance (4,5). Moreover, high circulating IL-6 concentrations have been found to predict the development of type 2 diabetes (6).

It has been reported that the common C-174G polymorphism in the promoter of the human IL-6 gene regulates its transcription in vitro with the G allele, showing increased transcriptional activity both under basal condition and in response to inflammatory stimuli such as lipopolysaccharides or IL-1 (7). However, data on the effects of this polymorphism on IL-6 levels in vivo have led to conflicting results. Some studies have shown that individuals with the *G/G* genotype have higher circulating IL-6 levels, as would be expected from in vitro data (7,8), whereas other studies reported no differences among the genotypes or increased IL-6 levels in subjects carrying the *C/C* genotype (9,10). Similarly, the impact of the C-174G polymorphism on type 2 diabetes and insulin resistance is controversial. Thus, two studies have shown that the *G/G* genotype was associated with type 2 diabetes in Spanish individuals and Pima Indians and in a German Caucasian population (11,12), whereas another study reported that obese German subjects carrying the *C/C* genotype have increased risk of developing type 2 diabetes (13). Furthermore, this polymorphism did not predict the conversion from impaired glucose tolerance to type 2 diabetes in the Finnish Diabetes Prevention Study (14). In a

Table 1—*Clinical and biochemical characteristics according to the C-174G polymorphism of the IL-6 gene in 275 nondiabetic individuals*

	IL-6 promoter genotypes				
	G/G	G/C	C/C	*P	†P (G/G vs. C/G + C/C)
Male/female (*n*)	42/100	36/76	7/14	0.88	0.62
Age (years)	39 ± 12 (37)	37 ± 11 (37)	39 ± 10 (41)	0.48	0.43
BMI (kg/m^2)	31.2 ± 8.9 (28)	29.4 ± 8.8 (27)	28.5 ± 4.5 (28)	0.17	0.13
Waist-to-hip ratio	0.87 ± 0.10 (0.87)	0.86 ± 0.09 (0.86)	0.85 ± 0.09 (0.85)	0.80	0.80
Total fat mass (%)	37 ± 15 (36)	36 ± 14 (35)	32 ± 8 (31)	0.31	0.23
Fasting glucose (mg/dl)	95 ± 23 (92)	91 ± 12 (91)	91 ± 10 (92)	0.33	0.29
2-h glucose (mg/dl)	120 ± 42 (110)	110 ± 31 (107)	104 ± 26 (105)	0.08	0.09
Fasting insulin (μU/ml)	12 ± 8 (10)	11 ± 9 (10)	11 ± 7 (10)	0.34	0.09
2-h insulin (μU/ml)	66 ± 55 (47)	62 ± 75 (47)	64 ± 85 (43)	0.27	0.19
Total cholesterol (mg/dl)	199 ± 42 (197)	195 ± 36 (192)	196 ± 36 (203)	0.73	0.43
HDL cholesterol (mg/dl)	53 ± 13 (52)	53 ± 14 (52)	50 ± 13 (49)	0.50	0.57
Triglycerides (mg/dl)	122 ± 83 (94)	123 ± 71 (107)	116 ± 47 (105)	0.84	0.32
Fibrinogen (mg/dl)	303 ± 83 (294)	282 ± 63 (275)‡	289 ± 56 (270)	0.08	0.02
White blood cell count (× 10^9/ml)	7,444 ± 1,998 (7,100)	6,872 ± 1,468 (7,020)§	6,171 ± 1,457 (6,140)‖	0.04	0.03
Family history of T2DM (*n* yes/no)	102/39	88/24	19/2	0.14	0.11
NGT/IGT (*n*)	114/28	94/18	19/2	0.22	0.30
Glucose disposal (mg · kg^{-1} · min^{-1})	6.8 ± 2.9 (7.0)	7.8 ± 3.3 (7.7)¶	8.2 ± 3.3 (7.8)#	0.01	0.004
Fasting IL-6 (pg/ml)	2.5 ± 3.2 (1.7)	1.6 ± 1.4 (1.1)**	1.5 ± 0.8 (1.4)	0.07	0.02

Data are means ± SD (median) unless otherwise indicated. *P values for comparisons of differences of continuous variables between the three genotypes using ANOVA. †P values for comparisons of differences of continuous variables between two genotypes using unpaired Student's t or Mann-Whitney U test. Categorical variables were compared by χ^2 test. ‡$P = 0.03$ vs. G/G, §$P = 0.05$ vs. G/G, ‖$P = 0.03$ vs. G/G, ¶$P = 0.01$ vs. G/G, #$P = 0.05$ vs. G/G, **$P = 0.03$ vs. G/G genotype after least significant difference correction for multiple comparisons. IGT, impaired glucose tolerance; NGT, normal glucose tolerance; T2DM, type 2 diabetes.

Spanish population, the *C/C* genotype was reported to be associated with increased insulin sensitivity (15), whereas in a Finnish population subjects carrying the *C/C* genotype were found to be characterized by lower insulin sensitivity (10).

In the present study, the possible association of the common C-174G polymorphism in the promoter of the human IL-6 gene with insulin resistance was evaluated in a cohort of 275 Italian nondiabetic subjects and in a cohort of 77 morbidly obese patients in whom subcutaneous abdominal adipose tissue mRNA IL-6 levels were measured and correlated with insulin resistance.

RESEARCH DESIGN AND METHODS

— Clinical, anthropometric, and metabolic characteristics of the two cohorts studied are provided in Tables 1 and 2, respectively.

In cohort 1, 275 unrelated nondiabetic white subjects were consecutively studied and recruited by oral glucose tolerance test and euglycemic-hyperinsulinemic clamp study at the Department of Internal Medicine of the University of Rome-Tor Vergata according to previously reported inclusion criteria (16).

In cohort 2, abdominal subcutaneous adipose tissue samples (1.5–3 g) were obtained from 77 white patients with morbid obesity (i.e., grade III obesity according to World Health Organization criteria) during laparoscopic adjustable gastric banding (LAGB) at the San Raffaele Hospital, Milan. Inclusion and exclusion criteria, metabolic characteristics, and adipose tissue depot measurements by ultrasound or computed tomography have been reported previously in detail (17–18).

All studies were approved by institutional ethics committees, and informed consent was obtained from each subject in accordance with principles of the Declaration of Helsinki.

DNA analysis

Genomic DNA was isolated from peripheral blood according to standard procedures. The C-174G polymorphism in the promoter of human IL-6 gene was determined as previously described (15).

Adipose-tissue gene expression

Total RNA was extracted using Trizol (Life Technologies, Gaithersburg, MD) from fat samples immediately frozen in liquid nitrogen. The amount of total RNA was quantified spectrophotometrically at 260 nm. The ratio of absorption (260/280 nm) of all preparations was between 1.8 and 2.0. cDNA was prepared using TaqMan reverse transcription reagents, and expression of human IL-6 mRNA was measured quantitatively by the TaqMan Real-Time PCR technique (Applied Biosystems, Foster City, CA) according to the manufacturer's protocol. The Ct value for every sample was measured in duplicate, and IL-6 expression levels were determined by comparative Ct method using glyceraldehyde-3-phosphate dehydrogenase as an endogenous reference.

Analytical determinations

Plasma glucose was measured in duplicate by the glucose oxidation method (Beckman Glucose Analyzer II; Beckman Instruments, Milan, Italy). Total and HDL cholesterol and triglyceride concentrations were measured by enzymatic methods (Roche Diagnostics, Mannheim, Germany). Plasma fibrinogen concentration and white blood total cell count were determined by routine laboratory tests using a Sysmex CA-7000 Automated Coagulation Analyzer and Sysmex SE9500 (DASIT SpA, Milan, Italy), respectively. IL-6 concentration was measured by an enzyme-linked immunosorbent assay

Table 2—*Clinical and biochemical characteristics according to the C-174G polymorphism of the IL-6 gene in 77 obese subjects who underwent LAGB*

	IL-6 promoter genotypes				
	G/G	G/C	C/C	*P	†P (G/G vs. G/C + C/C)
Male/female (*n*)	4/33	4/30	2/4	0.30	0.58
Age (years)	39 ± 9 (39)	39 ± 10 (38)	44 ± 10 (41)	0.56	0.60
Weight (kg)	116 ± 17 (113)	127 ± 24 (124)‡	119 ± 10 (122)	0.08	0.04
BMI (kg/m^2)	43.6 ± 5.3 (44.0)	47.5 ± 8.1 (45.6)‡	42.8 ± 4.0 (43.1)	0.04	0.09
Waist-to-hip ratio	0.86 ± 0.07 (0.85)	0.87 ± 0.07 (0.86)	0.89 ± 0.1 (0.87)	0.68	0.26
Ultrasound thickness of VAT (mm)	84.3 ± 23.3 (79)	89.3 ± 26.9 (88)	95.3 ± 30.1 (108)	0.71	0.46
Ultrasound thickness of SAT (mm)	46.0 ± 8.7 (46)	48.9 ± 8.7 (51)	44.3 ± 15.5 (38)	0.56	0.50
CT scan area of VAT (mm^2)	170 ± 26 (126)	203 ± 55 (162)	215 ± 60 (188)	0.66	0.74
CT scan area of SAT (mm^2)	493 ± 83 (480)	503 ± 80 (452)	523 ± 90 (497)	0.79	0.81
VAT-to-SAT ratio	0.55 ± 0.45 (0.23)	0.44 ± 0.51 (0.32)	0.41 ± 0.70 (0.25)	0.82	0.58
Fasting glucose (mg/dl)	110 ± 26 (102)	113 ± 36 (102)	113 ± 36 (105)	0.96	0.82
2-h glucose (mg/dl)	143 ± 50 (137)	174 ± 90 (155)	174 ± 90 (156)	0.14	0.11
Fasting insulin (μU/ml)	21 ± 9 (17)	17 ± 7 (15)‡	11 ± 3 (10)§	0.02	0.04
2-h insulin (μU/ml)	78 ± 50 (75)	88 ± 75 (65)	81 ± 95 (54)	0.85	0.55
A1C (%)	6.0 ± 1.3 (5.7)	6.4 ± 1.4 (6.2)	5.9 ± 1.4 (5.8)	0.45	0.23
Total cholesterol (mg/dl)	189 ± 41 (182)	220 ± 39 (210)‖	204 ± 19 (198)	0.006	0.007
HDL cholesterol (mg/dl)	47 ± 12 (46)	51 ± 22 (46)	37 ± 7 (40)	0.25	0.90
Triglycerides (mg/dl)	135 ± 69 (124)	179 ± 139 (148)¶	179 ± 139 (123)#	0.006	0.04
HOMA	6.4 ± 5.9 (4.5)	4.5 ± 2.1 (4.1)	3.0 ± 0.5 (3.1)**	0.08	0.07
NGT/IGT/IFG/T2DM (*n*)	24/7/2/4	18/8/1/7	3/2/0/1	0.99	0.79
IL-6 mRNA levels (arbitrary units)	21 ± 19 (13.3)	10 ± 12 (7.0)††	13 ± 20 (2.4)	0.059	0.04

Data are means ± SD (median) unless otherwise indicated. *P values for comparisons of differences of continuous variables between the three genotypes using ANOVA. †P values for comparisons of differences of continuous variables between two genotypes using unpaired Student's t or Mann-Whitney U test. Categorical variables were compared by χ^2 test. ‡$P = 0.05$ vs. G/G, §$P = 0.05$ vs. G/G, ‖$P = 0.002$ vs. G/G, ¶$P = 0.007$ vs. G/G, #$P = 0.002$ vs. G/G, **$P = 0.05$ vs. G/G, genotype after least significant difference correction for multiple comparisons, ††$P = 0.02$ vs. G/G. IFG, impaired fasting glucose; IGT, impaired glucose tolerance; NGT, normal glucose tolerance; SAT, subcutaneous adipose tissue; T2DM, type 2 diabetes; VAT, visceral adipose tissue.

(Quantikine kit; R&D Systems, Minneapolis, MN). All other biochemical parameters were measured by standard laboratory procedures.

Statistical analysis

The Kolmogorov-Smirnov test was used to test the normality of distribution and nonnormally distributed variables were natural log transformed. ANOVA was used to compare differences of continuous variables across three genotypes with post hoc least significant difference correction for multiple comparisons. Phenotypic differences between the genotype groups were also tested after adjusting for age, sex, BMI, and glucose tolerance status by ANCOVA (general linear model). Unpaired Student's t or Mann-Whitney tests were used to compare differences of continuous variables between two groups. Relationships between variables were determined by Pearson's correlation coefficient (r) or Spearman's rank correlations (ρ), as appropriate. Relationships between variables were sought by stepwise multivariate linear regression analysis with forward selection to assess the magnitude of their individual effect on insulin sensitivity. Categorical variables were compared by χ^2 test. Continuous data are expressed as means ± SD and median. A P value <0.05 was considered statistically significant. All analyses were performed using SPSS software program Version 10.0 for Windows.

RESULTS — The genotypes were consistent with Hardy-Weinberg equilibrium proportions (51.6% *G/G,* 40.8% *G/C* heterozygous, and 7.6% *C/C*). The clinical and laboratory features of the study subjects are shown in Table 1. No significant differences in age, sex, BMI, waist-to-hip ratio, fat mass, fasting and 2-h postload plasma glucose levels, fasting and 2-h postload plasma insulin concentrations, triglyceride levels, total and HDL cholesterol concentrations were observed between subjects carrying the three genotypes (Table 1). By contrast, insulin sensitivity assessed as whole-body glucose disposal by euglycemic-hyperinsulemic clamp was significantly reduced in subjects carrying the *−174G/G* genotype as compared with carriers of the *G/C* or the *C/C* genotype ($P = 0.004$). After correction for multiple comparisons, the difference in insulin sensitivity remained significant between carriers of the *G/G* genotype and carriers of the other two genotypes (Table 1).

A subgroup analysis combining the *G/C* and *C/C* carriers was also performed because their phenotype appeared identical. Carriers of the *G/G* genotype displayed significantly lower insulin sensitivity in comparison with carriers of the *C* allele. These differences remained significant ($P = 0.03$) after adjusting for sex, age, and BMI. Fasting plasma IL-6 concentrations were significantly higher

Table 3—*Independent predictors of insulin sensitivity after forward stepwise linear regression analysis*

	Partial r^2 (%)	Total r^2 (%)	*P* (ANOVA)
Model A*			
BMI (kg/m^2)	45.8	45.8	0.0001
Triglyceride (mg/dl)	3.2	49.0	0.0001
C-174G polymorphism	2.0	51.0	0.0001
Age (years)	0.9	51.9	0.0001
Fasting plasma glucose (mg/dl)	1.7	53.6	0.0001
Model B†			
BMI (kg/m^2)	49.8	49.8	0.0001
Triglyceride (mg/dl)	3.9	53.7	0.0001
Waist-to-hip ratio	1.8	55.5	0.0001
Fasting IL-6 concentration (pg/ml)	1.6	57.1	0.0001

*Model A includes C-174G polymorphism, sex, age, BMI, waist-to-hip ratio, fasting plasma glucose, HDL cholesterol, and triglycerides levels. †Model B includes C-174G polymorphism, plasma IL-6 concentrations, sex, age, BMI, waist-to-hip ratio, fasting plasma glucose, HDL cholesterol, and triglycerides levels.

in subjects carrying the *−174G/G* genotype as compared with subjects carrying the *C* allele (Table 1). In addition, subjects carrying the *−174G/G* genotype displayed higher levels of fibrinogen ($P = 0.02$) and an increased white blood cell count ($P = 0.03$) in comparison with carriers of the *C* allele. In the whole cohort, insulin sensitivity assessed by euglycemic-hyperinsulinemic clamp significantly correlated with plasma IL-6 concentrations ($\rho = -0.37$, $P = 0.0001$), peripheral white blood cell count ($r = -0.29$, $P = 0.0001$), and fibrinogen levels ($r = -0.19$, $P = 0.004$). Plasma IL-6 concentrations significantly correlated with both peripheral white blood cell count ($\rho = 0.25$, $P = 0.01$) and fibrinogen levels ($\rho = 0.27$, $P = 0.002$).

To estimate the independent contribution of the C-174G polymorphism to insulin sensitivity assessed by euglycemic-hyperinsulinemic clamp, we carried out forward stepwise linear regression analysis in a model that also included well-known modulators of insulin sensitivity such as sex, age, BMI, waist-to-hip ratio, fasting plasma glucose, HDL cholesterol concentrations, and triglyceride levels (Table 3). The results of the multivariate analysis revealed that BMI was the strongest independent variable associated with insulin sensitivity, accounting for 45.8% of its variation ($P < 0.0001$). The C-174G polymorphism appeared to be independently associated with insulin sensitivity, accounting for 2.0% of the variation in insulin sensitivity ($P < 0.0001$). Also, age, fasting glucose, and triglycerides levels were independently associated with insulin sensitivity. By contrast, sex, waist-to-hip ratio, and HDL cholesterol concentrations were not independently associated with insulin sensitivity. The model accounted for 53.6% of the variation in insulin sensitivity. Interestingly, after plasma IL-6 concentrations were included in the regression model, the variables that remained independently associated with insulin sensitivity were BMI, waist-to-hip ratio, triglycerides levels, and plasma IL-6 concentrations, whereas the C-174G polymorphism was excluded. The model accounted for 57.1% of the variation in insulin sensitivity.

It has been shown that adipose tissue is a major source of circulating IL-6. There is also evidence that adipose tissue IL-6 content correlates with whole-body glucose disposal (5). To investigate whether the C-174G polymorphism affected IL-6 expression in adipose tissue, we examined IL-6 mRNA expression by real-time RT-PCR in abdominal subcutaneous fat obtained from a cohort consisting of 77 unrelated obese individuals who underwent LAGB. Table 2 shows the clinical features of this study group. Subjects carrying the *C* allele were heavier than subjects carrying the *−174G/G* genotype. Subjects carrying the *−174G/G* genotype showed a significant decrease in triglyceride and total cholesterol concentrations as compared with subjects carrying the *C* allele (Table 2). No significant differences in age, sex, waist-to-hip ratio, body fat distribution, fasting and 2-h postload plasma glucose levels, HbA_{1c} (A1C), HDL cholesterol levels, 2-h postload plasma insulin levels, and glucose tolerance status were observed among the three genotypes (Table 2). Carriers of the *−174G/G* genotype showed a significant increase in adipose IL-6 mRNA levels as compared with subjects carrying the *C* allele ($P = 0.04$).

In addition, subjects carrying the *−174G/G* genotype showed an increase in both fasting plasma insulin concentrations ($P = 0.04$) and homeostasis model assessment index ($P = 0.07$) as compared with carriers of the *C* allele, although this latter difference did not reach the significance. However, the differences in homeostasis model assessment index were significant ($P = 0.03$) after adjusting for sex, age, BMI, and glucose tolerance status. In the whole group, there was a significant correlation between adipose IL-6 mRNA expression and insulin resistance assessed by homeostasis model assessment ($\rho = 0.28$, $P = 0.014$) (Fig. 1). The correlation remained significant after correction for age, sex, and BMI ($\rho = 0.23$, $P = 0.04$).

Adipose IL-6 mRNA expression was also significantly correlated with fasting insulin levels ($r = 0.29$; $P = 0.012$) and cholesterol concentrations ($r = -0.27$; $P = 0.016$). There was no significant relationship between IL-6 mRNA levels and age, BMI, waist-to-hip ratio, ultrasound thickness of visceral and subcutaneous adipose tissue, computed tomography scan areas of visceral adipose tissue and subcutaneous adipose tissue, fasting plasma glucose levels, A1C, triglycerides, and HDL cholesterol concentrations.

CONCLUSIONS — Increasing evidence suggests that low-grade inflammation could be one of the determinants in the pathogenesis of insulin resistance and type 2 diabetes (1–6). Elevated plasma and adipose tissue levels of IL-6 have been associated with insulin resistance (4,5). The identification of the C-174G polymorphism in the promoter of IL-6 gene, which regulates its transcriptional activity (7), makes this variant a credible candidate for association with insulin resistance.

The present data demonstrate that the *G/G* genotype is associated with impaired insulin sensitivity. In a stepwise linear regression analysis, the C-174G polymorphism was significantly associated with insulin sensitivity independent of age, sex, BMI, waist-to-hip ratio, fasting glucose, triglyceride levels, and HDL cholesterol levels. Notably, inclusion of plasma

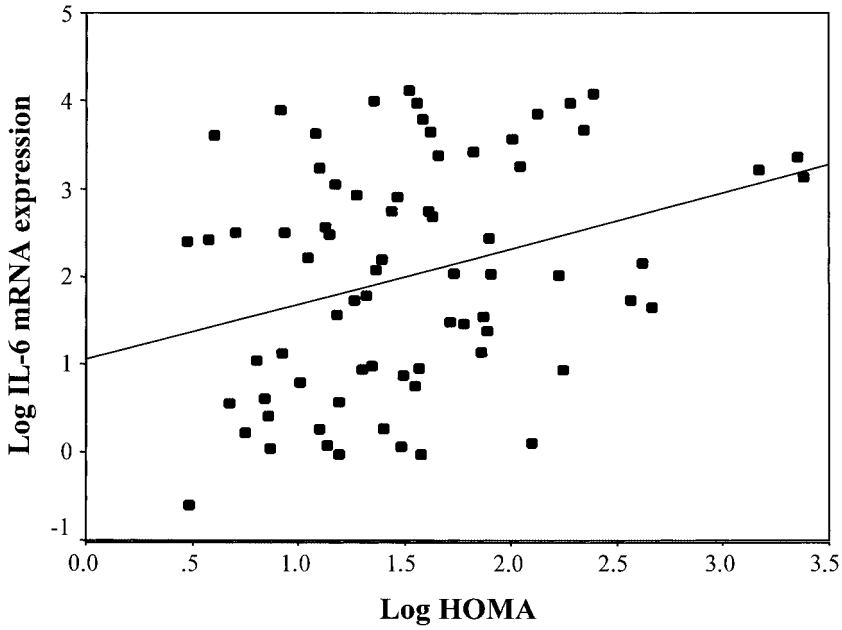

Figure 1—*Correlation between log-transformed adipose IL-6 mRNA expression and log-transformed homeostasis model assessment (HOMA) in the cohort of unrelated obese individuals who underwent LAGB (Spearman's rank correlation coefficient $\rho = 0.28$,* $P = 0.014$*).*

IL-6 concentrations in the regression model resulted in the exclusion of the C-174G polymorphism from the variables explaining the variability in insulin sensitivity, thus suggesting that the polymorphism in the promoter of the IL-6 gene was affecting insulin sensitivity by regulating IL-6 plasma levels. The *G/G* genotype is likely to affect IL-6 expression in vivo because it has been shown that the *G* allele has a stronger transcriptional activity than the *C* allele in vitro model (7). In agreement with this notion, we found that obese individuals carrying the *G/G* genotype exhibited higher expression of IL-6 in adipose tissue than carriers of the *C* allele. This different transcription rate was also supported by the in vivo observation that circulating concentrations of both IL-6 and fibrinogen, an acute-phase response protein whose expression is induced by IL-6, were higher in a group of nondiabetic subjects carrying the *G/G* genotype.

In addition, we found that carriers of the *G/G* genotype exhibited higher peripheral white blood cell counts. A higher peripheral white blood cell count has been repeatedly associated with insulin resistance and reduction of insulin resistance is associated with a reduction of white blood cell count (15,19,20). Interestingly, knockout mice lacking IL-6 showed reduced numbers of peripheral blood neutrophils (21). Accordingly, we observed a highly significant association between whole-body glucose disposal during a euglycemic-hyperinsulinemic clamp and plasma IL-6 levels or inflammatory markers such as fibrinogen and white blood cell count. Taken together, the present data indicate that the *G/G* genotype confers susceptibility to develop insulin resistance and increased plasma markers of the acute-phase response.

IL-6 has been shown to impair insulin signaling in both rats and cellular models such as adipocytes and hepatocytes through a mechanism that involves, at least in part, the upregulation of the inhibitory protein SOCS-3 (22–26). Thus, it is plausible that increased expression of IL-6 associated with the *G/G* genotype may locally induce insulin resistance at the skeletal muscle, liver, or adipose tissue level. However, our results are in contrast with one previous report showing that the *G/G* genotype of the IL-6 gene was associated with higher insulin sensitivity (10). We have no direct explanation for this discrepancy. Obviously, the apparent disparity in results might partly be due to interethnic variation in the allele frequency because the *G* allele appears more frequently in the Italian population as compared with the Finnish population (72 vs. 48%, respectively). Furthermore, differences in age between the Italian and the Finnish cohorts might contribute to explaining these divergent results. Indeed, it has been shown that the C-174G polymorphism affects IL-6 release by cultured peripheral blood mononuclear cells in an age-related manner, with the *C* allele but not the *G* allele showing an increased capacity to produce IL-6 with aging (26). Because subjects in the Finnish study were older than the subjects of present investigation (10), it is possible that the age-related increased production of IL-6 in subjects carrying the *C* allele may have masked the effect of the polymorphism on insulin sensitivity. Finally, we cannot rule out the possibility that C-174G IL-6 polymorphism is not itself responsible for the observed association with insulin resistance, but instead it is in linkage disequilibrium with an unknown causative variant in a distal regulatory site.

We found that obese patients carrying the *−174G/G* genotype have a significant decrease in total cholesterol and triglycerides concentrations as compared with subjects carrying the *C* allele. These differences were not observed in cohort 1 including nondiabetic subjects. It is possible that the differences in lipid profile observed in cohort 2 comprising morbidly obese subjects may be due in part to the lower body weight of obese patients carrying the *−174G/G* genotype as compared with carriers of the *C* allele. It has been shown that subjects with the *G* allele at −174 of the IL-6 gene have higher energy expenditure than subjects with the *C* allele, and thus it was not unexpected that they have lower BMI (10).

In summary, our results suggest that the *−174G/G* genotype in the promoter of the IL-6 gene is associated with insulin resistance. The present findings in two Italian cohorts need to be replicated in other populations to confirm whether this IL-6 polymorphism negatively influences insulin action.

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