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The interleukin 6 −174G/C Polymorphism is associated with indices of obesity in men

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Abstract Obesity represents an expansion of adipose tissue (AT) mass and is closely related to insulin resistance and cardiovascular disease. Several hormonal signals have been shown to originate from AT, one of them being interleukin 6 (IL6), for which one third of circulating levels is accounted for by AT. To study the impact of the IL6 −174G/C polymorphism on obesity-related phenotypes, we genotyped a cohort of 270 French-Canadian men from the greater Quebec City area selected to cover a wide range of body fatness values. The IL6 −174G allele was more commonly observed among lean subjects (body mass index $<25\,kg/m^2$, $\chi^2 = 7.27$, $P = 0.007$ or waist-line $<100\,cm$, $\chi^2 = 6.63$, $P = 0.01$). When men were subdivided according to insulin and glucose levels at 180 min following the glucose load, using 160 pmol/l and 4.6 mmol/l, respectively, as cutoff points, the −174G allele was more frequently observed in groups with low concentrations of either insulin or glucose, $P = 0.03$ and $P = 0.01$, respectively. When comparisons between genotype groups were performed, −174G/G homozygotes presented the lowest waist circumference ($P < 0.05$). In summary, this study showed that, in men, the IL6 −174G/C polymorphism is associated with some indices of body composition and parameters of glucose and insulin homeostasis.

Key words Interleukin 6 · Obesity · Glucose homeostasis · Genomic DNA · Complex traits

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Introduction

Metabolic alterations in adipose tissue signaling may contribute to obesity and insulin resistance. Adipocytes possess the capacity to vary in size, and during periods of caloric excess, they store triacylglycerols, complements of enzymes, and regulatory proteins needed to carry out lipolysis and lipogenesis (Fruhbeck et al. 2000). The regulation of these processes is responsive to hormones, cytokines, and other factors involved in energy metabolism. On the other hand, adipose tissue (AT) is actively involved in cell function regulation through a complex network of endocrine, paracrine, and autocrine signals (Fruhbeck et al. 2000) that may influence the responses of many tissues such as adipose tissue itself, the hypothalamus, pancreas, liver, skeletal muscle, and kidneys, as well as the endothelial and immune systems (Flier 1995; Serrero and Lepak 1996). In blood circulation, one third of total interleukin 6 (IL6) concentration has been estimated to originate from AT (Mohamed-Ali et al. 1997; Xing et al. 1997). In addition to its properties as an inflammatory or immune mediator and as a stress-induced cytokine (Yudkin et al. 2000), IL6 is also involved in glucose and lipid metabolism (Mohamed-Ali et al. 1998). Indeed, IL6 possesses pleiotropic effects on a variety of metabolic processes, including down-regulation of adipocyte lipoprotein lipase in mice (Greenberg et al. 1992) and stimulation of acute protein synthesis (Papanicolaou et al. 1998), and it may cause oxidative stress damage (Baeuerle et al. 1996). In consequence, IL6 has been perceived as an auto/paracrine regulator of adipocyte function. Increased plasma IL6 concentrations observed in obesity (Fried et al. 1998) and type 2 diabetes (Vgontzas et al. 2000) have been closely correlated with indices of adiposity and

insulin resistance such as body mass index (BMI) (Vgontzas et al. 1997; Kern et al. 1995) and fasting insulin concentrations (Bastard et al. 2000). Recently, a polymorphism 174 bp upstream of the transcription initiation site of the IL6 gene has been shown to be associated with insulin sensitivity (Fernandez-Real et al. 2000a), plasma triglyceride levels, postglucose load free fatty acid (FFA) concentrations (Fernandez-Real et al. 2000b), and an increased risk of coronary artery disease (Humphries et al. 2001). Although high plasma levels of IL6 have been associated with an increased risk of future myocardial infarction in healthy subjects (Ridker et al. 2000), the impact of the −174G/C variant on IL6 concentrations remains controversial. Fishman et al. (1998) observed that the −174C allele was associated with decreased plasma IL6 levels in healthy subjects. However, in other studies, genetic influences on plasma IL6 concentrations were only observed among patients with abdominal aortic aneurysm or after acute severe coronary injury such as coronary artery bypass grafting (Brull et al. 2000).

To our knowledge, the impact of IL6 gene variants on abdominal obesity and related metabolic complications has not been studied yet. Thus, the objective of this study was to verify whether the IL6 −174G/C polymorphism may modulate indices of abdominal obesity and concomitant metabolic alterations in a sample of French-Canadian men selected to cover a wide range of adiposity.

Subjects and methods

Subjects

A total of 270 men recruited from the greater Quebec City area and selected to cover a wide range of body fatness values participated in this study. These men were all nonsmokers and free from metabolic disorders such as hypertension, type 2 diabetes, and coronary heart disease. None of them was using medications known to affect insulin action or plasma lipoprotein levels. Solicitation was made through the media. All subjects gave their written consent to participate in this study, which was approved by the Ethics Committee of Laval University.

Anthropometric measurements

Waist circumference, body weight, and height were measured following standardized procedures (Van der kooy and Seidell 1993). Visceral and subcutaneous AT areas at the L_4L_5 level were measured by computed tomography on a Siemens Somatom DRH scanner (Erlagen, Germany), as previously described (Després et al. 1991; Ferland et al. 1989).

Lipoprotein and lipid measurements

Blood cholesterol and triglyceride concentrations were enzymatically measured on a Technicon RA-500 analyzer (Bayer, Tarrytown, NY, USA). Very low density lipoproteins (VLDLs) were isolated by ultracentrifugation ($d <$ 1.006 g/ml) and the high-density lipoprotein cholesterol (HDL-C) fraction was obtained after precipitation of low-density lipoprotein (LDL) in the infranatant ($d >$ 1.006 g/ml) with heparin and $MnCl_2$ (Després et al. 1991; Moorjani et al. 1987). Apolipoprotein (apo) B concentrations were measured in plasma and infranatant (LDL–apo B; $d >$ 1.006 g/ml) by the rocket immunoelectrophoretic method of Laurell (1966), as previously described (Krasinski et al. 1990). Serum standards were prepared in our laboratory and calibrated against reference sera obtained from the Center for Disease Control (Atlanta, GA, USA), which had been lyophilized and stored at −80°C until use (Ruotolo et al. 1992).

Free fatty acids, glucose, and insulin measurements

A 75-g oral glucose tolerance test (OGTT) was performed in the morning after an overnight fast. Blood samples were collected in ethylenediaminetetraacetate-containing tubes (Miles Pharmaceuticals, Resedale, ON, Canada) through a venous catheter placed in an antecubital vein at −15, 0, 30, 45, 60, 90, 120, 150, and 180 min for the measurement of plasma glucose, insulin, and FFA concentrations (Richterich and Dauwalder 1971). Plasma glucose was enzymatically measured (Ferland et al. 1989), plasma insulin levels were assessed by radioimmunoassay with polyethylene glycol separation (Desbuquois and Aurbach 1971), and plasma FFA levels were measured using a colorimetric method (Noma et al. 1973). The total glucose, insulin, and FFA areas under the curve (AUC-glucose, AUC-insulin, and AUC-FFA, respectively) during the OGTT were calculated with the trapezoid method.

Genotype determination

Conditions for polymerase chain reaction (PCR) amplification of IL6 −174G/C have been previously described by Fishman et al. (1998). Following amplification, PCR fragments were digested with the restriction enzyme *Nla*III (Jones et al. 2001), and digestion products were size separated on 8% polyacrylamide electrophoresis gels and pictured on a UV light box.

Statistical analysis

The gene-counting method with a χ^2 test was used to compare the frequency of the −174C allele among the different subgroups defined according to either BMI, waist girth, subcutaneous AT, visceral AT, glucose, or insulin at 180 min during the OGTT. To reduce the skewness of their distribution, we $\log_{10}$ transformed triglyceride, VLDL–apoB, HDL-C, VLDL-C, fasting glucose, insulin, and FFA concentrations at 0, 90, 120, 180 min during the OGTT. Comparisons among genotype groups were performed using analysis of variance. The Duncan multiple-range com-

parison test was used in cases in which a significant group effect was noted. Analysis of covariance was also used to adjust variables for age, BMI, or waist circumference, and differences between groups were tested with the LSMEANS procedure. Statistical analyses were performed with the SAS statistical package (SAS, Cary, NC, USA) and a value of $P < 0.05$ was considered significant.

Results

The genotyping of the IL6 −174G/C polymorphism resulted in the identification of 80 −174G/G homozygotes, 150 −174G/C heterozygotes, and 41 −174C/C homozygotes. The relative frequency of the −174C allele was 0.43.

To assess the contribution of the −174G/C polymorphism to the presence of obesity and obesity-related metabolic perturbations, we compared allele frequencies of that variant in subjects who were subdivided on the basis of BMI using a cutoff point of 25 kg/m^2, which is the value commonly used to classify subjects as overweight. As indicated in Table 1, the IL6 −174G allele was more commonly observed among lean subjects ($\chi^2 = 7.27$, $P = 0.007$). Similar results were observed when men were classified on the basis of waist circumference using a cutoff point of 100 cm, a critical value above which atherogenic metabolic disturbances are more likely to be observed (Pouliot et al. 1994). Again, the −174G allele was more frequent in the group characterized by a smaller waist girth ($\chi^2 = 6.63$, $P = 0.01$). Finally, men were subdivided according to insulin and glucose levels at 180 min following the glucose load. Because there are no values recognized as common limit values, the median values were used as cutoff points (160 pmol/l and 4.6 mmol/l, respectively). The −174G allele was more frequently observed in subgroups with low concentrations of either insulin or glucose ($P = 0.03$ and $P = 0.01$, respectively). There was no difference in allele frequencies when men were subdivided into two groups using the median value of fasting glucose or insulin concentrations (data not shown).

Table 1. Distribution of alleles into different subgroups of obesity-related indices

	G allele	C allele	χ^{-2} value	P value
Body weight (kg)				
< 89.25	53.85	48.72	1.41	0.23
≥89.25	46.45	51.28		
BMI (kg/m^2)				
<25	70.00	30.00	7.27	0.007
≥25	54.61	45.39		
Fat-free mass (g)				
<63.04	52.56	50.43	0.24	0.62
≥36.04	47.44	49.57		
Fat mass (g)				
<25.42	50.64	47.01	0.70	0.40
≥25.42	49.36	52.99		
Waist girth (cm)				
<100	63.33	36.67	6.63	0.01
≥100	52.32	47.68		
Subcutaneous AT (cm^2)				
<280.98	60.14	39.86	1.98	0.15
≥280.98	54.07	45.93		
Visceral AT (cm^2)				
<130	59.14	40.86	0.46	0.50
≥130	56.11	43.89		
Glu 180 (mmol/l)				
<4.6	61.97	38.03	6.02	0.01
≥4.6	51.27	48.73		
Ins 180 (pmol/l)				
<160	61.57	38.43	4.42	0.03
≥160	52.59	47.41		

Results are expressed as percentage and represent the distribution of the population between the two groups created with variables written in the first column. For waist circumference, BMI, and visceral adipose tissue, the common limit values of obesity were chosen (Pouliot et al. 1994). Regarding other variables, the cutoff points were median values. Chi-square test *P* values are provided. BMI, Body mass index; visceral AT, visceral adipose tissue area; subcutaneous AT, subcutaneous adipose tissue area; Glu 180, glucose concentration at 180 min after an oral glucose tolerance test (OGTT); Ins 180, insulin concentration at 180 min after OGTT

Subjects' characteristics according to the −174G/C genotype are shown in Table 2. Although trends toward higher body weight, fat mass, BMI, and subcutaneous AT area among carriers of the −174C allele were noted, none of them was statistically significant. However, carriers of the −174C allele in either the heterozygous or homozygous state had larger waist girths than did −174G/G homozygotes. This difference remained statistically significant after adjustment for age ($P = 0.02$). Plasma insulin, glucose, and FFA levels were also compared between genotype groups (Table 2). Fasting FFA and insulin concentrations, as well as insulin, glucose, and FFA response to the OGTT, expressed by the AUC, tended to be higher in −174C/C homozygotes than in the other two groups, but differences did not reach statistical significance. They remained non-statistically significant, even though they were adjusted for age, age and waist girth, or age and BMI. However, as shown in Fig. 1, the kinetics of glucose concentrations dur-

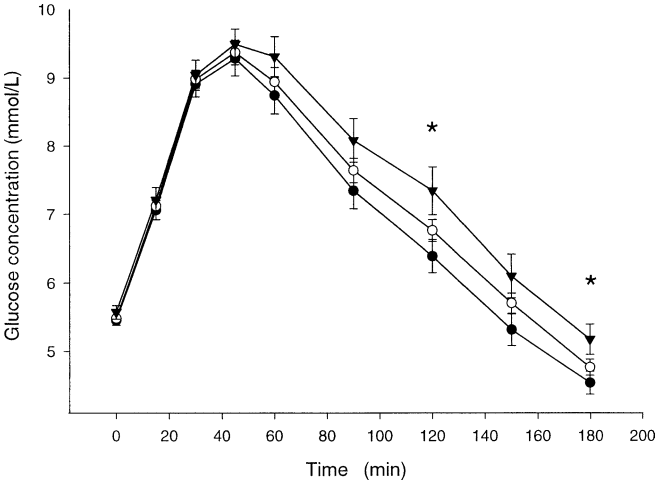

Fig. 1. Glucose concentrations during an oral glucose tolerance test for −174G/−174G homozygotes (*black circles*), −174C/G heterozygotes (*white circles*), and −174C/C homozygotes (*black triangles*). Results are presented as means ± SEM. *Asterisks* represent the significant difference ($P < 0.05$) between the −174C/C homozygotes and the −174G/G homozygotes

Table 2. Subjects' characteristics according to the −174G/C genotype

	Genotype and group number						
	GG (1)	GC (2)	CC (3)	*P* value	*P**	*P***	*P****
Age (years)	41.83 ± 7.39 (80)	42.64 ± 7.89 (150)	43.64 ± 8.77 (41)	0.48			
Weight (kg)	86.03 ± 13.71 (79)	89.21 ± 12.75 (150)	91.75 ± 15.04 (41)	0.06	0.053		
BMI (kg/m^2)	28.44 ± 4.46 (79)	29.57 ± 3.82 (150)	30.09 ± 4.55 (41)	0.06	0.06		
Fat percentage (%)	27.07 ± 6.77 (79)	28.82 ± 5.99 (150)	28.82 ± 7.10 (40)	0.13	0.13		
Fat mass (g)	23.95 ± 8.69 (79)	26.23 ± 8.09 (150)	27.31 ± 9.79 (40)	0.07	0.07		
Fat-free mass (g)	62.10 ± 7.33 (79)	62.98 ± 6.02 (150)	64.70 ± 7.15 (40)	0.16	0.11		
Waist girth (cm)	97.98 ± 11.36 (80)[2,3]	101.85 ± 10.62 (150)[1]	102.76 ± 11.19 (41)[1]	0.02	0.02		
Visceral AT (cm^2)	153.29 ± 56.74 (78)	163.47 ± 54.96 (149)	158.86 ± 54.09 (39)	0.42	0.43		
Subcutaneous AT (cm^2)	268.74 ± 110.68 (78)	299.72 ± 100.93 (149)	305.59 ± 113.86 (39)	0.08	0.051		
Fasting insulin[a] (pmol/l)	86.72 ± 53.42 (80)	101.94 ± 61.69 (149)	101.84 ± 79.29 (40)	0.10	0.10	0.16	0.11
Fasting glucose[a] (mmol/l)	5.45 ± 0.60 (80)	5.48 ± 0.54 (150)	5.57 ± 0.64 (40)	0.57	0.67	0.70	0.76
Fasting free fatty acids[a] (mmol/l)	5.75 ± 2.07 (56)	5.50 ± 1.70 (89)	7.33 ± 5.71 (21)	0.08	0.08	0.09	0.07
AUC-insulin (pmol/l/cm^2)	90723.83 ± 52213.93 (80)	110426.21 ± 71725.07 (149)	102368.20 ± 60276.72 (40)	0.12	0.14	0.56	0.31
AUC-glucose (mmol/l/cm^2)	1255.68 ± 303.36 (80)	1305.16 ± 302.19 (149)	1360.87 ± 267.20 (40)	0.10	0.15	0.43	0.42
AUC-FFA (mmol/l/cm^2)	50.86 ± 23.72 (56)	45.63 ± 13.70 (89)	69.88 ± 99.98 (21)	0.23	0.22	0.16	0.42

Results are expressed as mean ± SD. Number of subjects is shown in parentheses. Superscript numbers indicate group differences according to the Duncan test. Similar results were obtained with the LS MEANS procedure after adjustment for age.
AUC, Area under the curve; visceral AT, visceral adipose tissue area; subcutaneous AT, subcutaneous adipose tissue area; BMI, body mass index
*P**, *P* value adjusted for age; *P***, *P* value adjusted for age and waist circumference; *P****, *P* value adjusted for age and BMI
[a] Log_{10}-transformed prior to statistical analyses

ing the OGTT was slightly different between genotype groups, with −174C/C homozygotes having higher glucose concentrations at 120 min ($P = 0.003$, adjusted for age) and 180 min ($P = 0.04$, adjusted for age). However, this difference was partly mediated by the effect of obesity because adjustment for age and waist girth or for age and BMI abolished these differences (data not shown).

The effect of the IL6 polymorphism on plasma lipoprotein-lipid levels was also tested. No effect of the IL6 −174G/C polymorphism was observed when lipid levels were adjusted for known confounders such as age or age and BMI (data not shown).

Discussion

On the basis of the association between IL6 levels and obesity indices, as well as the association between IL6 genetic variants and plasma IL6 levels, we hypothesized that genetic variations in the IL6 gene that affect its plasma levels would in turn influence the development of abdominal obesity and its concomitant metabolic perturbations in humans. We then genotyped a cohort of French-Canadian men, recruited to cover a large range of body fatness values, and performed an association study. The frequency of the −174C allele observed in the present study was 0.43, which is similar to that observed in other Caucasian populations (Brull et al. 2000; Fishman et al. 1998; Humphries et al. 2001; Jones et al. 2001).

It is widely recognized that genetic variations in susceptibility genes may modulate obesity-related phenotypes. Accordingly, Humphries et al. (2001) have been interested in the impact of the −174G/C polymorphism on the BMI of healthy men. The −174C allele was not associated with the BMI, but was associated with a significantly higher blood pressure. The effect on blood pressure was of greater magnitude in men in the top two tertiles of BMI (>24.86 kg/m^2; Humphries et al. 2001). In the present study, we did observe a significant effect of the IL6 variant on obesity, as the −174G allele was more commonly observed among lean subjects (low BMI and low waist circumference), with carriers of the −174C allele being characterized by a larger waist line. Similar trends were observed for weight, BMI, and subcutaneous AT, but not with visceral AT when our population was subdivided into three subgroups according to IL6 genotype. Consequently, the −174G/C IL6 polymorphism was related to some indices of obesity, particularly to subcutaneous adiposity, in the population under study.

Several hypotheses have been postulated linking IL6 to obesity. Purohit et al. (1995) hypothesized that IL6 modulates the reaction of aromatase, a key regulatory enzyme for estrogen metabolism, influencing satiety and adipose tissue distribution. IL6 was also reported to be the second most secreted protein "hormone" to be released by human subcutaneous AT, supporting the hypothesis of the role of this

cytokine as a systemic regulator of body weight (Mohamed-Ali et al. 1997). IL6 production by human subcutaneous AT has been demonstrated in vitro (Fried et al. 1998) and in vivo (Mohamed-Ali et al. 1997). Moreover, the subcutaneous AT was found to release IL6, but not tumor necrosis factor-alpha (TNFα; Mohamed-Ali et al. 1997).

Because IL6 is produced centrally by hypothalamic neurons regulating body composition in rats (Schobitz et al. 1993), it has thus been involved in the modulation of body composition. Indeed, IL6 plays a key role in body weight control and is influenced by hormones, TNFα, and the sympathetic nervous system. Consequently, the obesity condition exacerbates the overexpression of this cytokine and increases IL6 concentrations, which in turn diminishes food intake and lipogenesis, and increases energy expenditure and lipolysis (Fruhbeck et al. 2000). Thus, the action of IL6 was reported to be related to an increase of hepatic triglyceride secretion, contributing to hypertriglyceridemia associated with abdominal obesity (Nonogaki et al. 1995), and to an increase of plasma fatty acids in men (Stouthard et al. 1995). Table 2 shows a tendency to elevated FFA levels in the carriers of the −174C allele. Consequently, the small effect of this polymorphism on FFA seems to be independent of obesity. Fernandez-Real et al. (2000b) hypothesized that the alterations in lipid levels may be attributed to genetic differences in IL6 transcription rates. However, they found that FFA levels were higher in carriers of the −174G allele. In comparison to the sample of the present study, subjects included in the study of Fernandez-Real et al. (2000b) were men and women and were subdivided into two genotype groups, carriers of the −174G allele and −174C/C homozygotes. These major differences in analytical approaches may explain the discrepancies in the results (Fernandez-Real et al. 2000b).

An allelic prediction of glucose and insulin concentrations at 180 min after the glucose load was achieved by χ^2 test. We also observed an increased post-glucose response in carriers of the −174C allele, which disappeared after adjustment for BMI or waist girth. In consequence, we hypothesized that the effect of the IL6 polymorphism on plasma glucose levels during the OGTT may be mediated by genotype difference in adiposity. Accordingly, the association between glucose disposal and plasma IL6 concentrations was also eliminated by statistical control for percent body fat in nondiabetic Pima Indians (Vozarova et al. 2001). To our knowledge, only Fernandez-Real et al. (2000a) studied the impact of the IL6 −174G/C polymorphism on insulin sensitivity. They reported a lower area under the curve for glucose and a higher insulin sensitivity index in −174C/C homozygotes compared with carriers of the G allele, despite similar age and body composition (Fernandez-Real et al. 2000a). In contrast to subjects included in the present study, the sample of subjects studied by Fernandez et al. (2000a) was composed of both men and women, and was subdivided into two genotype groups, carriers of the −174G allele and −174C/C homozygotes. Again, these important methodological differences complicate the comparison between the two studies. The results of Yudkin et al. (2000), conducted in nondiabetic Caucasians, showed that a chronic inflammatory state may induce insulin resistance and endothelial dysfunction and, therefore, may link the latter phenomena with obesity and cardiovascular disease (Yudkin et al. 1999). In this study, although no significant effect of IL6 genotype on fasting insulin and glucose concentrations was found, trends for higher fasting insulin and insulin response to OGTT were observed for carriers of the C allele. In the Rotterdam study, IL6 levels were strongly associated with fasting insulin, and the investigators suggested that some markers of inflammation contribute to the relationship between insulin and cardiovascular diseases (Hak et al. 2001).

Finally, we found no significant difference in the plasma lipoprotein-lipid profile between the three genotype groups before and after adjustments for age, BMI, or waist girth (data not shown). In contrast, Fernandez-Real et al. (2000b) found higher triglyceride and VLDL-triglyceride concentrations together with slightly lower HDL_2-C levels in carriers of the −174G allele in their study sample of 32 Caucasian men and women. Some investigators showed relationships between IL6 concentration and plasma HDL-C levels, but only in women (Ziccardi et al. 2002). An increase of triglyceride and VLDL-C concentrations was observed in female but not male mice IL6 $^{-/-}$ compared with wild-type IL6 $^{+/+}$ (Wallenius et al. 2002). Consequently, gender may explain the lack of association between the IL6 polymorphism and plasma lipoprotein-lipid levels observed in the present study. Further studies will be needed to establish whether a gender-specific effect exists for the IL6 −174G/C polymorphism on the plasma lipoprotein-lipid profile.

In summary, this study showed that, in men, the IL6 −174G/C polymorphism is a predictor of subcutaneous abdominal adiposity and of parameters of glucose and insulin homeostasis. Further studies will be necessary to replicate these results on a larger scale and in populations with different genetic backgrounds.

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