Interleukin-6 (IL-6) is a pleiotropic cytokine expressed in many tissues. IL-6 null mice show low energy expenditure, but the effect of the variants of the IL-6 gene on energy expenditure has not been previously studied in humans. Therefore, we investigated the effect of the C-174G promoter polymorphism of the IL-6 gene on energy expenditure, measured by indirect calorimetry in healthy Finnish subjects (n = 124). We also measured insulin sensitivity by the hyperinsulinemic-euglycemic clamp. Subjects with the C-174C genotype of the IL-6 gene had significantly lower energy expenditure than subjects with the G-174C or G-174G genotypes both in fasting (CC 13.68 ± 1.98, CG 14.73 ± 1.57, GG 14.81 ± 2.01 kcal·kg⁻¹·min⁻¹; P = 0.012) and during the euglycemic-hyperinsulinemic clamp (CC 15.24 ± 2.05, CG 16.62 ± 2.06, GG 16.66 ± 2.50 kcal·kg⁻¹·min⁻¹; P = 0.007). Moreover, subjects homozygous for the C allele had lower rates of whole-body glucose uptake than carriers of the G allele (CC 50.95 ± 13.91, CG 59.40 ± 14.17, GG 59.21 ± 15.93μmol·kg⁻¹·min⁻¹; P = 0.016). The rates of both oxidative (P = 0.013) and nonoxidative (P = 0.016) glucose disposal were significantly affected by the IL-6 promoter polymorphism. In conclusion, the C-174C promoter polymorphism of the IL-6 gene influences energy expenditure and insulin sensitivity in healthy normoglycemic subjects. Whether this polymorphism is a risk factor for obesity or type 2 diabetes can be estimated only in prospective population-based studies. Diabetes 52:558–561, 2003

Interleukin-6 (IL-6) is a multifunctional cytokine expressed in many tissues, including adipose tissue, skeletal muscle and hypothalamus, which are involved in the regulation of body energy balance. In IL-6 null mice, the lack of circulating IL-6 was associated with obesity and low energy expenditure (1). Moreover, IL-6–deficient mice exhibited high leptin levels and leptin insensitivity, and did not lose weight or decrease food intake after leptin treatment. The promoter of the IL-6 gene is dynamically regulated at many sites, including the multiple response element (−173 to 145), which responds to interleukin-1, tumor necrosis factor-α, and other factors (2). The recently described C-174G promoter polymorphism of the IL-6 gene has been found to influence transcriptional regulation (2,3) and plasma IL-6 levels in patients with systemic-onset juvenile chronic arthritis and in patients with primary Sjögren’s syndrome (3,4). Moreover, the C-164G polymorphism has been found to be associated with insulin resistance measured by a frequently sampled intravenous glucose tolerance test in one previous study (5).

In humans, the effect of the C-174G promoter polymorphism of the IL-6 gene on energy expenditure has not been previously studied. Therefore, we investigated the effect of this polymorphism on energy expenditure and insulin sensitivity during fasting and during the euglycemic-hyperinsulinemic clamp in 124 healthy normoglycemic subjects.

The frequency of the CC genotype was 30%, the CG genotype 44%, and the GG genotype 26% in our study subjects. Age, BMI and waist-to-hip ratio, systolic and diastolic blood pressure, fasting plasma glucose and insulin levels, and IL-6 concentration (n = 72) did not differ among the genotypes (Table 1).

Fasting energy expenditure was 8% lower in subjects with the CC genotype than in subjects with the CG or GG genotypes (13.68 ± 1.98, 14.73 ± 1.57, 14.81 ± 2.01 kcal·kg⁻¹·min⁻¹, respectively, P = 0.012 over the genotypes, Fig. 1). Similarly, the −174 IL-6 promoter polymorphism was associated with energy expenditure during the hyperinsulinemic clamp (CC 15.24 ± 2.05, CG 16.62 ± 2.06, GG 16.66 ± 2.50 kcal·kg⁻¹·min⁻¹; P = 0.007). Both fasting energy expenditure and energy expenditure during the clamp were significantly lower among subjects with the CC genotype compared with carriers of the G allele, even after adjustment for BMI, age, and sex (P = 0.035 and P = 0.024, respectively). Basal metabolic rate (BMR) estimated by the O₂ consumption during indirect calorimetry was lowest in subjects with the CC genotype, both in fasting (decreased by 7.4%) and during the hyperinsulinemic clamp (decreased by 8%) (fasting: CC 2.94 ± 0.42, CG 3.17 ± 0.34, GG 3.18 ± 0.43 ml·min⁻¹·kg⁻¹, P = 0.009 among the three genotypes; during the clamp: CC 3.18 ± 0.42, CG 3.46 ± 0.42, GG 3.46 ± 0.51 ml·min⁻¹·kg⁻¹, P = 0.008). The polymorphism did not affect the respiratory quotient (RQ) during the clamp (CC 0.96 ± 0.05, CG 0.96 ± 0.04, GG 0.97 ± 0.04; P = 0.505), indicating that similar
TABLE 1
Clinical and biochemical characteristics according to the C-174G polymorphism of the IL-6 gene in 124 normoglycemic healthy subjects

<table>
<thead>
<tr>
<th>IL-6 promoter genotypes</th>
<th>CC</th>
<th>CG</th>
<th>GG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F)</td>
<td>23/14</td>
<td>38/17</td>
<td>21/11</td>
</tr>
<tr>
<td>Age (years)</td>
<td>51 ± 9</td>
<td>52 ± 7</td>
<td>52 ± 8</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.6 ± 4.0</td>
<td>25.7 ± 3.6</td>
<td>25.3 ± 4.7</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.94 ± 0.08</td>
<td>0.93 ± 0.08</td>
<td>0.94 ± 0.06</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>138 ± 17</td>
<td>132 ± 14</td>
<td>135 ± 14</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>86 ± 8</td>
<td>84 ± 7</td>
<td>84 ± 7</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/l)</td>
<td>62.3 ± 36.1</td>
<td>54.1 ± 35.3</td>
<td>49.2 ± 17.8</td>
</tr>
<tr>
<td>Fasting IL-6 (pg/ml)</td>
<td>1.6 ± 1.16</td>
<td>1.80 ± 2.07</td>
<td>2.93 ± 3.79</td>
</tr>
</tbody>
</table>

Data are n or means ± SD. None of the comparisons was statistically significant over the three genotypes. BP, blood pressure.

proportions of fat and carbohydrates as fuel substrates were used independently of the genotypes.

The rates of whole-body glucose uptake were significantly lower in the subjects with the CC genotype (by 14%) when compared with the G allele carriers (CC 50.95 ± 13.91, CG 59.40 ± 14.17, GG 59.21 ± 15.93 μmol · kg⁻¹ · min⁻¹, P = 0.016; Fig. 2). Even after adjustment for BMI, age, and sex, the subjects with the CC genotype had lower rates of whole-body glucose uptake than carriers of the G allele (P = 0.041). When the results were expressed as μmol · kg⁻¹ · fat-free mass (FFM) · min⁻¹ in 30 subjects who had their fat percentage measured, the differences between the subjects with the CC genotype and subjects with the G allele were statistically significant (CC 71.70 ± 14.1, CG 78.70 ± 11.6, GG 94.49 ± 26.6 μmol/kg of FFM/min; P = 0.032). The polymorphism affected both the rates of glucose oxidation (16.79 ± 3.06, 18.67 ± 3.25, 18.80 ± 3.38 μmol · kg⁻¹ · min⁻¹; P = 0.013) and the rates of nonoxidative glucose disposal (33.83 ± 12.94, 40.73 ± 12.59, 40.41 ± 13.73 μmol · kg⁻¹ · min⁻¹; P = 0.034). Insulin sensitivity and energy expenditure correlated strongly in the fasting state (Pearson correlation coefficient r = 0.544, P < 0.001) and during the clamp (r = 0.769, P < 0.001) in all study subjects. When calculated according to the genotypes, the correlations remained significant (fasting: CC r = 0.499, P = 0.002; CG r = 0.478, P < 0.001; GG r = 0.545, P < 0.001; during clamp: CC r = 0.752, P < 0.001; CG r = 0.703, P < 0.001; GG r = 0.769, P < 0.001; Fig. 2). IL-6 levels did not correlate with fasting energy expenditure (r = −0.046), energy expenditure during the clamp (r = 0.024), or the rates of whole-body glucose uptake (r = 0.015). During the clamp, the rates of lipid oxidation and free fatty acid levels (data not shown) were unaffected by the IL-6 promoter polymorphism.

The novel finding of our study was that the C-174G IL-6 promoter polymorphism affects energy expenditure and

![FIG. 1. Energy expenditure in the fasting state (A) and during the hyperinsulinemic clamp (B) in 124 normoglycemic subjects according to the C-174G polymorphism of the IL-6 gene (CC, CG, and GG genotypes).](image)

![FIG. 2. The rates of whole-body glucose uptake, glucose oxidation (■), and nonoxidative glucose disposal (□) during the hyperinsulinemic clamp (A) in 124 normoglycemic subjects according to the C-174G polymorphism of the IL-6 gene (CC, CG, and GG genotypes). Correlation between energy expenditure and the rates of whole-body glucose uptake during the hyperinsulinemic clamp (B) (■ = CC, ■ = CG, □ = GG).](image)
BMR both in the fasting state and during hyperinsulinemia in healthy subjects. Moreover, the subjects with the C-174C genotype had lower insulin sensitivity measured by the hyperinsulinemic-euglycemic clamp compared with the subjects with the C-174G and G-174G genotypes, and the rates of glucose oxidation and nonoxidative glucose disposal were similarly affected by this polymorphism.

The CC genotype is likely to affect IL-6 expression and its physiological regulation. In agreement with this notion is that the CC genotype has been shown to be a weaker inducer of IL-6 gene expression than the G allele (2,3). Many tissues, including adipose tissue, secrete IL-6, and the levels of IL-6 correlate with BMI (6). However, tissue-specific expression may be more important, and thus the measurement of circulating IL-6 level may not reflect biological significance at tissue level. This notion was supported by the fact that we did not find significant correlations between circulating IL-6 levels and energy expenditure or insulin sensitivity measured with the euglycemic-hyperinsulinemic clamp.

Several mechanisms can be considered to explain the association of the C-174G polymorphism and energy expenditure. First, IL-6 can regulate energy expenditure centrally, as it is expressed in hypothalamus. In IL-6 knockout mice, a central injection of IL-6 caused a significant increase in energy expenditure, which was not mediated by peripheral injection (1). In humans, high cytokine levels (including IL-6) and cytokine brain synthesis were found to increase resting energy expenditure and induce cachexia (7). Additionally, a subcutaneous injection of IL-6 increased resting metabolic rate and hypothalamic-pituitary-adrenal axis activity in a dose-dependent fashion, suggesting that hypothalamic corticotrophin-releasing hormone may mediate both of these actions in humans (8).

A second possibility by which IL-6 may affect energy expenditure is enhanced adrenergic stimulation. In humans, IL-6 has been shown to increase heart rate and norepinephrine levels (9) and to stimulate the sympathetic nervous system (10,11), which is the primary efferent pathway regulating energy expenditure. Moreover, sympathetic neurons have been shown to secrete IL-6, express IL-6 receptors, and respond to IL-6 (12). In patients with renal cell carcinoma, the IL-6 infusion increased the plasma norepinephrine level and resting energy expenditure (13). Adrenergic agonists in humans increase energy expenditure, and skeletal muscle seems to be the most important tissue in energy metabolism (14).

A third possibility is that IL-6 may exert its effects through the involvement of leptin. IL-6 knockout mice are obese, have high leptin levels, and are leptin insensitive. Leptin action on energy expenditure is mediated by the corticotrophin-releasing factor, which increases energy expenditure and stimulates the sympathetic nervous system in rats (15). In humans, energy expenditure has been shown to increase during leptin administration (16).

BMR was decreased by ~8% in subjects with the CC genotype compared with subjects carrying the G allele. Thus, the caloric consumption was 134 kcal/day less in these subjects, and could lead to weight gain (17) and increased risk of diabetes. Because our study was cross-sectional, we cannot evaluate the effect of the CC genotype of the IL-6 gene on long-term weight gain.

We also found an association of the IL-6 polymorphism with insulin sensitivity. Because both glucose oxidation and nonoxidative glucose metabolism were similarly affected, the effect of the IL-6 promoter polymorphism must locate at the proximal site of insulin signaling. Subjects with the CC genotype had lower energy expenditure than subjects with the G allele, and thus it was not unexpected that they had somewhat higher BMI, which potentially could explain lower insulin sensitivity among subjects with the CC genotype. Obesity cannot explain the decrease in insulin sensitivity among subjects with the CC genotype, because in these subjects the rates of whole-body glucose uptake were significantly lower than in subjects with the G allele—even after adjustment for BMI, age, and sex. It is also possible that increased insulin sensitivity could increase energy expenditure since insulin stimulates hypothalamic neurons leading to the activation of the sympathetic nervous system and to an increase in energy expenditure (18). Finally, the effect of the IL-6 polymorphism on insulin sensitivity could be independent of its effect on energy expenditure. Because insulin sensitivity and energy expenditure correlated very strongly (Fig. 2), this possibility is not very likely. However, it cannot be determined on the basis of this study which of these three possible mechanisms is the most important one.

Our results are in contrast with one previous report showing that the CC genotype was associated with high insulin sensitivity (5). However, the number of subjects in that study was only 32 and the evaluation of insulin sensitivity was based on a frequently sampled intravenous glucose tolerance test. We studied a large number of subjects (n = 124) with the hyperinsulinemic-euglycemic clamp, which is the gold standard to measure insulin sensitivity.

In conclusion, the C-174G promoter polymorphism of the IL-6 gene affects energy expenditure, basal metabolic rate, and insulin sensitivity in healthy Finnish subjects. Most likely, the effect of the C-174G variant on energy expenditure is mediated centrally, as suggested by the IL-6 null mouse model. Whether this polymorphism of the IL-6 gene is a risk factor for obesity or type 2 diabetes can be evaluated only in prospective population-based studies.

RESEARCH DESIGN AND METHODS

All subjects participating in the study were randomly selected from healthy normoglycemic Finnish subjects, as previously described in detail (19,20). A total of 124 subjects (82 men, 42 women, age 52 ± 8 years, BMI 26.4 ± 4.1 kg/m²) participated in the euglycemic-hyperinsulinemic clamp (insulin infusion rate 80 mU·kg⁻¹·min⁻¹; blood glucose level was kept at 5.0 mmol/l) and indirect calorimetry (measurement of O₂ consumption and CO₂ production) to evaluate the energy expenditure and the degree of insulin sensitivity, as previously described (21). The subjects did not have any chronic diseases, hypertension, or abnormality in an oral glucose tolerance test (OGTT) and were not receiving any continuous drug treatment. Concentrations of plasma glucose and insulin in the fasting state and in an OGTT were measured using standard methods (21). IL-6 concentration was measured by an enzyme-linked immunosorbent assay (Quantikine kit; R&D Systems, Minneapolis, MN). The minimum detectable concentration using this assay is 0.094 pg/ml. Genomic DNA was extracted from peripheral blood leukocytes by the proteinase K-phenol-chloroform extraction method. The IL-6 promoter was amplified by PCR with published primers (5), and the C-174G polymorphism was screened by the single-strand conformation polymorphism, as previously described in detail (22).

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REFERENCES


