Interleukin-1 System Gene Polymorphisms Are Associated with Fat Mass in Young Men

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Context: There is growing evidence for interactions between the regulation of body fat and the immune system. Studies of knockout mice indicate that IL-1 has an antiobesity effect.

Objective: The objective of the study was to investigate our hypothesis that common polymorphisms of the IL-1 system, which are associated with IL-1 activity, also are associated with fat mass.

Design, Setting, and Study Subjects: The Gothenburg Osteoporosis and Obesity Determinants (GOOD) study is a population-based cross-sectional study of 18- to 20-yr-old men (n = 1068), mostly Caucasian, from the Gothenburg area (Sweden). Three different polymorphisms, IL-1β +3953 C/T, IL-1β-31 T/C, and IL-1 receptor antagonist (IL-1RN) variable number tandem repeat of 86 bp, were investigated in relation to body fat mass.

Main Outcome Measure: The main outcome measures were genotype distributions and their association with body fat mass in different compartments, measured with dual-energy x-ray absorptiometry.

Results: Carriers of the T variant (CT and TT) of the +3953 C to T (F = 0.25) IL-1β gene polymorphism had significantly lower total fat mass (P = 0.013) and also significantly reduced arm, leg, and trunk fat, compared with CC individuals. IL-1RN*2 carriers with two repeats of the IL-1RN variable number tandem repeat polymorphism had increased total fat (P = 0.036), serum leptin, and fat of trunk and arm as well as serum levels of IL-1RN and IL-1RN production in vivo. The IL-1β-31 polymorphism did not correlate with the fat measurements.

Conclusions: The IL-1 system, recently shown to affect fat mass in experimental animals, contains gene polymorphisms that are associated with fat mass in young men. (J Clin Endocrinol Metab 91: 2749–2754, 2006)

There is growing evidence of interactions between the regulation of body fat and the immune system. The cytokine IL-6 suppresses body fat mass and enhances energy expenditure in both mice and men (1–3). Another cytokine, IL-1β, also exerts proinflammatory effects, and there is some overlap between the effects of IL-1 and IL-6 on immune functions. We and others have recently found indications that the IL-1 system, like IL-6, influences body fat mass. Mice with depleted IL-1 signaling due to knockout of the gene coding for the biologically active IL-1 receptor I (IL-1RI) develop obesity (4). Conversely, mice with enhanced IL-1 activity due to IL-1 receptor antagonist (IL-1RN) gene knockout are lean and resistant to diet-induced obesity (5).

The IL-1 system has several components, including two agonists, IL-1β and the less potent IL-1α. The biological effects are exerted via the IL-1RI. The binding of IL-1 to IL-1RI can be inhibited by the endogenous receptor antagonist IL-1RN. The effect of IL-1 can also be inhibited by binding a second type of IL-1 receptor, IL-1RII. This receptor acts as a decoy and prevents IL-1 from binding IL-1RI. A delicate balance between IL-1 and IL-1RN is of importance for regulation of immune function (6, 7).

There are some common polymorphisms that appear to be associated with differences in the activity of the IL-1 system. The C to T single nucleotide polymorphism (SNP) at nucleotide +3953 from the transcription start of the IL-1β gene seems to be functional because it has been associated with increased production of IL-1β in vitro, worsened rheumatoid arthritis, enhanced inflammatory serum parameters, and decreased risk of certain infections (8–11). The T to C SNP at nucleotide −31 from the transcription start of the IL-1β gene has also been associated with changes in biological parameters in vivo (12). The IL-1RN gene contains a polymorphic region in the second intron, which has an 86-bp variable number tandem repeat (VNTR). The presence of two repeats, IL-1RN*2, in this polymorphism has been reported to be associated with serum IL-1RN levels, production of IL-1β in vitro, and occurrence of inflammatory diseases in vivo (7, 12–14).

In the present study, we aimed to investigate the association between genetic differences in the IL-1 system and the regulation of body fat. Therefore, common and functional polymorphisms of the IL-1 system were examined in association with several measures of fat mass in a homogenous...
cohort of young Swedish men. To further investigate the IL-1 system, we also measured serum levels of IL-1RN as well as IL-1RN and IL-1β levels in cell culture medium from lipopolysaccharide (LPS)-stimulated leukocytes.

Subjects and Methods

Subjects
The present study was conducted on subjects from the Gothenburg Osteoporosis and Obesity Determinants (GOOD) study. The subjects were 18- to 20-yr-old men (n = 1068), mostly Caucasian (98%), from the Gothenburg area (Sweden) who were randomly selected from national population registers (15). The GOOD study was approved by the Ethics Committee at Göteborg University. Written and oral informed consent was obtained from all study participants.

Measurement of body composition
Lean tissue mass and fat masses for total body, arm, leg, and trunk were determined by using dual-energy x-ray absorptiometry (DXA; Lunar Prodigy DXA, GE Lunar Corp., Madison, WI). The coefficient of variation (CV) values for the DXA measurements was 1.8% for lean tissue mass and 3.4% for fat measures.

Blood chemistry
Serum was obtained from whole blood using standard procedures, frozen without delay, and stored at ~70°C. Leptin was analyzed in serum samples (that had not undergone additional freeze-thaw cycles) using a commercially available kit (active human leptin ELISA, Diagnostic Systems Laboratories Inc., Webster, TX) with a detection limit of 0.05 ng/ml. Intra- and interassay CVs were 6.2 and 5.3%, respectively. IL-1RN levels in serum were measured with Fluorokine MAP human IL-1ra kit (R&D, Minneapolis, MN) with a detection limit of 0.05 ng/ml and a commercially available kit (active human leptin ELISA, Diagnostic Systems Laboratories Inc., Webster, TX) with a detection limit of 0.05 ng/ml. Intra- and interassay CVs were 6.2 and 5.3%, respectively. IL-1RN levels in serum were measured with Fluorokine MAP human IL-1ra kit (R&D, Minneapolis, MN) with a detection limit of 27.6 pg/ml and a coefficient of variation (CV) of 14%.

Isolation of leukocytes and stimulation of cytokine release
This experiment was performed in 148 individuals drawn at random from the GOOD study. Mononuclear cells from blood collected in heparinized tubes were prepared by centrifugation on Ficoll-Hypaque from the GOOD study. Mononuclear cells from blood collected in hep- 

TABLE 1. Characteristics of the study population

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean ± 2σ (n = 1068)</th>
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<tbody>
<tr>
<td>Age (yr)</td>
<td>18.9 ± 0.68</td>
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<tr>
<td>Total fat (kg)</td>
<td>13.4 ± 8.0</td>
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<tr>
<td>Total lean tissue (kg)</td>
<td>57.4 ± 6.2</td>
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<tr>
<td>Serum leptin (ng/ml)</td>
<td>7.7 ± 8.5</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>22.4 ± 3.2</td>
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<td>Trunk fat (kg)</td>
<td>6.8 ± 4.3</td>
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<tr>
<td>Arm fat (kg)</td>
<td>0.56 ± 0.41</td>
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<tr>
<td>Leg fat (kg)</td>
<td>2.5 ± 1.4</td>
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</table>
Results

General characteristics of the study population consisting of a homogenous group of well-characterized young men are shown in Table 1. To investigate whether the C to T +3953 polymorphism of the IL-1β gene is affecting body fat, we analyzed this polymorphism in relation to fat mass determined by DXA in this cohort. Allele frequencies for C and T in the +3953 IL-1β polymorphism were 0.75 and 0.25, respectively. The CC, CT, and TT genotypes were present in 591 (56.8%), 382 (36.7%), and 67 (6.4%) subjects, respectively. The CC, CT, and TT genotypes were present in 130 (12.7%), 448 (43.8%), and 445 (43.5%) subjects, respectively. The chi² analysis showed no deviation from Hardy-Weinberg equilibrium (P = 0.6, n = 1040). Multiple linear regression analysis of different fat parameters in relation to the IL-1β +3953 polymorphism showed that carriers of the T variant had significantly lower total body fat mass. The surrogate parameters body mass index (BMI) and serum leptin were also significantly decreased in T carriers. In contrast, there was no association at all between the polymorphism and total lean tissue mass (Fig. 1). Univariate analysis of total body fat also showed a significant association with the IL-1β +3953 polymorphism (beta = −0.071, P = 0.023). Additionally, the decrease in fat mass was also observed in regional measures of fat in trunk, leg, and arm (Fig. 2), whereas lean tissue mass in the same regions was not changed (data not shown). No significant differences between the CT and TT genotypes were found in any of the fat variables, indicating a lack of dose effect of the T allele. There was no association between the IL-1β gene polymorphism and any of the covariates.

Allele frequencies for C and T in the −31 IL-1β SNP were 0.35 and 0.65, respectively. The CC, CT, and TT genotypes were present in 130 (12.7%), 448 (43.8%), and 445 (43.5%) subjects, respectively. The chi² analysis showed no deviation from Hardy-Weinberg equilibrium (P = 0.300, n = 1023). This polymorphism did not correlate with total fat mass [CC = 13.3 ± 8.4, CT = 13.3 ± 7.3, TT = 13.3 ± 8.3, beta = −0.030, P = 0.332, data are mean (picograms per milliliter) ± sd] or any other of the investigated fat parameters (data not shown).

The IL-1RN*2 allele was present in 437 of 955 individuals. The allele frequencies for the most common alleles, IL-1RN*1 and IL-1RN*2, were 0.72 and 0.27, respectively. The combined frequency for the other alleles was less than 0.02. There was a slight deviation from Hardy-Weinberg equilibrium (P = 0.042, n = 955). Total body fat, leptin, and trunk and arm fat were significantly increased, whereas total lean tissue was decreased in IL-1RN*2 carriers (Table 2). Univariate analysis of total body fat showed no significant association with the IL-1RN*2 polymorphism (beta = −0.046, P = 0.16). No covariates other than lean tissue were associated with the IL-1RN 86-bp VNTR polymorphism (data not shown).

Studies of linkage disequilibrium (LD) showed that IL-1β +3953 and IL-1RN are in LD (D' = 0.52, P < 0.0001) and that the IL-1β +3953 C allele is associated with the IL-1RN*2 allele. Additionally, there is a moderate association between this haplotype and increased total body fat (P = 0.031). The frequency of this IL-1β +3953 C/IL-1RN*2 haplotype was 0.24. There were also LD between IL-1β −31 and IL-1β +3953 (D' = 0.69, P < 0.0001) and some LD between IL-1β-31 and IL-1RN (D' = 0.39, P < 0.0001). However, IL-1β-31 itself did, as previously mentioned, not correlate with any obesity parameters and was therefore not investigated further.

Serum IL-1RN levels showed a significant positive dose-dependent association with the number of IL-1RN*2 alleles.
The release of IL-1β and IL-1RN into the cell culture medium from primary human leukocyte cultures was measured after treatment with LPS. The levels of IL-1RN in the medium were positively associated with the number of IL-1RN*2 alleles [IL-1RN*2/−/− = 631 ± 2599, IL-1RN*2+/−/− = 6993 ± 3037, IL-1RN*2+/−/− = 8316 ± 4226, β = 0.187, P = 0.030, data are mean (picograms per milliliter) ± SD] in a similar way as the total body fat (β = 0.216, P < 0.001).

There was no correlation between serum IL-1RN levels and the IL-1 system, indicating that both of these polymorphisms are functional, affecting production of IL-1β and IL-1RN, inflammation levels, and susceptibility to infections (7–11, 13, 14).

The present results suggest a link between body fat mass and genetically determined changes in the function of the IL-1 system in humans. However, there are several studies that indicate that IL-1RI activation can affect body fat accumulation in humans. The present findings support this hypothesis.

The possible mechanism for the fat-reducing effect of IL-1 could involve mediation of the effects of leptin at the hypothalamic level as indicated by studies by Rothwell and coworkers (19). IL-1β is expressed in the hypothalamus, and the levels are enhanced by leptin and reduced by fasting (20, 21). In humans, it has been shown by Meier et al. (22) that the plasma levels of IL-1RN are enhanced in obese individuals. Based on their own findings and those of Rothwell and co-workers, these authors suggested that decreased IL-1 activity due to elevated IL-1RN production could contribute to leptin resistance in obese individuals (22). It remains to be investigated whether the IL-1RN*2/−/− or IL-1RN VNTR polymorphisms affect leptin sensitivity in humans.

Confirming earlier reports (22), we found a clear-cut positive correlation between IL-1RN levels and fat mass. The IL-1RN in serum of humans is probably to a large extent produced by adipose tissue, mainly by adipocytes but also by stromal cells (23, 24). It is possible that some of the IL-1RN in the stromal cells is produced by macrophages that accumulate in adipose tissue of obese individuals (25, 26). Although the major hypothesis is that IL-1 suppresses fat tissue mass via a hypothalamic effect (see above), it is also possible that IL-1 suppresses obesity via direct effects in the adipose tissue. IL-1β has been reported to inhibit adipocyte differentiation from preadipocytes and also to decrease the lipid content in mature adipocytes (27). Moreover, IL-1 may increase leptin secretion from preadipocytes and seems essential for inflammation-induced release of leptin from adipose tissue (27, 28). IL-1 may also decrease the release of the
antidiabetic hormone adiponectin (29), possibly due to decreased adipocyte differentiation (27).

The results of the present study and another (30) report indicate that BMI is decreased in IL-1\(\beta\) + 3953 T carriers. BMI is a nonspecific measure that is influenced by fat mass, lean tissue mass, and even height (31), and during certain pathophysiological conditions, IL-1 may decrease nonfat mass (32). However, by use of DXA, we could show that IL-1\(\beta\) + 3953 T carriers did not have different lean tissue mass. In contrast, the fat mass was clearly decreased in several compartments. Based on our DXA data, it might be assumed that the differences in BMI in the Korean population also are mainly due to changes in body fat. Therefore, it could be concluded that the IL-1\(\beta\) + 3953 T carriers have changed body composition, probably including decreased fat mass, in two very different populations: Korean women aged 18–47 yr and males, mostly Caucasian, aged 18–20 yr. This suggests that the association between IL-1 and body fat regulation in humans is robust and not substantially affected by ethnicity, gender, or age. Finally, the fact that IL-1\(\beta\) + 3953 T is associated with both decreased fat mass and increased IL-1 activity (8) fits well with the finding that IL-1RI stimulation suppresses fat mass in mice (4).

This study shows a significant association between the IL-1RN*2 allele and obesity variables, such as total fat and serum leptin levels. Um et al. (18) reported that IL-1RN*2 carriers of a Korean population tended to have a higher risk of being obese, although this effect was not significant. A possible reason for the significance in our study but not the Korean study may be that our study group was three to four times larger. Alternatively, there may be gender, age, or ethnic differences.

We found that the IL-1RN gene variant IL-1RN*2, in addition to its association with obesity, was associated with a slight but clearly significant increase in circulating IL-1RN levels, in line with an earlier report in a smaller material (14). Moreover, the IL-1RN*2 variant was associated with increased IL-1RN production in vitro. However, we could not confirm the results of an earlier study on a material one third the size of ours (13) that this gene variant is associated with increased IL-1\(\beta\) production. Based on the data from our study, we hypothesize that the IL-1RN*2 gene variant is associated with increased IL-1RN production, which in turn decreases IL-1 bioactivity and thereby contributes to obesity. This assumption mirrors the hypothesis that IL-1\(\beta\) + 3953T+ is associated with increased IL-1 bioactivity and thereby contributes to decreased obesity. In the present study, there was a LD resulting in an association between the IL-1\(\beta\) + 3953C and the IL-1RN*2 alleles in line with an earlier study (14). This haplotype was associated with obesity, as well as being expected from our hypothesis. Thus, there is some evidence that the IL-1 system genes and their protein products can affect obesity. However, the increased adipose tissue in obese individuals may itself cause increased production of IL-1RN (22–24). More studies are needed to investigate these complex interactions.

Earlier results from our group show that another proinflammatory cytokine with overlapping effects to those of IL-1\(\beta\), IL-6, can influence fat mass. IL-6-deficient mice develop mature-onset obesity (1), and the weaker variant of a functional polymorphism of the IL-6 promoter is associated with increased fat mass and decreased energy expenditure in humans (2, 3). In mice, the interactions between the effects of IL-1 and IL-6 on body fat have been described in a recent report (33). Finally, knockout mice that lack granulocyte macrophage-colony stimulating factor display a phenotype very similar to IL-6 knockout and IL-1RI knockout mice with a moderate degree of mature-onset obesity (34). All these cytokines seem to act in the central nervous system, possibly the hypothalamus (1, 21, 34, 35). Therefore, an emerging pattern seems to be that several different cytokines are regulating body fat via central effects in the absence of clinical inflammation in experimental animals and possibly also in humans.

In conclusion, this study on a cohort of more than 1000 young men shows that polymorphisms of the IL-1 system are associated with measures of fat mass in humans. This opens the possibility that the IL-1 system is linked to the development of obesity and obesity-related morbidities in humans.

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