Gender-based dimorphic pattern for interleukin-1 receptor antagonist in type 2 diabetes mellitus

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Abstract

Adipose tissue secretes a variety of cytokines, some of which are increased in the serum of obese patients. The anti-inflammatory interleukin-1 receptor antagonist (IL-1Ra) is the most highly elevated known cytokine in human obesity, and its serum levels are strongly associated with the degree of insulin resistance in non-diabetic patients.

Aim. – The present study examined serum levels of IL-1Ra in type 2 diabetic patients (T2DM) and their relationships with three other adipokines (leptin, interleukin-6 [IL-6], adiponectin). Their correlation with anthropometric and biochemical variables was examined, as well as their intraindividual fluctuations.

Methods. – Fifty T2DM patients, aged 58 ± 13 years, were consecutively recruited among those electively hospitalized for a one-week intensive training course with our Diabetes Education Service. Anthropometric measurements and blood samples were taken after an overnight fast on admission (baseline) and after four days.

Results. – Mean serum levels of IL-1Ra and leptin, but not of IL-6 and adiponectin, were significantly higher in women than in men (P < 0.0006), and this difference persisted after correction for body mass index (BMI) (P < 0.0004). In addition, IL-1Ra and leptin were strongly correlated with the BMI (P < 0.0004). By contrast, no significant correlations were observed between IL-1Ra and glucose-control parameters. Finally, all four adipokines exhibited wide interindividual variability, but with limited intraindividual fluctuations over the short time period.

Conclusion. – IL-1Ra, leptin and adiponectin serum levels exhibit marked interindividual variation with high intraindividual consistency. A gender-based dimorphic pattern for IL-1Ra, independent of the degree of adiposity and glucose control, was also found.

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Résumé

Dysmorphisme sexuel de l’interleukine-1 récepteur antagoniste dans le diabète de type 2.

Le tissu adipeux sécrète une variété de cytokines dont certaines sont augmentées dans le sérum des patients obèses. Le récepteur antagoniste interleukine-1 (IL-1Ra) est la cytokine connue comme étant la plus élevée chez les patients obèses et sa concentration sérique est fortement corrélée avec le degré de résistance à l’insuline chez les patients non diabétiques.

Objectifs. – Cette étude a analysé les concentrations sériques de l’IL-1Ra dans un groupe de patients diabétiques de type 2 (T2DM) et l’association de IL-1Ra avec trois autres adipokines (la leptine, l’interleukine-6 [IL-6] et l’adiponectine). Leur corrélation avec des variables anthropométriques et biochimiques a été examinée, ainsi que leurs fluctuations intraindividuelles.

Méthodes. – Cinquante patients diabétiques de type 2 (n = 50), âgés de 58 ± 13 ans, ont été recrutés lors d’une hospitalisation pour une semaine de formation intensive en éducation thérapeutique pour le diabète. Les mesures anthropométriques et les prélèvements sanguins ont été réalisés à jeun à l’état basal et quatre jours après.

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Adipose tissue secretes a wide variety of factors with pro- or anti-inflammatory cytokines that, in addition to their classical roles, also control metabolic events such as adipogenesis, energy homeostasis and insulin sensitivity [1].

We have previously reported that the anti-inflammatory cytokine interleukin-1 receptor antagonist (IL-1Ra) was elevated sevenfold in the serum of morbidly obese patients. This marked elevation of serum IL-1Ra in obese patients, together with its prodiabetic and adipogenic effects observed in rodents, led to the hypothesis that IL-1Ra might be linked to acquired resistance to insulin and leptin, the hallmarks of obesity and type 2 diabetes mellitus (T2DM) [2–5]. T2DM manifests in individuals with insulin resistance coupled with a beta-cell defect. Human beta cells produce IL-1β in response to high glucose concentrations, independent of an immune-mediated process. The latter is antagonized by IL-1Ra, a naturally occurring anti-inflammatory cytokine also found in beta cells. Thus, the balance between IL-1β and IL-1Ra may play a crucial role in the pathogenesis of diabetes [6–7]. While the elevated IL-1Ra serum levels present in human obesity might be protective for islet cells, excess IL-1Ra in humans is associated with an increase in insulin resistance, with a causal relationship having been established in rodents treated with IL-1Ra [8].

Over the past few years, inflammation has received considerable attention in the pathogenesis of both T2DM and atherosclerosis. IL-1Ra is a major negative modulator of the IL-1–proinflammatory pathway. In T2DM, a significant association has been reported between coronary artery disease and lipidaemia, diabetes, hypertension and vascular complications [23,24].

The aim of the present study was to assess the hypothetical relationships between IL-1Ra, IL-6, adiponectin and leptin levels as well as their intraindividual variability over a short time period in T2DM patients admitted through elective hospitalization for diabetes education.

2. Subjects and methods

2.1. Subjects

Fifty patients with T2DM according to World Health Organization (WHO) criteria and aged 58 ± 2 years (range: 31–97 years) were consecutively recruited from those entering hospital for one week at the Service of Therapeutic Education for Chronic Diseases of the University Hospital of Geneva. Patients gave their written informed consent after a thorough explanation of the aims and procedures of the study, which was approved by the local ethics committee. Inclusion criteria were the following: T2DM with an HbA1c less than 8.5%; stable body weight over the past two months; fasting plasma total cholesterol and triglyceride levels less than 6.5 mmol/l and less than 2.5 mmol/l, respectively. Exclusion criteria were:

- recently diagnosed (< 2 years) or uncontrolled (HbA1c > 8.5%) T2DM;
- prior bariatric surgery, intragastric balloon placement and/or liposuction;
- treatment with peroxisome proliferator agonists (fibrates, glitazones);
• active smoking; treatment with Aspirin®, warfarin, low-molecular-weight heparin, clopidogrel, statins, steroids or non-steroidal anti-inflammatory drugs (NSAIDs) within the past four weeks;
• recent or chronic infectious or inflammatory disorder.

Patients were apparently in a good state of health and underwent an elective hospital admission for a thorough medical check-up, including screening for diabetic micro- and macroangiopathic complications, and to improve their daily metabolic control through dietary counselling and patient education.

Extensive review of the participants’ medical records disclosed a diabetes duration of 8.8 ± 4.6 years and the absence of micro-/macroangiopathic complications. Half the patients (n = 25) were treated with diet and insulin while the remainder was treated with diet and oral antidiabetic drugs (metformin and/or sulphonylureas). Thirty of the 50 patients (60%) had high blood pressure, according to the Joint National Committee on Prevention, Detection, Evaluation, and the Treatment of High Blood Pressure (JNC) VI guidelines, that was under satisfactory control with either angiotensin-converting enzyme inhibitors or angiotensin receptor-II antagonists. None of the patients had evidence of hypertensive target-organ damage, or kidney or liver disease.

During the hospital stay, all patients benefited from the same interdisciplinary cognitive–behavioural–nutritional counselling programme, including therapeutic interactive-teaching lessons on nutrition, body-weight maintenance, self-esteem, long-term disease-coping, diabetes management, and the importance of treatment compliance and physical activity. Details of the programme have been described elsewhere [25,26]. The mean duration of hospital stay was 4.8 ± 0.9 days.

2.2. Anthropometric and serum measurements

Anthropometric measurements (weight, height) and blood samples (routine venous puncture) were obtained at baseline (day of admission) and on the day of discharge, both times after an overnight fast.

HbA1c was measured using an immunological method (Synchron LX Helix System), erythrocyte sedimentation rate (ESR) was measured by Westergen’s method, and serum IL-1Ra and IL-6 levels were determined using commercially available enzyme-linked immunosorbent assays (Quantikine ELISA, R&D Systems Europe Ltd, Abingdon, UK). Mean normal serum levels were 418 (range: 106–1552) pg/ml for IL-1Ra, and less than 3.12 (range: < 3.12–12.5) pg/ml for IL-6. Intra- and interassay coefficients of variation were 4.5% for IL-1Ra, and 4.2% and 6.4% for IL-6, respectively.

Plasma leptin concentrations were measured using a commercial human leptin radioimmunoassay kit (Linco, Labodia SA, Yens, Switzerland) with a detection limit, and intra- and interassay coefficients of variation of 0.5 ng/ml, 5.0% and 4.5%, respectively. Plasma adiponectin concentrations were measured using a commercial human adiponectin RIA kit (Linco, Labodia SA, Yens, Switzerland) with a detection limit, and intra- and interassay coefficients of variation of 2.0 ng/ml, 5.0% and 5.0%, respectively.

2.3. Statistical methods

Sample size was calculated as follows: assuming an α risk of 0.05, a β risk of 0.10 and a unilateral test, 40 subjects were needed to detect a statistically significant correlation, with r: 0.30. A greater number of patients than this was included to further decrease the α and β risks. Descriptive results of continuous variables were expressed as means and S.D. Before statistical analysis, normal distribution and homogeneity of the variances were tested. Parameters that did not fulfil these tests (IL-1Ra, leptin, adiponectin, IL-6, ESR) were logarithmically transformed. The differences between variables were analyzed by unpaired t test. In addition, differences between men and women were tested using Fisher’s PLSD (protected least-significant-difference) test. Multiple linear-regression analyses were used to calculate the gender difference for the log-transformed cytokine concentrations after correction for body mass index (BMI) values. Simple correlations between variables were assessed using Pearson’s correlation coefficient (r). The chi-square test was employed to compare gender differences in the percentage of individuals disclosing normal, moderately elevated and markedly elevated IL-1Ra levels. The level of statistical significance was set at P < 0.05. Data were analyzed and figures constructed using the SPSS 12.0 statistical package (SPSS Inc.).

3. Results

Table 1 summarizes the baseline anthropometric and metabolic parameters for the study population stratified by gender. Mean age, body weight, BMI, fasting capillary blood glucose, HbA1c and ESR values were similar for both men and women (P = NS).

Serum cytokine and adipokine levels were determined, and Table 2a presents the baseline of both absolute values, and the log-transformed concentrations of IL-1Ra, IL-6, leptin and adiponectin stratified by gender. Mean log IL-1Ra levels were higher in women (P < 0.0006), and this difference persisted after correction for individual BMI values (Table 2b; P < 0.0004). Moreover, 46% of all patients had normal IL-1Ra values (< 500 pg/ml), 46% had moderately elevated values

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Baseline anthropometric and metabolic parameters stratified by gender</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All</td>
</tr>
<tr>
<td>N</td>
<td>50</td>
</tr>
<tr>
<td>Age (year)</td>
<td>58.3 ± 12.9</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>83.6 ± 16.8</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30.5 ± 5.7</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>8.3 ± 1.6</td>
</tr>
<tr>
<td>FPG (mmol/l)</td>
<td>9.7 ± 2.7</td>
</tr>
<tr>
<td>Log ESR (log mm/h)</td>
<td>0.9 ± 0.4</td>
</tr>
</tbody>
</table>

Age in years (year); Weight in kilograms (kg); Body mass index (BMI) (kg/m²); HbA1c (%); fasting plasma glucose (FPG) (mmol/l); Log ESR: log of erythrocyte sedimentation rate (log mm/h); Values are expressed as mean ± S.D.
(500–1500 pg/ml) and 8% had markedly elevated values (> 1500 pg/ml). Men and women differed in this respect, with 62% versus 24% being normal, 38% versus 57% being moderately elevated, and 0% versus 19% being markedly elevated, respectively ($P < 0.001$).

Leptin levels were lower in men than in women ($P < 0.00007$), and this difference persisted after correction for BMI (Table 2b; $P < 0.0004$), whereas no significant gender differences were observed for serum IL-6 and plasma adiponectin (Tables 2a and 2b). However, a significant correlation was found between age and plasma adiponectin levels ($r: 0.550; P < 0.0005$) (data not shown).

Baseline and discharge IL-1Ra and leptin concentrations displayed significant correlations ($r: 0.475$ and $r: 0.566$, respectively; $P: 0.0004$ for both). The other cytokines showed no significant correlations (data not shown).

Fig. 1 shows the gender-stratified correlations between BMI versus IL-1Ra (Fig. 1A), BMI versus leptin (Fig. 1B), BMI versus adiponectin (Fig. 1C) and BMI versus IL-6 (Fig. 1D). IL-1Ra and leptin concentrations were significantly correlated with BMI in both men and women whereas, for IL-6, such a correlation was observed only in women. In addition, no correlation was observed between BMI and adiponectin.

To investigate the intraindividual variations of cytokines and adipokines, their concentrations were measured in blood samples obtained on admission and at discharge. As illustrated in Fig. 2, the IL-1Ra (Fig. 2A), leptin (Fig. 2B), IL-6 (Fig. 2C) and adiponectin (Fig. 2D) serum levels at baseline (admission) did not differ significantly from those on the day of discharge (after 4.8 ± 0.9 days of hospitalization), and the individual values were well intercorrelated. The intraindividual correlation for the four parameters studied yielded correlation coefficients ($r^2$) between 0.42 and 0.72, indicating that the baseline level explains 42–72% of the variance in the second blood sample. Also, patients’ body weight did not change significantly (data not shown).

### 4. Discussion

In the last few years, type 2 diabetes has been increasingly recognized as an inflammatory state as a result of concomitant peripheral insulin resistance and decreased beta-cell function. Moreover, insulin resistance and cardiovascular disease share common pathophysiological mechanisms in that both reflect chronic activation of the immune system.

Interleukin-1 (IL-1) is a proinflammatory cytokine implicated in the pathophysiology of numerous diseases as well as in the maintenance of homeostasis in a number of tissues. To prevent the damaging effects of IL-1, the naturally occurring anti-inflammatory cytokine IL-1Ra is produced to counteract the effects of IL-1 [27].

Beta cells producing IL-1β have been observed in pancreatic sections obtained from patients with type 2 diabetes [6]. Intra-islet production of inflammatory mediators has a role in the pathogenesis of type 2 diabetes, and IL-1β is a potential therapeutic target for preserving beta-cell mass and function in patients with this condition [6, 7]. Hence, a recent paper by Larsen et al. [10] reported improved glycaemic control in patients with type 2 diabetes treated with low-dose recombinant human IL-1 receptor antagonist (Anakinra® 100 mg/d) for 13 weeks, most likely through enhanced beta-cell secretory function. Interestingly, no effect on either BMI or insulin sensitivity was found.

### Table 2a

Logarithmically transformed concentrations of IL-1Ra (pg/ml), IL-6 (µg/l), leptin (pg/ml) and adiponectin (mg/l) stratified by gender at baseline (first day of educational stay in hospital).

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log IL-1Ra (log pg/ml)</td>
<td>2.8 ± 0.3</td>
</tr>
<tr>
<td>BMI (µg/l)</td>
<td>690 ± 520</td>
</tr>
<tr>
<td>Log leptin (log mcg/l)</td>
<td>1.1 ± 0.4</td>
</tr>
<tr>
<td>Leptin (mcg/l)</td>
<td>18.9 ± 14.0</td>
</tr>
<tr>
<td>Log IL-6 (log pg/ml)</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>4.8 ± 3.0</td>
</tr>
<tr>
<td>Log adiponectin (log mg/l)</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>Adiponectin (mg/l)</td>
<td>4.4 ± 2.3</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.D.

* $P < 0.0005$ (gender differences).

** $P < 0.0005$ (gender differences after correction for BMI).

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### Table 2b

Multiple linear-regression analyses

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Independent variables</th>
<th>Coefficients ($\beta$)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log IL-1Ra ($r^2 = 0.403; P = 0.0004$)</td>
<td>Constant</td>
<td>2.368</td>
<td>&lt; 0.0005</td>
</tr>
<tr>
<td></td>
<td>Gender ($0 = g/l = c'$)</td>
<td>−0.226</td>
<td>&lt; 0.0005</td>
</tr>
<tr>
<td></td>
<td>BMI</td>
<td>0.017</td>
<td>0.002</td>
</tr>
<tr>
<td>Log leptin ($r^2 = 0.452; P = 0.0004$)</td>
<td>Constant</td>
<td>0.343</td>
<td>0.201</td>
</tr>
<tr>
<td></td>
<td>Gender ($0 = g/l = c'$)</td>
<td>−0.353</td>
<td>&lt; 0.0005</td>
</tr>
<tr>
<td></td>
<td>BMI</td>
<td>0.032</td>
<td>&lt; 0.005</td>
</tr>
<tr>
<td>Log IL-6 ($r^2 = 0.237; P = 0.258$)</td>
<td>Constant</td>
<td>0.310</td>
<td>0.144</td>
</tr>
<tr>
<td></td>
<td>Gender ($0 = g/l = c'$)</td>
<td>−0.014</td>
<td>0.853</td>
</tr>
<tr>
<td></td>
<td>BMI</td>
<td>0.010</td>
<td>0.118</td>
</tr>
<tr>
<td>Log adiponectin ($r^2 = 0.020; P = 0.634$)</td>
<td>Constant</td>
<td>0.698</td>
<td>&lt; 0.0005</td>
</tr>
<tr>
<td></td>
<td>Gender ($0 = g/l = c'$)</td>
<td>−0.058</td>
<td>0.362</td>
</tr>
<tr>
<td></td>
<td>BMI</td>
<td>−0.002</td>
<td>0.670</td>
</tr>
</tbody>
</table>

Dependent variables: log-transformed concentrations of IL-1Ra, IL-6, leptin and adiponectin; Independent variables: gender, BMI.
The main finding of the present study was the existence of a gender-based dimorphism for IL-1Ra and leptin levels in T2DM individuals independent of the degree of obesity (BMI) and glucose control (HbA1c). This dimorphism should be taken into account in the design and future implementation of novel therapeutic strategies (such as human recombinant IL-1 receptor antagonists) currently in the developmental stages. Last, but not least, the present study explored the hypothetical relationships between four pro- or anti-inflammatory hormones (IL-1Ra, IL-6, adiponectin and leptin) secreted exclusively or partially by adipose tissue [2,5,28,29], and BMI and gender levels, as well as their intraindividual variability over a short time period, in T2DM patients admitted electively to hospital for diabetes education.

Various IL-1Ra cut-off levels have been reported for healthy individuals, depending on the assay used. Using the same method as in the present study, we found an IL-1Ra range of 256–800 pg/ml in 32 healthy blood donors. Thus, a direct comparison of absolute values is not feasible with our results, which display somewhat higher IL-1Ra concentrations. The two IL-1Ra measurements were not performed simultaneously and pertain to different studies. Otherwise, and taking into account the characteristics of our T2DM cohort, their mean IL-1Ra levels are in agreement with previous findings by our group [3] of raised IL-1Ra levels in obese individuals and correlations with BMI values. In fact, the values in our study cohort covered a wide range, from 20.7 to 47.2 kg/m$^{-2}$, and this for both men and women. To the best of our knowledge, the latter findings have not been previously reported in diabetic patients. Moreover, our results reveal that women have higher IL-1Ra levels than men. This difference is somewhat discrepant from previous reports [30], where other factors such as gender, age and mens-
tration status did not affect IL-1Ra levels \textit{in vivo}; this could be explained by the fact that those study volunteers were healthy non-diabetic blood donors. Also, markedly higher IL-1Ra urine levels have been described in healthy women compared with healthy men [31]. However, to the best of our knowledge, there are no reports concerning such a gender bias in IL-1Ra plasma levels.

Furthermore, in our study, the gender difference in IL-1Ra concentrations cannot simply be accounted for by differences in BMI, as the difference persisted even after correction for BMI values.

Leptin plasma levels were somewhat above normal values on average in our diabetic population, which is in agreement with other reports [32]. As expected, women also showed higher leptin levels than men and, again, this difference persisted after correction for BMI. As reported previously [19], leptin concentrations are highly correlated with body-fat storage and exhibit gender-based dimorphism, with women having higher concentrations at every level of relative or absolute adiposity. In our study population, leptin dimorphism persisted after correction for BMI, consistent with previous reports [33]. Hence, this gender-based dimorphism cannot be accounted for, simply by the difference in BMI values.

IL-6 and adiponectin levels were within the norm [34], and no significant differences in serum IL-6 and plasma adiponectin values were observed between genders, a finding that is not consistent with other reports [19,35]. However, the repeatedly reported [36] significant correlation between age and adiponectin was observed in our study.

Also, for BMI versus either IL-1Ra or leptin, both gender-stratified correlations were significant, in agreement with earlier studies [30,37–39] whereas, for BMI versus IL-6, only women showed a significant correlation, which is in contradiction to previous findings [3]. In particular, leptin levels were found to correlate with those of IL-1Ra, in agreement with previous reports [1,4].

As summary, our results support the existence of a gender-based dimorphism for IL-1Ra and leptin levels in T2DM individuals independent of the degree of obesity and glucose control. In addition, IL-1Ra, leptin and adiponectin serum levels exhibited both a marked interindividual variation and a high intraindividual consistency. Further studies of longer duration and involving a larger number of patients are warranted.
Acknowledgments

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