PLASMA LEVELS OF TUMOR NECROSIS FACTOR-ALPHA (TNF-α) ARE ESSENTIALLY DEPENDENT ON VISCERAL FAT AMOUNT IN TYPE 2 DIABETIC PATIENTS

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SUMMARY - TNF-α is considered as one of the potential determinants of insulin resistance. However several data suggest that TNF-α expression itself, could be modulated by the degree of adiposity and/or plasma insulin levels. To clarify the determinants of plasma TNF-α levels in type 2 diabetes mellitus, we studied the impact of intensive insulin treatment on plasma TNF-α levels in 16 type 2 diabetic subjects with failure to oral antidiabetic medication (HbA1c: 10.8 ± 1.2 %). Furthermore, we analyzed the relationship between plasma TNF-α levels and total or regional body fat measurements using anthropometry, bienergetic absorptiometry and computed tomography in a cohort of 33 caucasian obese type 2 diabetic subjects (BMI: 32.2 ± 4.4 kg/m²). The plasma TNF-α level was neither affected by plasma glucose level variations nor intensive insulin treatment despite a 37 % decrease in daily insulin needs at the end of insulin therapy (total duration: 11.5 ± 2.0 days). The plasma TNF-α level was similar in men and women and unrelated to age, fasting glycemia or HbA1c. A relationship was highlighted with BMI (r = 0.39, p < 0.02), but not with total fat mass. This relationship was only dependent on the intra-abdominal fat mass amount as assessed by the waist-to-hip circumference ratio (r = 0.52, p < 0.01) and the visceral adipose tissue area (r = 0.56, p < 0.01). These results show that plasma TNF-α levels depend essentially on visceral fat amount, thus suggesting that TNF-α could be one of the factors mediating insulin resistance and cardiovascular risk in obese type 2 diabetic patients.

Key-words: TNF-α, diabetes, visceral adipose tissue, human.

RÉSUMÉ - Les taux plasmatiques de TNF-α dépendent principalement de la quantité de graisse viscérale chez les diabétiques de type 2. Le TNF-α est considéré comme un des déterminants potentiels de l’insulinorésistance. Un certain nombre de travaux suggèrent cependant que l’expression de TNF-α est susceptible d’être modulée à son tour par le degré d’adiposité et/ou le niveau d’insulinémie. Pour mieux identifier les déterminants des taux plasmatiques de TNF-α dans le diabète de type 2, nous avons étudié l’impact d’une insulinothérapie intensive sur les taux plasmatiques de TNF-α chez 16 diabétiques de type 2 présentant un échec au traitement antidiabétique oral (HbA1c : 10,8 ± 1,2 %). De plus, nous avons analysé la relation entre les taux plasmatiques de TNF-α et les mesures de masse grasse globale et régionale obtenues par anthropométrie, absorptiométrie bi-énergétique et tomodensitométrie dans une cohorte de 33 diabétiques de type 2 obèses (BMI : 32,2 ± 4,4 kg/m²), d’origine caucasienne. Le taux plasmatique de TNF-α n’apparaît influencé ni par les variations de la glycémie, ni par l’insulinothérapie intensive malgré une diminution de 37 % des besoins en insuline à la fin de la séquence d’insulinothérapie (durée moyenne : 11,5 ± 2,0 jours). Le taux de TNF-α est similaire chez les hommes et les femmes et non corrélé à l’âge, la glycémie ou l’HbA1c. Il est corrélé à l’indice de masse corporelle (r = 0,39 ; p < 0,02), mais pas à la masse grasse totale. Cette corrélation est uniquement dépendante de la quantité de masse grasse intra-abdominale : r = 0,52 ; p < 0,01 avec le rapport tour de taille/tour de hanche, et r = 0,56 ; p < 0,01 avec la quantité de graisse viscérale mesurée par tomodensitométrie. Ces résultats montrent que les taux plasmatiques de TNF-α dépendent essentiellement de la quantité de graisse viscérale, suggérant ainsi que le TNF-α pourrait être un des facteurs intervenant dans la physiopathogénie de l’insulinorésistance et du risque cardiovasku-ulaire chez les diabétiques de type 2 obèses.

Mots-clés : TNF-α, diabète, graisse viscérale, humains.
Numerous data suggest that cytokine tumor necrosis factor-alpha (TNF-α) could play a role in insulin resistance. It down-regulates the signal transduction of the insulin receptor by a mechanism which is not yet clearly defined (1, 2). This cytokine was shown to be overexpressed in the adipose tissue of obese rodents and humans was shown to be correlated with the amount of visceral adipose tissue, a strong associated component of insulin resistance, in overweight Japanese subjects with type II diabetes mellitus (3), and a significantly higher GLUT-4 protein level was observed in muscle tissue of TNF-α deficient obese mice (4). Targeted mutations in the TNF-α and TNF-receptors genes resulted in an improved insulin sensitivity in animal models of obesity (4, 5).

On the other hand, the overexpression of TNF-α in the adipose tissue of obese rodents and humans was shown to be correlated with weight loss (6) and by an insulin-sensitizing drug (7). These data raise the hypothesis that TNF-α expression could also be modulated by the degree of adiposity and/or plasma insulin levels. Despite low plasma levels due to a prevailing paracrine and autocrine regulation, the circulating TNF-α could also play a significant role in the genesis of insulin resistance in obesity (8). Moreover, the plasma TNF-α level was recently reported to be related to the amount of visceral adipose tissue, a strong associated component of insulin resistance, in overweight Japanese subjects with type II diabetes mellitus. Available human data on circulating TNF-α expression could also be modulated by the degree of adiposity and/or plasma insulin levels. Despite low plasma levels due to a prevailing paracrine and autocrine regulation, the circulating TNF-α could also play a significant role in the genesis of insulin resistance in obesity (8). Moreover, the plasma TNF-α level was recently reported to be related to the amount of visceral adipose tissue, a strong associated component of insulin resistance, in overweight Japanese subjects with type II diabetes mellitus. Available human data on circulating TNF-α levels in this physiopathological condition are scarce and rather contradictory (9-11), and the respective role of plasma glucose and insulin variations on TNF-α production have only been studied in vitro (12). Moreover, the analysis of plasma TNF-α levels according to the whole body fat mass, has never been performed so far therefore. The purpose of the present study was to analyze the relationship between plasma level of TNF-α and glycemic control, body fat content and its distribution, both in male and female patients with type 2 diabetes mellitus. The impact of the correction of chronic hyperglycemia, as obtained by an intensive subcutaneous insulin therapy, on the cytokine level was also defined.

**RESEARCH DESIGN AND METHODS**

**Subjects**

*Study 1* — Sixteen patients with type 2 diabetes mellitus (10 women and 6 men, mean age : 52 ± 7.9 years, BMI : 30.4 ± 4.5 kg/m²) presenting a confirmed secondary failure to oral antidiabetic medication (HbA1c : 10.8 ± 1.2 %) were administered an intensive subcutaneous insulin therapy with a specific pump for 11.5 ± 2.0 days. Home caloric intake and oral antidiabetic medication (sulfonylurea and metformin) were unchanged during hospitalization, the only modification being insulin therapy. Fasting plasma levels of fructosamine, glucose and C-peptide were measured before insulin therapy (day 0) and at the end of the sequence (day 12). Plasma TNF-α and plasma triglycerides levels were measured in the same conditions and as soon as a strict glycemic control assessed by 6 daily capillary glycemia was obtained (day 3 : 2.8 ± 1.0 days after starting insulin therapy).

*Study 2* — The plasma TNF-α level was measured in 33 obese type 2 diabetic subjects (11 women and 22 men, mean age : 55 ± 9.1 years, BMI : 32.2 ± 4.4 kg/m², HbA1c : 9.8 ± 1.9 %, Glycemia : 12.2 ± 3.2 nmol/l) analyzed for body composition using anthropometry (BMI, waist-to-hip circumference ratio) and dual energy X-ray absorptiometry. Visceral fat amount was also assessed in 21 of these patients (4 women and 17 men) by using computed tomography. Subjects were treated with an oral antidiabetic medication but not with insulin.

All subjects gave their informed consent in agreement with the Helsinki Declaration of 1975 as revised in 1983. They were caucasian, free of any medication interfering with monocyte functions, and did not present either auto-immunity, or infectious or inflammatory state (including ectodermal mycosis). This was further confirmed by the dosage of C-reactive protein (CRP), of erythrocyte sedimentation rate (ESR), and by bacterial examination of the urines. Patients with renal insufficiency were excluded from the study.

**Analytical procedures** — Samples for TNF-α and other biological parameters measurements were taken after an overnight fast, between 8 : 00 and 8 : 30 AM. Blood sample for TNF-α was drawn under EDTA and immediately dispatched to the laboratory to be centrifuged and stored at -80°C until assayed. TNF-α level was measured using a two-step immunoenzymatic assay (TNF-α-Elisa”, Medgenix Diagnostics, Belgium) according to the manufacturer’s recommendations. HbA1c was measured by high-pressure liquid chromatography on a Diamat analyzer (Bio-Rad, Ivry-sur-Seine, France), and fructosamine on a BM/Hitachi 911 analyzer (Boehringer Mannheim). C-peptide was measured by a RIA assay (RIA coat C-peptide, Byk Sangted, SWEDEN).

**Body composition analysis** — Total body fat mass (FM) and its percentage (%FM) were measured by dual energy X-ray absorptiometry (DXA) using a total body scanner (QDR 2000/W HOLOGIC, software version 5.67, Waltham, MA) (13). Fat mass distribution was further assessed at the abdominal level (visceral and subcutaneous adipose tissue area : VAT and SAT) by computerized tomography (CT) using a slice at the midplane of the fourth lumbar vertebra (14). The waist-to-hip circumference ratio was measured as previously reported (15).
**Statistical analysis** — Data were expressed as mean ± SD. Pearson’s correlation coefficients were used, and forward stepwise multiple regression models permit to identify independent variables potentially related to the plasma TNF-α levels. The Student’s t test and the Wilcoxon signed rank-sum test were respectively used for unrelated and related samples. All statistics were done with a computer program software (Statview™ SE, V1.03 ; Abacus Concepts, Berkeley, CA).

**RESULTS**

**Study 1** — Changes in plasma TNF-α level, total body weight, and daily insulin needs during insulin therapy are shown in **Table 1**. TNF-α was not significantly different at day 3 or at day 12 of subcutaneous insulin therapy when compared to day 0, whereas body weight significantly changed between day 0 and day 12 (830 ± 910 g less at day 12, *p* < 0.01). Duration of insulin therapy, delay of normoglycemia achievement, daily amount and nycthemeral pattern of insulin needs, glycaemic control parameters (fasting glucose, fructosamine, HbA1c), triglycerides values before treatment, were unrelated to the weak relative variations of circulating TNF-α.

**Study 2** — The plasma TNF-α level was similar between the two genders (14.5 ± 6.4 pg/ml in women and 15.3 ± 5.6 pg/ml in men) and unrelated to age. As in the above results (study 1), no relationship with fasting glycaemia or HbA1c, triglycerides values before treatment, were unrelated to the weak relative variations of circulating TNF-α. A relationship was present with total body weight and especially with BMI (r = 0.39, *p* < 0.02) (Fig. 1), but not with total body fat mass or fat-free mass obtained by DXA. The waist-to-hip circumference ratio, which is a simple clinical predictor of adipose tissue distribution, was more closely and independently associated with the plasma level of TNF-α (r = 0.52, *p* < 0.01) (Fig. 2). When adjusting TNF-α on BMI, the relationship with waist-to-hip circumference ratio still persisted (R = 0.42, *p* < 0.03). Stepwise multiple regression analysis showed that the waist-to-hip circumference ratio was the unique variable independently associated with TNF-α. When analyzing the relationship between plasma TNF-α level and adipose tissue distribution assessed by CT at the abdominal level, the visceral adipose tissue area was shown to be closely associated with TNF-α (r = 0.56, *p* < 0.01) independently from the subcutaneous adipose tissue area (Fig. 3).

**Table I.** Changes in fasting plasma TNF-α level, body weight and metabolic parameters during insulin therapy in 16 insulin-requiring type 2 diabetic subjects (day 0 = just before, day 3 = first day of normoglycemia : 2.8 ± 1.0 days, day 12 = 11.5 ± 2.0 days after start)

<table>
<thead>
<tr>
<th></th>
<th>day 0</th>
<th>day 3</th>
<th>day 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>83.4 ± 14.7</td>
<td>-</td>
<td>82.6 ± 14.2*</td>
</tr>
<tr>
<td>Plasma glucose (mmol/l)</td>
<td>13.0 ± 2.6</td>
<td>-</td>
<td>7.2 ± 2.0*</td>
</tr>
<tr>
<td>Fructosamine (µmol/l)</td>
<td>341 ± 38</td>
<td>-</td>
<td>263 ± 34*</td>
</tr>
<tr>
<td>C-peptide (ng/ml)</td>
<td>2.8 ± 0.7</td>
<td>-</td>
<td>2.7 ± 1.2</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>3.2 ± 1.5</td>
<td>2.3 ± 0.6</td>
<td>2.1 ± 0.7*</td>
</tr>
<tr>
<td>Insulin needs (UI/day/kg)</td>
<td>-</td>
<td>0.62 ± 0.16</td>
<td>0.39 ± 0.17</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>9.3 ± 3.6</td>
<td>9.4 ± 3.8</td>
<td>10.7 ± 3.5</td>
</tr>
</tbody>
</table>

Results are presented as mean ± SD ; *p* < 0.01 versus day 0

**Table II.** Correlation coefficients (r) between body composition parameters and plasma TNF-α level in subjects with type 2 diabetes mellitus

<table>
<thead>
<tr>
<th></th>
<th>r</th>
<th>p</th>
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<tbody>
<tr>
<td>Body weight (kg)</td>
<td>0.37</td>
<td>&lt; 0.03</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.39</td>
<td>&lt; 0.02</td>
</tr>
<tr>
<td>WHR</td>
<td>0.52</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>% Fat Mass</td>
<td>0.05</td>
<td>-</td>
</tr>
<tr>
<td>Fat Mass (kg)</td>
<td>0.23</td>
<td>-</td>
</tr>
<tr>
<td>VAT (cm²)</td>
<td>0.56</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>SAT (cm²)</td>
<td>0.06</td>
<td>-</td>
</tr>
<tr>
<td>TAT (cm²)</td>
<td>0.37</td>
<td>-</td>
</tr>
</tbody>
</table>

*p* = degree of significance ; WHR = Waist-to-Hip Circumference Ratio ; Fat Mass and % Fat Mass were determined from DXA total body measurements ; VAT, SAT and TAT = Visceral, Subcutaneous and Total Adipose Tissue areas obtained from CT measurements.
DISCUSSION

In the first part of our study (study 1), we demonstrated for the first time that plasma TNF-α level was unchanged either by plasma glucose level variations or intensive insulin treatment in caucasian obese subjects with type 2 diabetes mellitus. The remarkable stability in the plasma TNF-α levels was further confirmed in some type 2 diabetic subjects over one year (personal unpublished data in several patients with stable body weight). Thus these data suggest that TNF-α which contributes to insulin resistance would not be in return influenced by insulin when considering its blood concentration. Moreover, our results are in agreement with those of Kroder et al. who reported evidence for different mechanisms and different effects between TNF-α and hyperglycemia on insulin signaling (16). We indeed observed that our patients experienced a great decrease in their daily insulin needs related to lower insulin resistance several days (day 12) after the obtention of normoglycemia (day 3), independently of changes in plasma TNF-α levels. We should also note that the glucose-dependent modulation of TNF-α production from human peripheral blood monocytes previously reported in vitro (12) is undetectable in vivo. This observation depends on the respective contribution of adipocytes and monocytes to regulate the plasma TNF-α levels, and we cannot exclude a detectable effect of hyperglycemia on TNF-α production from monocytes in lean patients.

In the second part of the present study (study 2), we analyzed the potential relationships between body fat content or distribution and the plasma TNF-α level in obese subjects with type 2 diabetes mellitus. This was particularly important because whole body fat mass and especially visceral fat mass is known to be related to insulin resistance and thus to type 2 diabetes mellit...
tus (17). This approach was possible since metabolic variations did not contribute to significant changes in the plasma level of the studied cytokine.

The whole body fat mass, as an absolute value or a percentage of total body weight, was not associated with plasma TNF-α levels. Our data would then exclude the contribution of the whole body fat mass to regulate the plasma TNF-α level as it was recently suggested by Katsuki et al. (11). However, we confirmed the correlation between plasma TNF-α levels and visceral adipose tissue area found by these authors in an overweight (but non obese) Japanese population. We also highlighted an independent relationship with the waist-to-hip circumference ratio which is a simple clinical indicator of visceral adipose tissue amount.

The bases of this relationship between plasma TNF-α level and visceral fat amount have yet to be clarified. It would suggest that the secretion of TNF-α by the adipose tissue may differ according to the site concerned with a higher secretion in the visceral adipose tissue when compared to the subcutaneous one. The absence of difference in the expression of TNF-α mRNA between omental and subcutaneous adipocytes in a recent study does not exclude this hypothesis (18), since no significant blood release of TNF-α could be detected in vivo from subcutaneous adipose tissue, in a recent study (19). The higher plasma TNF-α levels found in men than in women by Pfeiffer et al. (9) are in agreement with our data, since the visceral fat amount is classically higher in men than in women. Otherwise, the increased expression of PAI-1 by the adipose tissue after TNF-α treatment reported in a murine model, may suggest that the relationship between circulating PAI-1 level and android obesity could be partly related to an increased plasma level of TNF-α in this patho-physiological situation (20). These data and the other functions of TNF-α on endothelial cells and hepatocytes could also partly explain the increased cardiovascular risk associated with visceral obesity (21-23).

In conclusion, our results suggest that plasma TNF-α levels are essentially dependent on the visceral fat amount in obese type 2 diabetic patients. In addition, we demonstrate that plasma TNF-α levels of poorly controlled type 2 diabetic patients are not acutely influenced by intensive insulin therapy and consecutive rapid correction of hyperglycaemia.

REFERENCES