The association between leptin and insulin levels in adults with cystic fibrosis

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Abstract

Cystic fibrosis (CF)-related diabetes is an important complication of CF caused by a decrease in insulin secretion that is associated with weight loss, poor nutritional status and increased mortality. Leptin, a hormone secreted from white adipose tissue, has an important role in energy homoeostasis by inhibiting food intake and increasing energy expenditure. Leptin secretion can be increased by nutrient signals such as insulin.

Aims. – Considering that leptin plays a role in energy homoeostasis and that CF is associated to poor weight gain and decreased insulin secretion, leptin levels in CF patients with different glucose tolerances were investigated and compared with those of healthy control subjects.

Methods. – Two-hour oral glucose tolerance tests (OGTT) were performed in 82 patients with CF and various glucose tolerances as well as in 17 healthy control subjects during which blood was withdrawn every 30 min to measure glucose and insulin. Fasting leptin, fibrinogen and fat mass were also measured, and body mass index (kg/m\textsuperscript{2}) calculated for all participants. Early and late insulin secretion was separated by calculating the area under the curve from time 0 to 30 min and 30 to 120 min of the OGTT (AUC\textsubscript{0–30} and AUC\textsubscript{30–120}).

Results. – Leptin levels were comparable between CF patients and healthy control subjects. Interestingly, correlations were observed between leptin levels and insulin (AUC\textsubscript{0–120} and AUC\textsubscript{30–120}) after adjusting for gender and fat mass ($P<0.05$).

Conclusion. – This study suggests a potential role of insulin in regulating leptin levels in adults with stable CF.

Keywords: Cystic fibrosis; Cystic fibrosis-related diabetes; Leptin; Insulin

Résumé

La relation entre l’insuline et la leptine chez les adultes atteints de mucoviscidose est associée à la masse grasse et le sexe.

Le diabète, complication importante de la mucoviscidose (MV) causée par une diminution de la sécrétion d’insuline, est associé à une perte de poids et une augmentation de la mortalité. La leptine, hormone secrétée par le tissu adipeux, joue un rôle important dans l’homéostasie énergétique. L’insuline régule en partie la production et la sécrétion de leptine. La leptine pourrait donc jouer un rôle important dans le contrôle du poids en particulier chez les sujets diabétiques.

Objectif. – Examineraux niveaux de leptine de patients atteints de MV à différents stades de la tolérance au glucose comparativement à des témoins non diabétiques.

Méthodes. – Nous avons réalisé chez 82 patients atteints de MV et 17 témoins une hyperglycémie provoquée par voie orale (HGPO), un dosage de la leptine à jeun et du fibrinogène, une mesure de la masse grasse, et nous avons calculé l’indice de masse corporelle (kg/m\textsuperscript{2}). Nous avons étudié la sécrétion d’insuline précoce et tardive en calculant l’aire sous la courbe (AUC) du temps 0 à 30 minutes du temps 30 à 120 minutes de l’HGPO (AUC\textsubscript{0–30} et AUC\textsubscript{30–120}).

Résultats. – Les concentrations plasmatiques de leptine des patients atteints de MV et des témoins étaient semblables. Nous avons observé des corrélations significatives entre les concentrations plasmatiques de leptine et d’insuline (AUC\textsubscript{0–120} et AUC\textsubscript{30–120}) même après ajustement pour le sexe et la masse grasse ($P<0.05$).
Conclusions. – Cette étude suggère que l’insuline pourrait jouer un rôle dans la régulation des concentrations plasmatiques de leptine chez les adultes atteints de MV.

Mots clés : Mucoviscidose ; Diabète secondaire à la mucoviscidose ; Leptine ; Insuline

1. Introduction

1.1. Cystic fibrosis

Cystic fibrosis (CF) is the most common fatal autosomal-recessive disease in individuals of European descent [1,2]. CF is often associated to nutrient malabsorption, pancreatic exocrine and endocrine dysfunction, poor weight gain and chronic pulmonary infections [3–5]. In Canada, advances in CF treatment have increased the median life expectancy to over 40 years in 2009 [6]. Consequently, new complications associated with the disease have emerged, such as CF-related diabetes (CFRD). Symptoms of CFRD include weight loss, poor nutritional status and decline in lung function, and are often confused with those of pulmonary exacerbations [3,7]. CFRD is caused mainly by a decrease in insulin secretion [8,9].

1.2. Leptin

Leptin is a hormone that is mainly secreted by adipocytes in proportion to the amount of body fat [10,11]. It regulates thermogenesis, angiogenesis, immune responses and bone metabolism, among other processes [12]. It also has an important role in energy homoeostasis by inhibiting food intake and increasing energy expenditure [11,13]. Leptin secretion can be increased by nutrient signals such as insulin and by cytokines such as tumour necrosis factor (TNF)-α [14].

Given that leptin plays a role in energy homoeostasis, and that CF is associated with poor weight gain and decreased insulin secretion [3,7], the present study investigated leptin levels in patients with CF and different glucose tolerances, and compared them with healthy control subjects. In addition, the study examined whether factors other than fat mass, such as insulin, might also modulate leptin levels in CF adults. It was hypothesized that, independent of fat mass, leptin levels would be decreased in CF patients and particularly in those with CFRD because of their impaired insulin secretion.

2. Patients and methods

Eighty-two CF adults from the Montreal Cystic Fibrosis Cohort (MCFC), and 17 healthy controls of comparable age and BMI, were recruited into the study from the University of Montreal’s Hospital Centre (Centre hospitalier de l’université de Montréal [CHUM]) through advertisements. The MCFC cohort was established in 2004 as part of an ongoing systematic CFRD screening programme, using an oral glucose tolerance test (OGTT) [8]. All CFRD patients included in the study were newly diagnosed. The main objectives of this prospective observational cohort were to study the mechanisms leading to glucose intolerance as well as the association of prediabetic states with CF outcomes. CF patients included in this analysis were enrolled consecutively at baseline. The protocol was approved by the CHUM Research Ethics Committee, and all participants received a signed copy of the consent form. No patients included in the study were using any medication that could interfere with glucose metabolism, including oral antidiabetic drugs and insulin, nor diagnosed with a pulmonary exacerbation in the previous month by a pulmonologist [8]. All CF patients awaiting or having undergone lung transplantation were also excluded from the study.

2.1. Oral glucose tolerance test (OGTT)

All participants underwent a 2-h OGTT to confirm glucose tolerance. After at least 8 h of fasting, the subjects ingested 1.75 g of glucose per kg of body weight up to a maximum of 75 g. Venous blood samples were taken at time 0, 30, 60, 90 and 120 min. Glucose tolerance was determined according to criteria of the Canadian Diabetes Association [15].

2.2. Biochemical dosages

Immediately after sampling, plasma glucose was measured in duplicate by the glucose oxidase method (Beckman Coulter, Fullerton, CA, USA). Radioimmunoassay kits were used to quantify, in duplicate, insulin levels during the OGTT and fasting plasma leptin (Linco, St. Charles, MO, USA). For all experiments, intra- and interassay variation coefficients did not exceed 15%.

2.3. Clinical parameters

Using an electronic scale (Tanita Corporation, Arlington Heights, IL, USA) to record body weight and a standard wall stadiometer to measure height, the participants’ body mass index (BMI) scores (kg/m²) were calculated. Fat mass was determined for all subjects by bioimpedance [16] (Tanita Corporation) after an overnight fast. Forced expiratory volume in 1 s (% FEV1) for CF patients was obtained by spirometry (Medgraphics 1870, St. Paul, MN, USA).

2.4. Insulin secretion and sensitivity

Insulin secretion was quantified using the insulin area under the curve (AUC0–120). Early and late insulin secretion were then separated by calculating two AUCs, one from baseline to 30 min (AUC0–30) and the other from 30 to 120 min (AUC30–120). The AUCs were calculated using GraphPad Prism 4.0 (GraphPad Software Inc., La Jolla, CA, USA) [17].

2.5. Statistics

Data are expressed as means ± standard deviation (SD) and percentages (%). Statistical analysis was done with SPSS 16.0.
for Windows (Chicago, IL, USA). Normality of all variables was determined by the Kolmogorov–Smirnov test. Fasting leptin, 2-h glucose, fasting insulin, and insulin AUC$_{0-30}$ and AUC$_{30-120}$ were not normally distributed and, for this reason, were log-transformed. Two-way analysis of variance (Anova) was used to determine whether leptin levels were different between men and women, and the glucose tolerance groups compared to the controls. If statistically significant differences were found between group means, a least significant difference (LSD) post-hoc test was then used. Also, Pearson’s correlations, corrected for gender and/or fat mass, were used to examine the association between leptin and different variables. Statistical significance was accepted at $P \leq 0.05$.

3. Results

3.1. Characteristics of controls and Cystic fibrosis (CF) patients

The characteristics of the 17 control subjects and 82 CF patients (49 with normal glucose tolerance [NGT], 22 with impaired glucose tolerance [IGT] and 11 with CFRD) included in the present study are presented in Table 1. There were no significant differences in age, BMI, percent fat mass and leptin between the healthy controls and CF men or CF women. On the other hand, significant gender differences were observed for these parameters. Women in the healthy control, CF-NGT and CF-IGT groups had approximately two times the amount of body fat as the men, and around two to five times more leptin. Men with CF had higher levels of fibrinogen (an inflammatory marker) compared with their healthy counterparts ($P < 0.05$).

3.2. Leptin levels and body composition

As shown in Figs. 1 and 2, leptin levels in the CF patients correlated with BMI ($P = 0.002$; $r = 0.32$) and even more so with percent fat mass ($P < 0.001$; $r = 0.82$).

3.3. Insulin and glycaemia levels

Insulin and glucose levels obtained during the OGTT are shown in Table 2. The mean fasting glucose in CFRD patients was significantly higher than in the healthy controls. By design, all CF glucose tolerance groups had different mean 2-h glucose values. CF-NGT patients had a higher mean 2-h glucose value than the controls, while CFRD patients had more than twice the glucose AUC$_{0-120}$ of their healthy counterparts and higher values than the other CF groups. As expected, CF patients exhibited lower early-phase insulin secretion (AUC$_{0-30}$) than the control subjects. Similarly, within CF patient groups, a progressive trend

Table 1

Physical and metabolic parameters of healthy controls and patients with cystic fibrosis (CF).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Controls ($n=17$)</th>
<th>NGT ($n=49$)</th>
<th>IGT ($n=22$)</th>
<th>CFRD ($n=11$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (n)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>♂</td>
<td>7</td>
<td>29</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>♀</td>
<td>10</td>
<td>20</td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td>Age (years)</td>
<td>26.1 ± 4.5</td>
<td>26.2 ± 7.5</td>
<td>26.6 ± 6.9</td>
<td>30.0 ± 8.0</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.7 ± 3.7</td>
<td>28.5 ± 8.8</td>
<td>32.3 ± 10.3</td>
<td>31.5 ± 9.7</td>
</tr>
<tr>
<td>Fat mass (%)</td>
<td>23.1 ± 1.87</td>
<td>21.6 ± 2.85</td>
<td>21.4 ± 3.02</td>
<td>23.6 ± 2.33</td>
</tr>
<tr>
<td></td>
<td>21.9 ± 1.45</td>
<td>21.0 ± 2.54</td>
<td>21.8 ± 3.67</td>
<td>19.5 ± 2.43</td>
</tr>
<tr>
<td>FEV1 (%)</td>
<td>14.0 ± 3.32 *</td>
<td>13.6 ± 5.91 *</td>
<td>14.1 ± 6.50 *</td>
<td>18.9 ± 5.72</td>
</tr>
<tr>
<td></td>
<td>23.5 ± 5.14</td>
<td>23.4 ± 6.87</td>
<td>25.1 ± 8.49</td>
<td>18.7 ± 6.76</td>
</tr>
<tr>
<td>Fibrinogen (g/L)</td>
<td>2.08 ± 0.22</td>
<td>2.85 ± 0.55 **</td>
<td>2.81 ± 0.65 **</td>
<td>2.99 ± 0.41 **</td>
</tr>
<tr>
<td></td>
<td>2.43 ± 0.98</td>
<td>3.18 ± 0.54</td>
<td>3.07 ± 0.65</td>
<td>3.17 ± 0.89</td>
</tr>
<tr>
<td>Leptin*** (pg/mL)</td>
<td>1.57 ± 0.73 *</td>
<td>2.14 ± 1.90 *</td>
<td>1.81 ± 1.56 *</td>
<td>4.10 ± 3.66</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SD; NGT: normal glucose tolerance; IGT: impaired glucose tolerance; CFRD: cystic fibrosis-related diabetes; ♂: men; ♀: women; BMI: body mass index; FEV1: % forced expiratory volume in 1 s.

* $P < 0.05$ vs. women for the same parameter.
** $P < 0.05$ vs. controls.
*** Log-transformed data used in the statistical analyses.
Fig. 1. Correlation between the log of leptin and body mass index (BMI) of adults with cystic fibrosis (n=82; r=0.32; P<0.002).

Fig. 2. Correlation between the log of leptin and fat mass in adults with cystic fibrosis (n=82; r=0.82; P<0.001).

Table 2
Glycaemia and insulin values obtained from oral glucose tolerance tests in healthy controls and patients with cystic fibrosis (CF).

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=17)</th>
<th>NGT (n=49)</th>
<th>IGT (n=22)</th>
<th>CFRD (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fasting glucose (mmol/L)</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>♂</td>
<td>5.08 ± 0.75</td>
<td>5.53 ± 0.48***</td>
<td>5.47 ± 0.36</td>
<td>6.60 ± 0.77***</td>
</tr>
<tr>
<td>♀</td>
<td>4.74 ± 0.42</td>
<td>5.18 ± 0.34*</td>
<td>5.40 ± 0.41*</td>
<td>6.13 ± 0.76***</td>
</tr>
<tr>
<td><strong>2-h glucose (mmol/L)</strong>**</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>♂</td>
<td>4.56 ± 1.12</td>
<td>5.65 ± 1.19*</td>
<td>8.85 ± 0.76***</td>
<td>14.4 ± 1.5***</td>
</tr>
<tr>
<td>♀</td>
<td>5.22 ± 0.87</td>
<td>5.99 ± 0.96*</td>
<td>9.55 ± 0.98***</td>
<td>14.1 ± 2.6***</td>
</tr>
<tr>
<td><strong>Glucose AUC0–120</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>♂</td>
<td>665 ± 210</td>
<td>993 ± 169*</td>
<td>1190 ± 155***</td>
<td>1615 ± 265***</td>
</tr>
<tr>
<td>♀</td>
<td>359 ± 275</td>
<td>946 ± 199*</td>
<td>1151 ± 89***</td>
<td>1480 ± 249***</td>
</tr>
<tr>
<td><strong>Fasting insulin (pmol/L)</strong>***</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>♂</td>
<td>18.7 ± 15.6</td>
<td>10.3 ± 3.9*</td>
<td>8.83 ± 4.02*</td>
<td>12.0 ± 1.8</td>
</tr>
<tr>
<td>♀</td>
<td>13.4 ± 7.37</td>
<td>10.2 ± 3.7</td>
<td>10.7 ± 3.5</td>
<td>8.74 ± 2.83*</td>
</tr>
<tr>
<td><strong>Insulin AUC0–120</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>♂</td>
<td>5177 ± 2257</td>
<td>4444 ± 2148***</td>
<td>4189 ± 2111</td>
<td>3963 ± 1305</td>
</tr>
<tr>
<td>♀</td>
<td>6538 ± 3227</td>
<td>5671 ± 2339</td>
<td>4346 ± 1434</td>
<td>3775 ± 2198</td>
</tr>
<tr>
<td><strong>Insulin AUC0–30</strong>***</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>♂</td>
<td>1156 ± 738</td>
<td>590 ± 224*</td>
<td>491 ± 234*</td>
<td>534 ± 109*</td>
</tr>
<tr>
<td>♀</td>
<td>1236 ± 607</td>
<td>729 ± 276*</td>
<td>584 ± 184*</td>
<td>389 ± 120***</td>
</tr>
<tr>
<td><strong>Insulin AUC30–120</strong>***</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>♂</td>
<td>4020 ± 1740</td>
<td>3854 ± 1967***</td>
<td>3698 ± 1914</td>
<td>3428 ± 1337</td>
</tr>
<tr>
<td>♀</td>
<td>5303 ± 2688</td>
<td>4942 ± 101</td>
<td>3761 ± 1302</td>
<td>3386 ± 2088</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SD; NGT: normal glucose tolerance; IGT: impaired glucose tolerance; CFRD: cystic fibrosis-related diabetes; ♂: men; ♀: women; AUC: area under the curve.

* P<0.05 vs. controls.
** P<0.05 vs. NGT
*** P<0.05 vs. IGT.
**** P<0.05 vs. women for the same parameter.
***** Log-transformed data used in the statistical analyses.
towards insulin deficiency was observed from CF-NGT to CFRD patients.

Associations between leptin and insulin are presented in Table 3. All parameters were significantly correlated with leptin levels when adjusted for gender ($P \leq 0.001$). These associations were maintained for insulin AUC$_{0-120}$ and AUC$_{30-120}$ when adjusted for both gender and fat mass ($P \leq 0.022$), whereas a trend towards fasting insulin and insulin AUC$_{0-30}$ was observed. On the other hand, there were no associations between leptin and glucose values from the OGTT (fasting glucose, 2-h glucose and glucose AUC$_{0-120}$) and fibrinogen (data not shown).

### 4. Discussion

Despite reduced insulin levels and contrary to our hypothesis, our studied CF adults displayed leptin levels comparable to those of their healthy counterparts. There was also a positive association between leptin and insulin levels during an oral glucose challenge in stable CF adults with moderate disease. Leptin is an essential hormone for energy homoeostasis [11,13] and, thus, could be of critical importance in CF, a disease in which body weight is closely associated with life expectancy [18]. Leptin is proportional to fat mass [10,11], but additional factors also contribute to modulate levels of the hormone [14]. In CF patients and especially in those with CFRD, reduced plasma insulin concentrations [8,9] could be contributing to lower levels of leptin.

Our finding that CF individuals have similar levels of leptin compared with controls is similar to that of Arumugam et al. [19], who reported that the physiological regulation of leptin in relation to body fat is maintained in CF. However, most studies investigating leptin levels in CF were undertaken in children and adolescents, and reported higher levels of leptin in these populations compared with their healthy peers [20–23]. In CF adults, Cohen et al. [16] reported higher levels of leptin than in controls. The discordance between their results and our present findings could be due to the fact that, while Cohen et al. found elevated leptin levels in individuals with severe disease, the subjects in our study were at an earlier stage of the disease. This is supported by the fact that, in our study, controls were selected because of their similar BMI and age range, and no differences in body composition between the CF patients and healthy controls were observed. However, there is still the possibility that, in a more advanced stage of the disease, CF patients—and especially those with CFRD—have decreased insulin secretion, increased inflammation [12] and a negative energy balance [24], that leptin levels would be increased in CF patients compared with healthy controls. As was previously reported in CF patients [19], gender differences in leptin levels, as well as their association with fat mass, were also observed in the present study.

Nevertheless, this is the first study to examine whether insulin is associated with leptin levels in CF. Animal and in vitro studies have demonstrated that insulin has a positive effect on leptin secretion and, in some studies, on mRNA expression [14,25]. Thus, our study examined the association between insulin and leptin in CF patients. As hypothesized, strong associations between leptin levels and different insulin values obtained during a glucose challenge, even after correcting for confounding factors such as fat mass and gender, were observed (Table 3). This suggests that insulin and fat mass may possibly be involved in regulating leptin levels in CF adults, as was previously established in healthy controls [10]. On the other hand, it fails to explain why CF patients and particularly those with CFRD, who are affected by abnormal insulin secretion, have similar levels of leptin as do the controls.

Apart from inflammation due to repeated bacterial infections, subclinical inflammation is a hallmark of CF [3]. Inflammatory cytokines such as TNF-$\alpha$ and interleukin (IL)-6 have been shown to increase leptin levels in cellular and animal models [16]. Therefore, the impaired insulin secretion associated with lower leptin levels, counterbalanced by the proinflammatory state of CF patients [3] that is associated with higher leptin levels, might explain why CF adults have similar levels of leptin compared with their healthy peers.

### 5. Conclusion

Thus, the present study demonstrates the potential role of insulin in regulating leptin levels in adults with stable CF and moderate disease. However, the clinical relevance of this association remains to be established in more advanced disease, where weight maintenance is crucial. The evaluation of leptin levels after the initiation of insulin therapy in CF patients might also help to clarify the relationship between leptin and insulin.

### Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.
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