Circulating glucagon is associated with inflammatory mediators in metabolically compromised subjects

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Abstract

Background: Acute phase mediators promote metabolic changes by modifying circulating hormones. However, there is virtually no data about the link between glucagon and inflammatory parameters in obesity-related chronic low-grade inflammation.

Study design: We performed both cross-sectional and longitudinal (diet-induced weight loss) studies.

Methods: Circulating glucagon concentrations (ELISA), parameters of glucose and lipid metabolism, interleukin 6 (IL6), and complement factor B (CFB) were analyzed in 316 subjects (250 men and 66 women). The effects of weight loss were investigated in an independent cohort of 20 subjects.

Results: Circulating glucagon significantly correlated with glucose \( r = 0.407, P < 0.0001 \), HbAlc \( r = 0.356, P = 0.001 \), and parameters of innate immune response system such as IL6 \( r = 0.475, P = 0.003 \) and CFB \( r = 0.624, P = 0.001 \) in obese subjects with altered glucose tolerance, but not in individuals with normal glucose tolerance (NGT). In obese and NGT subjects, glucagon was associated with fasting triglycerides \( r = 0.475, P = 0.003 \) and CFB \( r = 0.624, P = 0.001 \). In obese subjects, glucagon \( P = 0.019 \) and CFB \( P = 0.002 \) independently contributed to 26% of fasting triglyceride variance \( P < 0.0001 \) after controlling for the effects of age and fasting serum glucose concentration in multiple linear regression models. Moreover, concomitant with fat mass, fasting triglycerides, and CFB, weight loss led to significantly decreased circulating glucagon \( -23.1\%, P = 0.004 \).

Conclusions: According to the current results, acute phase reactants such as IL6 and CFB are associated with fasting glucagon in metabolically compromised subjects. This suggests that glucagon may be behind the association between inflammatory and metabolic parameters in obesity-associated chronic low-grade inflammation.

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Introduction

On a day-to-day basis, both energy and protein systemic metabolism is mainly controlled by two key pancreatic hormones, insulin and glucagon (1). Both are responsible for the energetic status in many tissues, operating against the background of the hormonal environment to maintain the balance between catabolism and anabolism. However, some situations such as the early phase after surgical trauma (2) or the metabolic response to severe acute phase sepsis (3, 4) change this balance dramatically.

Glucagon is a potent stimulator of gluconeogenesis in the liver (5). Patients with trauma, burn, or sepsis normally exhibit increased plasma levels of this counter-regulatory hormone to promote gluconeogenesis, increase circulating glucose, and compensate the energetic demand of the body during these extreme situations (6–8). Otherwise, the deregulation of glucagon levels (i.e. hyperglucagonemia) could lead to hyperglycemia and aggravate their negative effects in individuals with glucose intolerance (impaired glucose tolerance (IGT)) and type 2 diabetes (T2D). Indeed, several studies have focused on the deleterious effect of hyperglucagonemia, especially in subjects with altered glucose tolerance (AGT) and insulin resistance (9–12).

In recent years, it has become evident that alterations in the function of the innate immune system are intrinsically linked to metabolic pathways (13–16). Obesity, associated with glucose intolerance, insulin resistance, hypertension, and hypertriglyceridemia, is an integral feature of the so-called metabolic syndrome (14, 16). The altered production of pro-inflammatory cytokines seems to be directly implicated in obesity-related...
metabolic complications. Interleukin 6 (IL6), for example, induces important changes on the major endocrine axis and the intermediary metabolism (17) and acts on pancreatic β-cells inducing several hormonal mediators such as glucagon, norepinephrine, and insulin (18–20). The systemic hypermetabolism resulting in this relationship may promote or aggravate comorbidities in chronic diseases (2, 21).

The secretion of complement factor B (CFB) seems to be regulated by pro-inflammatory cytokines such as IL1β, tumor necrosis factor α (TNFα), and interferon γ (IFNγ) (22). The activation of the alternative pathway of the complement system could be a link between obesity and obesity-related metabolic disorders since CFB is expressed by adipose tissue and upregulated in obese and/or IGT subjects (23–25). CFB may influence the production of acylation-stimulating protein (ASP), which could play an important role in the regulation of fatty acid uptake and triglycerides formation (26).

Despite the extensive bibliography focusing on circulating glucagon levels, there are virtually no data about the link with inflammatory parameters. Therefore, we aimed to analyze fasting glucagon concentration in a cohort of subjects with a wide range of body mass index (BMI) and glucose tolerance, in whom we explored the associations with acute phase mediators such as IL6 and CFB. The main effects of diet-induced weight loss on fasting glucagon concentration were also evaluated in a cohort of obese individuals.

**Materials and methods**

**Patient recruitment**

In total 316 Caucasian subjects were studied. Of them 175 were randomly localized from a census and were invited to participate in the study. A 75 g oral glucose tolerance test (OGTT) according to the American Diabetes Association Criteria was performed on all subjects, as described previously (23). Insulin sensitivity ($S_i$) was measured using the frequently sampled intravenous GTT (FSIVGTT) in these subjects. The remaining 141 subjects were prospectively recruited from outpatient clinics of the Service of Diabetes, Endocrinology and Nutrition of the Hospital Dr Josep Trueta of Girona based on a stable metabolic control in the previous 6 months, as defined by stable HbA1c and fasting glucose values. Data from these patients were merged with those from the recently diagnosed T2D (120′ post-load glucose > 11.1 mM) and IGT (120′ post-load glucose between 7.8 and 11.1 mM in the OGTT) subjects and individuals with normal glucose tolerance (NGT). Therefore, estimated statistical power in obese subjects for single associations of circulating glucagon and, for example, fasting triglycerides increased from 77.6 to 96.3% in a bilateral approach. Insulin resistance was calculated in all subjects using the homeostasis model assessment of insulin resistance (HOMA-IR) value (glucose (mmol/l)×insulin (mU/l)/22.5), as earlier (27). All subjects gave written informed consent after

### Table 1 Clinical characteristics of subjects in the cross-sectional study. Data are mean±s.d. for Gaussian variables and median (interquartile range) for non-Gaussian variables (only circulating glucagon concentrations).

<table>
<thead>
<tr>
<th></th>
<th>NGT and non-obese subjects</th>
<th>AGT and non-obese subjects</th>
<th>NGT and obese subjects</th>
<th>AGT and obese subjects</th>
<th>P (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (men/women)</td>
<td>98 (89/9)</td>
<td>92 (80/12)</td>
<td>38 (22/16)</td>
<td>81 (54/27)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>48±12</td>
<td>56±11</td>
<td>45±12</td>
<td>56±10</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.7±2.6</td>
<td>26.5±2.3</td>
<td>32.9±3.1</td>
<td>33.7±3.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fat mass (%)</td>
<td>5.6±1.9</td>
<td>6.9±9.9</td>
<td>30.1±17.5</td>
<td>20.1±10.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>87.3±7.2</td>
<td>92.7±7.2</td>
<td>102.2±8.9</td>
<td>106.3±7.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Hip (cm)</td>
<td>96.6±8.0</td>
<td>97.3±5.4</td>
<td>107.7±6.7</td>
<td>104.0±17.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>WHR</td>
<td>0.90±0.06</td>
<td>0.95±0.07</td>
<td>0.95±0.08</td>
<td>1.00±0.06</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>122.2±16.5</td>
<td>134.2±19.4</td>
<td>131.1±15.3</td>
<td>136.5±18.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>76.7±11.6</td>
<td>80.4±8.4</td>
<td>84.1±10.3</td>
<td>83.0±10.0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fasting glucose (mg/dl)</td>
<td>92.3±8.1</td>
<td>141.6±65.7</td>
<td>93.5±7.6</td>
<td>150.2±79.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HbAlc (%)</td>
<td>4.77±0.34</td>
<td>6.17±1.72</td>
<td>4.92±0.31</td>
<td>6.54±1.79</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fasting insulin (mU/ml)</td>
<td>37.8±27.9</td>
<td>77.9±57.3</td>
<td>84.7±61.0</td>
<td>137.4±96.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Insulin sensitivitya</td>
<td>3.34±1.94</td>
<td>2.09±1.03</td>
<td>2.19±1.24</td>
<td>0.94±0.78</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.80±0.88</td>
<td>2.59±1.76</td>
<td>2.86±1.58</td>
<td>3.73±1.85</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>200.4±39.1</td>
<td>205.0±38.2</td>
<td>214.7±35.6</td>
<td>203.5±38.6</td>
<td>0.269</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dl)</td>
<td>54.4±13.7</td>
<td>52.5±14.0</td>
<td>51.7±12.3</td>
<td>49.3±12.7</td>
<td>0.094</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dl)</td>
<td>125.8±35.2</td>
<td>120.2±38.0</td>
<td>140.6±32.4</td>
<td>115.9±43.3</td>
<td>0.008</td>
</tr>
<tr>
<td>Fasting triglycerides (mg/dl)</td>
<td>102.0±89.8</td>
<td>163.0±135.2</td>
<td>112.8±63.7</td>
<td>176.9±91.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>C-reactive protein (mg/l)</td>
<td>0.25±0.24</td>
<td>0.48±0.72</td>
<td>0.48±0.41</td>
<td>0.58±0.65</td>
<td>0.001</td>
</tr>
<tr>
<td>IL6 (ng/l; n = 146)</td>
<td>1.05±0.63</td>
<td>1.04±0.39</td>
<td>0.94±0.34</td>
<td>1.47±0.73</td>
<td>0.002</td>
</tr>
<tr>
<td>CFB (µg/ml; n = 240)</td>
<td>236.6±62.8</td>
<td>282.9±92.1</td>
<td>260.9±79.2</td>
<td>346.1±127.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Glucagon (pg/ml)</td>
<td>361.3 (216.8–618.2)</td>
<td>369.9 (232.9–702.8)</td>
<td>340.4 (168.3–705.9)</td>
<td>450.1 (295.1–777.8)</td>
<td>0.038</td>
</tr>
</tbody>
</table>

NGT, normal glucose tolerance; AGT, altered glucose tolerance; BMI, body mass index; WHR, waist-to-hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; IL6, interleukin 6; CFB, complement factor B; HOMA-IR, homeostasis model assessment of insulin resistance. *P<0.05 and †P<0.0001 significance for Bonferroni’s post-hoc comparisons between each group and the control group (non-obese subjects with NGT).

*aInsulin sensitivity was measured in 175 subjects (112 subjects with NGT and 63 subjects with IGT) using the frequently sampled intravenous glucose tolerance test.
the purpose of the study was explained to them. The institutional review board of the Hospital Dr Josep Trueta of Girona (Girona, Spain) approved the protocol.

**Study of the effects of weight loss**

Twenty Caucasian obese volunteers with NGT (eight men and 12 women) attending the Endocrinology Department at the University Clinic of Navarra were recruited. Patients underwent a clinical assessment including medical history, physical examination, body composition analysis, comorbidity evaluation, as well as nutritional interviews performed by a multidisciplinary consultation team. Weight loss was achieved by prescription of a diet providing a daily energy deficit of 500–1000 kcal/day as calculated from the determination of the resting energy expenditure through indirect calorimetry (Vmax29, SensorMedics Corporation, Yorba Linda, CA, USA) and multiplication by 1.4 as indicated for sedentary individuals to obtain the patients’ total energy expenditure. This hypocaloric regime allows a safe and steady weight loss of 0.5–1.0 kg/week when followed and supplied 30, 54, and 16% of energy requirements in the form of fat, carbohydrates, and protein, respectively, as described previously (28). The institutional review board of the University Clinic of Navarra (Navarra, Spain) approved the protocol. The estimated statistical power for single comparisons between glucagon concentrations in serum before and after diet-induced weight loss was 50.9% (n = 20) in a bilateral approach.

**Anthropometric measurements**

Bioelectric impedance was used to estimate body fat composition as before (29). Subjects were then classified as non-obese (BMI < 30.0 kg/m²) and obese (BMI ≥ 30.0 kg/m²) with and without AGT. Clinical characteristics are shown in Table 1.

**Insulin sensitivity**

$S_i$ was measured using the FSIIVGTT in those subjects who agreed (n = 175), as described previously (23).

**Analytical determinations**

Fasting glucagon concentration was measured using a competitive enzyme immunoassay (Gentaur BVBA, Brussels, Belgium), with high specificity to pancreatic glucagon and no cross-reactivity with intestinal glucagon, GLP-1 or GLP-2. Analytical sensitivity was > 50 pg/ml. CFB concentrations were measured by a sandwich ELISA in a subpopulation of 240 subjects, as described previously (23). Serum IL6 concentrations were measured in a subgroup of 146 subjects using a solid-phase, enzyme-labeled, chemiluminescent sequential immunometric assay (DPC Dipesa, Madrid, Spain).

**Table 2** Correlations between circulating glucagon concentrations and study variables in the cross-sectional study.

<table>
<thead>
<tr>
<th></th>
<th>NGT and N-O subjects n = 102</th>
<th>AGT and N-O subjects n = 92</th>
<th>NGT and obese subjects n = 38</th>
<th>AGT and obese subjects n = 81</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting glucose (mg/dl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>0.081</td>
<td>0.201</td>
<td>−0.210</td>
<td>0.407</td>
</tr>
<tr>
<td>Fasting triglycerides (mg/dl)</td>
<td>0.129</td>
<td>0.010</td>
<td>0.105</td>
<td>0.475</td>
</tr>
<tr>
<td>IL6 (ng/l; n = 146)</td>
<td>−0.035</td>
<td>0.363</td>
<td>−0.194</td>
<td>0.624</td>
</tr>
<tr>
<td>CBP (µg/ml; n = 240)</td>
<td>−0.027</td>
<td>0.034</td>
<td>0.624</td>
<td>0.404</td>
</tr>
</tbody>
</table>

NGT, normal glucose tolerance; N-O, non-obese; AGT, altered glucose tolerance; IL6, interleukin 6; CFB, complement factor B. Significant data are shown in bold.

![Figure 1 Linear relationships (continuous line) between circulating glucagon concentration and fasting glucose (Fig. 1a; P < 0.0001), HbA1c (Fig. 1b; P < 0.0001), and fasting triglycerides (Fig. 1c; P = 0.001) in obese subjects with altered glucose tolerance (AGT). The linear relationships (discontinuous line) between circulating glucagon concentration and these parameters are also represented for the other subjects (non-obese and obese subjects with NGT or non-obese with AGT). Values for non-obese and NGT individuals are represented as empty big circles (open circle); non-obese but AGT as full small circles (filled circle); obese and NGT as empty big diamonds (open diamond); and obese and AGT subjects are full small diamonds (filled diamond).](via free access)
Glucagon (pg/ml) 715.5 (562.1–1090.7) 602.8 (460.6–757.4) 

KCFB (Leptin (ng/ml) 41.9 

Fasting triglycerides (mg/dl) 101.3 
LDL-cholesterol (mg/dl) 127.2 
HDL-cholesterol (mg/dl) 57.7 
Total cholesterol (mg/dl) 204.5 
HOMA-IR 2.9 
Fasting insulin (mUI/ml) 12.8 
Fasting glucose (mg/dl) 94.4 
DBP (mmHg) 80.4 
SBP (mmHg) 16.2 
BMI (kg/m²) 111.6 
Waist (cm) 30.4 
Weight (kg) 331.5 
Fat mass (kg) 16.8 
Age (years) 44 
Number of participants 20 (8 men and 12 women) 

Analytical sensitivity was <0.5 pg/ml. No cross-reactivity with other cytokines was detected. Intra- and inter-assay coefficients of variation were <10% for all determinations. Other biochemical measurements were performed by routine laboratory tests, as described previously (7).

Statistical analyses

Normal distribution and homogeneity of the variances were evaluated using Levene’s test. Unless otherwise stated, descriptive results of continuous variables are expressed as mean ± s.d. for Gaussian variables. The relation between variables was compared using Pearson’s test. General linear models were also used to calculate fasting triglyceride concentrations after adjusting for several variables. One-factor ANOVA with post hoc Bonferroni’s test was used for comparisons of quantitative variables. Paired Student’s t-tests were used to compare parameters at the baseline and post-weight loss. Levels of statistical significance were set at P<0.05. The statistical analyses were performed using the program SPSS (version 13.0).

Results

Cross-sectional study

Anthropometrical, biochemical, and clinical variables of the participants in the cross-sectional study are shown in Table 1. Circulating glucagon concentrations were associated with obesity and AGT, as far as obese and AGT subjects showed ~20% (P=0.039) more circulating glucagon than non-obese subjects with NGT (Table 1). In fact, circulating glucagon significantly correlated with fasting glucose (r=0.133, P=0.019), HbAlc (r=0.119, 0.038), fasting triglycerides (r=0.174, P=0.002), and CFB (r=0.197, P=0.002) in the whole cohort. It should be noted that these relationships were mainly due to the inclusion of subjects with obesity and AGT in the study (Table 2). However, no significant associations were found between circulating glucagon concentrations and Sf measures such as HOMA-IR and SI values.

In obese subjects with AGT, glucagon concentration significantly correlated with fasting glucose (r=0.407, P<0.0001; Fig. 1a), HbAlc (r=0.426, P<0.0001; Fig. 1b), and fasting triglycerides (r=0.356, P=0.001; Table 3). Changes in glucagon concentrations were not significantly associated with any other circulating cytokines.

Figure 2 Linear relationships (continuous line) between circulating glucagon concentration, IL6 (Fig. 2a; P=0.05), and complement factor B (Fig. 2b; P=0.002) in obese subjects with altered glucose tolerance (AGT). The linear relationships (discontinuous line) between circulating glucagon concentration and these parameters are also represented for the other subjects (non-obese and obese subjects with NGT or non-obese with AGT). Values for non-obese and NGT individuals are represented as empty big circles (open circle); non-obese but AGT as full small circles (filled circle); obese and NGT as empty big diamonds (open diamond); and obese and AGT subjects as full small diamonds (filled diamond).

Table 3 Subjects’ characteristics in the weight loss study. Data are mean ± s.d. for Gaussian variables and median (inter-quartile range) for non-Gaussian variables (only glucagon concentration).

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Post-weight loss</th>
<th>Percentage of reduction</th>
<th>Student’s t-test paired samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of participants</td>
<td>20 (8 men and 12 women)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>44 ± 16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>102.0 ± 30.5</td>
<td>86.6 ± 19.2</td>
<td>16.0 ± 10.0</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>36.9 ± 8.3</td>
<td>31.2 ± 5.8</td>
<td>15.5 ± 9.8</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>43.4 ± 7.9</td>
<td>38.9 ± 9.4</td>
<td>16.2 ± 8.7</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>111.6 ± 18.9</td>
<td>99.8 ± 14.9</td>
<td>10.6 ± 11.7</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>128.0 ± 16.2</td>
<td>119.3 ± 12.7</td>
<td>6.8 ± 8.8</td>
<td>0.033</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>80.4 ± 11.6</td>
<td>75.3 ± 6.2</td>
<td>6.4 ± 7.1</td>
<td>0.065</td>
</tr>
<tr>
<td>Fasting glucose (mg/dl)</td>
<td>94.4 ± 13.8</td>
<td>93.9 ± 15.5</td>
<td>0.5 ± 15.0</td>
<td>0.896</td>
</tr>
<tr>
<td>Fasting insulin (mU/ml)</td>
<td>12.8 ± 7.6</td>
<td>8.6 ± 6.4</td>
<td>32.8 ± 8.3</td>
<td>0.016</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.9 ± 2.2</td>
<td>1.8 ± 1.4</td>
<td>39.2 ± 12.0</td>
<td>0.042</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>204.5 ± 34.1</td>
<td>175.6 ± 23.7</td>
<td>14.1 ± 12.5</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dl)</td>
<td>57.7 ± 13.0</td>
<td>52.6 ± 9.9</td>
<td>8.8 ± 6.0</td>
<td>0.076</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dl)</td>
<td>127.2 ± 30.4</td>
<td>105.8 ± 23.5</td>
<td>16.8 ± 9.8</td>
<td>0.001</td>
</tr>
<tr>
<td>Fasting triglycerides (mg/dl)</td>
<td>101.3 ± 24.4</td>
<td>83.2 ± 28.4</td>
<td>17.8 ± 9.7</td>
<td>0.021</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>41.9 ± 18.3</td>
<td>24.8 ± 18.1</td>
<td>40.8 ± 10.6</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>CFB (µg/ml)</td>
<td>331.5 ± 71.2</td>
<td>291.8 ± 57.2</td>
<td>12.0 ± 8.9</td>
<td>0.032</td>
</tr>
<tr>
<td>Glucagon (pg/ml)</td>
<td>715.5 (562.1–1090.7)</td>
<td>602.8 (460.6–757.4)</td>
<td>23.1 ± 10.0</td>
<td>0.004</td>
</tr>
</tbody>
</table>

BMI, body mass index; WHR, waist-to-hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; HOMA-IR, homeostasis model assessment of insulin resistance; CFB, complement factor B.

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In obese and NGT subjects, circulating glucagon was only associated with fasting triglycerides \((r=0.475, P=0.003)\) and with CFB \((r=0.624, P=0.001; \) Table 2). Interestingly, glucagon concentration also correlated with parameters of innate immune response system such as IL6 \((r=0.342, P=0.050; \) Fig. 2a) and CFB \((r=0.404, P=0.002; \) Fig. 2b) in AGT subjects with obesity but not in non-obese individuals with NGT (Table 2). Circulating glucagon was associated with IL6 \((r=0.363, P=0.013)\) in non-obese but AGT subjects (Table 2).

Correlations between glucagon levels, inflammatory parameters, and parameters of glucose and lipid metabolism were strengthened when obesity was taken into account (Table 2). In obese (but not in non-obese) subjects, fasting glucagon \((P=0.019)\), and CFB \((P=0.002)\) independently contributed to 26% of fasting triglyceride variance \((P<0.0001)\) after controlling for the effects of age and fasting glucose in multiple linear regression models.

**Weight loss study**

Characteristics of the subjects are shown in Table 3. In this independent cohort of obese subjects with NGT and diet-induced weight loss led to significantly decreased circulating glucagon concentration \((-23.1\%, P=0.004)\). In agreement with this data, the decrease in leptin, CFB, total cholesterol, low-density lipid, and fasting triglyceride concentrations was parallel to that of fasting glucagon (Table 3). However, although no significant associations were found between circulating glucagon concentrations and \(S_1\) measures, associations of glucagon \((r=0.499, P=0.058)\) and CFB \((r=0.654, P=0.008)\) with fasting triglycerides were present. Both fasting glucagon \((P=0.018)\) and CFB \((P=0.003)\) significantly explained together 64.8% \((P=0.002)\) of fasting triglyceride variance in plasma.

**Conclusions**

Many pro-inflammatory cytokines such as IL6, IL1β, TNFα, and IFNγ have been described to modify both endocrine and exocrine pancreas secretions at pharmacological doses, (18, 22). The novel findings of this study are as follows: i) circulating IL6 was associated with fasting glucagon concentration in subjects with AGT but not in NGT subjects; ii) CFB concentration correlated with fasting glucagon in obese subjects; and iii) both circulating glucagon and CFB concentrations contribute to explain the circulating triglycerides in obese subjects. In agreement with these data, diet-induced weight loss led to concomitant decreases in circulating CFB, fasting glucagon, and triglycerides in obese subjects.

Pro-inflammatory cytokines such as IL6 play a pivotal role in maintaining the glucose homeostasis and avoiding hypoglycemia in extreme physiological processes. In situations such as the early phase after surgical trauma and in the metabolic response to severe acute phase sepsis, circulating pro-inflammatory cytokines are highly upregulated (30, 31). The dose-dependent acute metabolic responses to IL6 have been well analyzed in healthy subjects by administering recombinant IL6 to mimic the acute inflammatory state of sepsis (2, 21). IL6 seems to affect pancreatic β-cells by inducing the expression of glucagon (6–8). Patients with trauma, burn, or sepsis normally exhibit a concomitant increase in plasma of this counter-regulatory hormone and IL6 levels (32), probably to compensate the extreme energetic demands of these clinical situations. Otherwise, skeletal muscle cells release IL6 to activate hepatic gluconeogenesis by increasing circulating glucagon (33). Finally, in subjects with IGT, increased circulating glucagon levels have been reported (10, 11) and, in T2D individuals, pro-inflammatory cytokines also lead to upregulation of different counter-regulatory hormones such as glucagon. In fact, this finding is similar to the classical observation that hyperglycemia cannot suppress glucagon secretion in patients with T2D (34).

In the hormonal control of lipolysis, glucagon plays a pivotal role stimulating lipolysis in adipose tissue and promoting fatty acid oxidation in hepatocytes (35). In this study, we describe the relationship between fasting glucagon and triglyceride concentrations in obese subjects, especially those that had shown IGT. These results are in line with glucagon infusion leading to decreased basal lipid oxidation and enhanced ability of insulin to inhibit lipid oxidation and augment lipid synthesis in classical studies in healthy subjects (36, 37). However, IL6 also influenced lipid metabolism (38) and induced hypertriglyceridemia in experimental models. The joint effects of increased IL6 and glucagon concentrations could contribute to dyslipidemia and hypertriglyceridemia in subjects with AGT.

Otherwise, it has been suggested that the activation of the alternative pathway of the complement system could be a link between obesity and obesity-related metabolic disorders such as hypertriglyceridemia (23). Levels of CFB and C3 were also higher in subjects with insulin resistance and other features of the metabolic syndrome (25). CFB is produced by adipose tissue where they likely influence the production of the anaphylatoxin C3a and its carboxypeptidase B-anaphylatoxic-inactivated derivative C3adesArg (ASP). Both ASP/C3adesArg and C3a interact with the receptor C5L2 to effectively stimulate triglyceride synthesis in cultured adipocytes (39). C3 knockout (C3KO) mice are obligatorily ASP deficient and show lipid abnormalities (40). In humans, ASP levels are increased in obesity, T2D, and in individuals at risk of arterial disease, including those with hypertension, T2D, dyslipidemia, and coronary artery disease, whereas exercise or weight loss decreases ASP levels (41).
Current results, accordingly with these previous reports, suggest a relationship between the activation of the alternative pathway of complement and the obesity-related hypertriglyceridemia that commonly shows obese subjects. In agreement with this, significant associations were reported between circulating CFB, fasting glucagon, and triglycerides in a small group of obese subjects before diet-induced weight loss but not after the treatment.

In summary, inflammatory mediators such as IL6 and CFB were associated with fasting glucagon concentrations in plasma, but specifically in obese subjects with the highest serum glucose concentration and AGT. The higher triglyceride concentration in subjects with the highest glucagon levels suggests that the latter may contribute to dyslipidemia to some extent. The concentration of CFB, whose secretion has recently been demonstrated in the exocrine pancreas, seems to be regulated by pro-inflammatory cytokines, and may also contribute to hypertriglyceridemia and is therefore evaluated. Finally, diet-induced weight loss led to concomitant reduction in fat mass, CFB, glucagon, and fasting triglycerides in obese subjects, and then the relationship between these metabolic and inflammatory parameters disappeared. However, further investigations will be required to evaluate the functional consequences of these findings, the causality of the relationships reported here, and the specific participation of each factor in hypertriglyceridemia as a common feature of the metabolic syndrome, which remains elusive.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement
All authors of this manuscript have directly participated in the execution and analysis of the study. F J Ortega drafted the manuscript, designed the study, participated in the analysis of biochemical variables, and performed the statistical analysis. J M Moreno-Navarrete and M Sabater analyzed biochemical variables. G Frühbeck investigated the diet-induced weight loss study. W Ricart and J M Fernández-Real obtained the anthropometrical characteristics and the written consent of patients participated in the conception and the coordination of the study. J M Fernández-Real carried out the conception, design, and coordination of the study and helped with the statistical analysis.

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