Circulating glucagon is associated with inflammatory mediators in metabolically compromised subjects

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Running title: Glucagon, inflammation and metabolism.

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Summary

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 (IL-6), 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), glycated hemoglobin (r=0.426, p<0.0001), fasting triglycerides (r=0.356, p=0.001), and parameters of innate immune response system such as IL-6 (r=0.342, p=0.050) and CFB (r=0.404, p=0.002) in obese subjects with altered glucose tolerance (AGT), 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) contributed independently to 26% of fasting triglycerides variance (p<0.0001) after controlling for the effects of age and fasting serum glucose concentration in multiple linear regression models. Moreover, concomitantly with fat mass, fasting triglycerides, and CFB, weight loss led to significantly decreased circulating glucagon (-23.1%, p=0.004).

Conclusions: According to current results, acute phase reactants such as IL-6 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.
Introduction

One a day-to-day basis, both energy and protein systemic metabolism is mainly controlled by two key pancreatic hormones, insulin and glucagon (1). They are both 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 dramatically this balance.

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 (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 to 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 (IL)-6, for example, induces important changes on the major endocrine axis and the intermediary metabolism (17) and acts on pancreatic beta-cells inducing several hormonal mediators such as glucagon, norepinephrine, and insulin (18-20). The systemic hypermetabolism resulting in this relationship may promote or aggravate co-morbidities in chronic diseases (2; 21).

The secretion of complement factor B (CFB) seems to be regulated by pro-inflammatory cytokines such as interleukin (IL)-1 beta, tumor necrosis factor (TNF)-alpha, and interferon (IFN)-gamma (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 up-regulated 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. Thereby, 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 IL-6 and CFB. The main effects of diet-induced weight loss on fasting glucagon concentration were also evaluated in a cohort of obese individuals.
Research Design and Methods

Patient recruitment

Three hundred and sixteen Caucasian subjects were studied. 175 were randomly localized from a census and were invited to participate. A 75-g oral glucose tolerance test (OGTT) according to the American Diabetes Association Criteria was performed in all subjects, as previously described (23). Insulin sensitivity (SI) was measured using the frequently sampled intravenous glucose tolerance test (FSIVGTT) in these subjects. Otherwise, 141 subjects were prospectively recruited from outpatient clinics of the Service of Diabetes, Endocrinology and Nutrition of the Hospital Dr. Josep Trueta of Girona on the basis of 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 type 2 diabetes (T2D; 120’ post-load glucose > 11.1 mM) and impaired glucose tolerance (IGT; 120’ post-load glucose between 7.8 and 11.1 mM in the OGTT) subjects, and individuals with normal glucose tolerance (NGT). Thereby, estimate 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 HOMA-IR value [glucose (mmol/l) × insulin (mU/l)/ 22.5], as before (27). All subjects gave written informed consent after 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 (8 men, 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, co-morbidity 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 500-1,000 kcal/d as calculated from the determination of the resting energy expenditure through indirect calorimetry (Vmax29, SensorMedics Corporation, Yorba Linda, California) and multiplication by 1.4 as indicated for sedentary individual’s to obtain the patient’s total energy expenditure. This hypocaloric regime allows a safe and steady weight loss of 0.5-1.0 kg/wk when followed and supplied 30, 54 and 16% of energy requirements in the form of fat, carbohydrates and protein, respectively, as previously described (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**

Insulin sensitivity was measured using the frequently sampled intravenous glucose tolerance test (FSIVGTT) in those subjects who agreed (n=175), as previously described (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 nor GLP-2. Analytical sensitivity was > 50 pg/ml. Complement factor B (CFB) concentrations were measured by a sandwich ELISA in a subpopulation of 240 subjects, as previously described (23). Serum IL-6 concentrations were measured in a subgroup of 146 subjects using a solid-phase, enzyme-labelled, chemiluminescent sequential immunometric assay (DPC Dipesa; Madrid, Spain). Analytical sensitivity was < 0.5 pg/ml. No cross-reactivity with other cytokines was detected. Intra- and inter-assay coefficients of variation were under 10% for all determinations. Other biochemical measurements were performed by routine laboratory tests, as previously described (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 ± SD for Gaussian variables. The relation between variables was tested using Pearson's test. General linear models were also used to calculate fasting triglycerides concentrations after adjusting for several variables. One factor ANOVA with post-hoc Bonferroni’s test was used for comparisons of quantitative variables. Paired 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 altered glucose tolerance (AGT), as far as obese and AGT subjects showed ~20% (p=0.039) more circulating glucagon than non-obese subjects with normal glucose tolerance (NGT) (Table 1). In fact, circulating glucagon significantly correlated with fasting glucose (r=0.133, p=0.019), glycated hemoglobin (r=0.119, 0.038), fasting triglycerides (r=0.174, p=0.002), and complement factor B (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 insulin sensitivity measures such as HOMA-IR and $S_I$ values.

In obese subjects with altered glucose tolerance, glucagon concentration significantly correlated with fasting glucose (r=0.407, p<0.0001; Fig.1a), glycated hemoglobin (r=0.426, p<0.0001; Fig.1b), and fasting triglycerides (r=0.356, p=0.001; Fig.1c). 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 IL-6 (r=0.342, p=0.050; Fig.2a) and complement factor B (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 IL-6 (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 in account (Table 2). In obese (but not in non-obese) subjects, fasting glucagon (p=0.019) and CFB (p=0.002) contributed independently to 26% of fasting triglycerides variance (p<0.0001) after controlling for the effects of age and fasting glucose in multiple lineal regression models.
**Weight loss study**

Characteristics of the subjects are shown in *Table 3*. In this independent cohort of obese subjects with normal glucose tolerance, 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 lipids, and fasting triglycerides concentrations was parallel to that of fasting glucagon (*Table 3*). On the other hand, although no significant associations were found between circulating glucagon concentrations and insulin sensitivity measures, associations of glucagon (\( r=0.499, \ p=0.058 \)) and CFB (\( r=0.654, \ p=0.008 \)) with fasting triglycerides were also present. Both fasting glucagon (\( p=0.018 \)) and CFB (\( p=0.003 \)) significantly explained together 64.8% (\( p=0.002 \)) of fasting triglycerides variance in plasma.
Conclusions

Many pro-inflammatory cytokines such as IL-6, IL-1 beta, TNF-alpha, and IFN-gamma have been described to modify, at pharmacological doses, both endocrine and exocrine pancreas secretion (18; 22). The novel findings of this study are: 1) circulating IL-6 was associated with fasting glucagon concentration in subjects with altered glucose tolerance (AGT) but not in NGT subjects; 2) CFB concentration correlated with fasting glucagon in obese subjects; and 3) both circulating glucagon and CFB concentration contribute to explain the circulating triglycerides in obese subjects. In agreement with this data, diet-induced weight loss led in obese subjects to concomitant decreases of circulating CFB, fasting glucagon, and triglycerides.

Pro-inflammatory cytokines such as IL-6 play a pivotal role to maintain the glucose homeostasis and avoid 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 up-regulated (30; 31). The dose-dependent acute metabolic responses to IL-6 have been well analyzed in healthy subjects by administering recombinant IL-6 to mimic the acute inflammatory state of sepsis (2; 21). IL-6 seems to affect pancreatic α-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 IL-6 levels (32), probably to compensate the extreme energetic demands of these clinical situations. Otherwise, skeletal muscle cells releases IL-6 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 type 2 diabetes (T2D) individuals, pro-inflammatory cytokines also led to up-regulation 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 who shown impaired glucose tolerance. 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). On the other hand, IL-6 also influenced lipid metabolism (38) and induced hypertriglyceridemia in experimental models. The join effects of increased IL-6 and glucagon concentrations could contribute to dislipidemia and hypertriglyceridemia in subjects with altered glucose tolerance.

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 higher also 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 (acylation-stimulating protein [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 present 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 show 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 IL-6 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 suggest that the latter may contribute to dyslipidemia to some extent. The influence of CFB, whose secretion has been recently demonstrated in the exocrine pancreas, seems to be regulated by pro-inflammatory cytokines, and may also contribute to hypertriglyceridemia, is also 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 metabolical 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.

**Author contributions:** All authors of this manuscript have directly participated in the execution and analysis of the study. FJO drafted the manuscript, designed the study, participated in the analysis of biochemical variables, and performed the statistical analysis. JMM and MS analyzed biochemical variables. GF realized the diet-induced weight loss study. WR and JMFR obtained the anthropometrical characteristics and the written consent of patients and participated in the conception and the coordination of the study. JMFR carried out the conception, design and coordination of the study, and helped with the statistical analysis.

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**Conflicts of interest:** The authors have nothing to declare. All authors of this manuscript have directly participated in the execution, and analysis of the study. All authors are aware of and agree to the content of the manuscript, and all authors have approved the final version submitted and their being listed as an author on the manuscript. The contents of this manuscript have not been copyrighted or published previously. There are no directly related manuscripts or abstracts, published or unpublished, by one or more authors of this manuscript. The contents of this manuscript are not now under consideration for publication elsewhere. The submitted manuscript nor any similar manuscript, in whole or in part, will be neither copyrighted, submitted, or published elsewhere while the Journal is under consideration.
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20. Chrousos GP: The hypothalamic-pituitary-adrenal axis and immune-mediated
Figures

Figure 1 Linear relationships (continuous line) between circulating glucagon concentration and fasting glucose (Fig.1a; \( p<0.0001 \)), glycated hemoglobin (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 (○); non-obese but AGT as full small circles (●); obese and NGT as empty big diamonds (◊); and obese and AGT subjects are full small diamonds (♦).

Figure 2 Linear relationships (continuous line) between circulating glucagon concentration, IL-6 (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 (○); non-obese but AGT as full small circles (●); obese and NGT as empty big diamonds (◊); and obese and AGT subjects are full small diamonds (♦).
Table 1
Clinical characteristics of subjects in the cross-sectional study.

<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 ±3.3**</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fat mass (%)</td>
<td>5.6 ±13.2</td>
<td>6.9 ±9.8*</td>
<td>30.6 ±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>96.8 ±6.0</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 ±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>HbA₁c (%)</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 (mUI/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 sensitivity†</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>IL-6 (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 (ug/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</td>
<td>369.9</td>
<td>340.4</td>
<td>450.1</td>
<td>0.038</td>
</tr>
</tbody>
</table>

Data are means ±SD for Gaussian variables and median (inter-quartile range) for non-Gaussian variables (only circulating glucagon concentrations); 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; HbA₁c, glycated hemoglobin; IL-6, interleukin-6; CFB, complement factor B. HOMA-IR: homeostasis model assessment of insulin resistance; †Insulin sensitivity was measured in 175 subjects (112 subjects with NTG and 63 subjects with IGT) using the frequently sampled intravenous glucose tolerance test. * 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). Significant data are shown in bold.
Table 2

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

<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>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>r</td>
<td>p</td>
<td>N</td>
</tr>
<tr>
<td>Fasting Glucose (mg/dl)</td>
<td></td>
<td>0.081</td>
<td>0.421</td>
<td>0.043</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>0.129</td>
<td>0.197</td>
<td>0.010</td>
<td>0.923</td>
</tr>
<tr>
<td>Fasting triglycerides (mg/dL)</td>
<td>-0.035</td>
<td>0.726</td>
<td>0.060</td>
<td>0.573</td>
</tr>
<tr>
<td>IL-6 (ng/L; n=146)</td>
<td>-0.019</td>
<td>0.890</td>
<td>0.363</td>
<td>0.013</td>
</tr>
<tr>
<td>CBP (ug/mL; n=240)</td>
<td>-0.027</td>
<td>0.792</td>
<td>0.034</td>
<td>0.782</td>
</tr>
</tbody>
</table>

NGT, normal glucose tolerance; AGT, altered glucose tolerance; HbA1c, glycated hemoglobin; IL-6: interleukin-6; CFB: complement factor B. Significant data are shown in bold.
### Table 3

Subjects’ characteristics in the weight loss study.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Post-weight loss</th>
<th>% of reduction</th>
<th>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</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>36.9 ±8.3</td>
<td>31.2 ±5.8</td>
<td>-15.5</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</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</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</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</td>
<td>0.056</td>
</tr>
<tr>
<td>Fasting Glucose (mg/dL)</td>
<td>94.4 ±13.8</td>
<td>93.9 ±15.5</td>
<td>-0.5</td>
<td>0.896</td>
</tr>
<tr>
<td>Fasting Insulin (mUI/mL)</td>
<td>12.8 ±7.6</td>
<td>8.6 ±6.4</td>
<td>-32.8</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</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</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</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</td>
<td>0.001</td>
</tr>
<tr>
<td>Fasting triglycerides (mg/dL)</td>
<td>101.3 ±24.4</td>
<td>83.3 ±28.4</td>
<td>-17.8</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</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CFB (ug/mL)</td>
<td>331.5 ±71.2</td>
<td>291.8 ±57.2</td>
<td>-12.0</td>
<td>0.032</td>
</tr>
<tr>
<td>Glucagon (pg/mL)</td>
<td>715.5 (562.1-1,090.7)</td>
<td>602.8 (460.6-757.4)</td>
<td>-3.1</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Data are means ±SD for Gaussian variables and median (inter-quartile range) for non-Gaussian variables (only glucagon concentration); **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. Significant data are shown in bold.
Figure 1

1a

Fasting glucose (mg/dL) vs. Glucagon (pg/mL)

1b

HbA1C (mg/dL) vs. Glucagon (pg/mL)

1c

Triglycerides (mg/dL) vs. Glucagon (pg/mL)

RsQ Linear = 0.166
RsQ Linear = 0.182
RsQ Linear = 0.127
RsQ Linear = 0.014
RsQ Linear = 0.014
RsQ Linear = 0.03
Figure 2

2a

IL-6 (ng/L) vs. Glucagon (pg/mL)

R^2 Linear = 0.117
R^2 Linear = 0.003

2b

CFB (µg/mL) vs. Glucagon (pg/mL)

R^2 Linear = 0.163
R^2 Linear = 0.039