# Elevated Pancreatic Glucagon in Obesity

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**SUMMARY**

**Because of conflicting reports on plasma levels of total immunoreactive glucagon (IRG) in obesity, we investigated, by gel filtration, the individual IRG fractions in grossly obese patients (N = 16; BMI 40 ± 2) as compared with normal weight subjects (N = 18). Mean total IRG was 142 ± 12 pg/ml in normal subjects and 190 ± 15 pg/ml ($P < 0.02$) in obese subjects. $IRG^{3500}$ was 16 ± 2 pg/ml in normal subjects and 34 ± 5 pg/ml ($P < 0.005$) in the obese subjects. Obese patients with normal glucose tolerance demonstrated 30 ± 5 pg/ml $IRG^{3500}$ (N = 11; $P < 0.01$) as compared with those who were obese with impaired glucose tolerance (42 ± 12 pg/ml, N = 5; $P < 0.0025$), although the total IRG of the latter group (173 ± 31 pg/ml) was not different from that of normal subjects. $IRG^{9000}$ was of comparable magnitude in the entire groups of obese and normal subjects. A wide variability of big plasma glucagon levels was found in all three groups, mean levels not being significantly different. These data suggest that plasma $IRG^{3500}$ levels are elevated in grossly obese patients, particularly in those patients with impaired glucose tolerance, a finding that may be masked by mere measurements of total plasma IRG. DIABETES 33:277–280, March 1984.**

Conflicting results have been reported with regard to plasma glucagon levels in obesity: normal,[1–6] elevated,[4,6–9] and decreased[2,10–12] levels of total immunoreactive glucagon (IRG) have been demonstrated under various conditions. Because of the previously described[9] wide variability of basal total plasma IRG levels in 129 grossly obese subjects (56–440 pg/ml) we have attempted in this study to differentiate total immunoreactive glucagon in obese and normal-weight subjects by gel filtration chromatography.

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## METHODS

### SUBJECTS

Clinical data of the 18 normal-weight (9 men, 9 women) and 16 obese subjects (6 men, 10 women) are listed in Table 1. The body mass index was calculated according to Keys et al.[13] The obese subjects were selected at their first appointment to our obesity clinic. They had been on weight-maintaining diets; no attempts to lose weight had been made for at least 6 mo before the study. Before undergoing a 100-g oral glucose tolerance test, the baseline samples for gel filtration and total IRG measurements were drawn and immediately transferred into chilled tubes containing 1000 KIU of Trasylol and 1.2 mg of $Na_2$-EDTA per ml blood to be collected. The samples were centrifuged within 1 h at 40°C and stored at −20°C until assayed.

### ANALYTIC PROCEDURES

**Gel filtration chromatography.** Modifications of originally described methodology were used.[14–18] The chromatography of all samples was performed at 4°C. Four milliliters of plasma were loaded on 450 × 16-mm columns (LKB Instruments, Bromma, Sweden) packed with Biogel P30 (Biorad Lab, Richmond, California). The elution buffer was 0.05 M ammoniumbicarbonate ($NH_4HCO_3$), pH 8.8, containing 500 KIU Trasylol/ml of buffer, 0.25% human serum albumin, and 0.005% $NaN_3$ to prevent bacterial growth. For calibration of the columns, 3–4000 cpm of $^{125}$I-glucagon and $^{125}$I-insulin were used. The void volume was obtained by monitoring the protein content of each fraction collected by ultraviolet absorption at 280 nm. Eluates of 2.0 ml were collected into siliconized glass tubes, immediately counted after gel filtration, and stored at −20°C up to 2 wk before being assayed in duplicate.

TABLE 1
Mean clinical data of normal and obese subjects (± SEM)

| Subjects | N (M/F) | Age (yr) | Weight (kg) | Body mass index (BMI = wt/h²) | Glucose tolerance (mg/dl) 0/2 h | Glucagon (total IRG) (pg/ml) | $IRG^{3500}$ (pg/ml) | Insulin (μU/ml) 0/2 h |
|---|---|---|---|---|---|---|---|---|
| Normal subjects | 18 (9/9) | 26 ± 4 | 67 ± 3<br>M: 74 ± 2<br>F: 58 ± 3 | 21 ± 1<br>M: 23 ± 1<br>F: 20 ± 1 | — | 142 ± 12 | 16 ± 2 | 6 ± 1/— |
| Obese patients ($OB_t$) | 16 (6/10) | 34 ± 3 | 111 ± 7<br>M: 126 ± 14<br>F: 102 ± 5 | 40 ± 2<br>M: 40 ± 3<br>F: 40 ± 2 | 98 ± 5/135 ± 13 | 190 ± 15* | 34 ± 5* | 20 ± 3/84 ± 16 |
| $OB_n$ | 11 (4/7) | 32 ± 4 | 116 ± 9 | 42 ± 2 | 89 ± 3/105 ± 5 | 198 ± 16* | 30 ± 5* | 21 ± 3/70 ± 18 |
| $OB_i$ | 5 (2/3) | 40 ± 3 | 100 ± 5 | 37 ± 2 | 110 ± 16/199 ± 18 | 173 ± 31 | 42 ± 12* | 18 ± 6/107 ± 28 |

$OB_t$: entire group of obese patients; $OB_n$: obese patients with normal glucose tolerance; and $OB_i$: obese patients with impaired glucose tolerance.
*Statistically significant versus normal subjects ($P < 0.05$).

**Glucagon assay.** Samples were assayed using the antiserum 30K (pool 5, lot 9, 1981) at a final dilution of 1:250,000 as described in detail.[19]

Eluate samples (0.8 ml) were incubated together with 0.2 ml of glycine buffer, 15 pg of $^{125}$I-glucagon (Novo-Alle, Bagsvaerd, Denmark), 1000 KIU of Trasylol, and the antiserum 30K at a total volume of 1.2 ml. Standards (0.2 ml) or plasma (0.2 ml) were prepared in 0.8 ml of elution buffer eluted from the column to provide exactly the same incubation mixtures for standards, eluates, and plasmas. Total plasma IRG and corresponding eluates were always run in the same assay. Immediately before performing the charcoal-dextran separation technique, 0.2 ml of horse serum was added to all standards and eluates not containing serum proteins. The lower limit of sensitivity at the 97.5% confidence limit was 20 pg/ml for plasmas and 5 pg/ml for the eluates, 0.8 ml being assayed. Thus, as little as 10 pg/2-ml fraction or 2.5 pg/ml of plasma were read from the 20 pg/ml standard. For recovery calculations values of <5 pg/fraction were disregarded. The interassay coefficient of variation was 11.6% at low and high concentrations, and the intraassay coefficient of variation was 2.6 ± 0.2% at 20 pg/ml.

Recovery of highly purified porcine glucagon (100 pg, 200 pg, and 400 pg; Novo-Alle, Bagsvaerd, Denmark) loaded onto the columns in 2 ml of assay buffer was 92–115% within the 3500-dalton region. In three plasmas of normal-weight subjects drawn before and after a standard intravenous arginine infusion, which stimulated an increase of total IRG from 119 ± 11 pg/ml to 275 ± 76 pg/ml, $IRG^{3500}$ increased from 28 ± 19 pg/ml to 188 ± 73 pg/ml.

The amount of total IRG loaded was determined by triplicate measurements of plasma concentration multiplied by four (amount of plasma loaded onto the column). The sum of all fractions gave the total amount of recovered IRG. IRG components were calculated as absolute amounts recovered and divided by the amount of loaded plasma (4 ml) to give pg/ml of plasma.

Serum insulin was assayed with the use of a commercially available test system (Phadebas, Pharmacia Diagnostics AB, Uppsala, Sweden). The limit of sensitivity was 3 μU/ml and the interassay coefficient of variation was 10% at all concentrations. Blood glucose was measured by the glucose-oxidase method on a Beckman glucose analyzer (Beckman Instruments, Inc., Fullerton, California).

Standard statistical calculations were employed for mean, SEM and Student's *t* test, correlation coefficients, and analysis of variance testing.

## RESULTS

In the normal subjects studied, the range of total IRG in plasma was 62–249 pg/ml (mean 142 ± 12 pg/ml); for the entire group of obese subjects total IRG was between 84 and 320 pg/ml (mean 190 ± 15 pg/ml; $P < 0.02$). The quantity of total IRG in 4 ml of plasma of normal subjects averaged 566 ± 48 pg, and 395 ± 33 pg (73 ± 5%) was recovered. The quantity of total IRG in 4 ml of plasma of obese subjects averaged 762 ± 58 pg, and 583 ± 64 pg (75 ± 4%) of this was recovered. The mean basal IRG level of each fraction is plotted for each group in Figure 1 for the 18 normal subjects, the 11 obese subjects with normal glu-

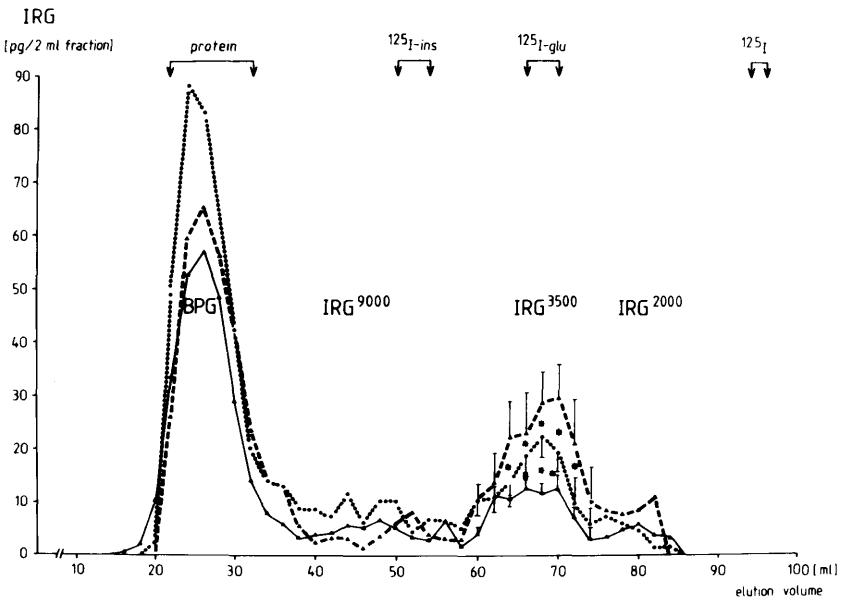


**FIGURE 1. IRG (30K) in 2-ml fractions obtained by gel filtration chromatography of 4 ml of plasma from 18 normal subjects (——), and from 11 obese patients with normal (●●●●●●) and 5 obese patients with impaired (— — —) glucose tolerance. *Indicates significantly different values ($P < 0.05$) of fractions in the $IRG^{3500}$ region comparing the obese groups with the normal subjects.**

cose tolerance ($OB_n$), and the 5 obese subjects with impaired glucose tolerance ($OB_i$).

Mean absolute amounts of each IRG component in each group are shown in Figure 2. In normal-weight subjects, $IRG^{3500}$ averaged 16 ± 2 pg/ml (16 ± 2% of recovered IRG). In the entire group of obese patients, big plasma glucagon and $IRG^{9000}$ levels were slightly higher, not reaching statistical significance, whereas $IRG^{3500}$ levels were significantly elevated (34 ± 5 pg/ml; $P < 0.005$; 24 ± 3%). The 5 patients with impaired glucose tolerance, whose total IRG levels (173 ± 31 pg/ml) were lower than the entire obese group (190 ± 15 pg/ml) and not different from the normal subjects, exhibited the highest $IRG^{3500}$ (42 ± 12 pg/ml; $P < 0.0025$ versus normal subjects; 30 ± 5%) as compared with the 11 $OB_n$ patients (30 ± 5 pg/ml; $P < 0.01$ versus normal subjects).

$IRG^{9000}$ sometimes seemed to consist of two distinguishable peaks and eluted from the columns as 18 ± 6 pg/ml in obese subjects as compared with 12 ± 2 pg/ml in normal subjects ($P < 0.05$).

The range of big plasma glucagon levels was similar in both groups though somewhat higher mean levels were obtained in the obese patients (90 ± 12 pg/ml as compared with 65 ± 7 pg/ml in normal subjects). $IRG^{2000}$ in most subjects was too low relative to the sensitivity limits of the assay procedure to differentiate between groups.

No correlation was found comparing BMI and total IRG. On the other hand, $IRG^{3500}$ and total IRG correlated significantly only in the normal subjects ($N = 18$, $r = 0.4954$, $P < 0.05$), whereas no such relationship was found in the obese subjects ($N = 16$, $r = 0.2829$, $P > 0.05$).

## DISCUSSION

Previous studies of total plasma IRG levels in obese subjects[1-12] have yielded conflicting results, even though all of these studies except one[5] were performed using the same antiserum (30K). Because of the known heterogeneity of plasma IRG and the wide variability of the total IRG,[21] it seemed possible that differences in $IRG^{3500}$ might be missed in determination of total IRG alone. Therefore, immunoreactive glucagon components in obese and normal-weight subjects in the basal overnight fasted state were compared by means of gel filtration chromatography. In the 11 obese subjects with normal glucose tolerance, the mean basal level of $IRG^{3500}$ was elevated to twice the basal levels of normal volunteers (+82 ± 29%). The patients with impaired glucose tolerance showed an even higher mean $IRG^{3500}$ level (+158 ± 71%), although their total IRG was not significantly different from that of the normal subjects. The finding that higher $IRG^{3500}$ levels in the obese subjects did not necessarily contribute to higher total IRG levels is further supported by the fact that $IRG^{3500}$ and total IRG were not significantly correlated in these patients.

Although recovery of known amounts of purified glucagon was approximately 100% and of comparable magnitude (75%) in both obese and normal subjects, it should be emphasized that nonequivalent losses of the different IRG

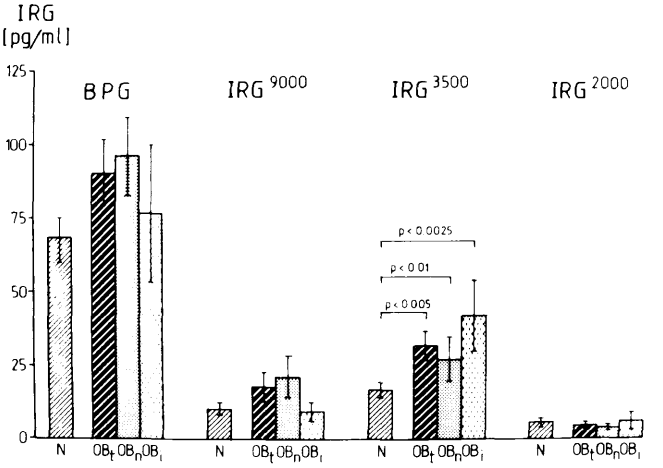


**FIGURE 2. Heterogeneity of IRG in normal and obese subjects expressed as pg IRG/ml of plasma. N = normal subjects, $OB_t$ = total group of obese patients, $OB_n$ = obese patients with normal glucose tolerance, and $OB_i$ = obese patients with impaired glucose tolerance.**

peaks cannot be totally excluded. But, unlike evaluating the reliability of the columns, the calculation of total IRG (100%) from plasma concentrations and the sometimes rather high amounts of big plasma glucagon in individual fractions might have influenced recovery calculations.

As $IRG^{3500}$ is predominantly degraded in the kidneys,[21] it is of importance that none of the obese subjects had any signs of renal failure by means of serum creatinine levels. Although the normal and obese subjects were not matched for age, basal glucagon levels are reported not to be significantly altered as a function of advancing years.[22] Furthermore, an artifactual elevation of basal glucagon levels due to antecedent carbohydrate restriction[20] could be excluded, since all of the obese subjects studied had been on a weight-maintaining diet for at least the previous 6 mo. From our data, it cannot be assessed whether elevated $IRG^{3500}$ levels found in obese patients are of any physiologic importance in the endocrine-metabolic syndrome of obesity. Elevated two- to threefold in the obese patients as compared with normal subjects, even relatively small absolute increases in circulating venous glucagon concentrations may reflect physiologically important increments in portal-vein glucagon. It has been shown recently, in the isolated, perfused dog pancreas,[23] that physiologic glucagon concentrations stimulated insulin and somatostatin release in the absence of hyperglycemia. A possible resistance of the A-cell to the inhibitory effect of insulin is unlikely, since it has been shown that plasma glucagon declined significantly in hyperinsulinemic obese subjects during an insulin infusion at clamped euglycemia.[24] Schade and Eaton[1] reported a decreased lipolytic and ketogenic response to glucagon in obesity, but it remains unknown whether insulin resistance or resistance to glucagon actions may account for these findings.

In summary, true pancreatic glucagon ($IRG^{3500}$) levels were elevated in obese patients, particularly in those with impaired glucose tolerance. Elevated $IRG^{3500}$ in obesity may be masked on determination of total IRG due to wide variations of glucagon components other than $IRG^{3500}$, e.g., big plasma glucagon, which may have yielded conflicting results on total plasma IRG in obesity. On the other hand, considering the heterogeneity of obesity and the different populations from which reported data were derived, our finding of elevated $IRG^{3500}$ in obesity might not necessarily reflect true pancreatic glucagon levels in large populations of obese subjects.

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