Variation in plasma glucagon levels according to obesity status in Japanese Americans with normal glucose tolerance

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Abstract. Japanese Americans living in the United States are genetically identical to Japanese people, but have undergone a rapid and intense westernization of their lifestyle. This study investigated variability in glucagon secretion after glucose loading among Japanese Americans with normal glucose tolerance (NGT) according to obesity status. The 75-g oral glucose tolerance test (OGTT) was performed for 138 Japanese Americans (aged 40–75 years) living in Los Angeles. Plasma glucagon levels measured using the sandwich enzyme-linked immunosorbent assay were compared according to body mass index (BMI) categories among 119 individuals with NGT. The individuals were classified into three categories according to their BMI values: <22 kg/m² (n = 37), 22–24.9 kg/m² (n = 46), and ≥25 kg/m² (n = 36). Fasting plasma glucagon levels and glucagon-area under the curve levels during the OGTT were the highest in the BMI ≥25 kg/m² group. Fasting glucagon levels were correlated with BMI (r = 0.399, p < 0.001), fasting insulin levels (r = 0.275, p = 0.003) and the homeostasis model assessment-insulin resistance (r = 0.262, p = 0.004). In conclusion, our findings suggest that fasting hyperglucagonemia is associated with obesity and insulin resistance even during the NGT stage in the Japanese American population.

Key words: Glucagon, Obesity, Insulin resistance

Materials and Methods

Subjects

Since 1970, we have been conducting medical surveys every few years for Japanese Americans living in the...
Participants diagnosed with DM (seven subjects: seven men and zero women) and IGT (12 subjects: six men and six women) using the 75-g OGTT were excluded. NGT participants (n = 119) were stratified according to their BMI values of <22 kg/m² (n = 37), 22–24.9 kg/m² (n = 46), and ≥25 kg/m² (n = 36). Significantly higher WC and HOMA-IR and significantly lower HDL cholesterol levels were observed in the BMI ≥25 kg/m² group than in the other groups (Table 1).

Serum insulin levels were significantly higher in the BMI ≥25 kg/m² group than in the other groups at 0 and 60 min, and they were non-significantly higher at 120 min (Fig. 1A). Furthermore, serum insulin-area under the curve (AUC) levels were significantly higher in the BMI ≥25 kg/m² group than in the other groups (Fig. 1B).

Plasma glucagon levels at 0 min were significantly higher in the BMI 22–24.9 kg/m² group than in the BMI <22 kg/m² group, and the levels at 0 and 60 min were significantly higher in the BMI ≥25 kg/m² group than in the other groups (Fig. 2A). However, the differences among the groups decreased after 60 min, and the values were similar at 120 min. Plasma glucagon-AUC levels were also significantly higher in the BMI ≥25 kg/m² group than in the other groups (Fig. 2B).
We also evaluated the relative glucagon levels compared to the fasting levels during OGTT in each group. Although there was no significant difference in relative glucagon change at 60 min, there was a significant difference at 120 min between the BMI <22 kg/m² and BMI ≥25 kg/m² groups (Supplementary Fig. 1A). The ratios of plasma glucagon levels to parameters which affect glucagon secretion, such as serum glucose or insulin levels, were also analyzed. Glucagon/glucose ratio in the BMI ≥25 kg/m² group was significantly higher at 0 min, but there was no significant difference at 60 min and 120 min (Supplementary Fig. 1B). Glucagon/insulin ratio was not significantly different among the three groups at each time point (Supplementary Fig. 1C).

### Table 1  Characteristics of the NGT subjects

| BMI group        | <22 kg/m² | 22–24.9 kg/m² | ≥25 kg/m² |
|------------------|-----------|---------------|-----------|
| N (men/women)    | 37 (10/27) | 46 (25/21)    | 36 (27/9) |
| Age (years)      | 61.6 ± 7.5 | 62.1 ± 6.7    | 61.3 ± 8.6 |
| BMI (kg/m²)      | 20.1 (18.7–21.2) | 23.8 (22.8–24.3)* | 26.9 (25.7–28.6)*† |
| Waist circumference (cm) | 78.0 (70.5–82.3) | 86.8 (83.8–90.0)* | 93.0 (88.9–96.8)*† |
| Systolic BP (mmHg) | 121.3 ± 16.1 | 126.4 ± 15.9 | 132.9 ± 16.8 |
| Diastolic BP (mmHg) | 75.4 ± 8.1 | 80.3 ± 8.9* | 83.6 ± 10.6* |
| Total cholesterol (mg/dL) | 217.1 ± 38.7 | 215.2 ± 34.8 | 209.4 ± 35.0 |
| HDL cholesterol (mg/dL) | 68.0 (57.0–78.5) | 58.0 (46.8–73.0) | 48.0 (41.8–55.5)*† |
| Triglycerides (mg/dL) | 103.0 (70.0–124.5) | 118.5 (90.3–165.8) | 150.0 (108.5–195.3)* |
| AST (U/L)        | 19 (17–25) | 21 (18–23)    | 23 (18–30) |
| ALT (U/L)        | 13 (10–18) | 16 (14–19)    | 19 (16–24) |
| γ-GTP (U/L)      | 19 (14–34) | 25 (17–42)    | 27 (22–53) |
| Crè (mg/dL)      | 0.68 (0.65–0.84) | 0.78 (0.63–0.88) | 0.85 (0.70–0.96)* |
| eGFR (mL/min/1.73 m²) | 67.9 (63.1–75.7) | 69.5 (62.9–78.2) | 66.6 (62.6–78.7) |
| HOMA-IR          | 0.8 (0.6–1.0) | 1.2 (0.8–1.5)* | 1.6 (1.2–1.9)*† |

Data are expressed as mean ± standard deviation or median (interquartile range).

* p < 0.05 vs. the <22 kg/m² group. † p < 0.05 vs. the 22–24.9 kg/m² group.

BP, blood pressure.

**Fig. 1** Differences in insulin variation after a 75-g oral glucose tolerance test according to obesity.

Each panel shows serum levels of insulin (A) and insulin-area under the curve (AUC) (B). In panel (A), the green circles indicate body mass index (BMI) <22 kg/m² (n = 37), orange triangles indicate BMI of 22–24.9 kg/m² (n = 46), red squares indicate BMI ≥25 kg/m² (n = 36), and error bars indicate standard deviation. In panel (B), the error bars indicate 95% confidence intervals.

* p < 0.05 vs. the BMI <22 kg/m² group. † p < 0.05 vs. the BMI 22–24.9 kg/m² group.
Plasma glucagon levels at 0 min (Fig. 3A) and glucagon-AUC levels (Fig. 3B) were significantly higher in men \((n = 62)\) than in women \((n = 57)\). However, glucagon-AUC/BMI ratio was not significantly different between men and women (Fig. 3C), and the sex difference was not an explanatory variable of glucagon-AUC levels when adjusted for BMI by multiple regression analysis \((\beta = 0.152, p = 0.103)\).

Accordingly, we compared plasma glucagon levels according to BMI values and sex. In men, plasma glucagon levels at 0, 60, and 120 min were significantly higher in the BMI \(\geq 25 \text{ kg/m}^2\) group than in the <22 kg/m\(^2\) group (Fig. 4A). Moreover, plasma glucagon-AUC levels were significantly higher in the BMI \(\geq 25 \text{ kg/m}^2\) group than in the BMI <22 kg/m\(^2\) group (Fig. 4B).

In women, plasma glucagon levels at 0 min were significantly higher in the BMI \(\geq 25 \text{ kg/m}^2\) group than in the other groups (Fig. 4C), although plasma glucagon-AUC levels were not significantly different among the three groups (Fig. 4D).

Finally, we investigated the correlation between fasting glucagon levels and insulin resistance. Fasting glucagon levels were correlated with BMI \((r = 0.399, p < 0.001;\text{ Fig. 5A})\), fasting insulin levels \((r = 0.275, p = 0.003;\text{ Fig. 5B})\) and HOMA-IR \((r = 0.262, p = 0.004;\text{ Fig. 5C})\). We also found the correlation between fasting glucagon levels and other parameters such as liver function, kidney function or lipid metabolism (Supplementary Fig. 2). Fasting glucagon levels were correlated positively with WC \((r = 0.333, p < 0.001)\) and negatively with eGFR \((r = -0.172, p < 0.001)\), total cholesterol \((r = -0.196, p = 0.033)\) and HDL cholesterol \((r = -0.339, p < 0.001)\).

**Discussion**

Based on the study results, plasma glucagon levels increased by gradually increasing obesity and insulin resistance during the NGT stage in Japanese Americans. A previous study in Denmark showed that fasting glucagon levels were higher in obese subjects than in lean subjects with NGT [2], and another study in Sweden reported that fasting glucagon levels and glucagon-AUC levels during OGTT were higher in obese adolescents than in lean adolescents [5]. Based on reports showing that glucagon levels were elevated with IGT and type 2 DM [2-9], these results indicated that glucagon secretion was not adequately suppressed after glucose loading with obesity as well as abnormal glucose tolerance.

Furthermore, a previous study reported a sex difference in the glucagon response during OGTT in healthy Japanese individuals [13]. In the current study, fasting glucagon levels were significantly higher in men than in women, and our results agreed with those of the previous study (Fig. 3A). Additionally, glucagon-AUC levels were significantly higher in men than in women (Fig. 3B), but if adjusted for BMI, the sex difference was not an independent variable of plasma glucagon levels, suggesting that body composition may affect the sex difference in glucagon secretion in this Japanese American population.

Recent studies have established the validity of sandwich ELISA for measuring glucagon levels in Japanese people [6, 7, 11, 14]. One study compared plasma glucagon levels during OGTT for healthy Japanese subjects using a liquid chromatography-high-resolution mass spectrometry assay and sandwich ELISA and revealed a
good correlation [14]; the finding supports the reliability of the values obtained using sandwich ELISA.

Compared with fasting glucagon levels measured using sandwich ELISA in healthy Japanese subjects [6, 7, 14], the current study revealed higher glucagon levels (11.7 ± 6.7 pM) in Japanese Americans with NGT. Our previous studies indicated that Japanese Americans have higher insulin resistance and significantly higher prevalence of obesity and DM than the Japanese living in Hiroshima, Japan, which can be attributed to a westernized lifestyle [15-18]. It is also possible that the difference in their lifestyle could influence glucagon secretion in the Japanese and Japanese American populations.

In addition, our study showed that fasting glucagon levels were significantly associated with insulin resistance in Japanese Americans (Fig. 5). A previous study also reported that insulin resistance measured by insulin clamp technique was associated with fasting glucagon levels in non-diabetic individuals in 14 European countries [19], which could be applicable to Japanese Americans belonging to Asians. Unfortunately, from our cross-sectional study, it is not possible to clarify the mechanism of this relationship between glucagon secretion and insulin resistance. In a previous study in Denmark, the nonlinear inverse relationship between fasting glucagon levels and insulin sensitivity suggests that basal α-cell secretion depends on insulin sensitivity, and hypersecretion of the α-cells (hyperglucagonemia), together with hyperinsulinemia, is tightly coupled to a reduction of insulin sensitivity in the early stages of glucose intolerance [4]. Another previous report suggests that the elevated fasting glucagon causes insulin resistance at the liver, and fasting insulin is elevated partly to compensate for glucagon-induced hepatic glucose output [6].

This study had some limitations. First, the OGTT sampling time points were only 60 and 120 min after glucose loading, which obscured the variation in plasma glucagon levels during the early phase (10–30 min) after glucose loading as previously reported in pre-DM or DM

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**Fig. 3** Differences in glucagon variation after a 75-g oral glucose tolerance test according to sex.

Each panel shows plasma levels of glucagon (A), glucagon-area under the curve (AUC) (B) and glucagon-AUC/BMI ratio (C). In panels (A), the blue circles indicate men ($n = 62$), pink triangles indicate women ($n = 57$), and error bars indicate standard deviation. In panels (B) and (C), the error bars indicate 95% confidence intervals.

* $p < 0.05$ vs. women.
condition compared with NGT [4, 6]. Second, non-carbohydrate nutrients, including amino acids and fatty acids, could affect insulin and glucagon secretions [20, 21], suggesting that it is more appropriate to evaluate these parameters after consuming a meal containing other nutrients rather than after a simple glucose load.

In conclusion, we obtained blood samples from Japanese Americans with NGT who were tested using sandwich ELISA, which is highly specific for glucagon. Our results indicate that high plasma glucagon concentrations were associated with obesity and insulin resistance even during the NGT stage.

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Disclosure

The authors declare no conflict of interest associated with this study.
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