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SHORT REPORT

Sex differences in insulin and glucagon responses for glucose homeostasis in young healthy Japanese adults

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ABSTRACT

It has been reported that glucose responses during the oral glucose tolerance test differ between healthy women and men. However, it remains unknown what factors contribute to these differences between the sexes. The present study analyzed the insulin and glucagon responses during the oral glucose tolerance test in 25 female and 38 male healthy young adults aged 22–30 years. The plasma glucose levels at 120 min were significantly higher in women than men. Insulin secretion was significantly greater at 30, 90 and 120 min from baseline in women than men. Glucagon suppression was greater at 30 and 120 min from baseline in men than women when determined by a sandwich enzyme-linked immunosorbent assay glucagon kit. These results suggest that the differences in glucose responses during the oral glucose tolerance test are mediated by the difference between the sexes in bi-hormonal responses in healthy individuals.

INTRODUCTION

Fasting plasma glucose levels and plasma glucose (PG) excursions during the oral glucose tolerance test (OGTT) are known to be different between healthy women and men with normal glucose tolerance[1–5]. PG was reported to be higher from fasting to approximately 30 min during the OGTT in men, whereas it was higher at approximately 120 min in women[1–3]. Greater muscle mass and/or faster gut glucose absorption in men than women has been suggested to be responsible for the difference[1,2,5].

Recently, it has been advocated that diabetes is caused not only by an insulin action deficiency, but also by insufficient glucagon suppression; therefore, diabetes is a bi-hormonal disorder[6]. The secretion of insulin and glucagon might be responsible for the difference in the OGTT between the sexes; however, this has not been fully examined because of the unreliability of the conventional glucagon assay[7]. Recently, a new quantitative glucagon assay was developed[8] in which the plasma glucagon concentration is determined by two monoclonal antibodies against the N- and C-terminal regions of the glucagon peptide with much less cross-reactivity against other proglucagon fragments than by the conventional kits[9].

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Here, we investigate the sex differences in the insulin and glucagon responses during 75-g OGTT in non-obese Japanese young adults with normal glucose tolerance.

METHODS

Participants

The present study was carried out with healthy Japanese adults aged 22–30 years at Nagasaki University Hospital, Nagasaki, Japan, from April 2015 to July 2017. We recruited volunteers from the community and a few employees in our institute. Individuals with glucose intolerance, any underlying diseases, alcohol abuse, pregnancy or a body mass index (BMI) >25 kg/m^2 were excluded. Among 69 volunteers who agreed to participate in the study, six were excluded from the analysis. Two individuals were excluded because they had impaired glucose tolerance, and four were excluded due to defective data. Informed consent was obtained from all participants. This study was approved by the ethical committee of Nagasaki University Hospital (the approval No. 14032483-2), and carried out in accordance with the declaration of Helsinki.

Study design

The OGTT was carried out after overnight fasting using a 75-g glucose formulation, Trelan-G75 (AY Pharma, Tokyo, Japan). Blood specimens were obtained before (expressed as 0 min),

and 30, 60, 90 and 120 min after the loading of the 75-g glucose. The levels of PG and immunoreactive insulin (IRI) were measured by the hexokinase ultraviolet method and chemiluminescent enzyme immunoassay, respectively. Blood sampling for immunoreactive glucagon (IRG) was carried out using BD P800 tubes (BD, Franklin Lakes, NJ, USA), and IRG was measured using a sandwich enzyme-linked immunosorbent assay kit (Mercodia, Uppsala, Sweden)[8].

Statistical analysis

The *t*-test and repeated-measures analyses of variance (ANOVA) were used to test differences between women and men. Statistical analysis was carried out using JMP pro version 11.2 (SAS Institute, Cary, NC, USA). *P*-values <0.05 were considered significant.

RESULTS

A total of 25 women and 38 men were enrolled in the study. The characteristics of participants and the results of OGTT are shown in Table 1. There were significant differences in the constitutional parameters between women and men. The PG levels at 120 min (2hPG) were significantly higher in women than men. The levels of serum IRI were significantly higher in women than men at 30, 90 and 120 min. The levels of plasma IRG tended to be higher after fasting and lower at all points after the glucose load was administered in men than in women; however, these differences were not significant. There were no

Table 1 | Anthropometric characteristics and results of the 75-g oral glucose tolerance test

	Males	Females	*P*-value
n	38	25	
Age (years)	24.4 ± 1.9	25.4 ± 2.5	0.072
Height (cm)	172.5 ± 5.6	160.1 ± 6.4	<0.001
Weight (kg)	63.8 ± 7.5	51.4 ± 5.4	<0.001
BMI (kg/m^2)	21.4 ± 2.2	20.1 ± 2.7	0.032
PG 0 min (mg/dL)	84.4 ± 6.3	82.2 ± 6.2	0.64
PG 30 min (mg/dL)	130.4 ± 21.3	123.7 ± 23.5	0.15
PG 60 min (mg/dL)	106.4 ± 23.3	110.7 ± 25.3	0.35
PG 90 min (mg/dL)	101.1 ± 15.6	106.0 ± 17.9	0.29
PG 120 min (mg/dL)	92.8 ± 14.9	102.7 ± 17.3	0.034
IRI 0 min (μU/mL)	5.3 ± 2.3	5.6 ± 2.3	0.96
IRI 30 min (μU/mL)	47.8 ± 23.6	59.5 ± 39.8	0.033
IRI 60 min (μU/mL)	34.4 ± 21.4	44.3 ± 31.2	0.072
IRI 90 min (μU/mL)	29.4 ± 14.4	41.2 ± 26.4	0.031
IRI 120 min (μU/mL)	23.7 ± 14.2	34.6 ± 17.4	0.047
IRG 0 min (pg/mL)	32.6 ± 14.1	28.2 ± 17.0	0.11
IRG 30 min (pg/mL)	13.1 ± 7.2	16.1 ± 11.1	0.28
IRG 60 min (pg/mL)	13.3 ± 8.7	13.9 ± 10.1	0.84
IRG 90 min (pg/mL)	13.9 ± 7.5	14.6 ± 9.2	0.81
IRG 120 min (pg/mL)	13.6 ± 7.6	15.5 ± 10.4	0.48
HOMA-IR	1.13 ± 0.54	1.16 ± 0.50	0.84
HOMA-β	100.1 ± 69.5	103.2 ± 40.4	0.84
Matsuda index	9.68 ± 4.22	8.10 ± 2.93	0.11
Insulinogenic index	1.17 ± 0.98	1.60 ± 1.21	0.12

The results are given as means ± standard deviation. *P*-values for females vs males in plasma glucose (PG), immunoreactive insulin (IRI) and immunoreactive glucagon (IRG) were calculated by repeated-measures ANOVA and those in the other data were calculated by the *t*-test. BMI, body mass index; HOMA-β, homeostatic model assessment of β cell function; HOMA-IR, homeostatic model assessment of insulin resistance.

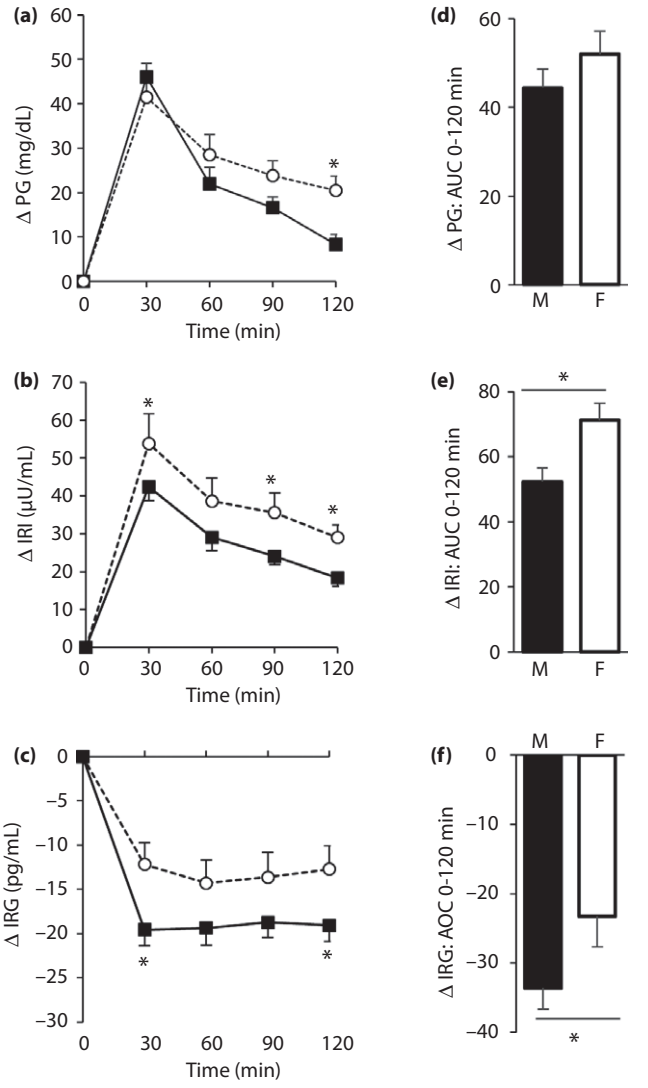

Figure 1 | Changes in the concentrations (mean ± standard error) of (a) plasma glucose (ΔPG), (b) serum immunoreactive insulin (ΔIRI) and (c) plasma glucagon (ΔIRG) between baseline (0 min) and the indicated time-point during 75-g oral glucose tolerance test in females (circle; $n = 25$) and in males (square; $n = 38$). The area under the curve of the increments during 0–120 min ($AUC_{0–120\ min}$) of (d) ΔPG and (e) ΔIRI, and the area over the curve of the reduction during 0–120 min ($AOC_{0–120\ min}$) of (f) ΔIRG in females (F; white; $n = 25$) and in males (M; black; $n = 38$). The bars indicate the standard error. *$P < 0.05$ for females vs males calculated using (a–c) repeated-measures ANOVA and (d–f) the *t*-test.

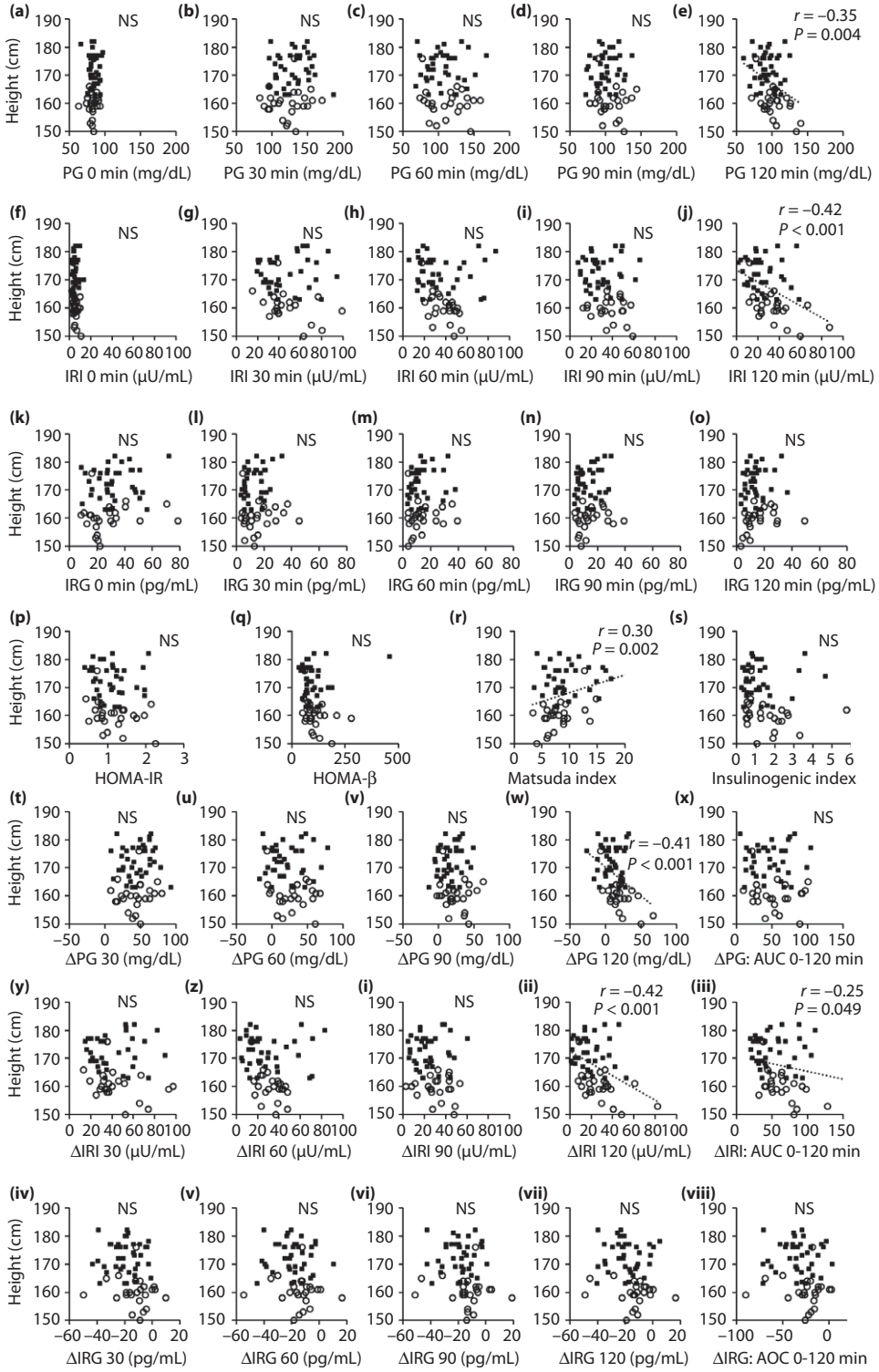

Figure 2 | Correlations between data obtained from the 75-g oral glucose tolerance test and height. Each correlation was determined based on Spearman's rank correlation coefficient. The circle and square indicate females (n = 25) and males (n = 38), respectively. ΔIRG:$AOC_{0–120\ min}$, the area over the curve of plasma glucagon reduction during 0–120 min in the oral glucose tolerance test; ΔIRI:$AUC_{0–120\ min}$, the area under the curve of the increment in serum insulin during 0–120 min; ΔPG:$AUC_{0–120\ min}$, the area under the curve of the change in plasma glucose during 0–120 min; HOMA-β, homeostatic model assessment for β-cell function; HOMA-IR, homeostatic model assessment for insulin resistance; IRG, immunoreactive glucagon; IRI, immunoreactive insulin; NS, not significant; PG, plasma glucose.

differences in the indexes of insulin sensitivity or insulin secretion calculated by homeostatic model assessment for insulin resistance[10], homeostatic model assessment for β cell function[10], Matsuda Index[11] or insulinogenic index[12] between women and men.

To assess the glucose metabolism and the responses of the hormone secretions after the glucose load, we studied the change in the levels of PG, IRI and IRG (shown as ΔPG, ΔIRI and ΔIRG) from baseline (0 min) to each time-point during OGTT (Figure 1). The increase in glucose concentrations at 120 min from baseline (ΔPG 120 min) was larger in women than men ($P = 0.006$; Figure 1a), whereas the increase in the area under the curve of ΔPG during OGTT ($\Delta PG{:}AUC_{0\text{–}120\ min}$) was comparable in both sexes (Figure 1d). There were significantly larger increases in IRI at 30, 90 and 120 min from the baseline in women than in men (Figure 1b), and similarly the increase in the area under the curve of ΔIRI ($\Delta IRI{:}AUC_{0\text{–}120\ min}$) was 36% larger in women than in men ($P = 0.022$; Figure 1e). In contrast, there was a significantly larger reduction in IRG at 30 and 120 min from baseline in men than in women during OGTT (Figure 1c), and the area over the curve of IRG reduction ($\Delta IRG{:}AOC_{0\text{–}120\ min}$) was 45% larger in men than in women ($P = 0.014$; Figure 1f).

To assess the association between body composition and hormonal responses, we studied the correlation between height and the data obtained from OGTT (Figure 2). The levels of 2hPG, IRI at 120 min, ΔPG 120 min, ΔIRI 120 min and $\Delta IRI{:}AUC_{0\text{–}120\ min}$ were inversely correlated with height, and the Matsuda Index was positively correlated with height. There were no correlations between all the values of IRG and height. We did not find correlations between IRG levels and PG levels or between ΔIRG and ΔPG obtained from the OGTT. Furthermore, there were no correlations between IRG levels and IRI levels or between ΔIRG and ΔIRI at each time-point during OGTT (Figure S1). Weight, BMI and age were not correlated with both glycemic and hormonal responses.

DISCUSSION

Several epidemiological studies from around the world have revealed that healthy men showed higher fasting plasma glucose and healthy women showed higher 2hPG levels into the OGTT[1–5,13]. Differences in height, which reflects muscle mass, are often considered to explain the sex difference in 2hPG[2–4], and we indeed observed that height was inversely correlated with 2hPG levels (Figure 2e). Other studies showed that fasting endogenous glucose production is higher in men than in women, and gut glucose absorption is slower in women than in men[1,5]. However, no clear explanation for these phenomena has been provided.

It has been also shown that the serum levels of active glucose-dependent insulinotropic polypeptide were significantly higher in young healthy men than in women during the early phase of the OGTT[14]. The higher glucose-dependent insulinotropic polypeptide secretion might explain the faster gut glucose absorption observed in the early phase of OGTT in men, because glucose-dependent insulinotropic polypeptide is secreted by K cells located in the proximal small intestine.

We found differences in insulin and glucagon responses during OGTT between the sexes among young healthy Japanese individuals. IRI levels after the glucose load were lower in men than in women (Figure 1b,e). This might be explained by the lower ratio of glucose load per muscular mass[4] in men than in women. Otherwise, it could be possible that the difference in insulin response between the sexes might be a secondary phenomenon for insulin sensitivity, rather than primary difference in insulin secretion. This was supported by our findings that the Matsuda Index, which is known to mainly reflect the insulin sensitivity on muscle, was positively correlated with height (Figure 2r). It should be noted that taller persons (mostly men) have more muscle mass[5].

We also found a stronger suppression of glucagon secretions after glucose load in men than in women (Figure 1c,f). In contrast with the insulin responses, the glucagon responses were not correlated with height (Figure 2k–o, 2iv–viii). It is known that insulin suppresses glucagon secretion through the paracrine mechanism. However, we observed no associations between insulin secretions and suppressions of glucagon (Figure S1k–t). Furthermore, the glucagon responses were not correlated with the levels of plasma glucose (Figure S1a–j). Considering these findings, the phenomenon we observed that a stronger suppression of glucagon after glucose load in men than in women might be due to an inherent difference between sexes for glucose homeostasis. Because glucagon is known to delay gut peristalsis[15], insufficient suppression of glucagon during OGTT might contribute to slower glucose absorption from the gut in women.

The present study had certain limitations. There was a significant difference in the mean BMI between women and men in this study. This seems to be a reflection of the fact that young women tend to be lean in Japan. The BMIs of the participants were almost equal to the mean values of Japanese individuals currently aged in their 20s[16]. The number of participants was small. The findings might have been skewed by some participants, although the data from each individual did not suggest this was the case. It is important that insulin and glucagon secretion during OGTT be re-examined using a larger cohort.

In summary, we showed sex differences in both insulin and glucagon responses during OGTT in non-obese young healthy adults. Further investigations are required to fully clarify the sex differences in glucose metabolism.

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DISCLOSURE

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1 | Correlation between glucagon responses and glucose metabolism, or between glucagon responses and insulin responses. Correlations (a–e) between plasma glucagon (IRG) levels and plasma glucose (PG) levels, (f–j) between ΔIRG and ΔPG, (k–o) between IRG levels and serum immunoreactive insulin (IRI) levels, (p–t) between ΔIRG and ΔIRI in the 75-g oral glucose tolerance test. Each correlation was not significant (N.S.) when determined based on the Spearman's rank correlation coefficient. The circle and square indicate women (n = 25) and men (n = 38), respectively.