Insulin Secretion and Insulin Sensitivity in Relation to Fasting Glucose in Healthy Subjects

BO AHRÉN, MD, PHD

OBJECTIVE — This study evaluated insulin secretion and insulin sensitivity in healthy subjects with normal fasting glucose.

RESEARCH DESIGN AND METHODS — A total of 148 healthy women (aged 53–70 years) underwent a glucose-dependent arginine stimulation test and a 2-h euglycemic-hyperinsulinemic clamp. In the arginine test, arginine (5 g) was injected intravenously under baseline (fasting) conditions and after raising the glucose levels to 15 and >28 mmol/l. From this test, the acute insulin response (AIR) to arginine during the three glucose levels (AIR_1, AIR_2, and AIR_3) were estimated. The subjects were divided into quartiles of fasting glucose ($n = 37$ in each group [range <4.32; 4.33–4.84; 4.85–5.22; and 5.23–6.1 mmol/l, respectively).

RESULTS — The results show that *1*) AIR_1 was higher in subjects in the two highest quartiles ($P = 0.004$), *2*) AIR_3 was higher in the quartile with the highest fasting glucose ($P = 0.012$), and *3*) insulin sensitivity was reduced in subjects in the highest quartile ($P = 0.026$) compared with the lower quartiles. The results also show, in contrast, that AIR_2 did not show a similar trend to be increased at higher fasting glucose.

CONCLUSIONS — It is concluded that *1*) raised fasting glucose (albeit still within normal values) augments baseline and maximal arginine-induced insulin secretion in healthy subjects, and 2) this is associated with reduced insulin sensitivity. This suggests that high, but still normal, fasting glucose may contribute to the augmented insulin secretion in subjects with low insulin sensitivity.

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Insulin secretion and insulin action are inversely related, and in glucose intolerance and type 2 diabetes there is an imbalance in that a combination of islet dysfunction (reduced insulin secretion) and insulin resistance (reduced insulin sensitivity) exists (1–7). Whether defective β-cell function and insulin resistance occur also in subjects in the upper range of normal glucose values is not known. It was previously thought that this was not the case because β-cell function was suggested to be defective only when fasting glucose was raised (8,9). Recent studies (10–12) have shown, however, that β-cell dysfunction is evident already before type 2 diabetes develops. A recent study (13) also has shown that in obese subjects, the glucose sensitivity of β-cell function during an oral glucose tolerance test is negatively related to glucose levels within the normal range. This would indicate that β-cell dysfunction contributes to a rise in fasting glucose also within the normal range. Glucose sensitivity of the β-cells during an oral glucose tolerance test is, however, a complex process that is regulated by many factors, the β-cell capacity only being one. Thus, the autonomic nerves and the incretin hormones in addition to β-cell capacity per se also contribute to insulin secretion after oral glucose (14). In this study, we evaluated β-cell capacity as determined by the glucose-dependent arginine stimulation test (15,16) in relation to fasting glucose in a large number of women having normal fasting glucose. The glucose-dependent arginine stimulation test determines the insulin response to intravenous arginine at three different glucose levels (fasting, 15 mmol/l, and >28 mmol/l). We also evaluated insulin sensitivity by the euglycemic-hyperinsulinemic clamp test (17) because insulin secretion needs to be related to insulin sensitivity for accurate estimation (1).

RESEARCH DESIGN AND METHODS

The study group included 148 nondiabetic women, aged 52–70 years (mean ± SD 62.2 ± 2.3 years), who were recruited from a health screening study in Malmö on the basis of normal glucose tolerance (18). All subjects were healthy, and none were taking any medication known to affect carbohydrate metabolism. None of the subjects had a first-degree relative with type 2 diabetes. All women had glucose levels determined after an overnight fast at three different occasions with at least 1 week in between.

Arginine test

Insulin secretion was determined with the intravenous arginine stimulation at three glucose levels (fasting, 15 mmol/l, and >25 mmol/l) as previously described (15,16). After an overnight fast, intravenous catheters were inserted into antecubital veins in both arms. One arm was used for the infusion of glucose and the other arm for intermittent sampling. The sampling catheter was kept patent by slow infusion of 0.9% saline when not in use. Baseline samples were taken at −5 and −2 min. A maximally stimulating dose of arginine hydrochloride (5 g) was then injected intravenously over 45 s. Samples were taken at 2, 3, 4, and 5 min. A variable rate 20% glucose infusion was then initiated to raise and maintain blood glucose at 15 mmol/l. Blood glucose was determined every 5 min bedside and the glucose infusion adjusted to reach the desired blood glucose level in 20–25 min. New baseline samples were taken, and

From the Department of Medicine, Lund University, Lund, Sweden.
Address correspondence and reprint requests to Dr. Bo Ahrén, Department of Medicine, Lund University, B11 BMC, SE-221 84 Lund, Sweden. E-mail: bo.ahren@med.lu.se.
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Abbreviations: AIR, acute insulin response.
A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.
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Table 1—*Characteristics of the 148 women participating in the study*

Variable (unit)	All subjects	Quartile 1	Quartile 2	Quartile 3	Quartile 4
n	148	37	37	37	37
Body weight (kg)	68.4 ± 9.5 (44–102)	67.4 ± 7.6	67.4 ± 8.9	66.4 ± 8.7	72.6 ± 11.3
BMI (kg/m^2)	25.5 ± 3.6 (17.0–35.3)	25.2 ± 3.1	24.7 ± 3.0	25.0 ± 3.6	26.9 ± 4.9
Fasting glucose (mmol/l)	4.8 ± 0.6 (3.6–6.0)	4.1 ± 0.2	4.6 ± 0.1	5.0 ± 0.1	5.6 ± 0.2
Fasting insulin (pmol/l)	66 ± 26 (16–166)	54 ± 13	61 ± 20	68 ± 27	82 ± 33
Insulin sensitivity (nmol glucose · kg^{-1} · min^{-1} per pmol insulin/l)	76.1 ± 32.6 (9.9–220)	79.3 ± 4.5	77.9 ± 4.6	79.0 ± 5.3	67.4 ± 6.8
AIR_1 (pmol/l)	346 ± 189 (79–1,216)	285 ± 138	301 ± 141	366 ± 162	454 ± 282
AIR_2 (pmol/l)	926 ± 555 (176–4,200)	922 ± 156	957 ± 384	938 ± 444	896 ± 402
AIR_3 (pmol/l)	1,105 ± 656 (56–4,085)	931 ± 330	1,104 ± 384	1,052 ± 606	1,419 ± 954

Data are means ± SD (range). Results are reported both for the entire cohort and for the four quartiles of subjects, when divided according to the fasting glucose.

then arginine (5 g) was again injected and new 2-, 3-, 4-, and 5-min samples were taken. A 2.5-h resting period was allowed, after which new baseline samples were obtained and a high-speed (900 ml/h) 20% glucose infusion during 25–30 min was used to raise blood glucose to >25 mmol/l, as determined bedside. At this blood glucose level, new baseline samples were taken and arginine (5 g) was injected followed by final 2-, 3-, 4-, and 5-min samples.

Insulin sensitivity

Insulin sensitivity was determined with the euglycemic-hyperinsulinemic clamp (17). After an overnight fast, intravenous catheters were inserted into antecubital veins in both arms. One arm was used for infusion of glucose and insulin. The contralateral arm was used for intermittent sampling, and the catheter was kept patent with slow infusion of 0.9% saline. A primed constant infusion of insulin (100 units/ml Actrapid; Novo Nordisk, Bagsvaerd, Denmark) with a constant infusion rate of 0.28 nmol · m^{-2} body surface · min^{-1} was started. After 4 min, a variable rate 20% glucose infusion was added and its infusion rate was adjusted manually throughout the clamp procedure to maintain the blood glucose level at 5.0 mmol/l. Blood glucose was determined every 5 min. Samples for analysis of the achieved insulin concentrations were taken at 60 and 120 min.

Analyses

Blood glucose concentration was determined bedside by the glucose dehydrogenase technique with a Hemocue (Hemocue, Ängelholm, Sweden) during the euglycemic-hyperinsulinemic clamp and with an Accutrend (Boehringer Mannheim Scandinavia, Bromma, Sweden) during the arginine test. Blood samples for insulin and glucose from the arginine study and for insulin from the clamp study were frozen immediately, and serum was frozen at −20°C. Serum insulin was analyzed with a double-antibody radioimmunoassay technique with the use of guinea pig anti-human insulin antibodies, mono-^{125}I-tyr-human insulin, and human insulin standard (Linco, St. Charles, MO). Plasma glucose concentrations were analyzed using the glucose oxidase method. All samples were analyzed in duplicate.

Calculations and statistics

Data are presented as means ± SE, unless otherwise stated. The subjects were divided into quartiles for fasting glucose, as determined by the mean of the three measures (n = 37 in each group; range <4.32, 4.33–4.84, 4.85–5.22, and 5.23–6.1 mmol/l). Mean glucose in the four groups was 4.2 ± 0.1, 4.6 ± 0.1, 5.0 ± 0.1, and 5.6 ± 0.1 mmol/l, respectively. For the determination of insulin secretion, the acute insulin response (AIR) to arginine was determined as the mean of the 2- to 5-min samples minus the mean prestimulus insulin concentration at fasting (AIR_1), at 15 mmol/l (AIR_2), and at >28 mmol/l (AIR_3) glucose. Insulin sensitivity was calculated as the glucose infusion rate per kilogram body weight during the 2nd h divided by the mean of the insulin levels at 60 and 120 min during the clamp (i.e., nmol glucose · kg body wt^{-1} · min^{-1} per pmol insulin/l). Differences between the four quartiles of fasting glucose were determined by ANOVA with Bonferroni post hoc analysis. Stepwise forward linear multiple regression was used to assess the independent effect of several variables.

RESULTS

Study group characteristics

Table 1 shows the characteristics of the 148 women who participated in the study and the characteristics of the four quartile subgroups of fasting glucose. Figure 1 shows the distribution of fasting glucose.

Glucose-dependent arginine stimulation test

When arginine was injected intravenously after an overnight fast, there was a sharp and rapid increase in circulating insulin in all subjects (Fig. 2). Glucose was then administered, which raised plasma glucose to 15 mmol/l. A new arginine administration resulted again in a rapid and marked increase in circulating insulin. Finally, glucose was administered again, which raised plasma glucose to >28 mmol/l. When arginine was injected, there was a marked further increase in insulin levels. AIR_3 is considered the maximal possible insulin response under these conditions (15).

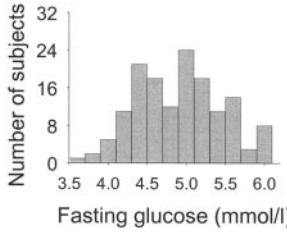

Figure 1—*Distribution profile of mean of three determinations of fasting glucose in the study population of 148 nondiabetic women.*

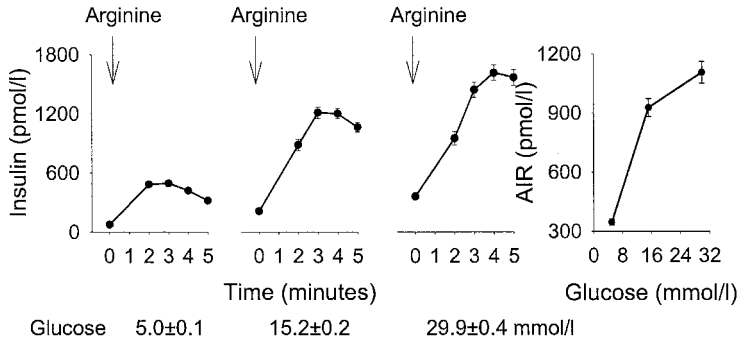

Figure 2—*Insulin levels before and after intravenous administration of arginine (5 g) under fasting conditions or after raising the glucose levels in 148 nondiabetic healthy women. Glucose levels at the three experimental conditions are shown at the* bottom. *The* right panel *shows the AIR (the mean suprabasal 2- to 5-min insulin response to arginine) versus the glucose levels. Means ± SE are shown.*

Relation between the insulin response to arginine, insulin sensitivity, and fasting glucose

The subjects were divided into four quartiles depending on their fasting glucose. Figure 3 shows the AIR_1, AIR_3, and insulin sensitivity in the respective quartile groups. AIR_1 increased by increasing fasting glucose. AIR_1 in subjects in the two quartile groups with the highest fasting glucose was significantly higher than AIR_1 in the two groups with the lowest fasting glucose ($P = 0.004$). In the quartile group with the highest fasting glucose (mean 5.6 mmol/l), AIR_1 was 445 ± 47 mmol/l vs. only 285 ± 23 pmol/l in the subjects in the lowest quartile (4.2 mmol/l). It is thus apparent that a glucose threshold for augmenting arginine-stimulated insulin secretion may exist between 4.6 and 5.0 mmol/l, although the data do not allow for a more specific estimation of a threshold. Similarly, the maximal insulin secretion (AIR_3) was significantly higher in the quartile with the highest fasting glucose ($P = 0.012$). In contrast, AIR_2 did not show a relation to fasting glucose and was not significantly different between the different groups. Insulin sensitivity, as determined by the euglycemic clamp technique, was not different between the groups of the three lowest glucose quartiles but was lower in the subjects with the highest fasting glucose than in the other three groups ($P = 0.026$).

Relation between insulin secretion and insulin sensitivity

Insulin secretion negatively correlated to insulin sensitivity. The correlations after logarithmic transformation of the data were for AIR_1: $r = -0.60$ ($P < 0.001$), AIR_2: $r = -0.46$ ($P < 0.001$), and AIR_3: $r = -0.51$ ($P < 0.001$). Figure 3*D* shows the relation between insulin sensitivity and AIR_3 in the four quartile subgroups of fasting glucose. It is seen that the quartile group with the highest glucose showed a clear increase in insulin secretion in association with the reduced insulin sensitivity.

CONCLUSIONS — It has been documented that insulin resistance is adapted by a compensatory increase in insulin secretion (1–6). This concept is of importance for the understanding of the key role of the islet β-cells for the development of impaired glucose tolerance and type 2 diabetes. If the β-cell compensation to insulin resistance fails, glucose homeostasis deranges, which will result in impaired glucose tolerance and type 2 diabetes. In this study, we examined whether β-cell dysfunction and insulin resistance exist in healthy subjects with high, but still normal, fasting glucose. Insulin secretion was evaluated by the glucose-dependent arginine stimulation test and insulin sensitivity was determined from the euglycemic-hyperinsulinemic clamp technique, and the study was undertaken in a large number of healthy postmenopausal women. From the glucose-dependent arginine stimulation test, three different insulin responses to arginine are estimated. The first response (AIR_1) is the insulin response to arginine at fasting glucose. The second response (AIR_2) is the response to arginine when glucose has been raised to 15 mmol/l. Finally, the AIR_3 is the maximal β-cell response when a combined arginine/glucose challenge is given. In accordance with previous reports (6), we found that AIR_1, AIR_2, and AIR_3 were negatively related to insulin sensitivity. Therefore, the islet capacity to respond to exogenous arginine and glucose negatively relate to insulin sensitivity.

It previously has been shown that the variables obtained by the glucose-dependent arginine stimulation test are reduced in subjects with type 2 diabetes (19) as well in those with impaired glucose tolerance (11,20). All subjects in the

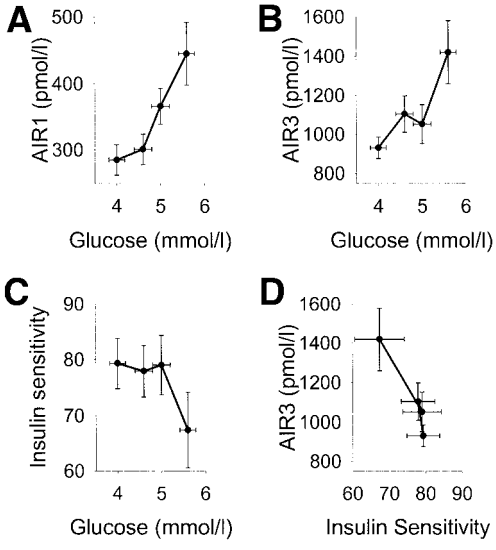

Figure 3—*The suprabasal 2- to 5-min insulin response to intravenous arginine at fasting glucose (AIR_1; mean 5.0 mmol/l glucose)* (A) *and at a mean of 30 mmol/l glucose (AIR_3)* (B) *and insulin sensitivity as determined by the euglycemic-hyperinsulinemic clamp* (C) *versus quartiles of fasting glucose in 148 nondiabetic healthy women.* D*: The relation between insulin sensitivity and AIR_3 in the four quartiles of fasting glucose. Means ± bidirectional SEs are shown.*

present study had normal fasting glucose (i.e., <6.1 mmol/l). We found a higher AIR_1 (i.e., the insulin response to intravenous arginine at fasting glucose in the subjects was augmented by higher fasting glucose within the normal limits). The higher AIR_1 in subjects with fasting glucose >~5 mmol/l shows how sensitive the β-cells are to minute changes in fasting glucose in healthy subjects. An apparent glucose threshold for arginine-stimulated insulin secretion in humans seems to exist between 4.6 and 5.0 mmol/l. A previous study in a small number of individuals showed that subjects with high (but still normal) fasting glucose had a tendency of a lower insulin response to intravenous glucose than subjects with lower fasting glucose (21). This would suggest that the insulin response to intravenous arginine, in contrast to the response to glucose, is preserved when fasting glucose is raised within the normal range (i.e., impaired sensitivity to glucose is an earlier phenomenon during development of type 2 diabetes).

We also found that the insulin response during the glucose-dependent arginine stimulation test at >28 mmol/l glucose is the maximal insulin response (AIR_3) and was increased in subjects in the quartile with the highest fasting glucose. Although not demonstrated experimentally, it is possible that this is due to increased β-cell mass. A potential mediator of this increased AIR_3 would therefore be the higher fasting glucose, since glucose is a powerful stimulator of β-cell growth as shown in experimental animals (rev. in 22).

Since insulin secretion is negatively related to insulin sensitivity, measures of insulin secretion need to be related to insulin sensitivity for a correct judgment of β-cell function. We found that insulin sensitivity was not different between the three groups with the lowest fasting glucose but was lower in subjects in the highest quartile of fasting glucose. This suggests that insulin resistance seems to exist in subjects with fasting glucose >~5.6 mmol/l. It is thus of interest that both AIR_1 and AIR_3 were augmented in this subgroup compared with the others. Figure 3 illustrates the relation between insulin sensitivity and AIR_3 in the four subgroups with different fasting glucose. Hence, in subjects with low insulin sensitivity, insulin secretion is higher, and this is evident in the subjects with the highest fasting glucose, although these subjects still have fasting glucose levels within the normal range. It is therefore a possibility that the slightly higher glucose in these subjects mediates the higher insulin secretion.

In contrast to the augmented AIR_1 and AIR_3 in the subjects with the highest fasting glucose, there was no significant difference in AIR_2 between the groups. These data might suggest a defective sensitivity of the β-cells for the action of glucose to augment arginine-induced insulin secretion at the level of ~15 mmol/l. However, this needs to be examined in more detail. A previous study (13) reported from oral glucose tolerance data in obese subjects that β-cell function had deteriorated by 70% in subjects with high, but still normal, 2-h glucose levels. It should be emphasized, however, that the glucose sensitivity of β-cell function during an oral glucose tolerance test includes the release and action of gut incretins and possibly also the action of the autonomic nervous system in addition to β-cell capacity per se, whereas the response to intravenous arginine and glucose is governed by β-cell function. This may explain the severe defect in β-cell response during oral glucose tolerance tests in subjects with high normal glucose, since these subjects might have reduced release of the gut incretin glucagon-like peptide-1, in analogue to reduced glucagon-like peptide-1 response to meal ingestion in subjects with impaired glucose tolerance (22).

In conclusion, this study has shown that reduced insulin sensitivity is seen in subjects with high, but normal, fasting glucose, that the rapid insulin response to intravenous bolus of arginine is dependent on the fasting glucose level with a glucose threshold of ~4.6 mmol/l, and that in subjects with fasting glucose levels >5.6 mmol/l there is an upregulation of the maximal insulin response to arginine and glucose. Based on these findings, we suggest that basal and maximal insulin secretion is upregulated by reduced insulin sensitivity and that a high, but still normal, fasting glucose may contribute to this regulation.

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