

Islet Dysfunction in Insulin Resistance Involves Impaired Insulin Secretion and Increased Glucagon Secretion in Postmenopausal Women With Impaired Glucose Tolerance

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OBJECTIVE — To characterize in detail the association between insulin sensitivity and islet function in relation to glucose tolerance in nondiabetic subjects.

RESEARCH DESIGN AND METHODS — The study included 108 postmenopausal women, aged 57–59 years, with normal glucose tolerance (NGT) or impaired glucose tolerance (IGT) and measured glucose tolerance (World Health Organization, 75 g glucose), insulin sensitivity (euglycemic-hyperinsulinemic clamp), and islet function (the 2–5 min insulin responses [AIR] and glucagon [AGR] responses to 5 g intravenous arginine at fasting, 14 and >25 mmol/l glucose levels). The product of insulin sensitivity and secretion was calculated (disposition index [DI]) and used to study the relationship between the two parameters.

RESULTS — Insulin sensitivity and insulin secretion were highly inversely correlated in a hyperbolic manner ($r > 0.64$, $P < 0.001$) in women with NGT ($n = 71$). Women with IGT ($n = 37$) had reduced insulin sensitivity compared with women with NGT ($P = 0.011$). The AIRs were not appropriately increased in relation to the reduced insulin sensitivity in the IGT women, demonstrated as reduced DI in IGT compared with NGT ($P < 0.001$). Further, women with IGT had an increased AGR ($P < 0.001$) and a reduced glucose inhibition of glucagon secretion (slope_{AGR}, $P = 0.014$) compared with women with NGT. In a multivariate regression model including all of the 108 women, 2-h glucose was independently determined by the DI, the AGR, and the slope_{AGR} ($r = 0.63$, $P < 0.001$).

CONCLUSIONS — We have shown that both the individual ability to adapt insulin secretion to the ambient insulin sensitivity and the level of glucagon secretion are important parameters for maintenance of NGT. Therefore, islet dysfunction in IGT involves low insulin and high glucagon secretion, which present potential targets for correcting impaired glycemia.

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Cross-sectional studies have demonstrated that, in normal healthy subjects, insulin secretion is inversely regulated by the degree of insulin sensitivity (1–4). To establish whether insulin secretion

is appropriate in an individual, the secretory capacity needs to be judged in relation to the degree of insulin sensitivity in the same individual. It is thought that diabetes results from an imbalance in the relationship

between these processes, because a poor islet compensation to insulin resistance has been documented in subjects with gestational diabetes (5,6). Individuals with insulin resistance, reduced insulin secretion, or impaired glucose tolerance (IGT) are known to have an increased risk of diabetes development (7–11). It has been documented that insulin-resistant subjects with IGT exhibit a reduced insulin secretion compared with normal subjects (12–14). To study in more detail the relationship between insulin sensitivity and islet function in these high-risk subjects, we have examined insulin sensitivity and insulin secretion in each individual in a large group of postmenopausal nondiabetic women. The women had normal glucose tolerance (NGT) or IGT and were randomly selected from a population with a high prevalence of glucose intolerance (15). Insulin sensitivity was measured with the euglycemic-hyperinsulinemic clamp (16) and islet function was studied using the glucose-dependent arginine stimulation test (17,18). This method enables the determination of several different aspects of insulin secretion, such as the baseline and maximal secretory capacity and the glucose potentiation of insulin secretion. The method also enables the determination of several aspects of glucagon secretion, such as baseline secretory capacity and the suppression of glucagon secretion by glucose. Therefore, in our study, we were able to evaluate not only β -cell but also α -cell function in relation to insulin resistance. This is important because, in contrast to insulin secretion, the role of glucagon secretion for the development of type 2 diabetes is not well characterized. In fully developed type 2 diabetes, increased glucagon levels are seen in the fasting state together with a reduced glucose suppression of glucagon secretion (17,19,20). However, it has not been established whether these changes are primary or, as suggested by some previous studies (21,22), secondary to the disease process.

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Abbreviations: AGR, acute glucagon response; AIR, acute insulin response; BG, blood glucose; CV, coefficient of variation; DI, disposition index; IGT, impaired glucose tolerance; IVGTT, intravenous glucose tolerance test; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test; RIA, radioimmunoassay; WHO, World Health Organization.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

The aim of our study was to characterize, in detail, the inverse relationship between islet function and insulin sensitivity in a large group of white postmenopausal women with NGT or IGT. The study focused on the hypothesis that IGT results from an inadequate increase of insulin secretion or inappropriately high glucagon secretion in relation to the ambient insulin sensitivity.

RESEARCH DESIGN AND METHODS

Study design

Examinations were performed in 1993–1994 and included a clinical examination, anthropometric measurements, an oral glucose tolerance test (OGTT), a euglycemic-hyperinsulinemic clamp (16) for determination of insulin sensitivity, and a glucose-dependent arginine stimulation test (17,18) to measure islet function. The Ethics Committee of Lund University approved the study and informed written consent was obtained from all participants before entry in the study. The studies of glucose tolerance, insulin sensitivity, and insulin secretion were all performed in the morning after an overnight fast, with at least 1 week between visits.

Subjects

A total of 108 women were randomly selected from a larger cohort of 841 postmenopausal women born in 1935, living in the city of Malmö, Sweden, who had previously participated in a health screening (1990–1991) that included an OGTT (15). The selection procedure was based on the 2-h blood glucose (BG) value after a standard World Health Organization (WHO) 75-g OGTT. Women with 2-h BG <11.1 mmol/l were stratified into 6 different ranges of 2-h BG so that all degrees of glucose tolerance, from NGT (2-h BG \leq 5.5, 5.6–6.2, 6.3–6.9, and 7.0–7.7 mmol/l) to IGT (2-h BG 7.8–9.0 and 9.1–11.0 mmol/l), were represented by 16–21 subjects in each range. No cases of diabetes were included and none of the women included in the study was taking any medication known to affect carbohydrate metabolism.

Anthropometric measurements

All measurements were performed with the subjects in light clothing and without shoes. Body weight was measured to the nearest 0.1 kg in the morning before breakfast. Height was measured to the nearest

centimeter. Both body weight and height were measured on 2 separate occasions. The BMI was calculated as the weight (kg) divided by height squared (m^2) for each separate measurement, and the mean BMI was calculated. Waist and hip circumferences were measured with the subjects standing. The waist circumference was measured at the level of the umbilicus, hip circumference at the level of the greater trochanter, and the waist-to-hip ratio was calculated as a measure of central adiposity. Body fat content was determined using a bioelectrical impedance analyzer (BIA-109; JRL Systems, Detroit, MI).

Glucose tolerance

Oral glucose tolerance was determined with a standard WHO 75-g glucose load (23), with capillary blood glucose samples taken before and 2 h after the glucose load. The subjects spent the 2 h in a semirecumbent position. According to WHO criteria, NGT was defined as a 2-h capillary blood glucose value <7.8 mmol/l and IGT was defined as a 2-h capillary blood glucose value of 7.8–11.1 mmol/l (23).

Insulin sensitivity

Insulin sensitivity was determined with the euglycemic-hyperinsulinemic clamp, performed according to DeFronzo et al. (16). 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. Baseline samples of glucose and insulin were taken. A primed constant infusion of insulin (Actrapid 100 U/ml; Novo Nordisk, Bagsvaerd, Denmark), with a constant infusion rate of $0.28 \text{ nmol} \cdot \text{m}^{-2} \text{ body surface} \cdot \text{min}^{-1}$, 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 bedside every 5 minutes. Samples for analysis of the achieved insulin concentration were taken at 60 and 120 min.

Islet function

Insulin and glucagon secretion were determined with intravenous arginine stimulation at 3 glucose levels (fasting and 14 and >25 mmol/l), as previously described (17,18). 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 a 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 initiated to raise and maintain blood glucose at 13–15 mmol/l. Blood glucose was determined every 5 min bedside and the glucose infusion adjusted to reach the desired blood glucose level of 13–15 mmol/l in 20–25 min. New baseline samples were taken, then arginine (5 g) was again injected and 2, 3, 4, and 5 min samples taken. A 2.5-h resting period was allowed to avoid the well-known priming effect of hyperglycemia (24,25). After the pause, baseline samples were again obtained. Then 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) injected, followed by final 2, 3, 4, and 5-min samples. At the baseline examination, the achieved plasma glucose levels, after the 2 glucose infusions, were 15.7 ± 2.1 and 31.2 ± 3.7 mmol/l.

Analyses

Blood glucose concentration was determined bedside by the glucose dehydrogenase technique with a Hemocue (Hemocue AB, Ångelholm, Sweden) during the euglycemic-hyperinsulinemic clamp, and with an Accutrend (Boehringer Mannheim Scandinavia AB, Bromma, Sweden) during the arginine test. Blood samples for analysis of insulin, glucagon, and glucose, from the arginine and clamp studies, and glucose from the OGTT, were immediately centrifuged at 5°C and serum or plasma frozen at -20°C. Serum insulin and plasma glucagon concentrations were analyzed with double-antibody radioimmunoassay (RIA) technique. For the insulin assay, guinea pig anti-human insulin antibodies, human insulin standard and mono- ^{125}I -Tyr-human insulin (Linco, St. Charles, MO) were used. The assay is specific for insulin, with no cross-reactivity (<0.2%) with intact proinsulin or des-31, 32-proinsulin. The intra- and interassay coefficients of variation (CVs) of the insulin assay are <3%. Samples for analysis of glucagon were obtained in prechilled test tubes containing 0.084 ml

EDTA (0.34 mol/l) and aprotinin (250 kallikrein inhibiting U/ml blood, Bayer AG, Leverkusen, Germany). Analysis of glucagon concentration was performed with double-antibody RIA using guinea pig anti-human glucagon antibodies specific for pancreatic glucagon, ^{125}I -glucagon as tracer, and glucagon standard (Linco). Plasma glucose concentrations were analyzed using the glucose oxidase method. Concentrations of insulin, glucagon, and glucose from the arginine and clamp studies were taken as means of the duplicate samples.

Calculations and statistics

Data are presented as means \pm SD, unless otherwise noted. For the calculation of insulin sensitivity, a steady-state condition was assumed during the 2nd h of the clamp. Calculations were performed according to DeFronzo et al. (16). Thus, insulin sensitivity ($\text{nmol glucose} \cdot \text{kg body weight}^{-1} \cdot \text{minute}^{-1} / \text{pmol insulin} \cdot \text{l}^{-1}$) was taken as the glucose infusion rate during the 2nd h of the clamp divided by the measured mean insulin concentration during the 2nd h of the clamp.

For determination of islet function, several parameters were calculated from results obtained in the glucose-dependent arginine stimulation test. The acute insulin responses to arginine (AIRs) were calculated as the mean of the 2 to 5-min samples minus the mean prestimulus hormone concentration. The slope between AIR at fasting blood glucose and blood glucose 14 mmol/l ($\text{slope}_{\text{AIR}} = \Delta\text{AIR} / \Delta\text{glucose}$) was calculated as a measure of glucose potentiation of β -cell secretion (17,18,26). It is known that arginine-stimulated insulin secretion is maximal when the glucose level exceeds 25 mmol/l (27). Therefore, the AIR, at the highest glucose level ($\text{AIR}_{\text{PG}>25}$), was taken as a measure of the maximal insulin secretory capacity of the B-cells. The plasma glucose level at which half of the maximal insulin secretion is achieved (PG_{50}), a measure of B-cell sensitivity to glucose, was calculated from $\text{AIR}_{\text{PG}>25}$ and $\text{slope}_{\text{AIR}}$. The acute glucagon responses (AGR) and the $\text{slope}_{\text{AGR}}$ (glucose inhibition of glucagon secretion) were calculated in the same manner.

Because it has previously been shown that the relationship between insulin sensitivity and insulin secretion is hyperbolic in nature (2), we fitted a hyperbolic regression to the data. Further, to quantify the relationship between insulin sensitivity and insulin secretion, we also calculated the

Table 1—Characteristics of women with NGT or IGT

	NGT	IGT	P*
<i>n</i>	71	37	—
Body weight (kg)	68.0 \pm 9.6	68.8 \pm 9.9	0.68
BMI (kg/m^2)	25.1 \pm 3.4	25.8 \pm 3.8	0.27
Body fat content (%)	31.7 \pm 4.2	32.9 \pm 4.6	0.17
Waist-to-hip ratio	0.78 \pm 0.05	0.79 \pm 0.06	0.095
Blood pressure (mmHg)			
Systolic	127 \pm 14	136 \pm 13	0.003
Diastolic	82 \pm 8	83 \pm 9	0.29
Blood glucose (mmol/l)			
Fasting	4.6 \pm 0.5	4.8 \pm 0.5	0.026
2-h	6.2 \pm 0.9	8.6 \pm 0.8	<0.001
Cholesterol (mmol/l)			
Total	6.18 \pm 1.00	6.51 \pm 1.13	0.11
LDL	4.16 \pm 0.91	4.38 \pm 0.99	0.25
HDL	1.57 \pm 0.42	1.49 \pm 0.38	0.33
LDL-to-HDL ratio	2.82 \pm 0.94	3.14 \pm 1.20	0.13
Triglycerides (mmol/l)	1.08 \pm 0.52	1.46 \pm 0.85	0.004
Insulin sensitivity ($\text{nmol glucose} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} / \text{pmol insulin l}^{-1}$)	77.0 \pm 30.5	61.7 \pm 27.0	0.011

Data are *n* or means \pm SD. *NGT vs. IGT.

product of insulin sensitivity times the AIRs or the $\text{slope}_{\text{AIR}}$. This product was termed the disposition index (DI). The DI measures the ability of the individual to adapt its insulin secretion to the prevailing degree of insulin sensitivity.

Statistical analyses were performed with the SPSS for Windows system (SPSS, Chicago). Differences between groups were tested with Student's *t* test for unrelated samples. Two-sided tests were used and a *P* value <0.05 was considered statistically significant. Pearson's product moment correlation coefficients were obtained to estimate linear correlation among variables. Linear multiple regression was used to assess the independent effect of several variables on 2-h glucose. The stepwise forward method was used. The models are described in detail in RESULTS. Hyperbolic regressions were calculated with the Sigmaplot 4.0 for Windows (SPSS).

RESULTS

Study group characteristics

Of the 108 women, 71 had NGT and 37 had IGT, according to the glucose tolerance test. Characteristics of the 2 study groups are shown in Table 1. The women with IGT and NGT were similar in body weight, BMI, waist-to-hip ratio and body fat content. In contrast, the women with IGT had slightly increased systolic blood pressure

compared with the women with NGT. Fasting and 2-h blood glucose levels were increased in the IGT versus the NGT group. The IGT women also manifested higher fasting triglyceride levels than the NGT women. However, the levels of total cholesterol, LDL and HDL cholesterol, as well as the LDL/HDL cholesterol ratio, did not differ between the 2 groups. Finally, the IGT group was insulin-resistant compared with the NGT group, because the glucose uptake during the clamp was significantly reduced in the IGT women by \sim 20%.

Islet function

Table 2 shows that the women with IGT had increased fasting insulin levels, while insulin levels after raising the glucose to PG14 and PG>25 did not differ between the groups. The calculated AIRs to arginine were numerically similar in the 2 groups, whereas the glucose potentiation of insulin secretion, the $\text{slope}_{\text{AIR}}$, was reduced in the IGT group compared with the NGT group. The fasting glucagon level did not differ between the groups, and neither did the glucagon levels after raising plasma glucose to 14 or >25 mmol/l. The AGR to arginine at fasting glucose was also similar in the women with IGT and NGT, whereas the AGRs to arginine at PG14 and PG>25 were significantly increased in the women with IGT. Finally, the IGT group had a slightly

Table 2—Results of the glucose-dependent arginine stimulation test in women with NGT and IGT

	NGT	IGT	P*
n	71	37	—
Glucose (mmol/l)			
At FPG	4.9 ± 0.1	5.2 ± 0.1	0.047
At PG14	15.4 ± 0.3	16.1 ± 0.5	0.39
At PG>25	31.3 ± 0.5	31.0 ± 0.9	0.74
Insulin (pmol/l)			
At FPG	57 ± 22	72 ± 25	0.001
At PG14	257 ± 163	206 ± 108	0.087
At PG>25	430 ± 316	457 ± 288	0.67
AIR _{FPG} (pmol/l)	340 ± 199	355 ± 171	0.71
AIR _{PG14} (pmol/l)	1,074 ± 638	925 ± 353	0.12
AIR _{PG>25} (pmol/l)	1,208 ± 694	1,269 ± 546	0.64
Slope _{AIR} (pmol/mmol)	72.2 ± 51.5	53.3 ± 25.8	0.038
PG ₅₀ (mmol/l)	8.5 ± 3.3	9.9 ± 3.0	0.044
Glucagon (ng/l)			
At FPG	69 ± 22	72 ± 19	0.43
At PG14	52 ± 18	55 ± 16	0.32
At PG>25	44 ± 17	45 ± 15	0.64
AGR _{FPG} (ng/l)	110 ± 52	110 ± 46	0.96
AGR _{PG14} (ng/l)	53 ± 22	72 ± 29	<0.001
AGR _{PG>25} (ng/l)	39 ± 21	48 ± 21	0.048
Slope _{AGR} (ng/mmol)	-5.45 ± 3.39	-3.77 ± 3.21	0.014

Data are n or means ± SD. *NGT vs. IGT.

higher PG₅₀ value, indicating a reduction in β -cell sensitivity to glucose.

Insulin secretion versus insulin sensitivity in NGT

In the subjects with NGT, the AIRs to argi-

nine, at the 3 glucose levels, and the slope_{AIR} were all negatively and nonlinearly related to the clamp insulin sensitivity. The relationships were, as previously described using the intravenous glucose tolerance test (IVGTT) (1,2), hyperbolic in nature (i.e., insulin sensitivity times insulin secretion =

constant). Figure 1A shows the hyperbolic regression of insulin sensitivity versus AIR_{PG14} (Fig. 1A, $r = -0.75$, $P < 0.001$). Similar relationships were seen for insulin sensitivity versus AIR_{FPG} ($r = 0.81$, $P < 0.001$), vs. AIR_{PG>25} ($r = -0.78$, $P < 0.001$) and versus slope_{AIR} ($r = -0.64$, $P < 0.001$). Thus, high insulin sensitivity was coupled with low insulin secretory responses and a low glucose potentiation of insulin secretion. In contrast, low insulin sensitivity, signifying a high insulin demand, was coupled with high insulin secretory responses and a high slope_{AIR}. The different aspects of insulin secretion that are measured by the arginine stimulation test were all related to insulin sensitivity in a similar manner. To examine whether the relationship between insulin secretion and insulin sensitivity was dependent on BMI, the women with NGT were stratified according to tertiles of BMI (range: lowest tertile 19.0–23.2, middle tertile 23.3–26.4, highest tertile 26.4–33.8 kg/m²). However, it was found that the relationship between insulin sensitivity and insulin secretion was similar in all 3 tertiles (data not shown in Fig. 1). Thus, the inverse association of insulin sensitivity and insulin secretion was not altered by the degree of obesity in subjects with NGT.

Insulin secretion versus insulin sensitivity in IGT

Figure 1A shows that, in women with IGT, the relationship between insulin sensitivity

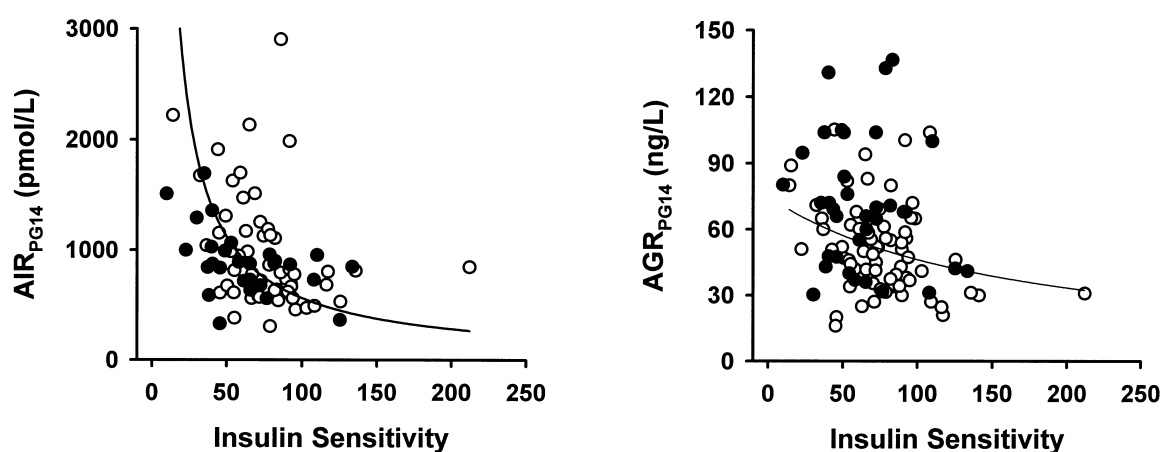


Figure 1—Insulin sensitivity versus islet function. Scatterplots of insulin sensitivity (measured by euglycemic-hyperinsulinemic clamp) versus the acute insulin (AIR, Fig. 1A) and glucagon (AGR, Fig. 1B) responses to 5 g intravenous arginine (2–5 min postload increase) at 14 mmol/l glucose (PG14) measured in the 108 women. Groups of NGT ($n = 71$, \circ) and IGT ($n = 37$, \bullet) are shown separately. Regression lines shown are the hyperbolic fit to the data for the NGT group.

Table 3—DIs in women with NGT or IGT

	NGT	IGT	P*
n	71	37	—
DI _{FPG} (μmol · kg ⁻¹ · min ⁻¹)	22.6 ± 8.9	19.7 ± 9.7	0.13
DI _{PG14} (μmol · kg ⁻¹ · min ⁻¹)	75.2 ± 42.0	53.1 ± 23.6	0.001
DI _{PG>25} (μmol · kg ⁻¹ · min ⁻¹)	81.8 ± 37.9	71.7 ± 31.4	0.17
DI _{Slope} (ml · kg ⁻¹ · min ⁻¹)	5.15 ± 3.85	3.07 ± 1.53	<0.001

Data are n or means ± SD. *NGT vs. IGT.

and the AIR_{PG14} was altered. Thus, the results of these women fell largely below the regression line calculated for the women with NGT for insulin sensitivity versus the AIR_{PG14} (χ² test for proportion of IGT or NGT women above vs. below the regression line P = 0.009). A similar relationship was seen for insulin sensitivity versus slope_{AIR} (χ² test P = 0.001). For AIR_{FPG} and AIR_{PG>25}, the proportional distribution of NGT or IGT subjects around the respective regression lines did not differ between the 2 groups.

Similarly, to combine the effects of insulin sensitivity and insulin secretion for an individual's glucose tolerance and to enable quantification of this relation, we calculated the product of insulin sensitivity times the AIRs or the slope_{AIR}. These products were termed DIs and they measure the ability of the individual to adapt their insulin secretion to the prevailing degree of insulin sensitivity. Table 3 shows that, in the IGT group, both the DI_{PG14} and the DI_{Slope} were significantly reduced compared with the NGT group. Therefore, this study shows that IGT is associated with an altered relationship between insulin secretion and insulin sensitivity both for the acute insulin response at mid-range glycemia and the glucose potentiation of insulin secretion.

Glucagon secretion versus insulin sensitivity

The AGRs were inversely related to insulin sensitivity. When including all subjects with NGT in the regression calculation, the regression displayed a nonlinear relationship (hyperbolic regression coefficients in the NGT subjects -0.63 to -0.68, P < 0.001; regression between AGR_{PG14} and insulin sensitivity is shown in Fig. 1B; r = -0.68, P < 0.001). However, when excluding the outlier with an insulin sensitivity of 212.1 nmol glucose kg⁻¹ · min⁻¹/pmol insulin · l⁻¹, a linear regression displayed higher coefficients (r = -0.49 to -0.56, P < 0.001) than hyperbolic regression coefficients (r = -0.36

to -0.42, P < 0.001). This suggests that the relationship between insulin sensitivity and glucagon secretion is inversely linear in most subjects. The relationship was, however, dependent on the insulin secretory capacity. Thus, in a partial correlation analysis correcting for the degree of AIR, the correlations between the AGRs and insulin sensitivity were no longer significant. In contrast, in a partial correlation analysis controlling for AGR, the correlations between insulin sensitivity and the AIRs were still highly significant (data not shown). This suggests that insulin sensitivity increases β-cell secretion in a manner that also induces an increased α-cell secretion.

Determinants of glucose tolerance

Using univariate correlation analysis, we found that several of the parameters of insulin sensitivity and islet function were related to glucose tolerance in the population. The 2-h glucose was negatively correlated to the insulin sensitivity as measured by the euglycemic-hyperinsulinemic clamp (r = -0.31, P = 0.001). The 2-h glucose was not significantly related to the AIRs, but was negatively

associated with the slope_{AIR} (r = -0.21, P = 0.032). Interestingly, the disposition indices were all negatively related to 2-h glucose (DI_{FPG}: r = -0.25, P = 0.01; DI_{PG14}: r = -0.34, P < 0.001; DI_{PG>25}: r = -0.22, P = 0.021; DI_{Slope}: r = -0.36, P < 0.001). Furthermore, the 2-h glucose was positively correlated to the AGR_{PG14} (r = 0.29, P = 0.002) and the slope_{AGR} (r = 0.31, P = 0.001). The PG₅₀ was positively associated with the 2-h glucose level (r = 0.24, P = 0.012).

In a stepwise forward multiple regression model (Table 4), we entered the sensitivity and secretion variables that were significantly (P < 0.05) related to 2-h glucose in the univariate analysis. For the disposition indices, only the DI_{Slope} was included in the demonstrated model, because the DIs are strongly interrelated. However, the same result was evident also with the DIs calculated for the other β-cell parameters. We found that independent determinants of 2-h glucose were DI_{Slope}, AGR_{PG14}, and slope_{AGR}, yielding a regression coefficient of 0.63. Thereby, ~40% of the variance in glucose tolerance was explained.

In a second model (Table 4), we also entered anthropometric variables that were related to 2-h glucose in univariate analyses. Thus, we entered body fat content (r = 0.27, P = 0.005), hip circumference (r = 0.23, P = 0.023), systolic blood pressure (r = 0.24, P = 0.015), triglycerides (r = 0.32, P = 0.001), and waist circumference (r = 0.23, P = 0.020). We found that, in this model, systolic blood pressure was also included as an independent predictor of glucose tolerance, increasing the regression coefficient to 0.66.

Table 4—Stepwise forward multiple regression model to determine which islet function, insulin sensitivity, and anthropometric parameters were predictors of the 2-h glucose in the 108 women included in the study

Model	Step	Variable entered*	B	SE (B)	R	R ²	P
A	1	DI _{Slope} (μmol · kg ⁻¹ · min ⁻¹)	-0.153	0.034	0.373	0.139	<0.001
	2	AGR _{PG14} (ng/l)	0.0263	0.004	0.516	0.267	<0.001
	3	Slope _{AGR} (ng/mmol)	0.164	0.035	0.630	0.396	<0.001
		Constant	6.995	0.304			<0.001
B	1	DI _{Slope} (μmol · kg ⁻¹ · min ⁻¹)	-0.147	0.034	0.374	0.140	<0.001
	2	AGR _{PG14} (ng/l)	0.0243	0.005	0.514	0.265	<0.001
	3	Slope _{AGR} (ng/mmol)	0.174	0.035	0.632	0.400	<0.001
	4	Systolic blood pressure (mmHg)	0.020	0.008	0.661	0.437	0.013
		Constant	4.547	1.021			<0.001

Model A: AGR_{PG14}, DI_{Slope}, insulin sensitivity, PG₅₀, slope_{AIR}, and slope_{AGR}. Model B: AGR_{PG14}, DI_{Slope}, insulin sensitivity, PG₅₀, slope_{AIR}, slope_{AGR}, body fat content, hip circumference, systolic blood pressure, triglycerides, and waist circumference. *Variables included.

CONCLUSIONS — Our study demonstrates that, in a population of postmenopausal women exhibiting a high prevalence (28%) of IGT (15), insulin sensitivity and secretory capacity are inversely related, both in women with NGT and IGT. This inverse association was initially suggested by Bergman et al. (1) after using the minimal model technique to determine insulin sensitivity and insulin secretion after an IVGTT. Further, the mathematical relationship between the 2 variables has been shown to be of a hyperbolic nature, i.e., displaying a function in which insulin sensitivity times insulin secretion is constant (2). Also, in our population, a hyperbolic regression fitted the data for normal subjects. In contrast to previous studies, our study group was randomly selected from the general population. This indicates that the findings regarding the relationship between insulin sensitivity and insulin secretion can be generalized for the background population. Furthermore, the relationship between insulin sensitivity and secretion is similar for several different aspects of islet function, which we were able to quantify. We found that insulin sensitivity was inversely related to the AIRs at different glucose levels and also to the glucose potentiation of insulin secretion. Thus, the more insulin resistant a subject is, the higher their maximal insulin secretion and the steeper their slope of glucose potentiation of insulin secretion. Therefore, to adequately judge an individual's insulin secretion, it has to be related to the insulin sensitivity in the same individual. Our study suggested that this relationship is independent of the degree of obesity, because we found that the upregulation of insulin secretion seen in insulin resistance was not different in subgroups of the subjects depending on BMI. However, this needs to be studied in more detail with larger groups of subjects, because this study had not statistical power enough to perform separate analysis of the relationship between insulin secretion and insulin sensitivity in different subgroups of BMI. Furthermore, when we divided the study population into subgroups depending on BMI, the glucose tolerance was not matched in these subgroups.

The hyperbolic relationship between insulin sensitivity and insulin secretion indicates that the product of these parameters is constant. This product is, therefore, of importance for the characterization of the relationship between these parameters. We

have used DI for this relationship. This index was initially derived from results obtained during an IVGTT (1). Our DI, however, used insulin secretory data after arginine stimulation and the glucose potentiation of insulin secretion. An advantage of DI as calculated in this study is that insulin secretion and insulin sensitivity were measured with independent methods. Our results show that the DIs, i.e., DI for all β -cell parameters obtained in this study, were correlated with 2-h glucose in univariate analysis and were found to be independent determinants of 2-h glucose in a multivariate regression. Other factors, such as insulin sensitivity and body adiposity, were controlled for. Our results, therefore, suggest that it is not the actual level of insulin sensitivity or insulin secretion that determines glucose tolerance, but rather the ability of the individual to adapt insulin secretion to the ambient insulin sensitivity. It could be emphasized that adiposity, whether included as BMI or waist circumference, although significantly correlated to 2-h glucose level in univariate analyses, did not remain significant when DI was also included in the model.

We showed that insulin-resistant women with IGT have a reduced insulin secretion compared with women with NGT (13,14). We now extend these findings to IGT women over a broad spectrum of insulin sensitivity and demonstrate that insulin secretion is indeed inadequate for the degree of insulin resistance in IGT. This is seen first as a different relationship between insulin sensitivity and secretion on the scatter plots, with a distribution mainly below the regression line seen in normal women. Second, the disposition indices are clearly reduced in IGT for both the AIR_{PG14} and the slope AIR , which indicates a deficient islet adaptation to insulin resistance in these women. Our results confirm previous studies pointing to reduced insulin secretion in subjects with IGT (12–14,28–31). However, in our present study, we compared insulin secretion in relation to insulin resistance at matched glucose levels in all individual subjects with NGT and IGT, which is required for a valid conclusion, and demonstrates that there is a reduced insulin secretion in women with IGT. The acute insulin responses at 14 mmol/l glucose and the glucose potentiation of insulin secretion were reduced in relation to the degree of insulin resistance in the IGT subjects, whereas the maximal insulin secretory capacity seemed to be less

affected in this prediabetic stage. Hence, a main pathophysiological event in IGT and diabetes is inadequate insulin secretion in relation to the ambient insulin sensitivity.

A novel finding in this study is that the AGRs were inversely related to insulin sensitivity. The relationship between the AGRs and insulin sensitivity was nonlinear when all subjects with NGT were included in the analysis. However, by excluding a single outlier with insulin sensitivity exceeding 200 nmol glucose \cdot kg⁻¹ min⁻¹/pmol insulin \cdot l⁻¹, a linear regression presented a better fit than a hyperbolic relation. Therefore, it seems that an inverse linear regression more closely explains the inverse relationship between glucagon secretion and insulin sensitivity in most subjects. The correlations between AGRs and insulin sensitivity were, however, dependent on insulin secretory capacity. This suggests that the influence of the degree of insulin resistance to upregulate β -cell function also upregulates α -cell function. Nevertheless, the multivariate model showed that glucagon secretion and the glucose inhibition of glucagon secretion are independent determinants of glucose tolerance. Therefore, this suggests that insulin resistance acts as a risk factor for diabetes in several aspects, both by increasing the demand for insulin and thereby causing glucose intolerance in subjects with low insulin secretory capacity, and by increasing the glucagon secretion. Furthermore, our results show that glucagon secretion is also an important parameter to consider in relation to glucose intolerance in prediabetic subjects and may be a novel risk factor for diabetes development. The mechanism for the impaired suppression of glucagon secretion by glucose in IGT, in view of the suggested inhibitory influence of local insulin on glucagon secretion, might be caused by the impaired insulin secretion (32). This would imply that the primary islet defect in IGT is the β -cell dysfunction and local insulinopenia exaggerates glucagon secretion, making the α -cell less sensitive to suppression by glucose. Follow-up studies on these women will be able to identify whether changes in insulin secretion will be followed by reciprocal changes in glucagon secretion, which would support that the increased glucagon secretion in IGT is due to β -cell dysfunction. Another interesting aspect is whether the impaired suppression of glucagon secretion by glucose in IGT contributes to the hyperglycemia by increasing hepatic glucose output. If found to be correct, this

would support the development of agents to inhibit glucagon action and secretion for early treatment of diabetes (33), which deserves further study.

In conclusion, we have demonstrated that in healthy postmenopausal women with NGT, insulin sensitivity and insulin secretion are hyperbolically related. In women with IGT, the relationship between insulin sensitivity and secretion is altered, with an insufficient adaptation of insulin secretion to the degree of insulin resistance. This is seen as a reduced product of insulin sensitivity and secretion, i.e., a low DI. Furthermore, women with IGT have increased levels of glucagon secretion. In a multiple regression model, the DI as well as glucagon secretion are independent determinants of glucose tolerance. Thus, both the individual's ability to adapt insulin secretion to the ambient insulin sensitivity and the level of glucagon secretion are important parameters for maintenance of NGT. Therefore, low insulin and high glucagon secretion present potential targets for correcting impaired glycemia.

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References

1. Bergman RN, Phillips LS, Cobelli C: Physiologic evaluation of factors controlling glucose tolerance in man: measurement of insulin sensitivity and β -cell glucose sensitivity from the response to intravenous glucose. *J Clin Invest* 68:1456–1467, 1981
2. Kahn SE, Prigeon RL, McCulloch DK, Boyko EJ, Bergman RN, Schwartz MW, Neifing JL, Ward WK, Beard JC, Palmer JP, Porte D Jr: Quantification of the relationship between insulin sensitivity and β -cell function in human subjects: evidence for a hyperbolic function. *Diabetes* 42:1663–1672, 1993
3. Ferrannini E, Natali A, Bell P, Cavallo-Perin P, Lalic N, Mingrone G: Insulin resistance and hypersecretion in obesity. *J Clin Invest* 100:1166–1173, 1997
4. Clausen JO, Borch-Jensen K, Ibsen H, Bergman RN, Hougaard P, Winther K, Ped-

- ersen O: Insulin sensitivity index, acute insulin response, and glucose effectiveness in a population-based sample of 380 young, healthy Caucasians: analysis of the impact of gender, body fat, physical fitness, and life-style factors. *J Clin Invest* 98:1195–1209, 1996
5. Ward WK, Johnston CL, Beard JC, Benedetti TH, Porte D Jr: Abnormalities of islet B-cell function, insulin action, and fat distribution in women with histories of gestational diabetes: relationship to obesity. *J Clin Endocrinol Metab* 61:1039–1045, 1985
6. Ryan EA, Imes S, Liu D, McManus R, Finegood DT, Polonsky KS, Sturis J: Defects in insulin secretion and action in women with a history of gestational diabetes. *Diabetes* 44:506–512, 1995
7. Lillioja S, Mott DM, Spraul M, Ferraro R, Foley JE, Ravussin E, Knowler WC, Bennett PH, Bogardus C: Insulin resistance and insulin secretory dysfunction as precursors of non-insulin-dependent diabetes mellitus: prospective studies of Pima Indians. *N Engl J Med* 329:1988–1992, 1993
8. Haffner SM, Miettinen H, Gaskill SP, Stern MP: Decreased insulin secretion and increased insulin resistance are independently related to the 7-year risk of NIDDM in Mexican-Americans. *Diabetes* 44:1386–1391, 1995
9. Keen H, Jarrett RJ, McCartney P: The ten-year follow-up of the Bedford survey (1962–1972): glucose tolerance and diabetes. *Diabetologia* 22:73–78, 1982
10. King H, Zimmet P, Raper LR, Balkau B: The natural history of impaired glucose tolerance in the Micronesian population of Nauru: a six-year follow-up study. *Diabetologia* 26:39–43, 1984
11. Saad MF, Knowler WC, Pettitt DJ, Nelson RG, Mott DM, Bennett PH: The natural history of impaired glucose tolerance in the Pima Indians. *N Engl J Med* 319:1500–1506, 1988
12. Haffner SM, Miettinen H, Gaskill SP, Stern MP: Decreased insulin action and insulin secretion predict the development of impaired glucose tolerance. *Diabetologia* 39:1201–1207, 1996
13. Larsson H, Ahrén B: Failure to adequately adapt reduced insulin sensitivity with increased insulin secretion in women with impaired glucose tolerance. *Diabetologia* 39:1099–1107, 1996
14. Larsson H, Ahrén B: Islet dysfunction in obese women with impaired glucose tolerance. *Metabolism* 45:502–509, 1996
15. Larsson H, Ahrén B, Lindgärde F, Berglund G: Fasting blood glucose in determining the prevalence of diabetes in a large, homogeneous population of Caucasian middle-aged women. *J Intern Med* 237:537–541, 1995
16. DeFronzo RA, Tobin JD, Andres R: Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* 237:E214–E223, 1979
17. Ward WK, Bolgiano DC, McKnight B, Halter JB, Porte D Jr: Diminished B cell secretory capacity in patients with noninsulin-dependent diabetes mellitus. *J Clin Invest* 74:1318–1328, 1984
18. Larsson H, Ahrén B: Glucose-dependent arginine stimulation test for characterization of islet function: studies on reproducibility and priming effect of arginine. *Diabetologia* 41:772–777, 1998
19. Dimitriadis GD, Pehling GD, Gerich JE: Abnormal glucose modulation of islet α - and β -cell responses to arginine in non-insulin-dependent diabetes mellitus. *Diabetes* 34:541–547, 1985
20. Baron AD, Schaeffer L, Shragg P, Kolterman OG: Role of hyperglucagonemia in maintenance of increased rates of hepatic glucose output in type II diabetics. *Diabetes* 36:274–283, 1987
21. Assan R, Efendic S, Luft R, Cerasi E: Dose-kinetics of pancreatic glucagon responses to arginine and glucose in subjects with normal and impaired pancreatic B cell function. *Diabetologia* 21:452–459, 1981
22. Hamaguchi T, Fukushima H, Uehara M, Wada S, Shirohani T, Kishikawa H, Ichinose K, Yamaguchi K, Shichiri M: Abnormal glucagon response to arginine and its normalization in obese hyperinsulinemic patients with glucose intolerance: importance of insulin action on pancreatic alpha cells. *Diabetologia* 34:801–806, 1991
23. World Health Organization: *Diabetes Mellitus: Report of a WHO Study Group*. Geneva, World Health Org., 1985 (Tech. Rep. Ser., no. 727)
24. Cerasi E: Potentiation of insulin release by glucose in man. *Acta Endocrinol* 79:511–534, 1975
25. Grill V: Time and dose dependencies for priming effect of glucose on insulin secretion. *Am J Physiol* 240:E24–E31, 1981
26. Halter JB, Graf RJ, Porte D Jr: Potentiation of insulin secretory responses by plasma glucose levels in man: evidence that hyperglycemia in diabetes compensates for impaired glucose potentiation. *J Clin Endocrinol Metab* 48:946–954, 1979
27. Porte D Jr: Banting Lecture 1990: β -Cells in type II diabetes mellitus. *Diabetes* 40:166–180, 1991
28. Ahrén B, Pacini G: Impaired adaptation of first-phase insulin secretion in postmenopausal women with glucose intolerance. *Am J Physiol* 273:E701–E707, 1997
29. Haffner SM, Miettinen H, Gaskill SP, Stern MP: Decreased insulin action and insulin secretion predict the development of impaired glucose tolerance. *Diabetologia* 39:1201–1207, 1996
30. Kanatsuka A, Makino H, Sakurada M, Hashimoto N, Iwaoka H, Yamaguchi T, Taira M, Yoshida S, Yoshida A: First-phase insulin response to glucose in nonobese or

- obese subjects with glucose intolerance: analysis by C-peptide secretion rate. *Metabolism* 37:878-884, 1988
31. Mitrakou A, Kelley D, Mokan M, Veneman T, Pangburn T, Reilly J, Gerich J: Role of reduced suppression of glucose production and diminished early insulin release in impaired glucose tolerance. *N Engl J Med* 326:22-29, 1992
32. Maruyama H, Hisatomi A, Orci L, Grodsky GM, Unger RH: Insulin within islets is a physiologic glucagon release inhibitor. *J Clin Invest* 74:2296-229, 1984
33. van Tine BA, Azizeh BY, Trivedi D, Phelps JR, Houslay MD, Johnson DG, Hruby VJ: Low level cyclic adenosine 3',5'-monophosphate accumulation analysis of [des-His¹, des-Phe⁶, glu⁹] glucagon-NH₂ identifies glucagon antagonists from weak partial agonists/antagonists. *Endocrinology* 137:3316-3322, 1996