Section 5: Methodology for Quantifying Insulin Release in Man

Quantification of Insulin Secretion in Relation to Insulin Sensitivity in Nondiabetic Postmenopausal Women

Bo Ahrén and Hillevi Larsson

To evaluate mechanisms underlying the close association between insulin secretion and insulin sensitivity, insulin sensitivity was evaluated by the euglycemic-hyperinsulinemic clamp technique (M/I_{clamp}) and insulin secretion was determined from the 75-g oral glucose tolerance test (OGTT) and from the glucose-dependent arginine-stimulation test in 81 nondiabetic postmenopausal women, all aged 61 years. M/I_{clamp} was normally distributed with mean ± SD of 69.9 ± 30.5 nmol glucose · kg^{-1} · min^{-1}/pmol insulin · l^{-1}. It was found that the several different measures of insulin secretion from the OGTT and the glucose-dependent arginine-stimulation test were all inversely related to M/I_{clamp}. However, measures determining the direct insulin responses were more markedly potentiated by low M/I_{clamp} than were measures determining glucose potentiation of insulin secretion. Moreover, the product of M/I_{clamp} times measures of insulin secretion (disposition index [DI]) was inversely related to the 2-h glucose value. Finally, surrogate insulin sensitivity measures quantified from OGTT and the glucose-dependent arginine-stimulation test only weakly correlated to M/I_{clamp} ($R^2 \approx 0.25$). Thus, *1*) insulin secretion is adaptively increased when insulin sensitivity is low in nondiabetic postmenopausal women; *2*) β-cell exocytotic ability shows more efficient adaptation than β-cell glucose recognition to low insulin sensitivity; *3*) impaired β-cell adaptation (i.e., low DI) is associated with higher 2-h glucose values during OGTT, although other regulatory mechanisms also exist; and *4*) indirect surrogate measures of insulin sensitivity only weakly correlate to insulin sensitivity as determined by the euglycemic-hyperinsulinemic clamp.
***Diabetes* 52 (Suppl. 1):202–211, 2002**

A syndrome linking cardiovascular diseases with glucose intolerance, hyperlipidemia, coagulation defects, and central obesity has been termed the insulin resistance syndrome, syndrome X, or the metabolic syndrome and has been estimated to affect at least 25% of middle-aged subjects in the industrialized part of the world (1–3). A common denominator in this syndrome is defective insulin sensitivity (1,2,4), which is due to a combination of genetic and environmental factors (5). The defective insulin sensitivity requires hyperinsulinemia for preservation of normoglycemia, and under normal conditions this is achieved by a compensatory and adequate increase in insulin secretion (6–8). This compensation is finely tuned, and in subjects with normal glucose tolerance, insulin sensitivity and insulin secretion are related to each other in a hyperbolic manner (6,7). This has been established using measures derived from both a single intravenous glucose tolerance test (6,7) and the euglycemic-hyperinsulinemic clamp for the determination of insulin sensitivity in combination with the glucose-dependent arginine-stimulation test for the determination of insulin secretion (8,9). This hyperbolic relationship has been quantified as the disposition index (DI), which is the product of insulin secretion times insulin sensitivity (6,7). Under abnormal conditions—i.e., when the islet β-cells function incorrectly—the increase in insulin secretion in relation to the demand is insufficient, resulting in an inadequate hyperinsulinemia (6,8–14). This leads to a low DI, which has been shown to be associated with impaired glucose tolerance (IGT) and type 2 diabetes (6–14). A low DI has also been shown to predict worsening of glucose tolerance over a 3-year period in middle-aged women (15). However, the mechanisms responsible for the adaptation of insulin secretion to changes in insulin sensitivity have not been established in humans. In the present study, the islet mechanisms were evaluated in 81 nondiabetic subjects with varying glucose tolerance using the hyperinsulinemic-euglycemic clamp technique for the determination of insulin sensitivity (16) and the glucose-dependent arginine-stimulation test for the evaluation of insulin secretion. The latter technique evaluates several aspects of β-cell function; by comparing these aspects,

From the Department of Medicine, Lund University, Lund, Sweden.
Address correspondence and reprint requests to bo.ahren@med.lu.se.
Accepted for publication 26 June 2001.

AIR, arginine-induced insulin release; BG, blood glucose; DI, disposition index; HOMA, homeostasis model assessment; IGT, impaired glucose tolerance; IGratio, insulin-to-glucose ratio; ins_{index}, insulinogenic index; IR, insulin response; ISI, insulin sensitivity index; M/I_{clamp}, insulin sensitivity by euglycemic-hyperinsulinemic clamp technique; OGTT, oral glucose tolerance test; WHO, World Health Organization.

The symposium and the publication of this article were made possible by an unrestricted educational grant from Servier, Paris.

TABLE 1
Characteristics of the 81 women examined in the present study and the linear correlation between each variable and insulin sensitivity as determined by the euglycemic-hyperinsulinemic clamp (M/I_{clamp}).

Variable	Means ± SD	Range	Correlation to M/I_{clamp}
Age	61 years, 9 months ± 6 months	60 years, 10 months to 62 years, 1 month	
Body weight (kg)	68.8 ± 9.3	48.8–96.0	-0.43, $P < 0.001$
BMI (kg/m^2)	25.7 ± 3.4	19.0–32.8	-0.46, $P < 0.001$
Waist circumference (cm)	82.5 ± 8.8	66–106	-0.51, $P < 0.001$
Waist-to-hip ratio	0.86 ± 0.05	0.79–0.98	-0.42, $P < 0.001$
Fasting plasma glucose (mmol/l)	4.8 ± 0.7	3.2–6.9	-0.27, $P = 0.015$
2-h plasma glucose (mmol/l)	7.7 ± 1.5	2.7–10.5	-0.45, $P < 0.001$
Fasting serum insulin (pmol/l)	82 ± 29	38–199	-0.55, $P < 0.001$

P indicates probability level of absence of correlation.

insight into the mechanisms of the adaptation of insulin secretion is achieved. Thus, the technique evaluates the β-cell response to a nonglucose stimulus (arginine) at different glucose levels and offers quantification of the baseline and maximal insulin response to a challenge over a wide glucose range, as well as the glucose potentiation of arginine-induced insulin release (AIR) (8–12,15,17). We also quantified insulin secretion as the insulin response during an oral glucose tolerance test (OGTT) in these subjects. The different insulin secretory measures were quantified in relation to the ambient insulin sensitivity. The results therefore offer insight into the relationship between insulin secretion, as judged by different techniques disclosing different β-cell mechanisms, and insulin sensitivity, in nondiabetic subjects.

RESEARCH DESIGN AND METHODS

Study design. Examinations were performed from 1996 to 1997 and included a clinical examination, anthropometric measurements, an OGTT, a euglycemic-hyperinsulinemic clamp (16), and a glucose-dependent arginine-stimulation test (17) in all subjects. All studies were performed in the morning after an overnight fast, with at least 1 week between the visits. The Ethics Committee of Lund University approved the study, and informed written consent was obtained from all participants before entry in the study.

Subjects. The subjects were all women born in 1935, i.e., they were 61 years of age at the time of the study. They were recruited from a larger cohort of 841 postmenopausal women born that year and living in the city of Malmö, Sweden. They had previously participated in a health screening (1990–1991) (18) and in a metabolic study on insulin secretion (1993–1994) (8,19). The selection procedure from the larger cohort was based on the 2-h blood glucose (BG) value after a standard World Health Organization (WHO) 75-g OGTT (20), where women with 2-h BG <11.1 mmol/l were stratified so that all degrees of glucose tolerance were studied. Thus, subjects from each of six strata (2-h BG ≤ 5.5, 5.6–6.2, 6.3–6.9, 7.0–7.7, 7.8–9.0, and 9.1–11.0 mmol/l) were included. All 81 subjects were healthy, and none was taking any medication known to affect carbohydrate metabolism (9). Table 1 shows the clinical characteristics of the study group.

Anthropometric measurements. All measurements were performed with the subjects in light clothing without shoes. Body weight was measured to the nearest 0.1 kg in the morning before breakfast. Height was measured to the nearest cm. BMI was then calculated as the weight (kg) divided by height squared (m^2). Waist and hip circumferences were measured with the subjects standing. The waist circumference was measured at the level of the umbilicus, and hip circumference, at the level of the greater trochanters.

Oral glucose tolerance test. An intravenous catheter was inserted into an antecubital vein. After a baseline sample was taken, an OGTT was performed with a standard WHO 75-g glucose load (20). Blood samples were taken after 30 and 120 min. The subjects spent 2 h in a semirecumbent position. Plasma or serum was immediately separated for analysis of glucose and insulin.

Hyperinsulinemic-euglycemic clamp. Insulin sensitivity was determined with the euglycemic-hyperinsulinemic clamp, performed according to De Fronzo 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 units/ml; Novo Nordisk, Bagsvaerd, Denmark) with a constant infusion rate of 0.28 nmol · m^{-2} body surface area · 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 at bedside every 5 min. Mean blood glucose during the second hour of the clamp test was 5.1 ± 0.1 (mean ± SE) mmol/l. Samples for analysis of the achieved insulin concentration were taken at 60 and 120 min.

Glucose-dependent arginine-stimulation test. Insulin secretion was determined with intravenous arginine stimulation at three glucose levels (fasting, 14 mmol/l, and >25 mmol/l), as previously described (12,17). Intravenous catheters were inserted into antecubital veins in both arms. One arm was used for 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 13–15 mmol/l. Blood glucose was determined every 5 min at bedside, and the glucose infusion was adjusted to reach the desired blood glucose level of 13–15 mmol/l in 15–20 min. New baseline samples were taken, then arginine (5 g) was again injected and samples were taken at 2, 3, 4, and 5 min. A 2.5-h resting period was then allowed to avoid the well-known priming effect of hyperglycemia (21,22). After the pause, baseline samples were again obtained. A high-speed (900 ml/h) 20% glucose infusion during 25–30 min was then used to raise blood glucose to >25 mmol/l, as determined at bedside. At this blood glucose level, new baseline samples were taken, arginine (5 g) was injected, and final samples were taken at 2, 3, 4, and 5 min. Fasting plasma glucose immediately before arginine injection was 4.7 ± 0.1 mmol/l. The first glucose infusion lasted for 17.5 ± 0.6 min, and the plasma glucose level before arginine injection after this first glucose infusion was 13.4 ± 0.2 mmol/l. The second glucose infusion lasted for 26.9 ± 0.6 min, and the plasma glucose level before arginine injection after this second glucose infusion was 27.6 ± 0.6 mmol/l.

Analyses. Blood glucose concentration was determined at bedside by the glucose dehydrogenase technique (Accutrend; Boehringer Mannheim Scandinavia AB, Bromma, Sweden). Blood samples for analysis of insulin and glucose were immediately centrifuged at 5°C, and serum or plasma was frozen at −20°C until analysis in duplicate. Serum insulin concentrations were analyzed with double-antibody radioimmunoassay technique using guinea pig anti–human insulin antibodies, human insulin standard, and mono-^{125}I-Tyr-human insulin (Linco Research, St. Charles, Mo). 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 of the insulin assay are <3%. Plasma glucose concentrations were analyzed using the glucose oxidase method. All concentrations were taken as means of the duplicate samples.

Calculations. Data are presented as means ± SE, unless otherwise noted. For estimation of insulin sensitivity from data obtained during the OGTT, several indexes were calculated using the insulin and glucose data at fasting or at 2 h (23–27). The insulin sensitivity index (ISI) was calculated by dividing insulin levels by glucose levels at fasting (ISI_0) or at 2 h (ISI_{120}). Insulin sensitivity was also calculated as the insulin-to-glucose ratio (IGratio) at fasting or at 2 h ($IGratio_0$ and $IGratio_{120}$, respectively), according to the homeostasis model assessment ($HOMA_{IR}$ = fasting insulin/[22.5 × $e^{-\ln \text{fasting glucose}}$]) using fasting levels of insulin and glucose. For calculation of insulin sensitivity from the hyperinsulinemic-euglycemic clamp test, a steady-state

TABLE 2
Variables used for estimation of insulin sensitivity as obtained from either the OGTT, the hyperinsulinemic-euglycemic clamp test, or the glucose-dependent arginine-stimulation test.

Variable	Abbreviation	Test	Definition
Fasting insulin	I_0	OGTT	Baseline serum insulin
Insulin sensitivity index at fasting	ISI_0	OGTT	$I_0 \times G_0$
Insulin sensitivity index at 2 h	ISI_{120}	OGTT	$I_{120} \times G_{120}$
Insulin-to-glucose ratio at fasting (pmol insulin/mmol glucose)	$IGratio_0$	OGTT	I_0/G_0
Insulin-to-glucose ratio at 2 h (pmol insulin/mmol glucose)	$IGratio_{120}$	OGTT	I_{120}/G_{120}
Homeostasis model assessment of insulin resistance	$HOMA_{IR}$	OGTT	Fasting insulin/$(22.5 \times e^{-\ln \text{fasting glucose}})$
Insulin sensitivity determined by clamp	M/I_{clamp}	Clamp	Glucose infusion rate/steady-state insulin
Insulin sensitivity at 14 mmol/l glucose	M/I_{14}	Arginine	Glucose infusion rate/insulin at 14 mmol/l glucose
Insulin sensitivity at >25 mmol/l glucose	M/I_{25}	Arginine	Glucose infusion rate/insulin at > 25 mmol/l glucose

G, glucose; I, insulin; M/I, insulin sensitivity.

condition was assumed during the second hour of the clamp. Calculations were performed according to DeFronzo et al. (16). Thus, insulin sensitivity (nmol glucose · kg body weight^{-1} · min^{-1}/pmol insulin · l^{-1}) was taken as the glucose infusion rate divided by the measured mean insulin concentration during the second hour of the clamp (M/I_{clamp}). Finally, for calculation of insulin sensitivity from the glucose-dependent arginine-stimulation test, the amount of glucose infused to raise the glucose level to the desired concentrations of 14 mmol/l (M/I_{14}) and >25 mmol/l (M/I_{25}) was divided by the serum insulin concentration achieved immediately before the arginine injection. Table 2 summarizes the variables used for estimation of insulin sensitivity as obtained from the three tests.

Three variables were used for estimation of insulin secretion from data obtained during the OGTT. First, the increase in insulin levels from baseline to 30 min was calculated by subtracting the baseline insulin from the 30-min insulin ($\Delta_{insulin}$). Second, the insulinogenic index (ins_{index}) was calculated by dividing $\Delta_{insulin}$ by the 30-min glucose level. Third, β-cell function as obtained by the homeostasis model assessment was calculated as $HOMA_B$ = fasting insulin × 3.33/(fasting glucose – 3.5) (23–25,27). For determination of insulin secretion from the glucose-dependent arginine-stimulation test, the arginine-induced insulin release (AIR) was calculated as the mean of the 2- to 5-min samples minus the mean prestimulus hormone concentration at fasting glucose (AIR_{FG}), at 14 mmol/l glucose (AIR_{14}), and at the maximal glucose level (AIR_{MAX}). The slope between AIR_{FG} and AIR_{14} ($slope_{AIR}$ = ΔAIR/Δglucose) was calculated as a measure of glucose potentiation of β-cell secretion, since it is known that augmentation of the insulin response to arginine is linearly related to the glucose level at levels below 17 mmol/l (12,17). It is known that arginine-stimulated insulin secretion is maximal when the glucose level exceeds 25 mmol/l (28). Therefore, the AIR at the highest glucose level (AIR_{MAX}) was taken as a measure of the maximal insulin secretory capacity of the β-cells. Finally, the insulin response (IR) to raising the glucose levels to 14 and >25 mmol/l ($IR_{glucose14}$ and $IR_{glucose25}$, respectively) was also calculated, by subtracting the baseline insulin levels from the levels obtained after raising the glucose levels, i.e., immediately before administration of arginine. Table 3 summarizes the variables used for estimation of insulin secretion as obtained from the two tests.

Statistics. Statistical analyses were performed with the SPSS for Windows system (SPSS, Chicago, Ill). Normality of distribution was tested with the Kolmogorov-Smirnov goodness-of-fit test. Pearson's product moment correla-

TABLE 3
Variables used for estimation of insulin secretion as obtained from the OGTT or the glucose-dependent arginine-stimulation test

Variable	Abbreviation	Obtained from test	Definition
30-min increase in insulin (pmol/l)	$\Delta_{insulin}$	OGTT	$I_{30} - I_0$
Insulinogenic index (pmol insulin/mmol glucose)	Ins_{index}	OGTT	ΔInsulin/G_{30}
Homeostasis model assessment for β-cell function	$HOMA_B$	OGTT	$I_0 \times 3.33/(G_0-3.5)$
Insulin secretion at fasting glucose (pmol/l)	AIR_{FG}	Arginine	Acute insulin response to arginine at fasting glucose
Insulin secretion at 14 mmol/l glucose (pmol/l)	AIR_{14}	Arginine	Acute insulin response to arginine at 14 mmol/l glucose
Insulin secretion at >25 mmol/l glucose (pmol/l)	AIR_{MAX}	Arginine	Acute insulin response to arginine at >25 mmol/l glucose
Glucose potentiation of arginine-stimulated insulin secretion (pmol insulin/mmol glucose)	$Slope_{AIR}$	Arginine	$(AIR_{14}-AIR_{FG})/\Delta$glucose
Insulin response to raising glucose to 14 mmol/l	$IR_{glucose14}$	Arginine	ΔSerum insulin after raising glucose to 14 mmol/l
Insulin response to raising glucose to > 25 mmol/l	$IR_{glucose25}$	Arginine	ΔSerum insulin after raising glucose to >25 mmol/l

G, glucose; I, insulin.

TABLE 4
Measures of insulin sensitivity and insulin secretion as obtained during the OGTT and the glucose-dependent arginine-stimulation test and their correlations to the insulin sensitivity value (M/I_{clamp}) obtained during the euglycemic-hyperinsulinemic clamp as judged by Spearman test.

Variable	Means ± SD	Correlation to M/I_{clamp}
$HOMA_{IR}$	17.9 ± 7.8	−0.54, $P < 0.001$
Fasting insulin (pmol/l)	82 ± 27	−0.55, $P < 0.001$
ISI_0 (nmol insulin · $mmol^{-1}$ glucose)	0.40 ± 0.2	−0.54, $P < 0.001$
$IGratio_0$ (pmol insulin · $mmol^{-1}$ glucose)	17.2 ± 5.9	−0.43, $P < 0.001$
ISI_{120} (nmol insulin · mmol glucose)	3.4 ± 3.1	−0.55, $P < 0.001$
$IGratio_{120}$ (nmol insulin/mmol glucose)	0.59 ± 0.36	−0.38, $P < 0.001$
M/I_{14} (nmol glucose · kg^{-1} · min^{-1}/pmol insulin · 1^{-1})	816 ± 50	0.43, $P < 0.001$
M/I_{28} (nmol glucose · kg^{-1} · min^{-1}/pmol insulin · 1^{-1})	978 ± 71	0.43, $P < 0.001$
AIR_{FG} (pmol/l)	339 ± 189	−0.29, $P = 0.008$
AIR_{14} (pmol/l)	880 ± 499	−0.07, NS
AIR_{MAX} (pmol/l)	1056 ± 617	−0.30, $P = 0.007$
$Slope_{AIR}$ (pmol insulin · $mmol^{-1}$ glucose)	63.4 ± 41.2	0.07, NS
$IR_{glucose14}$ (pmol/l)	143 ± 111	−0.29, $P = 0.009$
$IR_{glucose25}$ (pmol/l)	307 ± 205	−0.34, $P = 0.002$
$HOMA_B$	254 ± 262	0.09, NS
ins_{index} (pmol insulin · $mmol^{-1}$ glucose)	151 ± 133	−0.27, $P = 0.014$
$\Delta insulin_{30}$ (pmol/l)	21.6 ± 16.4	−0.29, $P = 0.009$

tion coefficients were obtained to estimate linear correlation between normally distributed variables, and Spearman regression coefficients were obtained to estimate correlation in the absence of a normal distribution. Linear stepwise forward multiple regression was used to assess the independent effect of several variables.

RESULTS

Insulin sensitivity. As determined by the hyperinsulinemic, euglycemic clamp technique, the insulin sensitivity (measured as the M/I_{clamp} value) showed a normal distribution with a mean ± SD value of 69.9 ± 30.5 nmol glucose · kg^{-1} · min^{-1}/pmol insulin · 1^{-1} ($P = 0.56$, Kolmogorov-Smirnov goodness-of-fit test). Insulin sensitivity showed a strong inverse correlation with body weight, BMI, waist circumference, 2-h plasma glucose, and fasting insulin (Table 1). Using fasting and 2-h postload glucose and insulin determinations from the OGTT, insulin sensitivity was quantified as $HOMA_{IR}$, fasting insulin, ISI_0, $IGratio_0$, ISI_{120}, and $IGratio_{120}$. Table 4 shows that these variables all exhibited a linear correlation with the M/I_{clamp} value, with an R^2 value of ~0.25.

Insulin sensitivity was also estimated by variables obtained from the glucose-dependent arginine-stimulation test, calculated as the amount of glucose infused to increase the blood glucose values to 14 and >25 mmol/l, respectively, divided by the insulin levels at these glucose concentrations (M/I_{14} and M/I_{25}, respectively). Table 4 shows that these two estimates also correlated with M/I_{clamp}. Therefore, these results show that insulin sensitivity as quantified by the euglycemic-hyperinsulinemic clamp can be indirectly estimated by several different variables obtained under fasting conditions, after oral glucose, and in the glucose-dependent arginine-stimulation test, although the correlation coefficients for these surrogate measures versus M/I_{clamp} were not high.

Insulin secretion. Insulin secretion measurements were derived using data obtained from the OGTT ($HOMA_B$, $\Delta insulin_{30}$, and ins_{index}) and from the glucose-dependent arginine-stimulation test (AIR_{FG}, AIR_{14}, AIR_{MAX}, $slope_{AIR}$, $IR_{glucose14}$, and $IR_{glucose25}$). Table 4 shows the means ± SD of these measures in the population. Of these data, only $slope_{AIR}$ displayed a normal distribution ($P > 0.05$, Kolmogorov-Smirnov goodness-of-fit test). Table 5 shows the relations between these measures of insulin secretion. They generally showed a good interrelation, except for $HOMA_B$, which did not correlate significantly to any of the other measures of insulin secretion. However, the regression coefficients for all variables were <0.6. Table 4 also shows the correlations between variables of insulin secretion with the insulin sensitivity as obtained in the clamp study. A significant negative relation was evident for M/I_{clamp} versus AIR_{FG}, AIR_{MAX}, $IR_{glucose14}$, $IR_{glucose25}$, and

TABLE 5
Correlations between measures of insulin secretion as obtained by the Spearman test

	AIR_{14}	AIR_{MAX}	$Slope_{AIR}$	$IR_{glucose14}$	$IR_{glucose25}$	$HOMA_B$	ins_{index}	$\Delta insulin_{30}$
AIR_{FG}	0.71*	0.68*	0.43*	0.37*	0.53*	0.01	0.40*	0.29†
AIR_{14}		0.72*	0.88*	0.64*	0.54*	0.13	0.50*	0.49*
AIR_{MAX}			0.54*	0.49*	0.74*	0.13	0.41*	0.31†
$Slope_{AIR}$				0.60*	0.45*	0.04	0.44*	0.48*
$IR_{glucose14}$					0.57*	0.21	0.60*	0.47*
$IR_{glucose25}$						0.17	0.39*	0.25‡
$HOMA_B$							0.22*	0.15
ins_{index}								0.69*

Probability level of random difference for the relations of *$P < 0.001$, †$P < 0.01$, or ‡$P < 0.05$.

$\Delta insulin_{30}$. In contrast, $slope_{AIR}$, AIR_{14}, $HOMA_B$, and ins_{index} did not correlate significantly to M/I_{clamp}.

Relationship between insulin sensitivity and insulin secretion. The relation between insulin sensitivity and insulin secretion was inverse for all the parameters studied and displayed the hyperbolic relation as previously documented (6–9). To compare the relation to insulin sensitivity for the various measures of insulin secretion, the entire study group was divided into quartiles with respect to M/I_{clamp}. Figure 1 shows the various insulin secretory measures in these four groups of subjects with varying M/I_{clamp}. It is clearly seen that insulin secretion was higher in subjects with low M/I_{clamp} than in those with high M/I_{clamp}. For most variables, a significant increase in insulin secretion required an M/I_{clamp} as low as ~60 nmol glucose · kg^{-1} · min^{-1}/pmol insulin · l^{-1}. For example, there was no significant difference in insulin secretion as determined by AIR_{14}, AIR_{MAX}, $slope_{AIR}$, $\Delta insulin_{30}$, or ins_{index} between quartiles 1 and 2 of M/I_{clamp}, despite a large difference in M/I_{clamp} between those two groups (107.3 ± 6.1 vs. 74.1 ± 1.2 nmol glucose · kg^{-1} · min^{-1}/pmol insulin · l^{-1}; $P < 0.001$). This would suggest that a threshold of ~60 nmol glucose · kg^{-1} · min^{-1}/pmol insulin · l^{-1} would be required for M/I_{clamp} to augment insulin secretion. Such a conclusion is supported by results in which, when including all subjects, the correlation between the logarithmic value of these variables versus the logarithmic value of M/I_{clamp} was not significant, because a hyperbolic relation through the entire range of M/I_{clamp} would result in significant linear relation between the logarithmic values. In contrast, when excluding the quartile with the highest M/I_{clamp}, these correlations, based on logarithmic values, were significant ($r = -0.40$ to $r = -0.60$). Therefore, for most measures of insulin secretion, it seems as if a threshold for M/I_{clamp} when augmenting insulin secretion exists, although this may also be explained by there being too few subjects under study. In contrast, for $logAIR_{FG}$, a significant correlation was evident with $logM/I_{clamp}$ over the entire M/I_{clamp} range in all subjects, suggesting a clear hyperbolic relation between AIR_{FG} and M/I_{clamp} over the entire range of insulin sensitivity. In any case, however, the magnitude of the adaptation in insulin secretion is weak in high insulin sensitivity ranges, which is expected from the hyperbolic nature of the relationship. However, in quartile 3, when M/I_{clamp} was only 56.8 ± 1.2 nmol glucose · kg^{-1} · min^{-1}/pmol insulin · l^{-1}, insulin secretion was significantly increased compared to quartiles 1 and 2, showing that, in subjects with low insulin sensitivity, the magnitude of the islet compensation is high. The degree of augmentation of insulin secretion in subjects with low insulin sensitivity, however, was different between the different measures of insulin secretion. This is shown in Fig. 2, which displays the percentage increase in insulin secretion versus quartiles of M/I_{clamp} when the mean insulin secretion values in the group with the highest quartile of M/I_{clamp} were defined as 100%. It is seen that AIR_{FG} showed the most potent adaptation from the highest to the lowest quartile of M/I_{clamp}, being 188 ± 18%, whereas $slope_{AIR}$ only increased by 121 ± 8% ($P = 0.021$).

Relationship between disposition index and glucose tolerance. The relation between insulin secretion and insulin sensitivity was quantified by calculating the DI, i.e., the product of the different measures of insulin secretion times M/I_{clamp}. To study the relationship between DI, as calculated using different measures of insulin secretion, and glucose tolerance, the population was divided into quartiles according to the 2-h glucose level in the OGTT. Figure 3 shows that the higher the 2-h glucose level, the lower the DI. In fact, there was a negative correlation between the 2-h glucose value and the DI for AIR_{FG} ($r = -0.34$, $P = 0.002$), AIR_{14} ($r = -0.41$, $P < 0.001$), AIR_{MAX} ($r = -0.29$, $P = 0.008$), $slope_{AIR}$ ($r = -0.41$, $P < 0.001$), $\Delta insulin_{30}$ ($r = -0.36$, $P = 0.001$), and ins_{index} ($r = -0.34$, $P = 0.002$). In contrast, the correlations between DI and fasting glucose levels were much lower and, in fact, significant only for DI for AIR_{14} ($r = -0.26$, $P = 0.018$) and DI for $slope_{AIR}$ ($r = -0.25$, $P = 0.025$). This suggests that DI is a more robust determinator of glucose tolerance than of fasting glucose in subjects with this small range of glucose levels. In fact, multivariate analysis using the 2-h glucose level as the dependent variable and the DI calculated from AIR_{14} times M/I_{clamp} as the independent variables revealed a significant contribution of both these variables to the 2-h glucose level, with an R^2 value of 0.34 ($P < 0.001$). However, the finding that the R^2 value was only ~0.3 between the DI and the 2-h glucose value shows that other control mechanisms are also involved in determining the degree of glucose tolerance. This is also evident from the wide range of DI for all measures in the various quartiles of 2-h glucose (Fig. 3). The significant overlap for DI between the different quartiles of 2-h glucose is illustrated in Fig. 4, showing the scatter of DI for AIR_{FG} in the highest and lowest quartile of 2-h glucose. It is seen that, in spite of a clear difference in mean values (25.9 ± 2.1 vs. 15.1 ± 1.5 μmol glucose · kg^{-1} · min^{-1}; $P < 0.001$), a considerable overlap exists.

DISCUSSION

Identifying mechanisms of the adaptation of β-cell function in relation to changes in insulin sensitivity will increase our understanding of basic mechanisms in the metabolic syndrome and in the pathophysiology of IGT and type 2 diabetes. Such analyses might also yield information of importance toward targeting treatment for inadequate β-cell adaptation. Increasing the knowledge of the β-cell adaptation to changes in insulin sensitivity requires reliable measures of the relationship between insulin sensitivity and insulin secretion. Development of such measures relies on the hyperbolic relation between insulin sensitivity and insulin secretion. This was first described by Bergman et al. in 1981 (6), using data obtained from an intravenous glucose tolerance test with minimal modeling of data of glucose disappearance. The concept of the hyperbolic nature between these variables was later elegantly characterized in detail by Kahn et al. in 1993 (7). The term "disposition index" was introduced to quantify the relation between insulin sensitivity and insulin secretion (6,7). The clinical importance of this was illustrated by the demonstration that the disposition index was lower in subjects with type 2 diabetes and IGT than in healthy subjects, implying that the relation between insulin sensitivity and insulin secretion is abnormal under these conditions (6,7). The original definition of disposition index

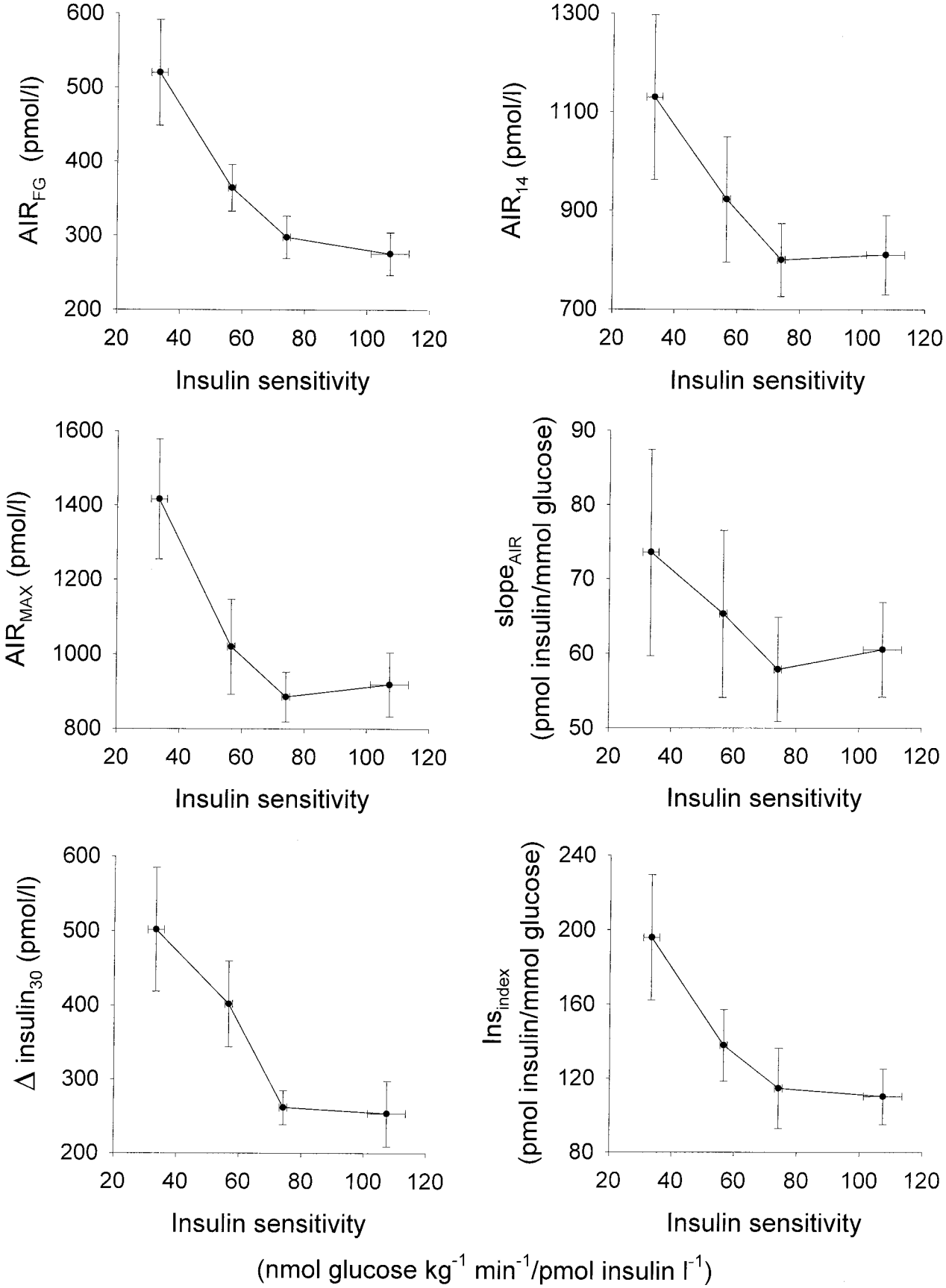

FIG. 1. Insulin secretion as determined by the glucose-dependent arginine-stimulation test (AIR_{FG}, AIR_{14}, AIR_{MAX}, and $slope_{AIR}$) and by the oral glucose tolerance test ($\Delta insulin_{30}$ and ins_{index}) versus insulin sensitivity as determined by the euglycemic-hyperinsulinemic clamp in the four quartiles of insulin sensitivity in 81 healthy, nondiabetic women aged 61 years (n = 20–21 in each quartile). Means ± SE are shown.

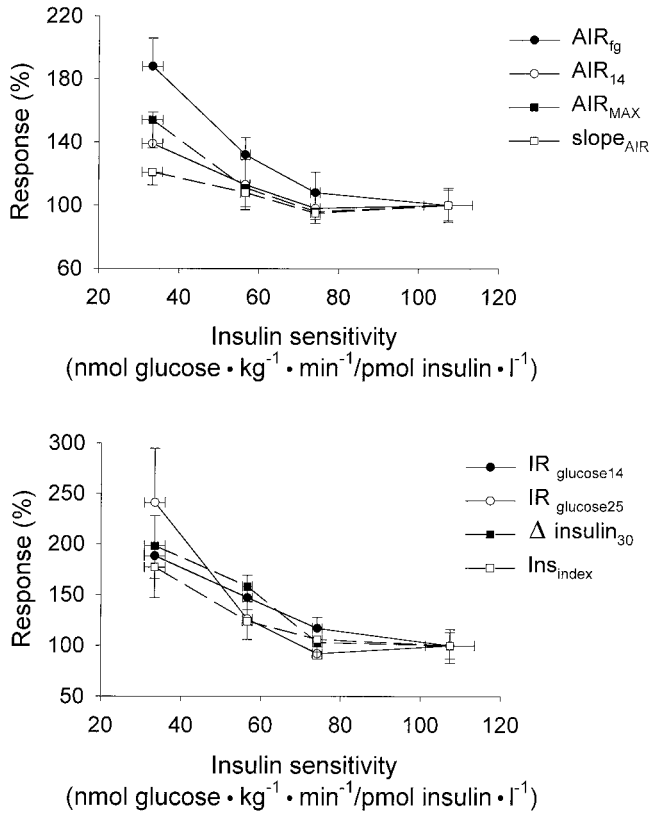

FIG. 2. Insulin secretion as determined by the glucose-dependent arginine-stimulation test (AIR_{FG}, AIR_{14}, AIR_{MAX}, $slope_{AIR}$, $IR_{glucose14}$, and $IR_{glucose25}$) and as determined by the oral glucose tolerance test ($\Delta insulin_{30}$ and insulinogenic index) versus insulin sensitivity in the four quartiles of insulin sensitivity as determined by the euglycemic-hyperinsulinemic clamp. Insulin secretion is expressed as percentage of mean of the respective values in the highest insulin sensitivity quartile in 81 healthy, nondiabetic women aged 61 years (n = 20–21 in each quartile). Means ± SE are shown. IR, insulin response.

used the potentiation by glucose of the second phase of circulating insulin levels after glucose challenge as an independent measure of insulin secretion and calculation of the insulin sensitivity index using minimal model analysis of data obtained from an intravenous glucose tolerance test with fast sampling as a measure of insulin sensitivity (6). This index therefore applies to the posthepatic insulin levels in relation to insulin sensitivity as determined in the same test. An index more directly using the β-cell secretion from the C-peptide response to glucose was later introduced and named "adaptation index," and this index was also found to be reduced in subjects with IGT (11). Finally, we have recently introduced an index called the "insulin effect index" for the quantification of insulin secretion as determined by the glucose-dependent arginine-stimulation test times insulin sensitivity as determined in an independent test (euglycemic-hyperinsulinemic clamp), and we found that this index was also reduced in IGT (15). Although the calculations of these various indexes are different, they all quantify the relationship between insulin secretion and insulin sensitivity, and therefore the rationale for using these different indexes is the same. To avoid introducing different names for the indexes when different variables are used, in the present study we have used the term disposition index throughout, regardless of whether insulin secretion was derived from the OGTT or the various measures obtained from the glucose-dependent arginine-stimulation test.

The evaluation of insulin sensitivity by the euglycemic-hyperinsulinemic clamp is laborious, and therefore more simple indexes derived from data obtained from the OGTT have been suggested to replace the M/I_{clamp} value (24,29). We found that such surrogate measures of insulin sensitivity from the OGTT and from the glucose-dependent arginine-stimulation test correlate significantly to the M/I_{clamp} value obtained from the clamp study. The highest correlation was obtained using the $HOMA_{IR}$, the fasting insulin, and the ISI_0. However, the R^2 values of the relation between these measures and M/I_{clamp} were not impressively high and reached only ~0.25. This confirms several previous studies of a regression coefficient for the regression between $HOMA_{IR}$ and M/I_{clamp} of approximately r = 0.5 to r = 0.6 (13,30,31). Hence, $HOMA_{IR}$ and the other surrogates seem to be only weak substitutes for the clamp measurement of insulin sensitivity when measuring insulin sensitivity in nondiabetic subjects. We also found that the M/I values obtained at 14 and 28 mmol/l glucose during the glucose-dependent arginine-stimulation test showed a correlation with the M/I_{clamp} of only r = 0.43, i.e., having an R^2 value of only ~0.18. This is lower than the correlation between M/I values from euglycemic versus hyperglycemic clamp tests (32), which is due to the steady-state conditions under which these clamp tests are performed in contrast to the glucose-dependent arginine-stimulation test, in which the hyperglycemic phase lasted for no more than ~20 to 30 min. Hence, the glucose-dependent arginine-stimulation test does not achieve the same duration of clamp as the euglycemic and hyperglycemic clamp tests, and therefore their values of M/I should be viewed cautiously.

For quantification of insulin secretion, several different techniques have been used. These techniques disclose different mechanisms of β-cell stimulation. The intravenous glucose tolerance test, as commonly used for the calculation of first and second phases of insulin secretion (6,7,11), studies the direct β-cell response to a glucose challenge, whereas the OGTT evaluates the combined β-cell action of increased glycemia, stimulated secretion of gastrointestinal incretins, and activated nerves during a cephalic phase. The technique used in the present study is the glucose-dependent arginine-stimulation test, which was initially described by Ward et al. (12) and later described in detail (17). This test offers the quantification of several aspects of β-cell function, of which insulin secretion at fasting glucose (AIR_{FG}), the maximal insulin secretion (AIR_{MAX}), and the glucose potentiation of insulin secretion ($slope_{AIR}$) are of most interest. The AIR_{FG} quantifies the direct and acute β-cell response to a sudden arginine challenge and is therefore related to the rapid efficiency of the exocytotic machinery of the β-cell. The AIR_{MAX} quantifies the maximal possible β-cell response under acute conditions, and $slope_{AIR}$ is a measure of the glucose potentiation of the arginine-induced insulin secretion. We found that all these variables showed strong interrelations and that they also correlated with indirect insulin secretion measures obtained from the OGTT, except for $HOMA_B$, which turned out to correlate only poorly to the other measures of insulin secretion. In fact, the

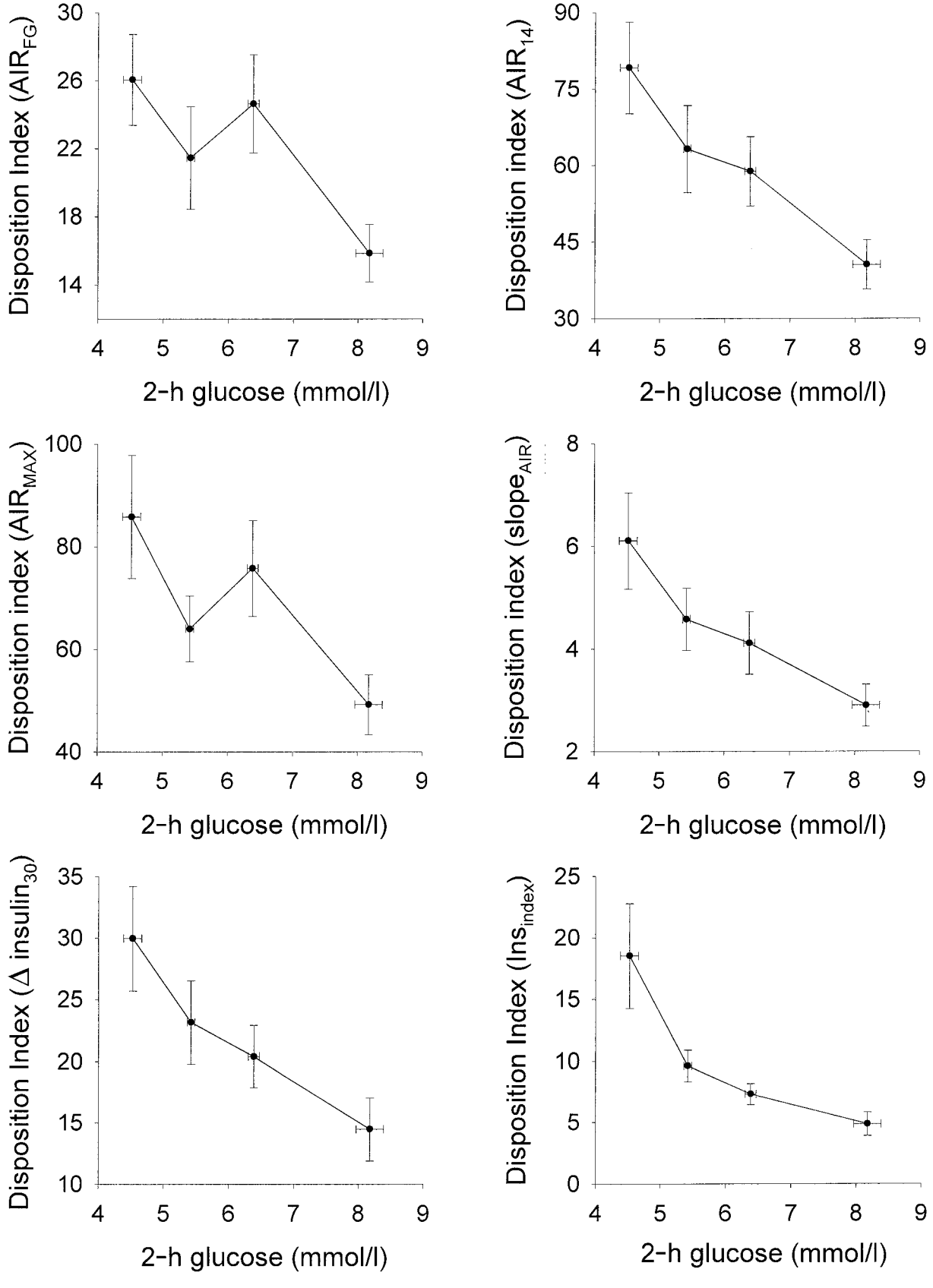

FIG. 3. Disposition index calculated as M/I_{clamp} during the euglycemic-hyperinsulinemic clamp times various measures of insulin secretion obtained during the glucose-dependent arginine-stimulation test (AIR_{FG}, AIR_{14}, AIR_{MAX}, and $slope_{AIR}$) and the OGTT ($\Delta insulin_{30}$ and insulinogenic index) versus the 2-h glucose level during the OGTT in the four quartiles of the 2-h glucose value in 81 healthy, nondiabetic women aged 61 years (n = 20–21 in each quartile). Means ± SE are shown.

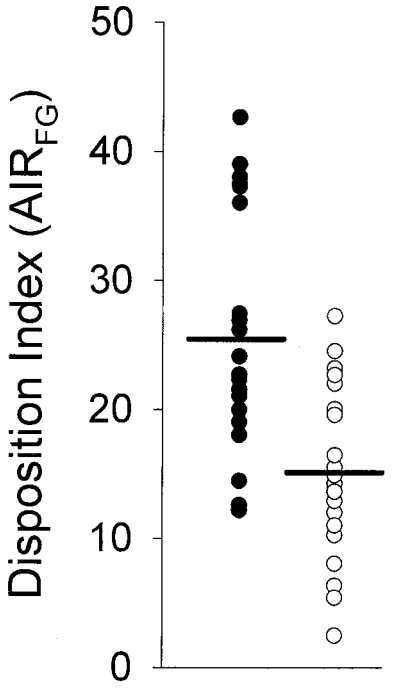

FIG. 4. Scatter of disposition index for AIR_{FG} in the lowest (plasma glucose 5.0 ± 0.1 mmol/l; n = 20; ●) and the highest (plasma glucose 9.7 ± 0.2 mmol/l; n = 20; ○) quartiles of the 2-h glucose among the 81 healthy, nondiabetic women aged 61 years.

$HOMA_B$ did not significantly correlate to postchallenge measures of insulin secretion obtained from the OGTT or the glucose-dependent arginine-stimulation test. Also, $HOMA_B$ did not correlate significantly to insulin sensitivity as the other measures of insulin secretion. Therefore, the results of the present study question the use of $HOMA_B$ as a surrogate measure of insulin secretion.

All measures of insulin secretion derived from OGTT and glucose-dependent arginine-stimulation test were inversely related to insulin sensitivity as obtained during the euglycemic, hyperinsulinemic clamp. This indicates that all the measured aspects of insulin secretion—i.e., the direct action of arginine, the combined action of arginine and hyperglycemia, or the complex challenge induced by oral glucose involving hyperglycemia, gastrointestinal incretins, and autonomic nerves—are sensitive to changes in insulin sensitivity. The β-cell adaptation to insulin resistance thus seems to involve both an increased secretory rate upon a given challenge as well as an increased sensitivity to extracellular glucose, suggesting that the adaptation involves both the exocytotic and the glucose recognition mechanisms in the β-cells. We found, however, that there was a quantitative difference between the responses of the various insulin secretory measures to reduced insulin sensitivity. The direct insulinotropic action to arginine showed a higher response than the glucose potentiation of the β-cells. Although the mechanism of this difference cannot be established from this work, the results indicate that the exocytotic adaptation (reflected as arginine-stimulated insulin secretion) appears to be more responsive to a given reduction in insulin sensitivity than the glucose recognition mechanisms (reflected as the glucose potentiation of arginine-stimulated insulin secretion). The mechanistic basis for the increase in insulin secretion in low insulin sensitivity remains, however, to be established. What also remains to be established is the signal generated by low insulin sensitivity and augmenting β-cell function. This signal may hypothetically reside in perturbations in fuel metabolism caused by insulin resistance, such as hyperglycemia or elevated levels of free fatty acids, which are well-established signals to the β-cells (33). Other possibilities exist, however, such as increased vagal activity, which has been shown to accompany insulin resistance in Pima indians (34); other putative mechanisms are mediation induced by adipocyte-derived factors or gastrointestinal hormones, which may affect β-cell function. Identifying the mechanisms behind the close relationship between insulin sensitivity and insulin secretion is important, since failure to adjust these processes to each other may be an underlying cause for the deterioration of glucose metabolism in IGT and type 2 diabetes.

Except for insulin secretion determined as AIR_{FG}, the increase in insulin secretion by reduced insulin sensitivity was not observed until insulin sensitivity was below ~60 nmol glucose · kg^{-1} · min^{-1}/pmol insulin · l^{-1}. This suggests that the β-cells do not sense a slight reduction of insulin sensitivity in subjects within the highest insulin-sensitive quartiles, although the size of the present study group might be too small for detecting an increased insulin secretion in these very insulin-sensitive subjects. In the groups having low insulin sensitivity, i.e., <60 nmol glucose · kg^{-1} · min^{-1}/pmol insulin · l^{-1}, insulin secretion was, however, clearly increased compared with subjects with higher insulin sensitivity, and this was seen regardless of which parameter was used for calculating insulin secretion. Therefore, a clear adaptation in insulin secretion is evident in nondiabetic subjects having reduced insulin sensitivity, and this adaptation involves several aspects of β-cell function. By calculating the DI for the various insulin secretion measures, it was found that this variable related to the 2-h glucose value, in that subjects in the quartile with the highest 2-h glucose value had lower DIs than subjects in the quartile with the lowest 2-h glucose value. Therefore, these subjects did not adequately increase insulin secretion in response to their low insulin sensitivity. This reduction in DI was the same regardless of which insulin secretory parameter was used in the calculation, which suggests that the failure to adequately increase insulin secretion in low insulin sensitivity is a global phenomenon involving both exocytotic mechanisms and glucose recognition. The multivariate analysis disclosed that insulin sensitivity and insulin secretion both independently contributed to the 2-h glucose value, showing that both of these mechanisms are of importance for glucose tolerance in nondiabetic subjects. However, the R^2 value in the multivariate analysis was only ~0.3. This confirms that these variables contribute only by 30% to the glucose tolerance (13), which indicates that insulin-independent mechanisms exert a major impact on glucose tolerance. This is also evident by the wide scatter of DI in the various quartiles of 2-h glucose levels, as illustrated in Fig. 4. Such insulin-independent factors may include glucose-dependent glucose disposal and glucagon. These insulin-independent mechanisms appear to be even more important for the regulation of fasting

glucose, which only weakly correlated to insulin sensitivity and insulin secretion. However, these nondiabetic subjects showed a very narrow range of fasting glucose, and therefore regression analyses including this parameter should be viewed cautiously.

In conclusion, this study shows that in nondiabetic postmenopausal women, insulin sensitivity causes an adaptive increase in insulin secretion, which involves both increased secretory rate as a sign of increased exocytotic ability, as well as increased glucose potentiation of the β-cells as a sign of increased β-cell glucose recognition capacity. The study also shows that impaired β-cell adaptation is associated with higher 2-h glucose values during OGTT, although it also shows that other regulatory mechanisms are involved. Finally, the comparison of different methods for the determination of insulin sensitivity and insulin secretion shows that the indirect measures of insulin sensitivity only weakly correlate to insulin sensitivity as determined by the euglycemic-hyperinsulinemic clamp, whereas the measures of insulin secretion as obtained by OGTT and glucose-dependent arginine-stimulation tests show a higher degree of interrelationship.

ACKNOWLEDGMENTS

The study was supported by the Swedish Medical Research Council (14X-6834), Novo Nordisk, and Albert Påhlssons Foundations, the Swedish Diabetes Association, Malmö University Hospital, and the Faculty of Medicine, Lund University.

The authors are grateful to Lilian Bengtsson, Lena Bryngelsson, Ulrika Gustavsson, Kerstin Nilsson, and Margaretha Persson for expert technical assistance.

REFERENCES

1. Reaven GM: Role of insulin resistance in human disease. *Diabetes* 37:1595–1607, 1988
2. Haffner SM: The insulin resistance syndrome revisited. *Diabetes Care* 19:275–276, 1996
3. Meigs JB, D'Agostino RB, Wilson PWF, Cupples LA, Nathan DM, Singer DE: Risk variable clustering in the insulin resistance syndrome. The Framingham Offspring Study. *Diabetes* 46:1594–1600, 1997
4. Modan M, Halkin H, Almog S, Lusky A, Eshkol A, Sefi M, Shitrit A, Fuchs Z: Hyperinsulinemia—a link between hypertension, obesity and glucose intolerance. *J Clin Invest* 75:809–817, 1985
5. Clausen JO, Borch-Jensen K, Obsen H, Bergman RN, Hougaard P, Winther K, Pedersen 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
6. 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
7. 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
8. 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
9. Larsson H, Ahrén B: Islet dysfunction in insulin resistance involves impaired insulin secretion and increased glucagon secretion in postmenopausal women with impaired glucose tolerance. *Diabetes Care* 23:650–657, 2000
10. Larsson H, Ahrén B: Islet dysfunction in obese women with impaired glucose tolerance. *Metabolism* 45:502–509, 1996
11. Ahrén B, Pacini G: Impaired adaptation of first-phase insulin secretion in postmenopausal women with glucose intolerance. *Am J Physiol* 273:E701–E707, 1997
12. 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
13. Van Haeften TW, Pimenta W, Mitrakou A, Krotkiewski M, Jenssen T, Yki-Järvinen H, Gerich JE: Relative contributions of β-cell function and tissue insulin sensitivity to fasting and postload glycemia. *Metabolism* 49:1318–1325, 2000
14. Gerich JE: Addressing the insulin secretion defect: a logical first-line approach. *Metabolism* 49 (Suppl. 2):12–16, 2000
15. Larsson H, Ahrén B: Glucose intolerance is predicted by low insulin secretion and high glucagon secretion: outcome of a prospective study in postmenopausal Caucasian women. *Diabetologia* 43:194–202, 2000
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. 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
18. Larsson H, Ahrén B, Lindgärde F, Berglund G: Fasting blood glucose in determining prevalence of diabetes in a large, homogeneous population of Caucasian middle-aged women. *J Intern Med* 237:537–541, 1995
19. Larsson H, Berglund G, Ahrén B: Glucose modulation of insulin and glucagon secretion is altered in impaired glucose tolerance. *J Clin Endocrinol Metab* 80:1778–1782, 1995
20. World Health Organization: *Diabetes Mellitus*: *Report of a WHO Study Group*. Geneva, Switzerland, WHO 1985
21. Cerasi E: Potentiation of insulin release by glucose in man. *Acta Endocrinol* 79:511–534, 1975
22. Grill V: Time and dose dependencies for priming effect of glucose on insulin secretion. *Am J Physiol* 240:E24–E31, 1981
23. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC: Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28:412–419, 1985
24. Hanson RL, Pratley RE, Bogardus C, Venkat Narayan KM, Roumain JML, Imperatore G, Fagot-Campagna A, Pettitt DJ, Bennett PH, Knowler WC: Evaluation of simple indices of insulin sensitivity and insulin secretion for use in epidemiologic studies. *Am J Epidemiol* 151:190–198, 2000
25. Sluiter WJ, Erkelans DW, Terpstra P, Reitsma VD, Doorenbos H: Glucose tolerance and insulin release, a mathematical approach. II. Approximation of the peripheral insulin resistance after oral glucose loading. *Diabetes* 25:245–249, 1976
26. Lakkso M: How good a marker is insulin level for insulin resistance? *Am J Epidemiol* 137:959–965, 1993
27. Sluiter WJ, Erkelens DW, Reitsma WD, Doorenbos H: Glucose tolerance and insulin release, a mathematical approach. I. Assay of the β-cell response after oral glucose loading. *Diabetes* 25:241–244, 1976
28. Porte D Jr: Banting lecture 1990: β-Cells in type II diabetes mellitus. *Diabetes* 40:166–180, 1991
29. Phillips DW, Clark PM, Hales CN, Osmond C: Understanding oral glucose tolerance: comparison of glucose and insulin measurements during the oral glucose tolerance test with specific measurements of insulin resistance and secretion. *Diabet Med* 11:286–292, 1994
30. Lausang MC, Williams GH, Carroll JS: Correlation between the glucose clamp technique and the homeostasis model assessment in hypertension. *Am J Hypertens* 14:51–53, 2001
31. McAuley KA, Williams SM, Mann JI, Walker RJ, Lewis-Barned NJ, Temple LA, Duncan AW: Diagnosing insulin resistance in the general population. *Diabetes Care* 24:460–465, 2001
32. Pimenta W, Korytkowski M, Mitrakou A, Jenssen T, Yki-Järvinen H, Evron W, Dailey G, Gerich J: Pancreatic beta-cell dysfunction as the primary genetic lesion in NIDDM: evidence from studies in normal glucose-tolerant individuals with a first-degree NIDDM relative. *JAMA* 273:1855–1861, 1995
33. McGarry JD, Dobbins RL: Fatty acids, lipotoxicity and insulin secretion. *Diabetologia* 42:128–138, 1999
34. Weyer C, Salbe AD, Lindsay RS, Pratley RE, Bogardus C, Tataranni PA: Exaggerated pancreatic polypeptide secretion in Pima Indians: can an increased parasympathetic drive to the pancreas contribute to hyperinsulinemia, obesity, and diabetes in humans. *Metabolism* 50:223–230, 2001