Islet Dysfunction in Obese Women With Impaired Glucose Tolerance

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Insulin sensitivity and islet function were examined in 22 obese women: 11 with normal glucose tolerance (mean ± SD body mass index [BMI], 32.2 ± 2.8 kg/m^2) and 11 with impaired glucose tolerance (BMI, 30.1 ± 2.2 kg/m^2). Thirteen non-obese women with normal glucose tolerance (BMI, 20.9 ± 1.3 kg/m^2) served as controls. All women were 58 to 59 years of age. Insulin sensitivity was measured with the euglycemic, hyperinsulinemic clamp. Insulin secretion was studied after intravenous arginine (5 g) at fasting, and at blood glucose levels of 14 and greater than 25 mmol/L. Insulin sensitivity was higher in non-obese (99.8 ± 11.5 nmol/kg/min/pmol insulin/L) than in obese subjects ($P \leq .002$), but did not differ between obese subjects with normal versus impaired glucose tolerance (47.2 ± 8.8 *v* 45.5 ± 5.2 nmol/kg/min/pmol insulin/L, difference not significant [NS]). Obese subjects with normal glucose tolerance had a higher insulin response to both glucose ($P \leq .004$) and arginine ($P \leq .02$) than nonobese women, and a higher glucose potentiation of insulin secretion, slope$_{AIR}$ ($P = .05$). Compared with obese subjects with normal glucose tolerance, the obese subjects with impaired glucose tolerance had a lower insulin response to glucose ($P = .03$) and to arginine at blood glucose levels of 14 mmol/L ($P = .03$), as well as a lower slope$_{AIR}$ levels ($P = .03$). Fasting glucagon was higher in obese subjects with normal glucose tolerance than in non-obese subjects with normal glucose tolerance ($P = .006$). In obese subjects with impaired glucose tolerance, the glucose inhibition of glucagon secretion, slope $_{AGR}$, was lower than in obese subjects with normal glucose tolerance ($P = .04$). Thus, obese subjects with impaired glucose tolerance have altered glucose modulation of islet function, mainly manifested as reduced slope$_{AIR}$ and slope$_{AGR}$, yet insulin sensitivity is not different than in equally obese subjects with normal glucose tolerance. We therefore conclude that islet dysfunction, and not a further reduction of insulin sensitivity, determines the development of impaired glucose tolerance in obesity.

ALTHOUGH IT IS KNOWN that in fully developed non–insulin-dependent diabetes mellitus (NIDDM), both decreased insulin sensitivity and failing insulin secretion are of importance for the deranged glucose metabolism, the temporal relation between these two events has not been established. Several studies have shown that a decrease in insulin sensitivity occurs early in the development of NIDDM.[1-5] For example, it is a common view that while insulin sensitivity decreases progressively from normal glucose tolerance via impaired glucose tolerance to mild NIDDM, insulin secretion increases progressively.[6] With further progression of an already established mild NIDDM to a more severe phase, a hypoinsulinemic phase ensues, causing rising blood glucose levels.[6] In contrast with this, some studies have shown a decreased insulin secretion already in subjects at risk for NIDDM,[7-12] indicating that failing β-cell function is of importance for the development of the disease. To establish the temporal relationship between impairment of insulin sensitivity and islet dysfunction for the development of NIDDM and the relative importance of them, it is of value to study subjects with increased risk for NIDDM, and to use parallel examinations of both insulin secretion and insulin sensitivity. Subjects with impaired glucose tolerance are of interest in this respect, since it is well known that this condition is accompanied by an increased risk of developing NIDDM,[13-16] as studied for example in Pima Indians[13] and caucasions.[16] Similarly, subjects with obesity are of interest, since obesity is a major risk factor for NIDDM development.[16-19] Therefore, to provide information on the involvement of changes in insulin sensitivity and secretion for the pathogenesis of NIDDM, we have studied obese subjects with and without a concurrent impaired glucose tolerance.

Obese subjects are known to exhibit a reduced insulin sensitivity,[20,21] and are generally found to have increased circulating insulin concentrations both in the fasting state and following a glucose load.[22,23] The hyperinsulinemia seems primarily to be caused by increased secretion rate as opposed to decreased insulin clearance, and the increased secretion is regarded as a compensation for the reduced peripheral insulin sensitivity.[24,25] It is not established whether development of impaired glucose tolerance or NIDDM in obese subjects is due to deterioration of insulin secretion, or worsening of insulin sensitivity. A recent study on this subject demonstrated that a group of obese subjects with impaired glucose tolerance had an insulin secretion in response to intravenous glucose that was not different from that of equally obese subjects with normal glucose tolerance exhibiting similar insulin sensitivity.[25] However, since glucose levels postload were higher in subjects with impaired glucose tolerance than in subjects with normal glucose tolerance, insulin secretion was not evaluated under isoglycemic conditions, and thus, the insulin secretion in the subjects with impaired glucose tolerance could have been overestimated due to their higher glucose levels. Therefore, to study in more detail how obesity affects insulin secretion and to characterize insulin secretion in obese subjects with impaired glucose tolerance, we have investigated obese subjects with normal or impaired glucose tolerance. Insulin

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sensitivity was measured with the euglycemic hyperinsulinemic clamp technique,[26] and insulin secretion was studied with the glucose-dependent arginine test.[27] This method determines insulin secretion at equal levels of glycemia in all subjects using both glucose and nonglucose secretory stimuli.

SUBJECTS AND METHODS

Oral glucose tolerance, insulin sensitivity, and insulin secretion were determined in 35 nondiabetic middle-aged caucasian women, constituting part of a stratified random sample of a population in which the prevalence of impaired glucose tolerance in women is 27.9%.[28] Thirteen were non-obese women with body mass index (BMI) in the range of 18 to 23 kg/m^2 (mean ± SD, 20.9 ± 1.3 kg/m^2) and with normal glucose tolerance. Eleven were obese women (BMI range, 29 to 37 kg/m^2; mean ± SD, 32.2 ± 2.8 kg/m^2) with normal glucose tolerance and 11 were obese women (BMI range, 27 to 33 kg/m^2; mean ± SD, 30.1 ± 2.2 kg/m^2) with impaired glucose tolerance, as judged by an oral glucose tolerance test. The women were 58 or 59 years of age (mean ± SD, 58.6 ± 0.4 years) and they were all healthy. None were taking any drugs known to affect carbohydrate metabolism. The study was approved by the Ethics Committee at Lund University, Malmö, Sweden. All subjects gave written informed consent before entrance in the study. The different studies were performed on 3 separate days, after an overnight fast, with at least 1 week between visits.

Glucose Tolerance

Oral glucose tolerance was determined with a standard World Health Organization 75-g glucose load,[29] with capillary blood glucose samples taken before and 2 hours after the glucose load. The subjects spent 2 hours in a semirecumbent position, and were not allowed to smoke during the test. Normal glucose tolerance was defined as a 2-hour capillary blood glucose value less than 7.8 mmol/L, and impaired glucose tolerance was defined as a 2-hour capillary blood glucose value of 7.8 to 11.1 mmol/L.[29]

Insulin Sensitivity

Insulin sensitivity was determined with the euglycemic, hyperinsulinemic clamp, performed according to DeFronzo et al.[26] 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 nmol/m^2 body surface area/min was started. After 4 minutes, 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 minutes.

Insulin and Glucagon Secretion

Insulin and glucagon secretion were determined with intravenous arginine stimulation at three blood glucose levels (fasting, and 14 and >25 mmol/L), a method introduced by Ward et al.[27] Intraveneous 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 used. Baseline samples were taken at −5 and −2 minutes. A maximally stimulating dose of arginine hydrochloride (5 g) was then injected intraveneously over 45 seconds. Samples were taken at +2, +3, +4, and +5 minutes. A variable-rate 20% glucose infusion was then initiated to increase and maintain blood glucose levels at 13 to 15 mmol/L. Blood glucose level was determined every 5 minutes bedside, and the glucose infusion adjusted to reach the desired blood glucose level of 13 to 15 mmol/L in 20 to 25 minutes. New baseline samples were taken, then arginine (5 g) was again injected and +2, +3, +4, and +5 samples taken. A 2.5-hour rest period was then allowed to avoid the well-known priming effect of hyperglycemia.[30,31] After the pause, baseline samples were again obtained. Then a high-speed (900 mL/h) 20% glucose infusion during 25 to 30 minutes was used to increase blood glucose levels to more than 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 samples.

Analyses

Capillary blood glucose samples from the oral glucose tolerance test were chilled at 4°C and analyzed with an automatic glucose oxidase method at the hospital central laboratory. Blood glucose concentration was determined bedside by the glucose dehydrogenase technique with a Hemocue (Hemocue AB, Ängelholm, Sweden) during the hyperinsulinemic, euglycemic clamp and with an Accutrend (Boeringer Mannheim Scandinavia AB, Bromma, Sweden) during the arginine test. Blood samples for analysis of insulin, glucagon, and glucose from the arginine and clamp studies were immediately centrifuged at 5°C and serum or plasma frozen at −20°C for analysis in duplicate. Serum insulin concentrations were analyzed with a double-antibody radioimmunoassay technique. Guinea-pig antihuman insulin antibodies and human insulin standard (Linco Res, St Louis, MO) were used, and mono-^{125}I-Tyr-human insulin was used as a tracer (Novo Nordisk). Samples for analysis of glucagon were obtained in prechilled test tubes containing 0.084 mL EDTA (0.34 mol/L) and aprotinin (250 KIU/mL blood; Bayer AG, Leverkusen, Germany). Analyses of glucagon concentration were performed with double-antibody radioimmunoassay using guinea-pig antihuman glucagon antibodies specific for pancreatic glucagon, ^{125}I-glucagon as tracer, and glucagon standard (Linco Res). 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 the mean ± SEM, unless otherwise noted. For calculation of insulin sensitivity, a steady-state condition was assumed during the second hour of the clamp. Calculations were performed according to DeFronzo et al.[26]

The acute insulin response to arginine (AIR) was calculated as the mean of the +2 to +5 minute samples minus the prestimulus insulin concentration. Previous studies have shown that arginine-stimulated insulin secretion is maximal when blood glucose concentration exceeds 25 mmol/L.[27] Therefore, AIR at blood glucose levels greater than 25 mmol/L was taken as a measure of the maximal insulin secretory capacity of the β cells (AIR_{MAX}). It is known that glucose potentiation of AIR to arginine is linear at blood glucose levels between 3.5 and 18 mmol/L.[32,33] The slope between AIR at fasting blood glucose and at blood glucose 14 mmol/L ($slope_{AIR} = \Delta AIR/\Delta glucose$) was thus calculated as a

measure of glucose potentiation of β-cell secretion. BG_{50} is the blood glucose level at which half maximal insulin secretion is achieved, a measure of β-cell sensitivity to glucose calculated from AIR_{MAX} and $slope_{AIR}$. Acute glucagon responses (AGR) and $slope_{AGR}$ (representing glucose inhibition of α cells) were calculated in the same manner.

Statistical analyses were performed with the SPSS for Windows system.[34] Differences between groups were tested with the Mann-Whitney *U* test for unrelated samples.

RESULTS

Characteristics of the three study groups are listed in Table 1. Body weight and BMI differed significantly between the non-obese subjects and the two groups with a high BMI. However, there was no difference with regard to body weight or BMI between the obese subjects with normal versus impaired glucose tolerance.

Non-Obese Versus Obese Subjects With Normal Glucose Tolerance

When comparing obese and non-obese subjects with normal glucose tolerance, the obese subjects had higher levels of fasting blood glucose than the non-obese subjects (Table 1). However, the 2-hour blood glucose values were not significantly different between the two groups. In the euglycemic, hyperinsulinemic clamp study, the glucose infusion rate was significantly lower in the obese group than in the non-obese group (Table 1). The insulin sensitivity was significantly lower in the obese subjects, as shown in Fig 1. Mean glucose concentration during the second hour of the clamp did not differ between the two groups, while the obese subjects had slightly higher insulin concentration during the clamp, as they had higher fasting insulin concentrations.

Insulin and glucagon concentrations during the glucose-dependent arginine test are listed in Table 2. Obese subjects had significantly higher prestimulus insulin levels at the three blood glucose levels. Fasting glucagon concentrations were higher in obese subjects than in non-obese subjects, whereas glucagon levels at blood glucose levels of 14 and greater than 25 mmol/L were not significantly different between the two groups.

The intravenous injection of arginine induced a rapid increase in insulin and glucagon secretion in all subjects studied. Figure 2 shows that obese subjects had significantly higher AIRs than non-obese subjects at all three glucose levels (fasting, 538 ± 87 *v* 230 ± 35 pmol/L, $P = .003$; blood glucose 14 mmol/L, 1,520 ± 299 *v* 828 ± 111 pmol/L, $P = .008$; and blood glucose >25 mmol/L, 1,910 ± 313 *v* 1,019 ± 128 pmol/L, $P = .02$). Glucose potentiation of insulin secretion, $slope_{AIR}$, was also higher in obese subjects (Table 2). In contrast, there was no difference in β-cell sensitivity to glucose, BG_{50}, between the groups ($P = .93$). There were no differences in AGR to arginine between obese and non-obese subjects either at fasting, or at blood glucose levels of 14 or greater than 25 mmol/L. Similarly, the glucose inhibition of glucagon secretion, $slope_{AGR}$, was not different between the two groups (Table 2).

Obese Subjects With Normal Versus Impaired Glucose Tolerance

The fasting levels of glucose did not differ between obese subjects with normal versus impaired glucose tolerance, whereas subjects with impaired glucose tolerance had, by definition, significantly higher 2-hour blood glucose values than subjects with normal glucose tolerance (Table 1). There were no differences in glucose infusion rate (Table 1) or insulin sensitivity (Fig 1) between normal and impaired glucose tolerance subjects. Neither were there any differences in their mean glucose or insulin concentration during the second hour of the clamp (Table 1).

During the glucose-dependent arginine injection test, fasting insulin levels did not differ between the two groups (Table 2). In contrast, the prestimulus insulin levels after increasing the blood glucose to 14 mmol/L were significantly lower in obese subjects with impaired glucose tolerance than in obese subjects with normal glucose tolerance. Also at blood glucose levels greater than 25 mmol/L, obese subjects with impaired glucose tolerance had numerically

Table 1. Characteristics of 35 Women Aged 58 to 59 Years Divided Into Three Groups Based on BMI and Glucose Tolerance

Characteristic	N-NGT (n = 13)	*P* (N-NGT *v* O-NGT)	O-NGT (n = 11)	*P* (O-NGT *v* O-IGT)	O-IGT (n = 11)
Weight (kg)	58.2 ± 1.4	<.001	85.0 ± 2.2	NS	79.6 ± 2.3
BMI (kg/m²)	20.9 ± 0.4	<.001	32.2 ± 0.9	NS	30.1 ± 0.7
W/H	0.77 ± 0.01	NS	0.79 ± 0.02	NS	0.83 ± 0.02
FBG (mmol/L)	4.5 ± 0.1	.05	4.9 ± 0.2	NS	5.0 ± 0.2
2-hour BG (mmol/L)	6.0 ± 0.3	NS	6.4 ± 0.2	<.001	9.3 ± 0.3
Glucose infusion rate (60-120 minutes, μmol/kg/min)	68.2 ± 7.4	.001	37.1 ± 4.7	NS	34.4 ± 3.8
Mean insulin concentration (60-120 minutes, pmol/L)	697 ± 30	.03	862 ± 49	NS	792 ± 48
IS (60-120 minutes, nmol glucose/kg/min/ pmol insulin/L)	99.8 ± 11.5	.002	47.2 ± 8.8	NS	45.5 ± 5.2
Mean glucose level (60-120 minutes, mmol/L)	5.22 ± 0.07	NS	5.05 ± 0.10	NS	5.02 ± 0.12

NOTE. Values are means ± SEM.

Abbreviations: NS, not significant; N-NGT, non-obese subjects with normal glucose tolerance; O-NGT, obese subjects with normal glucose tolerance, O-IGT, obese subjects with impaired glucose tolerance; BG, blood glucose; BMI, body mass index; W/H, waist to hip ratio; FBG, fasting blood glucose level; IS, insulin sensitivity.

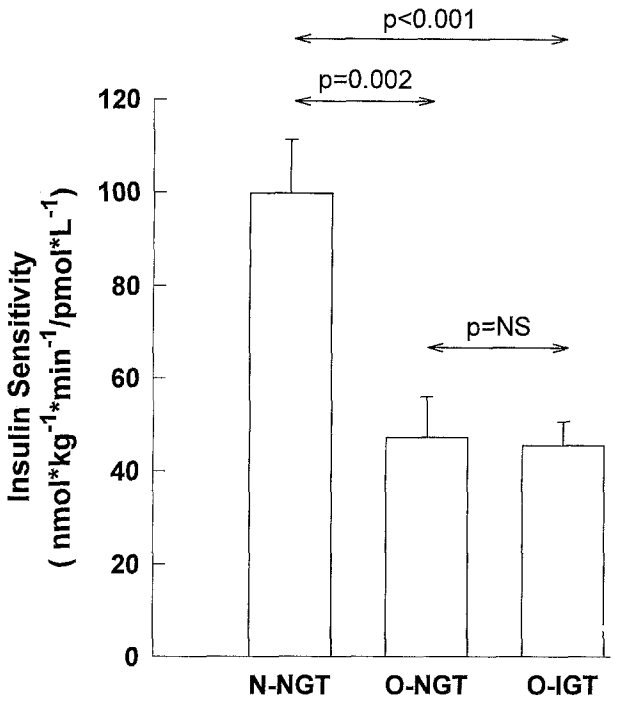

Fig 1. Insulin sensitivity as measured with the hyperinsulinemic, euglycemic clamp method in 35 women aged 58 to 59 years; 13 non-obese subjects with normal glucose tolerance (N-NGT), 11 obese subjects with normal glucose tolerance (O-NGT) and 11 obese subjects with impaired glucose tolerance (O-IGT). Values are the mean ± SEM. *P* indicates the probability level of random difference between groups. NS, not significantly different.

lower values than obese subjects with normal glucose tolerance, but at this glucose level, the difference was not statistically significant ($P = .09$). Fasting glucagon and prestimulus glucagon at blood glucose levels of 14 and greater than 25 mmol/L did not differ between normal and glucose-intolerant obese subjects.

The AIR to arginine was significantly lower in obese subjects with impaired glucose tolerance group than in obese subjects with normal glucose tolerance at blood glucose level 14 mmol/L (872 ± 94 *v* 1,520 ± 299 pmol/L, $P = .03$; Fig 3). Although there was a general trend towards lower AIRs at fasting and at blood glucose levels greater than 25 mmol/L in obese subjects with impaired glucose tolerance, the *P* values did not reach statistical significance (fasting blood glucose, 372 ± 63 *v* 538 ± 87 pmol/L, $P = .11$; blood glucose greater than 25 mmol/L, 1,453 ± 178 *v* 1,910 ± 313 pmol/L, $P = .34$). $Slope_{AIR}$, the glucose potentiation of insulin secretion, was significantly lower in obese subjects with impaired glucose tolerance than in obese subjects with normal glucose tolerance (Table 2), whereas there was no significant difference in BG_{50} between the two groups. The AGRs to arginine did not differ between the obese groups. However, the subjects with impaired glucose tolerance had a significantly lower $slope_{AGR}$ than subjects with normal glucose tolerance.

DISCUSSION

This study shows that impaired glucose tolerance in obese subjects is accompanied by reduced glucose potentiation of insulin secretion when compared with obese subjects with normal glucose tolerance. It should be emphasized that the two groups of obese subjects studied had the same degree of obesity and insulin sensitivity. The results therefore suggest that impairment of insulin secretion is a key factor responsible for the deterioration of glucose tolerance in obese subjects. In addition, the study shows that among subjects with normal glucose tolerance, obesity is accompanied by reduced insulin sensitivity and increased insulin secretion. These results are compatible with the view that in obese subjects with retained normal glucose tolerance, the reduced insulin sensitivity that accompanies the obesity is compensated by increased insulin secretion. Furthermore, impaired glucose tolerance develops when insulin secretion fails, which may occur without further deterioration of the reduced insulin sensitivity.

Our finding that obese subjects with normal glucose tolerance have increased glucose-stimulated insulin secretion when compared with non-obese subjects, confirms previous studies of obese subjects.[22-23,25] We also demonstrate that arginine-stimulated insulin secretion is increased significantly at both fasting and at blood glucose levels of 14 and greater than 25 mmol/L in obese subjects. The glucose potentiation of insulin secretion, ie, $slope_{AIR}$, was also significantly increased in obesity. This suggests that both the glucose modulation of β-cell function, ie, the glucose potentiation of insulin secretion, and the maximal insulin secretory capacity are increased in obesity, perhaps due to a longstanding hypertrophy of the islet cell mass.

A recent study on glucose-stimulated insulin secretion in

Table 2. Results of the Glucose-Dependent Arginine Test

Variable	N-NGT (n = 13)	*P* (N-NGT *v* O-NGT)	O-NGT (n = 11)	*P* (O-NGT *v* O-IGT)	O-IGT (n = 11)
Fasting insulin (pmol/L)	43 ± 5	<.001	99 ± 16	NS	80 ± 9
Insulin at BG 14 (pmol/L)	187 ± 25	.004	336 ± 55	.03	209 ± 19
Insulin at BG >25 (pmol/L)	351 ± 50	.003	932 ± 219	NS (.09)	563 ± 94
Fasting glucagon (ng/L)	60 ± 3	.006	78 ± 5	NS	80 ± 6
Glucagon at BG 14 (ng/L)	49 ± 3	NS	51 ± 3	NS	60 ± 6
Glucagon at BG >25 (ng/L)	37 ± 3	NS	43 ± 3	NS	50 ± 5
BG_{50} (mmol/L)	11.4 ± 1.5	NS	10.6 ± 1.0	NS (.08)	13.0 ± 0.9
$Slope_{AIR}$ (pmol insulin/mmol glucose)	57.2 ± 9.7	.05	94.4 ± 20.5	.03	49.8 ± 6.0
$Slope_{AGR}$ (ng glucagon/mmol glucose)	−4.3 ± 0.7	NS	−5.6 ± 1.2	.04	−2.2 ± 0.7

NOTE. Values are means ± SEM.

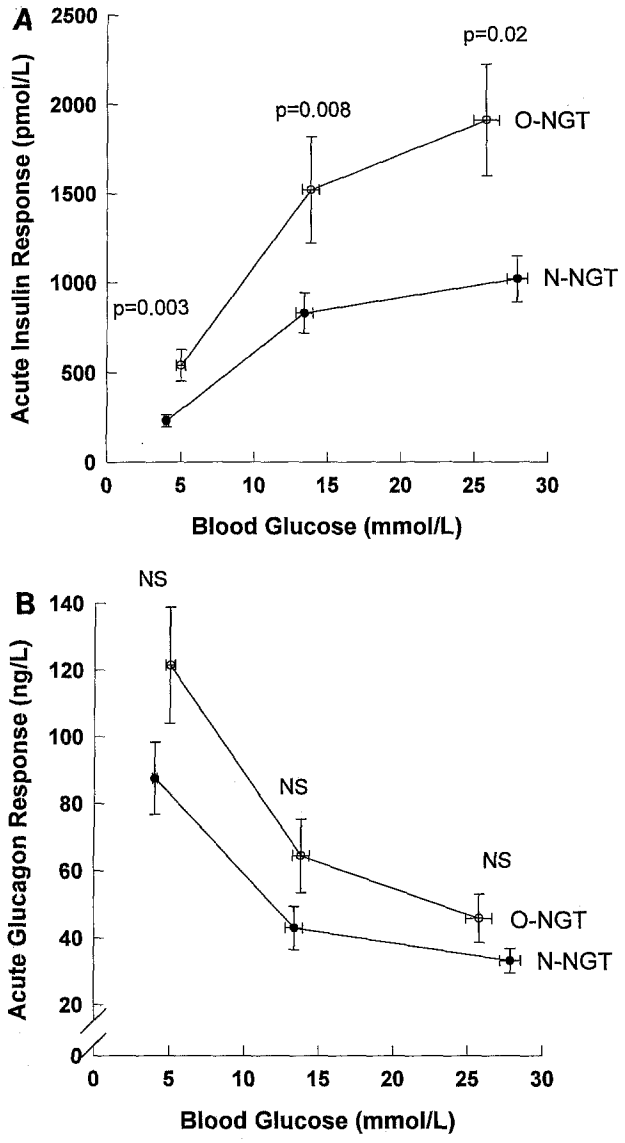

Fig 2. Calculated acute insulin (A) and glucagon (B) responses to arginine stimulation (5 g intravenously, 2 to 5 minutes postload increase) at 3 blood glucose levels (fasting, and 14 and >25 mmol/L) in women aged 58 to 59 years; 13 non-obese subjects with normal glucose tolerance (N-NGT) and 11 obese subjects with normal glucose tolerance (O-NGT). Values are the mean ± SEM. *P* indicates the probability level of random difference between the groups. NS, not significantly different.

obesity showed, as in the present study, that an exaggerated insulin secretion accompanies overweight, and that the hepatic extraction of insulin is unaltered in obesity.[25] The design of that study enabled separation of basal insulin secretion from that induced by a rapid glucose injection, and the results showed that the basal secretory rate was increased. This confirms another study demonstrating similar first-phase insulin secretion after a glucose load in obese and non-obese subjects.[35] Kautzky-Willer et al concluded that the exaggerated β-cell response to glucose in obesity is due to the elevated glucose level and not to a potentiated stimulation of the exocytosis process.[25] However, the results of the present study allow the conclusion that obesity indeed is accompanied by increased secretory rate in response to stimulation. Under isoglycemic conditions at fasting, and at 14 and greater than 25 mmol/L glucose, arginine-stimulated insulin secretion was markedly exaggerated in obesity. Therefore, provided that the hyperinsulinemic response seen in obesity is due to increased β-cell secretion and not to reduced hepatic extraction, for which recent evidence exists,[24,25] we conclude that the reduced insulin sensitivity in obesity is accompanied by potentiated secretion of insulin to identical stimuli.

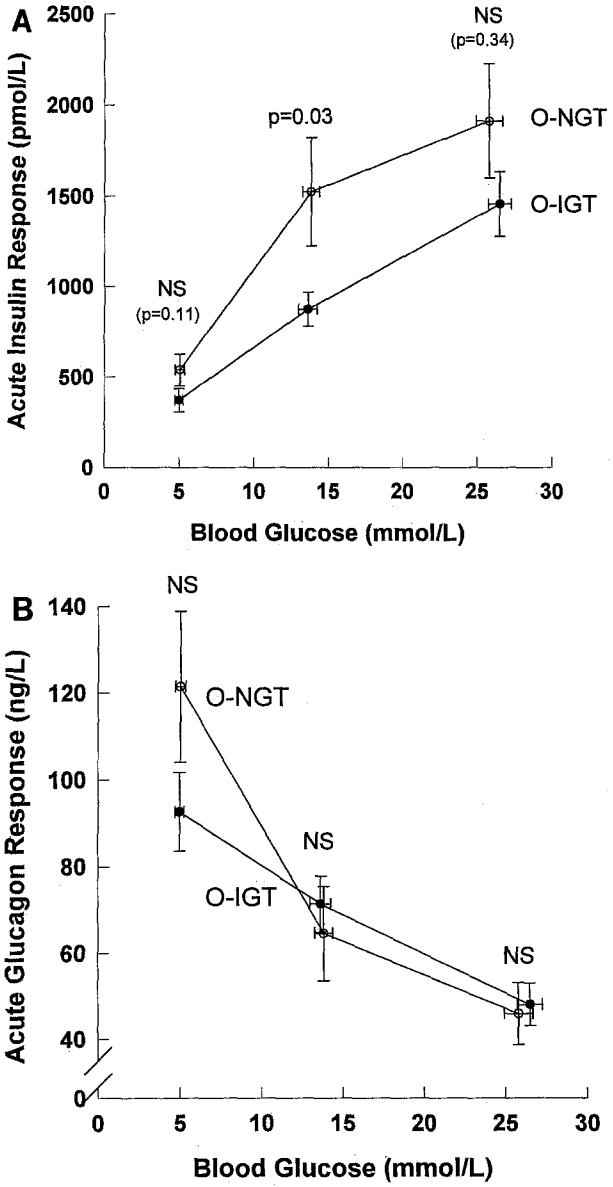

Fig 3. Calculated acute insulin (A) and glucagon (B) responses to arginine stimulation (5 g intravenously, 2 to 5 minutes postload increase) at 3 blood glucose levels (fasting, and 14 and >25 mmol/L) in obese women aged 58 to 59 years; 11 with normal glucose tolerance (O-NGT) and 11 with impaired glucose tolerance (O-IGT). Values are the mean ± SEM. *P* indicates the probability level of random difference between the groups. NS, not significantly different.

The present study confirms the results of a previous study in young men by Beard et al,[36] showing that subjects, equally obese as in the present study, had increased AIR to nonglucose stimuli, even though isoproterenol, and not arginine, was used as the insulin secretagogue in the Beard study. The obese young men in their study also had increased $slope_{AIR}$. Although it is known that isoproterenol and arginine exert their stimulatory β-cell actions by different mechanisms,[37] and different populations were examined, these two studies showed that insulin secretory capacity, as well as glucose potentiation of insulin secretion, are increased in obese subjects with normal glucose tolerance.

Obese subjects with impaired glucose tolerance had the same low degree of insulin sensitivity as comparably obese subjects with normal glucose tolerance, as measured with the euglycemic, hyperinsulinemic clamp, although differences in insulin sensitivity cannot be excluded at hyperglycemic levels. However, in accordance with the clamp results, the subjects had similar levels of fasting insulin, as an indirect measure of insulin sensitivity. This suggests that it is not a worsening of the insulin insensitivity that explains the glucose intolerance in these obese subjects with impaired glucose tolerance. Of particular interest is that the plasma insulin levels, after increasing the blood glucose level to 14 mmol/L, were significantly lower in obese subjects with impaired glucose tolerance compared with obese subjects with normal glucose tolerance. Also, arginine-stimulated insulin secretion was significantly lower in obese subjects with impaired glucose tolerance than in glucose tolerant obese subjects at a blood glucose level of 14 mmol/L, with a trend towards lower values at fasting and blood glucose levels greater than 25 mmol/L. Finally, $slope_{AIR}$ was lower in obese subjects with impaired glucose tolerance than in obese subjects with normal glucose tolerance. We conclude that insulin secretion is impaired and the glucose modulation of β-cell function is altered in impaired glucose tolerance in obesity, yet insulin sensitivity is not lower in subjects with impaired glucose tolerance than in comparably obese subjects with normal glucose tolerance. This suggests that in obese subjects with the same low level of insulin sensitivity, those with impaired glucose tolerance have a failing effect of glucose to potentiate insulin secretion. We have previously demonstrated that impaired glucose tolerance in non-obese subjects is accompanied by an altered glucose modulation of insulin secretion, as manifested by a decreased glucose potentiation of the arginine effects on β-cell secretion.[10] Therefore, we suggest that impaired glucose modulation of β-cell function is a main underlying mechanism for impaired glucose tolerance, regardless of the degree of obesity.

A previous study demonstrated that glucose-stimulated insulin secretion was not significantly different in obese subjects with impaired glucose tolerance, compared with obese subjects with normal glucose tolerance.[25] However, in that study, basal glucose levels were higher in subjects with impaired glucose tolerance, which could have increased the insulin secretory response to the glucose load, and also could have caused basal hyperinsulinemia compared with subjects with normal glucose tolerance. Even though the glucose levels in the subjects with impaired glucose tolerance of the study of Kautzky-Willer et al were elevated, glucose-stimulated insulin secretion was not, indicating a failure of the β cells to respond appropriately to glucose.

A recently published study by Pimenta et al[12] demonstrated a β-cell dysfunction in normoglycemic subjects with a first-degree NIDDM relative. This study used the hyperglycemic clamp to assess first- and second-phase insulin secretion to isoglycemic stimuli in subjects with and without NIDDM, showing lower insulin secretion in the relatives. These findings suggest that a β-cell dysfunction is the primary genetic defect in NIDDM, a hypothesis which correlates well with the results of our present study. Another study of Kautzky-Willer et al should be mentioned, showing that impaired glucose tolerance in primary hyperparathyroidism is accompanied by low first-phase glucose-stimulated insulin secretion.[38] Taken together, these data suggest that a β-cell defect is of importance early on in NIDDM development.

Besides higher fasting insulin levels, we also found that fasting glucagon values were increased in obese subjects with normal glucose tolerance compared with non-obese subjects. This hyperglucagonemia could be an explanation for the slightly, but significantly, increased fasting blood glucose level in obese subjects with normal glucose tolerance, and could suggest that the glucose inhibition of glucagon secretion is impaired in obesity. However, this latter explanation seems unlikely, since we found that when blood glucose was increased to 14 mmol/L, the inhibition of glucagon secretion was not different from that in normal subjects. Another more likely possibility is that the mechanisms that underly the compensatory exaggerated insulin secretion in obesity also result in an enhanced glucagon secretion. For example, if the hyperinsulinemic response that accompanies reduced peripheral insulin sensitivity is mediated by increased parasympathetic tone on the islets, as is suggested from studies in rats,[39] such an increased tone would also be expected to exaggerate glucagon secretion,[40] which could explain the fasting hyperglucagonemia. Nevertheless, the α-cell function seems to be normally regulated in obesity, since other parameters with regard to glucagon secretion during the glucose-dependent arginine test, including the $slope_{AGR}$, were not different between obese and non-obese subjects with normal glucose tolerance. We have previously demonstrated that the $slope_{AGR}$ is reduced in non-obese subjects with impaired glucose tolerance compared to those with normal glucose tolerance.[10] The same observation was now made in obese subjects with impaired glucose tolerance, who had a lower $slope_{AGR}$ than obese subjects with normal glucose tolerance, suggesting that besides β-cell dysfunction, also an impairment of glucose inhibition of α-cell secretion is present in glucose intolerance.

Since impaired glucose tolerance is accompanied by increased risk for the development of NIDDM,[13-16] our results imply that the decreased insulin secretion to glucose and nonglucose stimulation, and decreased glucose potentiation of insulin secretion are early defects in NIDDM in

caucasian populations. These findings are in line with recently published data from a study in the Pima Indians.[41] The cause of the β-cell defect is not established. It may be a primary β-cell event or a toxic effect secondary to actions of the hyperglycemia on the β cells.[42] The importance of β-cell dysfunction in NIDDM development has been studied and reviewed by Porte,[33] Efendic et al.[43] The hypothesis that impairment of insulin secretion is the important step when developing impaired glucose tolerance and NIDDM, as opposed to progressive decrease of insulin sensitivity, is also supported by the fact that short-term blood glucose control with, eg, insulin treatment improves insulin secretion but not insulin sensitivity.[44] However, our findings do not exclude that reduced insulin sensitivity is, as discussed by DeFronzo et al,[45] of major importance for NIDDM development, since the obese subjects with impaired glucose tolerance in our study had markedly low insulin sensitivity, compared with non-obese subjects. Nevertheless, since a main observation in our study was that the impaired and normal glucose tolerance in obese subjects were accompanied by equivalent insulin resistance, it has to be emphasized that the difference between these two groups was only the glucose modulation of islet function. Whether the development to NIDDM is accompanied by a further deterioration of islet dysfunction, or a further reduction in insulin sensitivity, remains now to be established.

In conclusion, we have demonstrated that obesity in subjects with normal glucose tolerance is accompanied by decreased peripheral insulin sensitivity and increased insulin secretion in response to glucose and arginine. Furthermore, obese subjects with impaired glucose tolerance, who are at greatly increased risk of developing NIDDM, have decreased insulin secretion both to glucose and to arginine, and a decreased glucose effect on α- and β-cell function, while insulin sensitivity is not lower than in glucose-tolerant obese subjects. Therefore, since impaired glucose tolerance is known to precede NIDDM, our results suggest that impaired islet function is an important early defect in the development of NIDDM in obese subjects.

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