Insulin Secretion and Incretin Hormones After Oral Glucose in Non-obese Subjects With Impaired Glucose Tolerance

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Subjects with impaired glucose tolerance (IGT) are usually overweight and exhibit insulin resistance with a defective compensation of insulin secretion. In this study, we sought to establish the interrelation between insulin secretion and insulin sensitivity after oral glucose in non-obese subjects with IGT and we also examined this interrelation in relation to the 2 main incretins, glucagon-like peptide (GLP-1) and gastric inhibitory polypeptide (GIP). To that end, 13 women with IGT and 17 women with normal glucose tolerance (NGT) underwent an oral glucose tolerance test (OGTT) with measurements of glucose, insulin, C-peptide, GLP-1, and GIP. Insulin secretion (TIS) and insulin sensitivity (OGIS) were assessed using models describing the relationship between glucose, insulin and C-peptide data. These models allowed estimation also of the hepatic extraction of insulin. The age (54.2 ± 9.7 [mean ± SD] years) and body mass index (BMI; 26.0 ± 4.0 kg/m^2) did not differ between the groups. Subjects with IGT displayed lower TIS during the initial 30 minutes after oral glucose (0.97 ± 0.17 [mean ± SEM] *v* 1.75 ± 0.23 nmol/L in NGT; *P* = .018) and lower OGIS (397 ± 21 *v* 463 ± 12 mL/min/m^2; *P* = .005). The incremental 30-minute TIS times OGIS (reflecting insulin secretion in relation to insulin sensitivity) was significantly reduced in IGT (359 ± 51 *v* 774 ± 91 nmol/min/m^2, *P* = .001). This measure correlated inversely to the 2-hour glucose level (*r* = −0.71; *P* < .001). In contrast, TIS over the whole 180-minute period was higher in IGT (26.2 ± 2.4 *v* 20.0 ± 2.0 nmol/L; *P* = .035). Hepatic insulin extraction correlated linearly with OGIS (*r* = 0.71; *P* < .001), but was not significantly different between the groups although there was a trend with lower extraction in IGT (*P* = .055). Plasma levels of GLP-1 and GIP increased after oral glucose. Total secretion of these incretin hormones during the 3-hour test did not differ between the 2 groups. However, the 30-minute increase in GLP-1 concentrations was lower in IGT than in NGT (*P* = .036). We conclude that also in non-obese subjects with IGT, when adiposity is controlled for in relation to NGT, defective early insulin secretion after oral glucose is a key factor. This defective beta-cell function is associated with, and may be caused by, a reduced early GLP-1 response.

TYPE 2 DIABETES is characterized by insufficient insulin secretion in relation to the increased demand created by the insulin resistance.[1-3] Also subjects with impaired glucose tolerance (IGT) exhibit a defective insulin secretion if the increased demand due to insulin resistance is taken into consideration.[4-6] The importance of controlling insulin secretion for the ambient insulin resistance is of relevance in studies in IGT, since these subjects often are overweight, and obesity is associated with insulin resistance. Furthermore, it has been suggested that subjects with type 2 diabetes or IGT exhibit mainly a defective early insulin release, ie, the insulin release seen during the initial 30 minutes after meal intake or oral glucose.[7] For example, loss of early insulin response results in glucose intolerance in healthy humans,[8] the 30-minute increase in insulin after oral glucose correlates negatively to 120-minute glucose,[9] and rapid restoration of the initial 30-minute insulin response in subjects with type 2 diabetes by exogenous administration of insulin restores normal glucose tolerance.[10] However, the underlying cause of the defective early insulin response in IGT is not known, and may be due to intrinsic beta-cell defects or to defective augmentation of insulin secretion by factors of importance for postprandial insulin secretion, such as gut incretin hormones.

Of most importance among postprandial factors for insulin secretion are the gut hormones, glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP). These hormones are released during meal intake and augment glucose-stimulated insulin secretion, being the main incretins.[11] Their importance for postprandial insulin secretion and glucose tolerance is evident by studies in mice with genetic deletion of their respective receptors, both having defective insulin secretion and glucose intolerance.[12,13] These hormones also seem to be of relevance for type 2 diabetes in humans. Thus, a low GLP-1 release is seen in type 2 diabetes[14] and, in a recent study we showed that insulin resistance, as determined by the euglycemic, hyperinsulinemic clamp, was associated with low GLP-1 and GIP responses to a mixed meal in healthy men.[15] Furthermore, the action of GIP to stimulate insulin secretion seems defective in diabetes.[16] However, whether altered release of GIP or GLP-1 contributes to the defective release of insulin in IGT is not known. In this study, we have therefore evaluated whether the defective insulin response during an oral glucose tolerance test (OGTT) in subjects with IGT is associated with defective beta-cell function or by altered release of GIP or GLP-1. To avoid misinterpretation of data with regard to changes induced by overweight or obesity, which is often the case in subjects with IGT, this study examined differences in

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insulin secretion, insulin sensitivity, and incretin hormones in non-obese subjects with IGT versus normal glucose tolerance (NGT), ie, in subjects of the 2 groups who were not different in regard to body weight or body mass index (BMI). Insulin secretion, clearance, and sensitivity were assessed by employing mathematical methods to evaluate glucose clearance[17] and to reconstruct prehepatic insulin secretion[18] from systematically measured glucose, C-peptide, and insulin concentrations. The methods also allow the calculation of the hepatic extraction of insulin, which together with insulin secretion contributes to the postprandial insulinemia.

MATERIALS AND METHODS

Subjects

Subjects were recruited from a population-based study in Northern Sweden, the World Health Organization (WHO)-conducted Multinational Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA) project.[19] From an original random sample of 2,815 women and men, 33 Caucasian women living in the health care districts of Umeå and Skellefteå, not using contraceptive pills or hormone replacement therapy, were selected representing a wide range of fasting insulin levels. Six women were smokers. Prevalence of diabetes mellitus and thyroid dysfunction was checked by routine laboratory tests, and one subject was excluded because of manifest type 2 diabetes mellitus. Two additional women were diagnosed as having type 2 diabetes when the OGTT was undertaken in this study; these two women were excluded from data analyses. None of the 30 others had clinical features of endocrine, hepatic or renal disease. One woman took inhalation steroids due to bronchial asthma, but the dose used (budesonide <400 μg/24 h) was considered not to influence test results.[20] Three women were on medication with acetylsalicylic acid after suspected transient ischemic attacks, due to arthralgia or migraine, respectively. Seventeen women were postmenopausal. The study was approved by the Ethical Committee of Umeå University and written informed consent was obtained from all individuals.

Protocol

Subjects attended the outpatient clinic at 12:30 PM without having ingested anything during the preceding 3 hours. Anthropometric measurements of height to the nearest centimeter and weight to the nearest 100 g were determined, and BMI was calculated. An antecubital vein cannula was inserted for blood sampling, and a 75-g oral glucose load was given. Venous samples for determination of glucose, insulin, C-peptide, GLP-1, and GIP were drawn at 5 and 2 minutes before glucose ingestion and at specific time points for the following 180 minutes.

Analyses

Samples were drawn in prechilled tubes containing 0.084 mL EDTA (0.34 mol/L) and aprotinin (450 kallikrein-inhibiting units/mL blood; Bayer AG, Leverkusen, Germany). Blood samples were immediately centrifuged at 5°C and plasma frozen at −80°C until analysis in duplicate. Plasma insulin and C-peptide concentrations were analyzed with double-antibody radioimmunoassay techniques (Linco Research, St Charles, MO), using guinea-pig anti-human insulin antibodies, human insulin standard, mono-^{125}I-Tyr–labeled human insulin, guinea-pig anti-human C-peptide antibody, human C-peptide standard, and ^{125}I–human C-peptide as tracer. GIP was analyzed with radioimmunoassay using rabbit antibodies (code no. R65) reacting with the C-terminus of the GIP molecule, thereby measuring sum of intact GIP and its primary metabolite, $GIP_{3\text{-}42}$, formed in the body by dipeptidyl-peptidase-4–mediated cleavage.[21] The results of the assay therefore accurately reflect the rate of secretion of GIP. The assay does not cross-react with GIP^{8000}, the relationship of which to the synthesis or secretion of GIP remains unclear. GLP-1 was determined with a radioimmunoassay after extraction with ethanol.[22] The antiserum is directed against the amidated C-terminus of GLP-1 and therefore measures GLP-1 of intestinal origin. Glucose was determined with the glucose oxidase technique.

Table 1. Clinical Characteristics and Fasting Plasma Levels of Glucose, Insulin, C-Peptide, GLP-1, and GIP and the 2-Hour Glucose Value During the OGTT in the NGT and IGT Groups

	NGT (n = 17)	IGT (n = 13)
Age (yr)	54.1 ± 2.2	54.3 ± 3.0
Body weight (kg)	72.9 ± 3.0	67.8 ± 2.9
BMI (kg/m^2)	27.1 ± 1.0	24.6 ± 0.9
Fasting glucose (mmol/L)	4.6 ± 0.1	4.6 ± 0.1
2-h glucose (mmol/L)	6.8 ± 0.2	8.7 ± 0.3 ($P < .001$)
Fasting insulin (pmol/L)	62.0 ± 10.3	55.0 ± 8.9
Fasting C-peptide (nmol/L)	0.50 ± 0.06	0.41 ± 0.06
Fasting GLP-1 (pmol/L)	11.4 ± 1.1	11.3 ± 2.1
Fasting GIP (pmol/L)	25.0 ± 8.7	22.6 ± 7.7

NOTE. Values are means ± SEM. *P* indicates probability level of random difference between the groups.

Calculations

Subjects were divided in those with NGT or IGT based on the 2-hour glucose value (cut-off value, 7.8 mmol/Ll). Areas under the curve (AUCs) for C-peptide, insulin, GIP, and GLP-1 were calculated from plasma levels by the trapezoid rule. Insulin secretion during OGTT was computed by using a model of insulin and C-peptide dynamics.[18] This model, described in detail elsewhere,[18] has been validated in experiments involving direct arteriovenous transhepatic measurements.[23] By analyzing systemic insulin and C-peptide concentrations, this method provides individualized time courses of C-peptide secretion rate, CPS(t), interpreted to equal prehepatic insulin secretion and of insulin appearance, IDR(t), which represents the posthepatic hormone delivery. Simultaneous assessment of CPS(t) and IDR(t) allows the estimation of the rate of insulin clearance in the liver during the whole OGTT and not just during the first pass.[18,23] The early insulin secretion and the total amount of insulin secretion (TIS, nanomoles per liter) were estimated by integrating CPS(t) over the first 30 minutes or the whole 180-minute period, respectively. Insulin sensitivity was obtained from the OGTT by predicting glucose clearance at fixed insulin concentration levels as it would be calculated from a euglycemic, hyperinsulinemic clamp experiment.[17] This method provides the parameter OGIS, derived from a comprehensive mathematical model, which takes into account the known relationships between glucose disappearance and insulin.[17] OGIS represents an insulin sensitivity index as glucose clearance normalized to body surface area (units: $mL \cdot min^{-1} \cdot m^{-2}$). Details of the model, and of its derivation and assumptions have been reported elsewhere.[17] OGIS has been validated against the euglycemic, hyperinsulinemic clamp in healthy, obese subjects and in subjects with type 2 diabetes,[17] has been exploited in several published studies on carbohydrate metabolism,[24,25] and was recently further validated by an independent group.[26] Another important metabolic parameter obtained in this study was the disposition index (DI), defined as insulin sensitivity times incremental insulin concentrations and characterizing the ability of changes in systemic insulin to compensate for increasing insulin resistance.[27] DI was calculated as OGIS times $AUC_{insulin}$ (during 30 or 180 minutes). Another parameter was the adaptation index, which describes the capacity of the beta cell to release increasing

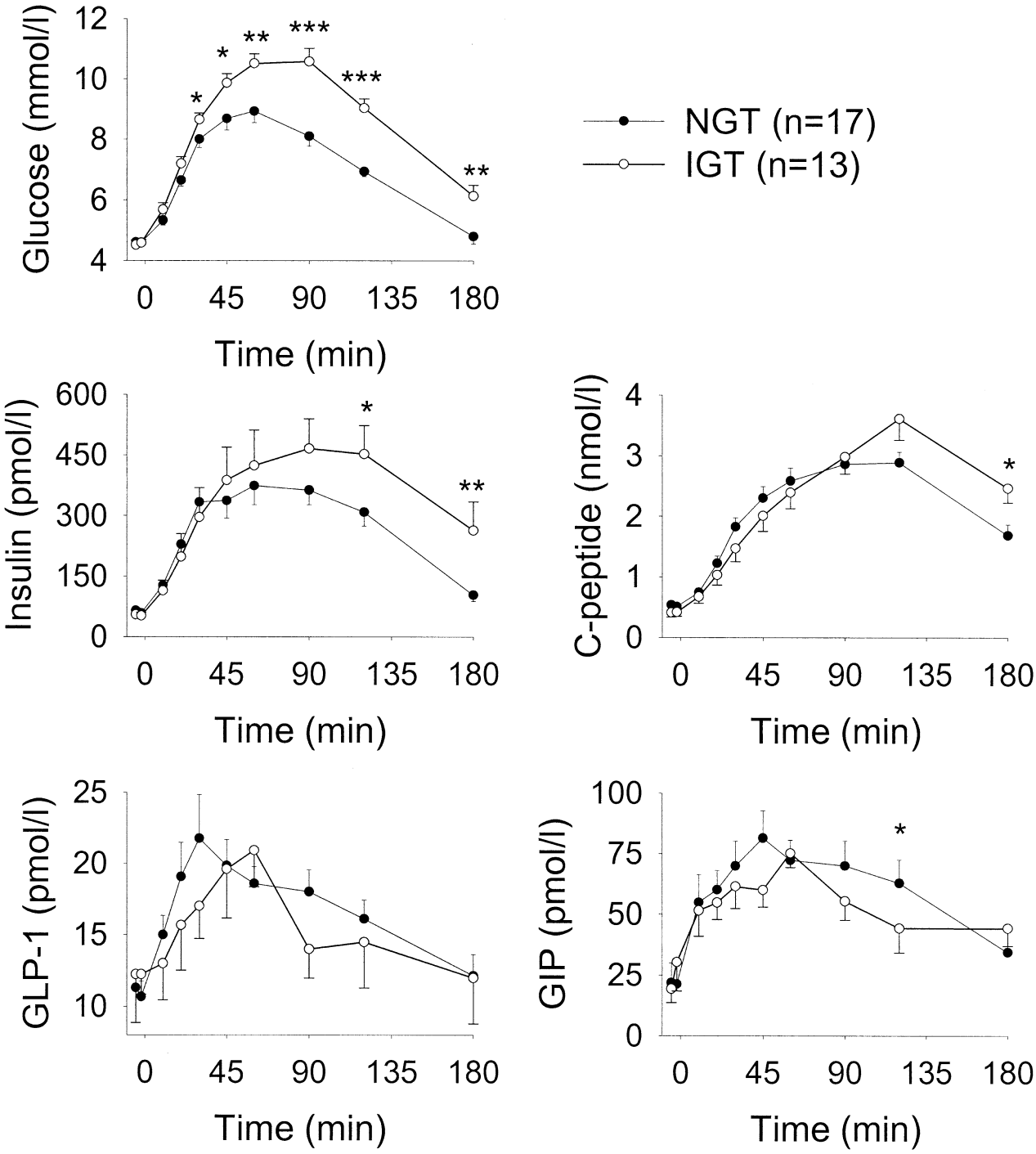

Fig 1. Plasma levels of glucose, insulin, C-peptide, GLP-1, and GIP in subjects with NGT or IGT before and for 180 minutes after a 75-g oral glucose load. Means ± SEM are shown. Asterisks indicate probability level of random difference between the group as assessed by Mann-Whitney nonparametric test for unpaired samples; *$P < .05$, **$P < .01$, ***$P < .001$.

amount of insulin to compensate for the increasing insulin resistance.[28] Adaptation index was calculated as OGIS times TIS (during 30 or 180 minutes). The difference between disposition and adaptation indices is therefore that DI is based on peripheral insulin concentrations whereas adaptation index is based on pancreatic (prehepatic) insulin secretion.

Statistics

Data are reported as mean ± SEM if not otherwise stated. Mann-Whitney *U* test for unpaired observations was used to compare differences between subjects with IGT versus NGT.

Table 2. AUCs for Glucose, Insulin, C-Peptide, GLP-1, and GIP During the Initial 30 Minutes and the Entire 180 Minutes After 75 g Oral Glucose in a 180-Minute OGTT in Women With NGT or IGT

	Total		Incremental	
	NGT (n = 17)	IGT (n = 13)	NGT (n = 17)	IGT (n = 13)
30-Minute AUC				
Glucose (mmol/L × 30 min)	179 ± 4.2	190 ± 3.9 (P = .057)	41.3 ± 4.1	53.4 ± 4.0 (P = .044)
Insulin (nmol/L × 30 min)	5.84 ± 1.22	5.53 ± 0.58	4.26 ± 0.94	3.68 ± 0.46
C-peptide (nmol/L × 30 min)	30.1 ± 2.5	26.8 ± 3.7	14.9 ± 1.6	14.3 ± 2.0
GLP-1 (pmol/L × 30 min)	510 ± 57	460 ± 60	172 ± 57	93 ± 55 (P = .078)
GIP (pmol/L × 30 min)	1,408 ± 218	1,491 ± 136	911 ± 129	747 ± 233
180-Minute AUC				
Glucose (mol/L × 180 min)	1.360 ± 0.037	1.628 ± 0.041 (P < .001)	0.0532 ± 0.046	0.815 ± 0.047 (P < .001)
Insulin (nmol/L × 180 min)	49.7 ± 4.6	74.8 ± 15.3	38.5 ± 4.2	65.2 ± 13.8 (P = .043)
C-peptide (nmol/L × 180 min)	403 ± 30	449 ± 39	309 ± 27	375 ± 31
GLP-1 (pmol/L × 180 min)	2,956 ± 200	3,098 ± 469	949 ± 270	893 ± 234
GIP (pmol/L × 180 min)	9,494 ± 1,159	9,507 ± 1,109	6,350 ± 1,190	5,044 ± 1,730

NOTE. Values are means ± SEM. P indicates probability level of random difference between the groups.

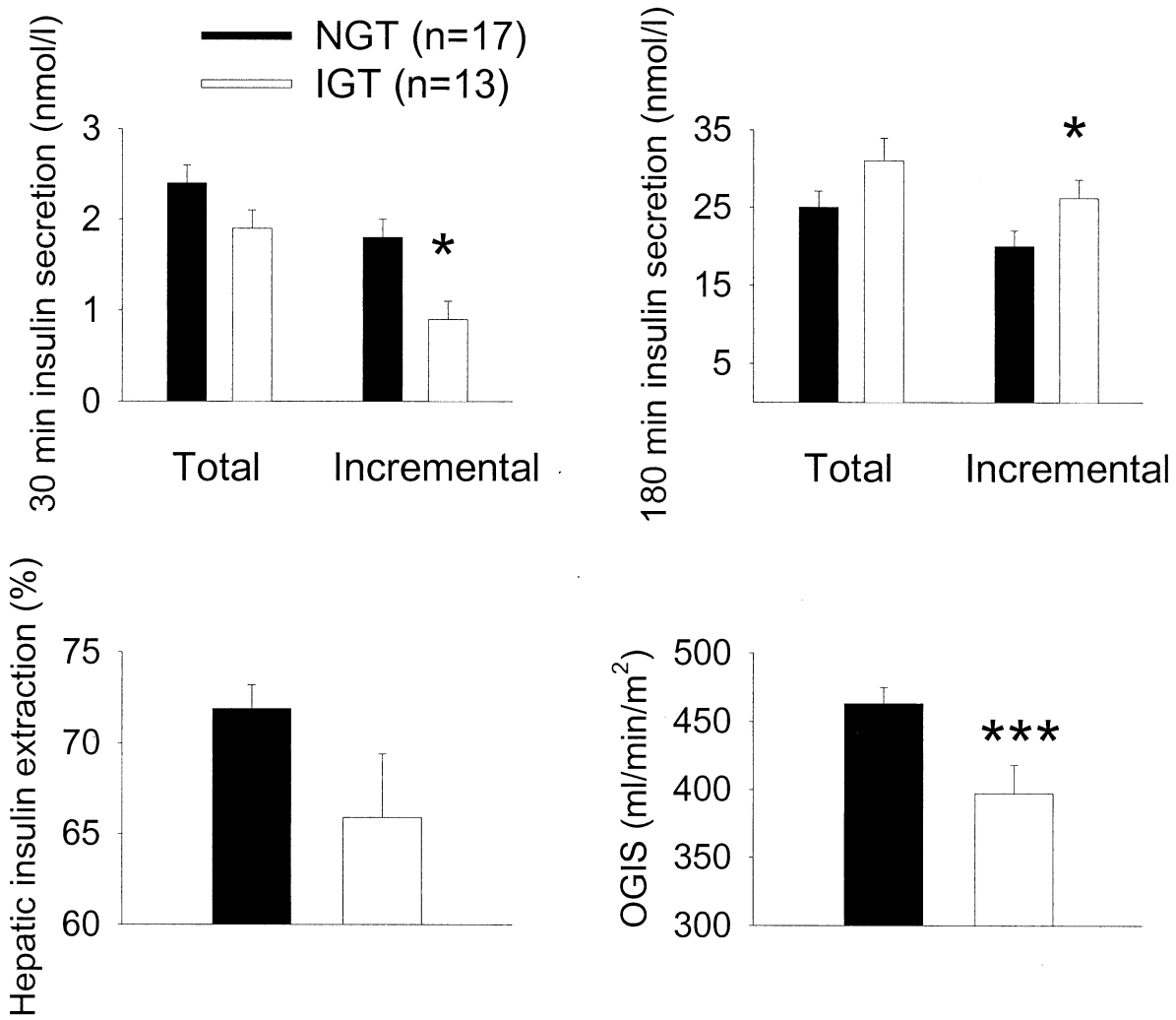

Fig 2. Early (30 minutes) and entire (180 minutes) insulin secretion (both as total insulin secretion and incremental insulin secretion), hepatic insulin extraction, and insulin sensitivity (OGIS) during a 180-minute OGTT after ingestion of 75 g oral glucose in subjects with NGT or IGT. Means ± SEM are shown. Asterisks indicate probability level of random difference between the group as assessed by Mann-Whitney nonparametric test for unpaired samples; *P < .05, ***P < .001.

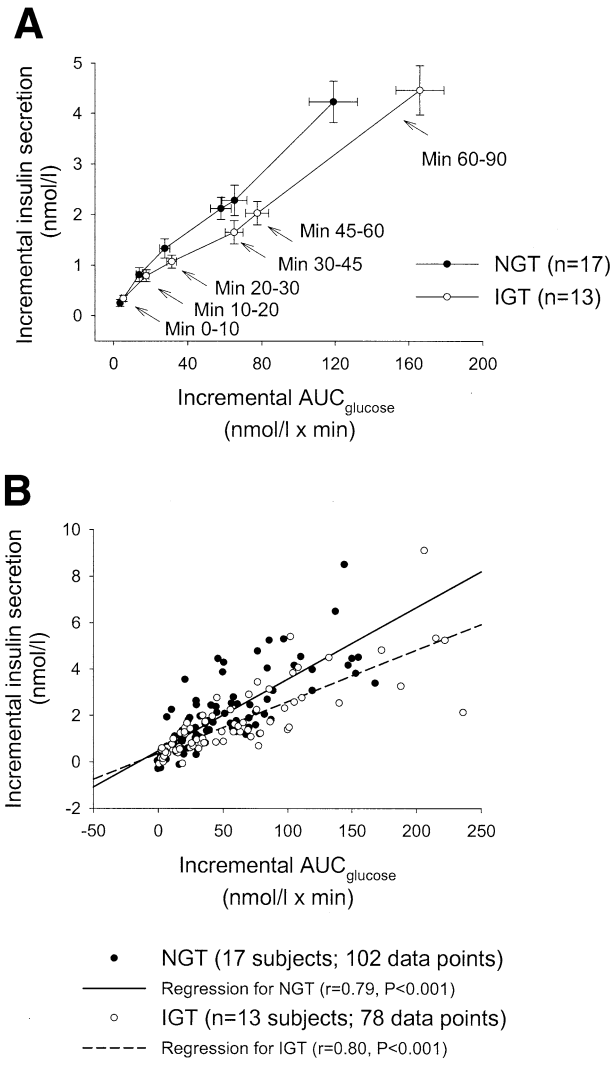

Fig 3. (A) Incremental insulin secretion (from C-peptide data) during 6 different time intervals of the OGTT as a function of the suprabasal increase in glucose levels during this particular time interval, expressed as AUC, in subjects with NGT and IGT. (B) Individual data points of the incremental insulin secretion (from C-peptide data) during the 6 different time intervals of the OGTT of (A), as a function of the suprabasal increase in glucose levels during these particular time intervals, expressed as AUC, in subjects with NGT and IGT. Regression lines are shown.

RESULTS

Baseline Samples

Table 1 shows that no difference was seen between subjects with NGT versus IGT regarding age, BMI, or fasting levels of glucose, insulin, C-peptide, GLP-1, or GIP. The 2-hour glucose levels were, by definition, significantly higher in IGT.

Glucose, Insulin, C-Peptide, GLP-1, and GIP Levels During the OGTT

Figure 1 shows glucose, insulin, C-peptide, GLP-1, and GIP concentrations during the OGTT in the 2 groups of subjects. Glucose levels were higher in IGT from minute 30 onwards ($P < .05$ or less), whereas insulin levels were higher at 120 ($P = .007$) and 180 minutes ($P = .003$). C-peptide levels were higher in subjects with IGT at minute 180 ($P = .006$). GLP-1 and GIP levels were not significantly different between the groups at any time point, except a lower GIP level in IGT at 120 minutes ($P = .028$). Table 2 shows total and incremental 30- and 180-minute AUCs of plasma levels for these variables. $AUC_{glucose}$ was higher in subjects with IGT, whereas $AUC_{insulin}$, $AUC_{C\text{-}peptide}$, $AUC_{GLP\text{-}1}$, and AUC_{GIP} were not different between the groups. However, the 30-minute incremental $AUC_{GLP\text{-}1}$ showed a tendency to be lower in the IGT group ($P = .072$) and the increase in GLP-1 concentrations during the first 30 minutes after meal ingestion was lower in IGT (4.5 ± 1.8 pmol/L) than in NGT (11.1 ± 2.1 pmol/L, $P = .036$).

Insulin Secretion and Sensitivity

Figure 2 shows the comparisons between groups as regards insulin secretion and sensitivity. The latter (OGIS) was significantly lower in subjects with IGT ($P = .005$). Although total insulin secretion during the first 30 minutes after oral glucose ingestion was not significantly different between the 2 groups, the dynamic incremental insulin secretion during the first 30 minutes was lower in subjects with IGT ($P = .018$). In contrast, the incremental entire insulin secretion over the 180-minute test was higher in subjects with IGT ($P = .035$). Figure 3A shows the incremental prehepatic insulin secretion rate in the intervals 0 to 10, 10 to 20, 20 to 30, 30 to 45, 45 to 60, and 60 to 90 minutes, respectively, during the OGTT as a function of the increase in glucose levels above basal (exprerssed as AUC) during these particular time periods for the 2 groups of subjects. It is seen that for similar increase in glucose levels, the increase in insulin secretion was lower in subjects with IGT than in those with NGT. From this representation, it is shown that the

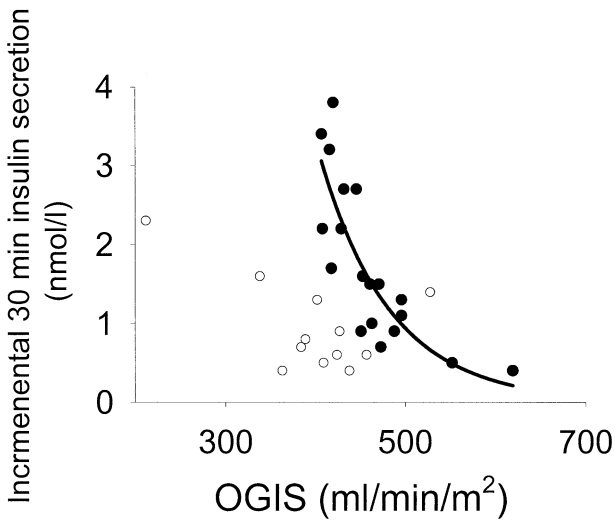

Fig 4. Relation between insulin sensitivity (OGIS) and the 30-minute incremental insulin secretion in subjects with NGT (●) or IGT (○). The inverse nonlinear, hyperbolic, regression line for the NGT subjects is indicated ($r = -0.86$, $P < .001$).

Table 3. Adaptation and Disposition Indices in Women With NGT or IGT

	NGT (n = 17)	IGT (n = 13)
Incremental 30-min adaptation index	774 ± 91	359 ± 51 (P = .001)
Total 30-min adaptation index	1,085 ± 105	743 ± 113 (P = .036)
Incremental 180-min adaptation index	9,163 ± 834	9,957 ± 577
Total 180-min adaptation index	11,787 ± 845	11,779 ± 674
Incremental 30-min DI	1,651 ± 189	1,517 ± 235
Total 30-min DI	2,495 ± 2,315	2,085 ± 287 (P = .278)
Incremental 180-min DI	17,637 ± 1,832	22,983 ± 2,739 (P = .120)
Total 180-min DI	22,723 ± 1,960	26,435 ± 2,961

NOTE. Values are means ± SEM. P indicates probability level of random difference between the groups.

estimate of the change in insulin secretion, which can be attributed to a change of 1 mmol/l × min of $AUC_{glucose}$, was 0.031 ± 0.002 (nmol insulin/L)/(mmol glucose/L × min) in the subjects with NGT versus only 0.022 ± 0.002 (nmol insulin/L)/(mmol glucose/L × min) in the subjects with IGT (P = .003). Therefore, during the first 90 minutes of the OGTT, there was a reduced insulin secretory response to the increase in glucose levels among subjects with IGT. This is also seen in Fig 3B, which depicts the individual values for each of the patients during each of these 6 time periods. For both groups, there was a significant correlation between incremental $AUC_{glucose}$ and incremental insulin secretion. The slopes of the regression lines were, however, significantly different, indicating again a reduced insulin response in IGT.

Disposition and Adaptation Indices

The relation between dynamic 30-minute insulin secretion and OGIS in subjects with NGT was nonlinear in nature (Fig 4). In contrast, regressions between model-reconstructed insulin secretion and OGIS in subjects with IGT was not significant when 30-minute insulin secretion was considered. Because of the inverse relation between insulin sensitivity and insulin secretion, and because insulin sensitivity was significantly lower in the subjects with IGT, OGIS has to be taken into consideration to accurately account for the changes of insulin secretion when comparing NGT versus IGT. This is accomplished by computing the DI. As usually defined, this index relates insulin sensitivity to insulin levels seen peripherally ($AUC_{insulin}$). DI was not different between the groups, both using the 30- and the 180-minute insulin levels (Table 3). However, if OGIS is related to prehepatic beta-cell secretion (TIS), the adaptation index is obtained. This index was lower in IGT when using the 30-minute insulin secreton but not when using the 180-minute value (Table 3). It was interesting to relate the 2-hour glucose value with these indices. No significant correlation was found between the 2-hour glucose value and the DI; in contrast, an inverse linear relation was found between the adaptation index and the 2-hour glucose value (r = −0.71, P < .001). This shows that higher values of the adaptation index reflect a better glucose tolerance. The different behavior between the adaptation index (related to prehepatic beta-cell secretion) and the disposition index (related to posthepatic insulin appearance) poses the focus on hepatic insulin extraction in IGT. Despite not reaching statistical significance between groups, there was a clear tendency for reduction in IGT (P = .055, Fig 2), and when insulin sensitivity was related to hepatic insulin extraction in all 30 studied women, a significant linear correlation was found (Fig 5), indicating that low insulin sensitivity is associated with a lower insulin clearance.

DISCUSSION

Non-obese subjects with IGT were compared with those with NGT in terms of insulin secretion, sensitivity, and clearance during OGTT. Prehepatic insulin secretion and posthepatic systemic appearance rates were assessed by simultaneously analyzing peripheral insulin and C-peptide concentrations by means of widely accepted and validated mathematical models.[18,23] We found that the incremental and total 30-minute insulin secretion were impaired in the subjects with IGT, and by estimating the increase in insulin as a function of increase in glucose, it is seen that glucose sensitivity of insulin secretion is reduced in IGT during the first hour of the OGTT. This confirms previous observations that defective early insulin secretion is a characteristic of subjects developing type 2 diabetes.[7] In contrast, we also found that the total and incremental insulin secretion during the entire 3-hour test was significantly increased in IGT. This augmented insulin secretion during the third hour after oral glucose in IGT is probably a consequence of the hyperglycemia at this stage of the test.

It needs to be emphasized that it was the model-recon-

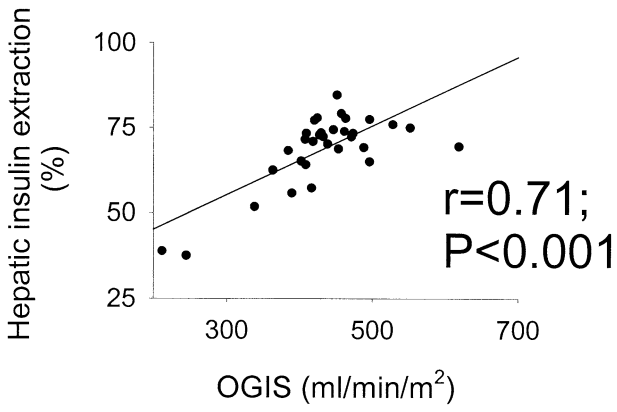

Fig 5. Relation between hepatic insulin extraction and insulin sensitivity (OGIS) in subjects with NGT or IGT. The linear regression line is indicated.

structed prehepatic insulin secretion that was different between the groups. In contrast, when simply calculating either $AUC_{insulin}$, or even $AUC_{C\text{-}peptide}$, or using insulin or C-peptide concentrations at individual time points, there were no differences between the groups. Hence, simply by using these empiric estimates (derived only from peripheral concentrations), it is not possible to determine that these subjects with IGT had impaired beta-cell function. However, for an accurate evaluation of insulin secretion it is not even sufficient to simply compare insulin secretion data between the groups, even if they are accurately determined, because insulin secretion is inversely related to insulin sensitivity.[7] Therefore, subjects with insulin resistance have a higher insulin secretion than subjects with normal insulin sensitivity. The actual observation of impaired insulin secretion in IGT, having low insulin sensitivity, therefore tends to underestimate the true defective beta-cell function. This problem is addressed by introducing an index that mirrors the interplay between insulin secretion and insulin sensitivity. This index, the DI, is obtained by multiplying insulin responses to a beta-cell–stimulating challenge with a measure of insulin sensitivity.[27] In the present study, we have calculated OGIS as a measure of insulin sensitivity.[17] This index is based on insulin action on glucose kinetics after the OGTT and identifies insulin sensitivity by evaluating glucose clearance under conditions of variable insulin.[17] OGIS has previously been validated in comparison with the euglycemic, hyperinsulinemic clamp[17] and has been used in other studies.[24-26] Interestingly, however, when calculating the DI, by multiplying the $AUC_{insulin}$ by OGIS, there was no significant difference between the groups. In contrast, when OGIS was related to the measure of prehepatic insulin secretion, there was a significant reduction of this index, named the adaptation index and suggested to more accurately reflect the relation between inulin secretion and insulin sensitivity. In a previous study, which used the intravenous glucose test, this adaptation index was found to be low in IGT,[28] and here, we show that also after oral glucose, it is low in IGT. The clinical importance of this measure is evident by the finding that the 2-hour glucose value inversely correlated with the adaptation index, as it theoretically should, given the meaning of this index.[28] Thus, relating insulin secretion to insulin sensitivity in each individual, it is clearly seen that subjects with IGT display impaired beta-cell function, which results in a higher 2-hour glucose value.

The finding that the adaptation index (relating prehepatic beta-cell secretion to OGIS) was lower in IGT than in NGT, whereas the DI (relating posthepatic insulin appearance to OGIS) was not significantly different between the groups, seems to indicate that a compensatory mechanism allowed insulin clearance to be retarded in IGT to compensate for the inhibited insulin secretion. We believe that this compensation is exerted as a reduction of the hepatic insulin degradation, as suggested by our finding that hepatic insulin extraction correlated with insulin sensitivity. Hence, insulin resistance seems to be associated with reduced insulin extraction, as demonstrated in elderly subjects[29] and, conversely, increased insulin sensitivity may be associated with increased insulin clearance, as demonstrated in athletes.[30] This would infer that the subjects with IGT had reduced insulin extraction, and although in this study the low hepatic extraction of IGT did not reach significance, there was a clear trend showing this ($P = .055$).

Another important aim of this study was to relate the incretin levels during the OGTT to the changes in insulin secretion in IGT. We found that the total 180-minute increase in GIP and GLP-1 levels were not different between the groups. This confirms a previous study where we also did not detect any significant difference in GLP-1 levels after oral glucose in subjects with IGT versus those with NGT.[31] In contrast, we previously observed a reduced GIP release after oral glucose in IGT.[31] This discrepancy is probably explained by differences in BMI between the studies. Thus, subjects in the previous study were older and had higher BMIs than subjects examined in the present study. However, we found that the early 30-minute GLP-1 response to oral glucose showed a tendency to be reduced in IGT, because the increase in concentrations at 30 minutes above baseline was significantly reduced and the incremental 30-minute $AUC_{GLP\text{-}1}$ showed a tendency to be reduced in IGT. This would support recent studies showing that the GLP-1 response to meal ingestion or oral glucose is defective in subjects with type 2 diabetes.[14,32,33] It also suggests that defective early GLP-1 response is associated with development of defective insulin secretion during the progression to diabetes, although in our study the difference between the groups was small. GLP-1 is secreted from the intestinal L-cells as an active $GLP\text{-}1_{7\text{-}36}$amide peptide, which is rapidly degraded to its inactive form ($GLP\text{-}1_{9\text{-}36}$amide) by the enzyme dipeptidyl peptidase-4.[34] It should be emphasized that in this study we used a C-terminally directed antibody for the detection of GLP-1. With this antibody, both active ($GLP\text{-}1_{7\text{-}36}$amide) and inactive ($GLP\text{-}1_{9\text{-}36}$amide) GLP-1 are determined.[22] The measurement is therefore valid for the estimation of GLP-1 secretion. The impaired GLP-1 release in type 2 diabetes has previously been thought to be a consequence of the diabetic condition, and not a primary event, due to the normal GLP-1 response in our previous study in obese subjects with IGT[31] and, similarly, the normal GLP-1 release in subjects with a family history of type 2 diabetes.[33] However, the present study together with a previous report[31] shows that subjects with IGT have reduced early release of GLP-1, suggesting that already before the onset of diabetes, the rapidity in the GLP-1 response might be delayed. This impaired early GLP-1 response may contribute to the impairment of insulin secretion.

In conclusion, non-obese subjects with IGT display defective early insulin secretion after oral glucose, as previously was demonstrated after intravenous challenges.[4,6] This study also shows that it is necessary to assess prehepatic beta-cell secretion and relate it to the changes of insulin sensitivity in IGT to reach this conclusion. In contrast, assessing posthepatic insulin appearance in relation to insulin sensitivity does not show reduced insulin response in IGT. Finally, this study also suggests a role of impaired early GLP-1 response for the islet dysfunction in IGT.

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