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Glucose intolerance is predicted by low insulin secretion and high glucagon secretion: outcome of a prospective study in postmenopausal Caucasian women

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Abstract

Aims/hypothesis. To study the pathophysiological importance of changes in insulin sensitivity and islet function over time for alterations in glucose tolerance in a randomly selected large group of non-diabetic women aged 57–59 years over a 3-year period.
Methods. At baseline and at the 3-year follow-up, glucose tolerance (WHO 75 g oral glucose), insulin sensitivity (euglycaemic, hyperinsulinaemic clamp) and insulin and glucagon secretion (2 to 5-min responses to 5 g i.v. arginine at fasting, 14 and > 25 mmol/l glucose) were measured.
Results. At baseline, women with impaired glucose tolerance (IGT, $n = 28$) had lower insulin sensitivity ($p = 0.048$) than normal women (NGT, $n = 58$). The arginine-induced insulin responses (AIR) were inversely associated with insulin sensitivity ($r \geq -0.55$, $p < 0.001$). When related to the 3-year follow-up, the baseline product of AIR at 14 mmol/l glucose times insulin sensitivity, insulin effect index (IE) ($r = -0.40$, $p < 0.001$) and the arginine-induced glucagon response at 14 mmol/l glucose (AGR, $r = 0.28$, $p = 0.009$) both correlated with follow-up 2-h glucose. In a multiple regression model, baseline 2-h glucose, insulin effect index and arginine-induced glucagon response independently predicted 2-h glucose at follow-up (total $r = 0.668$, $p < 0.001$). Furthermore, Δinsulin sensitivity (i.e. follow-up minus baseline) correlated with Δinsulin secretion ($r = -0.30$, $p = 0.006$), whereas Δglucagon secretion correlated with Δ2-h glucose ($r = 0.30$, $p = 0.006$) over the 3 years. In a multiple regression, alterations in 2-h glucose over the 3 years were independently determined by changes in fasting insulin and glucagon secretion ($r = 0.424$, $p < 0.001$).
Conclusion/interpretation. Low insulin secretion, when judged in relation to insulin sensitivity, and high glucagon secretion, determine glucose tolerance over time in the individual subject. These processes are therefore potential targets for prevention of deterioration in glucose tolerance. [Diabetologia (2000) 43: 194–202]

Keywords Normal glucose tolerance, impaired glucose tolerance, Type II diabetes, insulin sensitivity, insulin secretion, glucagon secretion.

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Abbreviations: AGR, Arginine-induced glucagon response; AIR, arginine-induced insulin response; FPG, fasting plasma glucose; IE, insulin effect index; PG14, 14 mmol/l plasma glucose; PG > 25, more than 25 mmol/l plasma glucose; PG_{50}, beta-cell sensitivity to glucose; Δ, delta (follow-up minus baseline).

Over the last decades cross-sectional studies have shown that the interplay between insulin sensitivity and the insulin secretory capacity is vital for maintaining normal glucose tolerance. Such studies have shown that in normal healthy subjects, insulin secretion is inversely regulated by the level of insulin sensitivity [1–3]. Consequently, factors that affect insulin sensitivity, such as body weight, sex, and physical activity [4], could determine the insulin secretory capacity for the individual person. Further, cross-sectional studies have suggested that this finely tuned regula-

tion is disturbed in subjects with impaired glucose tolerance (IGT), who are at increased risk of developing diabetes [5–7]. Thus, when comparing groups of subjects, the islet adaptation to insulin resistance is inadequate in IGT [8–10], resulting in insulin concentrations lower than required to maintain glucose concentrations in the normal range, causing the hyperglycaemia seen in IGT. It is, however, not known whether the development of glucose intolerance occurs due to impairment of the islet adaptation to insulin resistance. Therefore, prospective studies are necessary to establish the importance of insulin sensitivity and insulin secretion for the pathogenesis of glucose intolerance in the individual subject. Thus, although previous prospective studies have shown that both insulin resistance and a low insulin secretory capacity are risk factors for glucose intolerance [11–13], it is not known how the inverse relation between insulin sensitivity and insulin secretion is regulated in the individual subject over time and it has not been established whether alterations in insulin sensitivity or islet function or both contribute to the development of glucose intolerance in the individual patient.

In this study we prospectively examined insulin sensitivity and insulin secretion in a large group of postmenopausal non-diabetic women randomly selected from a larger cohort with a high prevalence of glucose intolerance [14]. Insulin sensitivity and islet function were studied both at baseline and at the 3-year follow-up, which enabled determination of changes in these variables in relation to alterations in glucose tolerance for the individual subject. Insulin sensitivity was measured with the euglycaemic, hyperinsulinaemic clamp [15]. Islet function was studied using the glucose-dependent arginine stimulation test [16, 17] which enables determination of several different aspects of insulin and glucagon secretion such as the baseline and maximum secretory capacity and the glucose dependence of insulin and glucagon secretion. Therefore, in our study we evaluated the importance of not only beta-cell but also alpha-cell function for deterioration of glucose tolerance which is important because, in contrast to insulin secretion, the role of glucagon secretion in the development of Type II (non-insulin-dependent) diabetes mellitus is not well characterized. In fully developed Type II diabetes, increased glucagon concentrations are seen in the fasting state together with a reduced glucose suppression of glucagon secretion [16, 18, 19]. Whether these changes are primary or, as suggested by some previous studies [20, 21], secondary to the disease process has, however, not been established.

The aims of our study were thus firstly, to evaluate changes in glucose tolerance in relation to alterations in islet function and insulin sensitivity over time and secondly to determine baseline risk factors for the deterioration of glucose tolerance in this homogeneous group of Caucasian postmenopausal women.

Subjects and methods

Study design. Baseline examinations which were done between 1993 and 1994 included a clinical examination, anthropometric measurements, an oral glucose tolerance test, a euglycaemic, hyperinsulinaemic clamp [15] for determination of insulin sensitivity and a glucose-dependent arginine stimulation test [16] to measure islet function. All these tests were repeated between 1996 and 1997 after a 3-year follow-up period (means ± SD follow-up time 3 years 1 ± 3 months, range 2 years 9 months – 4 years 2 months). The ethics committee of Lund University approved the study and written informed consent was obtained from all participants before the study. The studies of glucose tolerance, insulin sensitivity and insulin secretion were all done in the morning after an overnight fast, with at least 1 week between visits, both at the baseline and follow-up examinations.

Subjects. A total of 108 women were enrolled in the prospective study. The women were selected randomly from a larger cohort of 841 postmenopausal women born in 1935 and living in the city of Malmö, Sweden. All of them had previously participated in health screening (1990–1991) which included an oral glucose tolerance test [14]. The selection procedure was based on the 2-h blood glucose concentration after a standard WHO 75-g oral glucose tolerance test. Women with 2-h blood glucose concentration less than 11.1 mmol/l were stratified so that all degrees of glucose tolerance from normal (NGT, 2-h blood glucose concentration < 7.8 mmol/l) to impaired glucose tolerance (IGT, 2-h blood glucose concentration ≥ 7.8 and < 11.1 mmol/l) were represented but no cases of diabetes were included. None of the women included in the study was taking any medication known to affect carbohydrate metabolism. Of the 108 women, 86 participated in the follow-up. Of the 22 subjects lost to follow-up, 2 subjects had died, 1 was excluded because of corticosteroid treatment, another had moved out of town and in 1 it was impossible to establish the necessary intravenous lines at the follow-up examinations. The remaining 17 subjects did not want to take part in the follow-up for various reasons. The 22 women who did not participate in the follow-up did not differ from the 86 participants in baseline body weight or BMI, glucose tolerance, insulin sensitivity or insulin secretory capacity. This paper presents data on the 86 women who participated both in the baseline and follow-up examinations. At the baseline examination, the women were aged 57–59 years and at the 3-year follow-up they were aged 60–62 years (Table 1).

Anthropometric measurements. All measurements were done with the subjects in light clothing without shoes. Body mass index (BMI) was calculated as the weight (kg) divided by height (m^2). The waist circumference was measured at the level of the umbilicus and the hip circumference at the level of the greater trochanters, with the subjects standing.

Glucose tolerance. Oral glucose tolerance was determined with a standard WHO 75-g glucose load. Normal glucose tolerance was defined as a 2-h capillary blood glucose value less than 7.8 mmol/l and IGT was defined as a 2-h capillary blood glucose value of 7.8 –11.1 mmol/l [22].

Insulin sensitivity. Insulin sensitivity was determined with the euglycaemic, hyperinsulinaemic clamp as reported previously [15]. Intravenous catheters were inserted into antecubital veins in both arms. 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)$^{-1}$ · min^{-1} was started.

Table 1. Characteristics of the 86 women who participated in baseline examinations between 1993 and 1994 and follow-up examinations between 1996 and 1997

Characteristic	Baseline	Follow-up	*p* value
Age (years and months)	58 yr 8 mo ± 5 mo	61 yr 9 mo ± 6 mo	
Body weight (kg)	68.0 ± 9.7	68.6 ± 9.7	0.15
Height (cm)	164.3 ± 0.05	163.9 ± 0.05	< 0.001
Body mass index (kg/m^2)	25.2 ± 3.7	25.5 ± 3.6	0.044
Waist-to-hip ratio	0.78 ± 0.06	0.86 ± 0.05	< 0.001
Fasting blood glucose (mmol/l)	4.7 ± 0.5	5.0 ± 0.6	< 0.001
OGTT 2 h blood glucose (mmol/l)	7.0 ± 1.4	7.7 ± 1.7	< 0.001
Fasting insulin (mmol/l)	63 ± 26	77 ± 38	< 0.001
Fasting plasma glucagon (ng/l)	70 ± 21	75 ± 21	0.020

Data are shown as means ± SD

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 concentration at 5.0 mmol/l. Blood glucose was determined at bedside every 5 min. Samples for analysis of the insulin concentration achieved were taken at 60 and 120 min.

Islet function. Insulin and glucagon secretion were determined with intravenous arginine stimulation at three glucose concentrations (fasting, 14 and > 25 mmol/l), as described previously [16, 17]. Intravenous catheters were inserted into antecubital veins in both arms. 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 given for 20–25 min to increase and maintain blood glucose at 13 – 15 mmol/l. 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 hyperglycaemia [23, 24]. After the pause, baseline samples were again obtained. Then a high-speed (900 ml/h) 20 % glucose infusion lasting 25–30 min was used to increase blood glucose concentration to over 25 mmol/l, as determined at bedside. At this blood glucose concentration, new baseline samples were taken and arginine (5 g) injected, followed by final samples taken at + 2, + 3, + 4 and + 5 min. At the baseline examination, the plasma glucose concentrations achieved after the two glucose infusions were 15.7 ± 2.1 and 31.4 ± 3.7 mmol/l. At the follow-up, they were 15.0 ± 1.6 and 29.8 ± 4.6 mmol/l.

Analyses. Blood glucose concentration was determined at bedside by the glucose dehydrogenase technique with a Hemocue (Hemocue, Ängelholm, Sweden) during the hyperinsulinaemic, euglycaemic clamp and with an Accutrend (Boehringer Mannheim Scandinavia, Bromma, Sweden) during the baseline arginine test. During the follow-up arginine test, bedside glucose monitoring was done with the Accutrend alpha. Blood samples from the arginine and clamp studies and the oral glucose tolerance test were immediately centrifuged at 5 °C and serum or plasma frozen at –20 °C until analysis in duplicate. Serum insulin and plasma glucagon concentrations were analysed with the double-antibody RIA technique. For the insulin assay, guinea-pig anti-human insulin antibodies, human insulin standard and mono-^{125}I-Tyr-human insulin (Linco Research, St Charles, Mo., USA) were used. Samples for analysis of glucagon were obtained in pre-chilled test tubes containing 0.084 ml EDTA (0.34 mol/l) and aprotinin (250 kallikrein inhibiting units/ml blood, Bayer, Leverkusen, Germany). Analysis of glucagon concentration was done with double-antibody RIA using guinea-pig anti-human glucagon antibodies specific for pancreatic glucagon, ^{125}I-glucagon as tracer and glucagon standard (Linco). Plasma glucose concentrations were analysed using the glucose oxidase method.

Calculations and statistics. Data are presented as means ± SD, unless otherwise noted. For calculation of insulin sensitivity, a steady-state condition was assumed during the second hour of the clamp. Insulin sensitivity (nmol glucose · kg body weight^{-1} · min^{-1}/pmol insulin · l^{-1}) was taken as the glucose infusion rate during the second hour of the clamp divided by the mean insulin concentration measured during the second hour of the clamp [15].

From results obtained in the glucose-dependent arginine stimulation test, the arginine-induced insulin responses (AIR) were calculated as the mean of the + 2 to + 5 min-samples minus the mean prestimulus hormone concentration at the three different glucose concentrations (fasting, 14 mmol/l and > 25 mmol/l), i. e. AIR_{FPG}, AIR_{PG14} and $AIR_{PG>25}$. The slope between AIR at fasting plasma glucose concentration and plasma glucose 14 mmol/l ($slope_{AIR} = \Delta AIR/\Delta glucose$) was calculated as a measure of glucose potentiation of beta-cell secretion [25, 26]. It is known that arginine-stimulated insulin secretion is maximum when the plasma glucose concentration exceeds 25 mmol/l [26]. Therefore, the AIR at the highest glucose concentration ($AIR_{PG>25}$) was taken as a measure of the maximum insulin secretory capacity of the beta cells. The plasma glucose concentration at which half maximum insulin secretion is achieved (PG_{50}), a measure of betacell sensitivity to glucose, was calculated from $AIR_{PG>25}$ and $slope_{AIR}$. The arginine-induced glucagon responses (AGR) and the $slope_{AGR}$ (the glucose inhibition of glucagon secretion) were calculated in the same manner.

To quantify the relation between insulin sensitivity and insulin secretion, we also calculated the product of insulin sensitivity times the AIRs or the $slope_{AIR}$. This product was termed the insulin effect index (IE). The IE therefore measures the ability of the subject to adapt insulin secretion to the prevailing degree of insulin sensitivity.

Statistical analyses were done with the SPSS for Windows system. Differences between groups were tested with Student's *t* test for unrelated samples. Differences between baseline and follow-up within groups was tested with Student's *t* test for related samples. Two-sided tests were used and a *p* value less than 0.05 was considered statistically significant. Pearson's product moment correlation coefficients were obtained to estimate linear correlation between variables. Linear multiple regressions were used to assess the independent effect of several variables on follow-up 2-h glucose, as well as the delta (Δ) 2-h glucose (follow-up minus baseline). The stepwise forward method was used. The models are described in detail in the results section. Hyperbolic regressions were calculated with the Sigmaplot 4.0 for Windows program (SPSS, Chicago, Ill., USA).

Table 2. Results of the glucose-dependent arginine stimulation test in the 86 women who participated in baseline examinations between 1993 and 1994 and follow-up examinations between 1996 and 1997

Variable	Baseline	Follow-up	*p*-value
Serum insulin concentration at PG 14 (pmol/l)	227 ± 132	221 ± 143	0.61
Serum insulin concentration at PG > 25 (pmol/l)	433 ± 320	422 ± 428	0.71
AIR_{FPG} (pmol/l)	352 ± 197	395 ± 255	0.005
AIR_{PG14} (pmol/l)	983 ± 577	1026 ± 606	0.36
$AIR_{PG>25}$ (pmol/l)	1207 ± 679	1217 ± 746	0.86
$Slope_{AIR}$ (pmol/mmol)	61.5 ± 43.0	65.2 ± 44.1	0.34
PG_{50} (mmol/l)	8.9 ± 3.3	8.5 ± 4.7	0.43
Plasma glucagon concentration at PG 14 (ng/l)	53 ± 16	56 ± 18	0.011
Plasma glucagon concentration at PG > 25 (ng/l)	44 ± 16	44 ± 16	0.76
AGR_{FPG} (ng/l)	111 ± 49	114 ± 47	0.41
AGR_{PG14} (ng/l)	59 ± 26	70 ± 36	< 0.001
$AGR_{PG>25}$ (ng/l)	42 ± 21	46 ± 25	0.031
$Slope_{AGR}$ (ng/mmol)	–5.1 ± 3.3	–4.5 ± 3.3	0.14

Data are shown as means ± SD

Results

Changes in the entire study group: baseline compared with follow-up

Study group characteristics. Characteristics from the baseline and follow-up examinations in the entire group of 86 women are shown in Table 1. It is seen that during the 3-year period, both fasting and 2-h blood glucose concentrations increased significantly, as did fasting insulin and glucagon. At the baseline examination, 58 of the women had NGT according to the oral glucose tolerance test and 28 women had IGT. At the follow-up, 44 women had maintained NGT and 23 women had maintained IGT. The rest of the women had altered their glucose tolerance status. Of the women 15 worsened their glucose tolerance status; 13 NGT women progressed to IGT, whereas 1 NGT and 1 IGT woman progressed to diabetes. Improved glucose tolerance was seen in 4 women who had IGT at baseline and NGT at follow-up. Thus, at the follow-up examination, a total of 48 women had NGT, 36 had IGT and 2 had developed diabetes. The rate of progression from NGT to diabetes was 0.6 % a year and the progression from IGT to diabetes was 1.1 % a year. The rate of progression from NGT to IGT was 7.2 % a year.

Insulin sensitivity. In the entire group, insulin sensitivity did not change during the study (baseline 71.9 ± 30.8 vs follow-up 67.9 ± 30.9 nmol glucose · kg^{-1} · min^{-1} /pmol insulin · l^{-1}, $p = 0.11$). Further, insulin sensitivity measurements from the two clamps 3 years apart were highly correlated to each other ($r = 0.72$, $p < 0.001$). The variable was normally distributed in the cohort both at the baseline and the follow-up, as we have previously shown in a larger group of subjects [9].

Insulin and glucagon secretion. Table 2 shows the results of the glucose-dependent arginine stimulation test from the baseline and follow-up examinations. In the entire group, the arginine-induced insulin response at fasting plasma glucose (AIR_{FPG}) was increased at the follow-up. In contrast, the AIR_{PG14}, or the $AIR_{PG>25}$, the glucose-stimulated insulin concentrations at PG14 or PG > 25, the $slope_{AIR}$ or the $PG_{50,(PG14)}$ or the $AIR_{PG>25}$ did not differ between baseline and follow-up in the entire group. The glucagon concentration after increasing plasma glucose to 14 mmol/l increased slightly from baseline to follow-up, whereas at the highest glucose concentration glucagon concentrations were equally suppressed during the two tests. The AGR_{FPG} did not differ from baseline to follow-up but both AGR_{PG14} and $AGR_{PG>25}$ were statistically significantly higher at the follow-up examination. Finally, the $slope_{AGR}$ did not differ between baseline and follow-up in the entire study group.

Relation between insulin sensitivity and islet function

Insulin secretion in relation to insulin sensitivity. In the subjects with NGT, the insulin responses to arginine at the three glucose concentrations, and the $slope_{AIR}$ were all negatively and non-linearly related to the clamp insulin sensitivity at the baseline examination as well as follow-up. The association between insulin sensitivity and the AIR_{FPG} was as described previously using the IVGTT [1, 2], hyperbolic in nature (i.e. insulin sensitivity times insulin secretion = constant) (Fig. 1). The hyperbolic regression coefficients were for insulin sensitivity in relation respectively to AIR_{FPG}: $r = 0.70$, $p < 0.001$ (Fig. 1); AIR_{PG14}: $r = 0.60$, $p < 0.001$; $AIR_{PG>25}$: $r = 0.55$, $p < 0.001$; and $slope_{AIR}$: $r = 0.36$, $p < 0.001$.

Relation between changes in insulin secretion and insulin sensitivity. Although insulin sensitivity did not change statistically significantly in the group as a whole during the 3-year follow-up period, there was a negative association between the Δ (delta = follow-up minus baseline) insulin sensitivity and the $\Delta AIR_{PG>25}$ (Fig. 2, $r = -0.30$, $p = 0.006$) as well as the

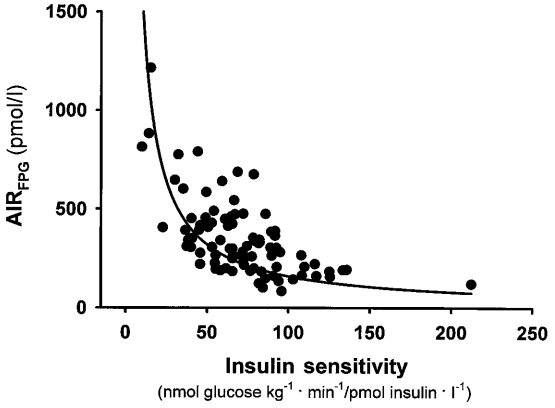

Fig. 1. Scatterplot of insulin sensitivity (measured by euglycaemic, hyperinsulinaemic clamp) in relation to the arginine-induced insulin responses (2–5 min post-load increase) at fasting glucose (AIR_{FPG}) measured at the baseline examination in the 86 women. The regression line shows the hyperbolic fit to the data. The regression coefficient of the hyperbolic curve is 0.79 ($p < 0.001$)

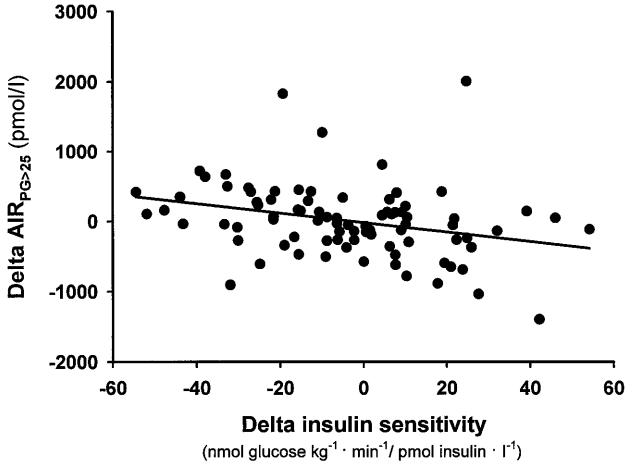

Fig. 2. Scatterplot of delta insulin sensitivity (follow-up value minus baseline value) in relation to delta $AIR_{PG>25}$ in the 86 women (insulin response to 5-g iv arginine, 2–5 min post-load increase, at > 25 mmol/l plasma glucose). The line represents the linear regression of the variables. The correlation coefficient is – 0.30 ($p < 0.0006$)

ΔAIR_{PG14} ($r = -0.24$, $p = 0.025$). Therefore, in subjects who had a change from baseline to follow-up in the insulin sensitivity, there was also a reciprocal inverse change in the insulin secretion.

Insulin effect index. To combine the effects of insulin sensitivity and insulin secretion for a subject's glucose tolerance and to enable quantification of this relation, we calculated the product of insulin sensitivity times the AIRs or the $slope_{AIR}$. These products were termed insulin effect indices (IEs) and measure the ability of the subject to adapt insulin secretion to the prevailing degree of insulin sensitivity. The results show that during the 3 years, there were no significant changes in

Table 3. Univariate correlation coefficients between alterations from baseline to follow-up of anthropometric and metabolic variables and alterations from baseline to follow-up of the 2-h glucose concentration (delta 2-h glucose concentration) in the 86 women who participated both in baseline and 3-year follow-up examinations

Δ Variable[a]	Δ 2-h glucose correlation coefficient	*p* value
Δ Body mass index (kg/m^2)	0.24	0.027
Δ Fasting blood glucose (mmol/l)	0.29	0.007
Δ Fasting insulin (mmol/l)	0.34	0.001
ΔAIR_{PG14} (pmol/l)	0.29	0.007
$\Delta AIR_{PG>25}$ (pmol/l)	0.31	0.005
$\Delta Slope_{AIR}$ (pmol/mmol)	0.28	0.009
ΔAGR_{PG14} (ng/l)	0.27	0.014
$\Delta AGR_{PG>25}$ (ng/l)	0.30	0.006

[a] For the following variables, the difference between baseline and follow-up did not correlate with the delta 2-h glucose concentration (*r* values shown in parenthesis): ΔAGR_{FPG} (0.05), ΔAIR_{FPG} (0.18), Δ fasting glucagon (0.03), ΔIE_{FPG} (–0.16), ΔIE_{PG14} (0.07), $\Delta IE_{PG>25}$ (0.11), ΔIE_{Slope} (0.06), Δ Insulin sensitivity (–0.10), $\Delta Slope_{AGR}$ (0.17)

the entire study group in either of the IEs (baseline vs follow-up IE_{FPG}: 21.7 ± 8.9 vs 23.6 ± 13.6, $p = 0.094$; IE_{PG14} 64.2 ± 36.3 vs 66.0 ± 42.2, $p = 0.60$; $IE_{PG>25}$ 75.6 ± 34.2 vs 74.5 ± 49.0 μmol · kg^{-1} · min^{-1}, $p = 0.79$; IE_{Slope} 4.1 ± 2.9 vs 4.3 ± 3.1 ml · kg^{-1} · min^{-1}, $p = 0.36$).

Determinants of alterations in glucose tolerance over 3 years.
Changes in the variables of insulin sensitivity and islet function were studied in relation to changes in 2-h glucose over the 3 years. An increase in 2-h glucose from baseline to follow-up was significantly correlated to increases over the 3 years in BMI, fasting glucose and fasting insulin concentration but not fasting glucagon concentration (Table 3). Furthermore, an increased 2-h glucose was related to increased AIRs and AGRs, as well as to an increased $slope_{AGR}$ (Table 3). In contrast, alterations in 2-h glucose over the 3 years were not related to alterations in the IEs from baseline to follow-up.

A multiple regression was done to ascertain which of the altered variables were independently associated with changes in glucose tolerance from baseline to follow-up (Table 4). We found that an increase in 2-h glucose over the 3 years was positively associated with an increased fasting insulin as well as a higher glucagon secretion. These two variables could, however, explain no more than about 20 % of the variance in the Δ2-h glucose, suggesting that other factors not included in the model are also of importance for increases in 2-h glucose over time.

Baseline predictors of follow-up glucose tolerance.
Table 5 shows the univariate correlations between baseline variables and follow-up glucose tolerance.

Table 4. Stepwise forward multiple regression model of determinants of alterations in glucose tolerance over 3 years in the 86 women who participated both in the baseline and follow-up examinations

Step	Variable entered[a]	B	SE (B)	r	r^2	p value
1	Δfasting insulin (pmol/l)	0.018	0.006	0.359	0.118	0.004
2	$\Delta AGR_{PG>25}$ (ng/l)	0.017	0.008	0.424	0.180	0.029
	Constant	0.399	0.164			0.017

[a] Variables included in the model: $\Delta AGR_{PG>25}$, $\Delta AIR_{PG>25}$, $\Delta slope_{AIR}$, Δfasting insulin, Δbody mass index. B indicates the slope of the regression, i.e., the estimation of the change in the dependent variable that can be attributed to a change of one unit in the independent variable. SE (B) indicates the standard error of the mean of variable B

Table 5. Univariate correlation coefficients between baseline anthropometric and metabolic variables and the follow-up 2-h glucose concentration in the 86 women who participated both in baseline and 3-year follow-up examinations

Baseline variable[a]	Follow-up 2-h glucose correlation coefficient	p value
Body mass index (kg/m^2)	0.23	0.036
Fasting blood glucose (mmol/l)	0.29	0.007
OGTT 2-h blood glucose (mmol/l)	0.60	< 0.001
Insulin sensitivity[b]	– 0.33	0.002
Fasting insulin (mmol/l)	0.38	< 0.001
AGR_{PG14} (ng/l)	0.28	0.009
IE_{FPG} (μmol · kg^{-1} · min^{-1})	– 0.31	0.004
IE_{PG14} (μmol · kg^{-1} · min^{-1})	– 0.40	< 0.001
$IE_{PG>25}$ (μmol · kg^{-1} · min^{-1})	– 0.37	< 0.001
IE_{Slope} (ml · kg^{-1} · min^{-1})	– 0.35	0.001

[a] The following baseline variables did not correlate with follow-up 2-h glucose concentration (r values shown in parentheses): waist-to-hip ratio (0.17), AIR_{FPG} (0.12), AIR_{PG14} (–0.05), $AIR_{PG>25}$ (0.03), $slope_{AIR}$ (–0.10), fasting glucagon (0.13), AGR_{FPG} (0.14), $AGR_{PG>25}$ (0.14), $slope_{AGR}$ (–0.02). [b] nmol glucose · kg^{-1} · min^{-1}/pmol insulin · l^{-1}

We found that the follow-up 2-h glucose was positively related to the baseline BMI, fasting glucose and insulin concentration. Similarly, there was a close positive correlation with the baseline 2-h glucose concentration. Further, there was a negative association with the baseline insulin sensitivity. The follow-up 2-h glucose concentration was not related to the baseline concentrations of the arginine-induced insulin responses as such. In contrast, the baseline IEs were all negatively related to the follow-up 2-h glucose concentration (Table 5; $r = -0.31$ to -0.40, $p < 0.004$). Finally, follow-up 2-h glucose concentration was positively correlated with the baseline AGR at the 14 mmol/l glucose concentration.

To determine which of these baseline variables could independently predict the follow-up 2-h glucose concentration, we entered the variables in a forward stepwise multiple regression (Table 6). Entered in the model were AGR_{PG14}, IE_{PG14}, fasting glucose, 2-h glucose, fasting insulin, and insulin sensitivity. For the IEs, only IE_{PG14} was selected since the four IEs were highly correlated with each other. The results (Table 6) show that baseline 2-h glucose concentration was by far the strongest predictor of follow-up 2-h glucose concentration. Independent predictors included in the model were also the IE_{PG14} and the AGR_{PG14}, and the impact of these two variables on 2-h glucose concentration was of similar magnitude. In all, the three variables yielded an r^2 of 0.45, thereby explaining nearly half of the variance in follow-up 2-h glucose concentration.

The independent effects of baseline AGR_{PG14} and IE_{PG14} on follow-up 2-h glucose concentration are illustrated in Figure 3, showing the 2-h glucose concentration in combined tertiles of AGR_{PG14} and IE_{PG14}. Tertile combinations were numbered groups 1 to 9. It is seen that 2-h glucose concentration was highest in the group with the lowest tertile of IE_{PG14} combined with the highest tertile of AGR_{PG14} (ANOVA $p = 0.002$; group 3 vs group 4: 9.8 ± 1.0 vs 6.9 ± 0.5 mmol/l, $p = 0.01$; vs group 5: 7.3 ± 0.4, $p = 0.033$; vs group 7: 6.9 ± 0.5, $p = 0.006$; vs group 8: 6.6 ± 0.2, $p = 0.005$).

Table 6. Stepwise forward multiple regression model to determine which baseline variables were the best predictors of the 3-year follow-up 2-h glucose concentration in the 86 women who participated both in the baseline and follow-up examinations

Step	Variable entered[a]	B	SE (B)	r	r^2	p value
1	OGTT 2-h glucose (mmol/l)	0.543	0.115	0.593	0.352	< 0.001
2	IE_{PG14} (μmol · kg^{-1} · min^{-1})	– 0.0136	0.004	0.629	0.396	0.002
3	AGR_{PG14} (ng/l)	0.0153	0.006	0.666	0.444	0.010
	Constant	3.841	0.904			< 0.001

[a] Variables included in the model: AGR_{PG14}, IE_{PG14}, fasting glucose concentration, 2-h glucose concentration, fasting insulin concentration and insulin sensitivity. B indicates the slope of the regression, i.e., the estimation of the change in the dependent variable that can be attributed to a change of one unit in the independent variable. SE (B) indicates the standard error of the mean of variable B

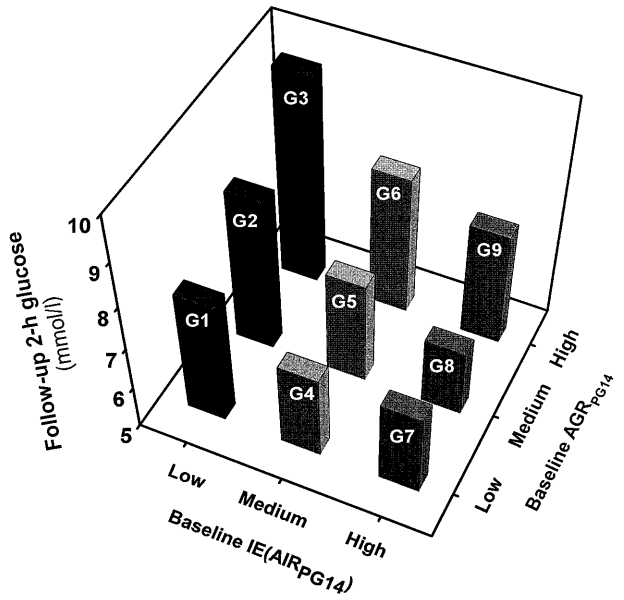

Fig. 3. Follow-up 2-h glucose concentration in combinations of tertiles of baseline IE_{PG14} and AGR_{PG14} in the 86 women included in the study. The 2-h glucose concentrations in the respective groups were (means ± SEM in mmol/l): G1: 7.8 ± 0.5 (n = 9), G2: 8.5 ± 0.6 (n = 11), G3: 9.8 ± 1.0 (n = 8), G4: 6.9 ± 0.5 (n = 9), G5: 7.3 ± 0.4 (n = 11), G6: 8.2 ± 0.4 (n = 9), G7: 6.9 ± 0.5 (n = 10), G8: 6.6 ± 0.2 (n = 7), G9: 7.6 ± 0.3 (n = 12). The 2-h glucose was highest in G3, significantly different from G4, G5, G7 and G8, $p < 0.033$

Discussion

This prospective study in Caucasian postmenopausal women has shown that during a 3-year follow-up, glucose tolerance is predicted by the insulin effect index and the glucagon secretion at baseline. Further, deterioration in glucose tolerance over the 3 years is determined by increased fasting insulin and heightened glucagon secretion from baseline to follow-up. This shows that an inadequate interplay between insulin sensitivity and insulin secretion is one important determinant for development of glucose intolerance. Moreover, our finding that increased glucagon secretion predicts glucose intolerance indicates that the high glucagon concentrations seen in diabetes could also be of importance for the pathogenesis of the disease.

The relative importance of insulin sensitivity compared with islet dysfunction in the pathogenesis of diabetes has been the topic of extensive research during the last decades [27–30]. Prospective studies in populations with high prevalence rates of diabetes, such as the Pima Indians and Mexican-Americans, have thereby shown that both reduced islet function and insulin resistance are risk factors for development of IGT [13] and diabetes [11, 12]. These studies did not, however, assess changes in insulin secretion or insulin sensitivity over time but studied baseline risk factors for development of glucose intolerance. Our approach was to study prospectively insulin sensitivity and islet function, using methods that give detailed information on these variables. Thus, we used the euglycaemic, hyperinsulinaemic clamp to measure insulin sensitivity [15] and islet function was studied using the glucose-dependent arginine stimulation test [16, 17]. We repeated the examinations after 3 years, a time point chosen to avoid too pronounced changes in the examined variables that could be generated secondarily due to the diabetic disease process. The study was thereby designed to allow characterization of even subtle changes in the relation between insulin sensitivity and islet function, which could cause glucose tolerance to deteriorate from normal to IGT to mild Type II diabetes.

We found that in our cohort, insulin sensitivity and secretory capacity are inversely related, both in women with normal glucose tolerance and those with IGT. This inverse association was initially suggested by Bergman using the minimal model technique to determine insulin sensitivity and insulin secretion after an intravenous glucose tolerance test (IVGTT) [1] and the mathematical relation between the two variables has been shown to be of a hyperbolic nature, i.e. displaying a function in which insulin sensitivity times insulin secretion is constant [2]. Also in our cohort, a hyperbolic regression fitted the data for normal subjects. In contrast to previous studies, our study group was randomly selected from a normal population. This indicates that the findings regarding the relation between insulin sensitivity and insulin secretion can be generalized to the background population. Furthermore, we show that this inverse relation also holds true for individual subjects over time because reductions in insulin sensitivity during the 3 years were significantly correlated to increases in insulin secretion and vice versa.

The hyperbolic relation between insulin sensitivity and insulin secretion indicates that the product of these variables is constant. We calculated this product and named it the islet insulin effect index. Our results show that this variable is of importance for the actual glucose tolerance since the IEs were negatively related both to baseline and follow-up 2-h glucose. Moreover, the IE_{PG14} could independently predict the 3-year follow-up 2-h glucose in a multivariate regression model. This important finding suggests that it is not the level of insulin sensitivity or insulin secretion themselves that are relevant for person's glucose tolerance but rather the ability to adjust the insulin secretory capacity to the ambient level of insulin sensitivity. The results also suggest that this ability is of importance for maintaining NGT over time.

Although the IE predicted the follow-up 2-h glucose, the strongest predictor was the baseline 2-h glucose concentration. This is in line with findings in several previous prospective studies [6, 31–33]. For example, in an analysis of six prospective studies in the USA [33], combining data from a large number of

subjects from varying populations, the 2-h glucose concentration was a strong predictor of follow-up glucose tolerance, along with fasting glucose concentration and indices of obesity. In contrast with these findings, in our study the fasting glucose concentration did not independently predict 3-year glucose tolerance. This could be due to the narrow range of fasting glucose concentrations seen in the cohort at baseline. Furthermore, obesity was not an independent predictor of glucose intolerance in our study, although the baseline body mass index correlated weakly with follow-up glucose tolerance. Obesity is, however, thought to be a main determinant of insulin sensitivity [4, 34], and BMI was highly and negatively correlated with insulin sensitivity as measured in the hyperinsulinaemic clamp both at baseline and follow-up (baseline; $r = -0.57$, $p < 0.01$). Because insulin sensitivity was included in the prediction model in the form of the insulin effect index, this could explain why the degree of obesity by itself was not an independent predictor of follow-up glucose tolerance in our study.

In previous studies on Pima Indians, the development of glucose intolerance was examined longitudinally with regard to insulin sensitivity and secretion, by measuring the insulin concentrations during an oral glucose tolerance test [35, 36]. It was shown that in NGT subjects who developed IGT, insulin concentrations increased concomitantly with increased fasting and 2-h glucose concentrations. This was taken to indicate that development of IGT is largely due to increased insulin resistance over time whereas beta-cell function remains normal until overt diabetes develops. It has, however, to be taken into account that insulin concentrations must be evaluated in relation to both the ambient glucose concentration and the individual degree of insulin sensitivity. This renders insulin secretory data from an OGTT difficult to interpret. Our study measured insulin secretion in relation to insulin sensitivity at matched glucose concentrations across NGT and IGT groups. Thereby, using multiple regression we found that deteriorations in 2-h glucose from baseline to follow-up were determined by increased fasting insulin combined with enhanced glucagon responses to arginine. These results indicate that deterioration of glucose tolerance over time is related both to a reduction in insulin sensitivity (seen as an increased fasting insulin concentration) and alterations in islet function consisting primarily of an enhanced glucagon secretion. It should be emphasized that even though statistically significant associations were observed between changes in islet function and insulin sensitivity compared with change in glucose tolerance, the r value in the multiple regression was 0.42. This suggests that changes in insulin sensitivity and islet function explain approximately 18 % of the variance in 2-h glucose values over the 3 years. The main conclusion is, however, that it is not solely a change in either sensitivity or islet function that causes deterioration of glucose tolerance but rather a combination of the two factors.

The change in fasting insulin was included in the prediction model for changes in glucose tolerance, whereas the change in insulin sensitivity as measured by the euglycaemic clamp was not statistically significant in relation to alterations in 2-h glucose concentrations. The reason for this is not known. Because fasting insulin was closely correlated with insulin sensitivity measurements from the clamp in the cohort ($r = -0.58, p < 0.001$), our results suggest that the two measurements are related but not interchangeable.

Although insulin secretion was not altered in the group as a whole during the study, glucagon secretion was, statistically, significantly increased over the 3 years. This could be of importance for the increases in fasting and 2-h glucose concentrations seen in the group during the study because the changes in the AGRs were related positively both to follow-up fasting and 2-h glucose concentrations. Further, in a multiple regression, alterations in glucose tolerance over the 3 years was independently predicted by alterations in glucagon secretion from baseline to follow-up. Moreover, baseline AGR_{PG14} was found to independently predict the follow-up 2-h glucose concentration when other factors were controlled for in the multivariate model. Thus, increased glucagon secretion seems to be an important factor to take into account when predicting the development of glucose intolerance over time. It has been shown earlier that glucagon secretion is increased [16, 19] and the glucose inhibition of glucagon secretion is reduced in Type II diabetes [16, 18].

In summary, our prospective study in Caucasian postmenopausal women has shown that glucose tolerance after 3 years is independently determined by the baseline insulin effect index, the glucagon secretion and the baseline 2-h glucose concentration. These three variables explain almost 50 % of the variation in follow-up glucose tolerance. Furthermore, changes in glucose tolerance from baseline to follow-up are determined by alterations in fasting insulin concentrations and glucagon secretion. We conclude that islet adaptation to the individual degree of insulin sensitivity is of paramount importance for glucose tolerance and that increased glucagon secretion is a risk factor for developing glucose intolerance in postmenopausal women. Hence, both low insulin secretion and high glucagon secretion are potential targets for prevention of progressive impairment of glucose tolerance.

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References

1. Bergman RN, Phillips LS, Cobelli C (1981) 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
2. Kahn SE, Prigeon RL, McCulloch DK et al. (1993) Quantification of the relationship between insulin sensitivity and β-cell function in human subjects. Evidence for a hyperbolic function. Diabetes 42: 1663–1672
3. Ferrannini E, Natali A, Bell P, Cavallo-Perin P, Lalic N, Mingrone G (1997) Insulin resistance and hypersecretion in obesity. J Clin Invest 100: 1166–1173
4. Clausen JO, Borch-Jensen K, Ibsen H et al. (1996) 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
5. Keen H, Jarrett RJ, McCartney P (1982) The ten-year follow-up of the Bedford survey (1962–1972): glucose tolerance and diabetes. Diabetologia 22: 73–78
6. King H, Zimmet P, Raper LR, Balkau B (1984) The natural history of impaired glucose tolerance in the Micronesian population of Nauru: a six-year follow-up study. Diabetologia 26: 39–43
7. Saad MF, Knowler WC, Pettitt DJ, Nelson RG, Mott DM, Bennett PH (1988) The natural history of impaired glucose tolerance in the Pima Indians. N Engl J Med 319: 1500–1506
8. Larsson H, Ahrén B (1996) Islet dysfunction in obese women with impaired glucose tolerance. Metabolism 45: 502–509
9. Larsson H, Ahrén B (1996) Failure to adequately adapt reduced insulin sensitivity with increased insulin secretion in women with impaired glucose tolerance. Diabetologia 39: 1099–1107
10. Ahrén B, Pacini G (1997) Impaired adaptation of first-phase insulin secretion in postmenopausal women with glucose intolerance. Am J Physiol 273: E701–E707
11. Lillioja S, Mott DM, Spraul M et al. (1993) Insulin resistance and insulin secretory dysfunction as precursors of non-insulin-dependent diabetes mellitus. Prospective studies of Pima Indians. N Engl J Med 329: 1988–1992
12. Haffner SM, Miettinen H, Gaskill SP, Stern MP (1995) Decreased insulin secretion and increased insulin resistance are independently related to the 7-year risk of NIDDM in Mexican-Americans. Diabetes 44: 1386–1391
13. Haffner SM, Miettinen H, Gaskill SP, Stern MP (1996) Decreased insulin action and insulin secretion predict the development of impaired glucose tolerance. Diabetologia 39: 1201–1207
14. Larsson H, Ahrén B, Lindgärde F, Berglund G (1995) Fasting blood glucose in determining the prevalence of diabetes in a large, homogeneous population of Caucasian middle-aged women. J Intern Med 237: 537–541
15. DeFronzo RA, Tobin JD, Andres R (1979) Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol 237: E214–E223
16. Ward WK, Bolgiano DC, McKnight B, Halter JB, Porte D Jr (1984) Diminished B cell secretory capacity in patients with noninsulin-dependent diabetes mellitus. J Clin Invest 74: 1318–1328
17. Larsson H, Ahrén B (1998) Glucose-dependent arginine stimulation test for characterization of islet function: studies on reproducibility and priming effect of arginine. Diabetologia 41: 772–777
18. Dimitriadis GD, Pehling GD, Gerich JE (1985) Abnormal glucose modulation of islet A- and B-cell responses to arginine in non-insulin-dependent diabetes mellitus. Diabetes 34: 541–547
19. Baron AD, Schaeffer L, Shragg P, Kolterman OG (1987) Role of hyperglucagonemia in maintenance of increased rates of hepatic glucose output in type II diabetics. Diabetes 36: 274–283
20. Assan R, Efendic S, Luft R, Cerasi E (1981) Dose-kinetics of pancreatic glucagon responses to arginine and glucose in subjects with normal and impaired pancreatic B cell function. Diabetologia 21: 452–459
21. Hamaguchi T, Fukushima H, Uehara M et al. (1991) Abnormal glucagon response to arginine and its normalization in obese hyperinsulinaemic patients with glucose intolerance: importance of insulin action on pancreatic alpha cells. Diabetologia 34: 801–806
22. World Health Organization Study Group on Diabetes mellitus (1985) Technical Report Series No 727, WHO
23. Cerasi E (1975) Potentiation of insulin release by glucose in man. Acta Endocrinol 79: 511–534
24. Grill V (1981) Time and dose dependencies for priming effect of glucose on insulin secretion. Am J Physiol 240: E24–E31
25. Halter JB, Graf RJ, Porte D Jr (1979) Potentiation of insulin secretory responses by plasma glucose levels in man: Evidence that hyperglycemia in diabetes compensates for impaired glucose potentiation. J Clin Endocrinol Metab 48: 946–954
26. Porte D Jr (1991) Banting lecture 1990. β-cells in type II diabetes mellitus. Diabetes 40: 166–180
27. Taylor SI, Accili D, Imai Y (1994) Insulin resistance or insulin deficiency. Which is the primary cause of NIDDM? Diabetes 43: 735–740
28. Cerasi E (1995) Insulin deficiency and insulin resistance in the pathogenesis of NIDDM: is a divorce possible? Diabetologia 38: 992–997
29. Ferrannini E (1998) Insulin resistance versus insulin deficiency in non-insulin-dependent diabetes mellitus: problems and prospects. Endocr Rev 19: 477–490
30. Gerich JE (1998) The genetic basis of Type II diabetes mellitus: Impaired insulin secretion versus impaired insulin sensitivity. Endocrine Reviews 19: 491–503
31. Kadowaki T, Miyake Y, Hagura R et al. (1984) Risk factors for worsening to diabetes in subjects with impaired glucose tolerance. Diabetologia 26: 44–49
32. Sicree RA, Zimmet PZ, King HOM, Coventry JS (1987) Plasma insulin response among Nauruans. Prediction of deterioration in glucose tolerance over 6 yr. Diabetes 36: 179–186
33. Edelstein SL, Knowler WC, Bain RP et al. (1997) Predictors of progression from impaired glucose tolerance to NIDDM. An analysis of six prospective studies. Diabetes 46: 701–710
34. Bogardus C, Lillioja S, Mott DM, Hollenbeck C, Reaven G (1985) Relationship between degree of obesity and in vivo insulin action in man. Am J Physiol 248: E286–E291
35. Lillioja S, Mott DM, Howard BV et al. (1988) Impaired glucose tolerance as a disorder of insulin action. Longitudinal and cross-sectional studies in Pima Indians. N Engl J Med 318: 1217–1225
36. Saad MF, Pettitt DJ, Mott DM, Knowler WC, Nelson RG, Bennett PH (1989) Sequential changes in serum insulin concentration during development of non-insulin-dependent diabetes. Lancet I: 1356–1359