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ARTICLE

# Natural history and physiological determinants of changes in glucose tolerance in a non-diabetic population: the RISC Study

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#### Abstract

*Aims/hypothesis* The natural history and physiological determinants of glucose intolerance in subjects living in Europe have not been investigated. The aim of this study was to increase our understanding of this area.

*Methods* We analysed the data from a population-based cohort of 1,048 non-diabetic, normotensive men and women (aged 30–60 years) in whom insulin sensitivity was measured by the glucose clamp technique (M/I index; average glucose infusion rate/steady-state insulin concentration) and beta cell function was estimated by mathematical modelling of the oral glucose tolerance test at baseline and 3 years later.

*Results* Seventy-seven per cent of the participants had normal glucose tolerance (NGT) and 5% were glucose

**Electronic supplementary material** The online version of this article (doi:10.1007/s00125-011-2112-x) contains supplementary material, which is available to authorised users. This includes a list of the RISC Study centres and investigators.

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Service of Therapeutic Education for Chronic Diseases, WHO Collaborating Centre, Department of Community Medicine, University Hospitals of Geneva, Geneva, Switzerland intolerant both at baseline and follow up; glucose tolerance worsened in 13% (progressors) and improved in 6% (regressors). The metabolic phenotype of the latter three groups was similar (higher prevalence of familial diabetes, older age, higher waist-to-hip ratio, higher fasting and 2 h plasma glucose, higher fasting and 2 h plasma insulin, lower insulin sensitivity and reduced beta cell glucose sensitivity with increased absolute insulin secretion). Adjusting for these factors in a logistic model, progression was predicted by insulin resistance (bottom *M/I* quartile, OR 2.52 [95% CI 1.51–4.21]) and beta cell glucose insensitivity (bottom quartile, OR 2.39 [95% CI 1.6–3.93]) independently of waist-to-hip ratio (OR 1.44 [95% CI 1.13–1.84] for one SD). At follow up, insulin sensitivity and beta cell glucose sensitivity were unchanged in the

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A. Mari CNR Institute of Biomedical Engineering, Padua, Italy stable NGT and stable non-NGT groups, worsened in progressors and improved in regressors.

*Conclusions/interpretation* Glucose tolerance deteriorates over time in young, healthy Europids. Progressors, regressors and glucose-intolerant participants share a common baseline phenotype. Insulin sensitivity and beta cell glucose sensitivity predict and track changes in glucose tolerance independently of sex, age and obesity.

**Keywords** Beta cell function · Diabetes · Dysglycaemia progression · Glucose tolerance · Insulin resistance

### Abbreviations

AIR	Acute insulin response
CVD	Cardiovascular disease
DPP	Diabetes Prevention Program
FFM	Fat-free mass
IFG	Impaired fasting glycaemia
IGT	Impaired glucose tolerance
IRAS	Insulin Resistance Atherosclerosis Study
NGT	Normal glucose tolerance
OGIS	Oral Glucose Insulin Sensitivity
RISC	Relationship between Insulin Sensitivity
	and Cardiovascular

### Introduction

Individuals with impaired glucose tolerance (IGT) or impaired fasting glycaemia (IFG) are at increased risk of developing overt type 2 diabetes [1]. On the other hand, individuals with normal glucose tolerance (NGT) may also progress to diabetes over time [2]. However, IGT and IFG may be transient dysglycaemic states [1] and progression of NGT to diabetes may be found to follow a non-linear time trajectory when glucose tolerance is tested serially [3–5]. Thus, it has been argued that the variability of glucose tolerance testing is such that regression from dysglycaemia to normoglycaemia is an equally likely occurrence as the reverse process, making the category of IGT a 'diagnostic ragbag' [1].

What factors influence spontaneous changes in glucose tolerance in non-diabetic individuals remains incompletely understood. Among 241 people with 'borderline diabetes' in the Bedford Survey [6], the levels of blood glucose at baseline were the main predictor of worsening to diabetes, but glucose tolerance improved in half of the participants. In an observational 8–10 year follow-up study of middle-aged Swedish participants, among 280 participants with IGT/IFG, lower glucose levels—fasting and post-glucose—predicted reversal to NGT [7]. In the Diabetes Prevention Program (DPP), lower glucose levels predicted restoration

of normal glucose regulation in participants with abnormal glucose levels [8]. Thus, fasting plasma glucose concentrations and glucose levels 2 h following a standard OGTT consistently track either progression or regression.

Prospective data on the chief physiological determinants of glucose tolerance, that is, insulin sensitivity and beta cell function, have been obtained using various techniques in diverse groups of people, thereby arriving at somewhat variable conclusions. Early studies in 155 white offspring of type 2 diabetic patients identified reduced glucose clearance on the IVGTT and hyperinsulinaemia as the antecedents of overt type 2 diabetes, implicating insulin resistance as the primary defect [9]. A 25 year follow-up analysis of the same cohort using minimal model analysis of the IVGTT likewise concluded that defects in both insulin-dependent and insulin-independent glucose uptake precede and predict diabetes and that the defects are detectable when patients are normoglycaemic [10]. At about the same time, a study carried out in 200 young, obese Pima Indians using the euglycaemic hyperinsulinaemic clamp to measure insulin sensitivity reported that insulin resistance was the strongest predictor of incident diabetes among both NGT and IGT individuals, with the acute insulin response (AIR) to glucose on the IVGTT being an additional significant predictor only after adjusting for obesity and insulin resistance [11]. In a subsequent report on 34 Pima Indians studied sequentially over ~5 years, both insulin resistance and AIR predicted deterioration of glucose tolerance independently of one another [12]. The Insulin Resistance Atherosclerosis Study (IRAS) [13] employed the frequently sampled IVGTT with minimal model analysis to estimate insulin sensitivity (as the S<sub>I</sub> index) and AIR in African-American and Hispanic-American families and reported that both insulin resistance and beta cell dysfunction predict incident type 2 diabetes [14]. Finally, detailed studies in 72 Hispanic women with gestational diabetes followed up for 15-30 months concluded that insulin resistance and a low insulin response to glucose were independently associated with subsequent development of diabetes [15].

Data in homogeneous, population-based cohorts of non-diabetic white subjects generated with the use of techniques directly measuring an array of physiological control variables are lacking. Moreover, no study has analysed reversal of glucose intolerance from the same dataset or has repeated physiological measurements at follow up. We report here the findings obtained in the follow-up phase of the Relationship between Insulin Sensitivity and Cardiovascular disease (RISC) Study [16], which assessed insulin sensitivity, using the clamp technique, and multiple aspects of beta cell function in 1,308 accurately phenotyped non-diabetic participants from Europe.

#### Methods

Study participants and design RISC is a prospective, observational, cohort study whose rationale and methodology have been published previously [16]. In brief, participants were recruited from those attending clinics and laboratory personnel at 19 centres in 13 countries in Europe (extending from Finland to Greece in latitude and from Spain to Serbia in longitude), according to the following inclusion criteria: men or women, aged between 30 and 60 years (stratified by sex and by age according to 10 year age groups) and clinically healthy. Initial exclusion criteria were: treatment for obesity, hypertension, lipid disorders or diabetes, pregnancy, cardiovascular or chronic lung disease, weight change of  $\geq 5$  kg in the last month, cancer (in the last 5 years) and renal failure. Exclusion criteria after screening were: arterial blood pressure ≥140/90 mmHg, fasting plasma glucose ≥7.0 mmol/l, 2 h plasma glucose (on a standard, 75 g OGTT, performed in each participant) ≥11.0 mmol/l or known diabetes, total serum cholesterol  $\geq$ 7.8 mmol/l, serum triacylglycerols  $\geq$ 4.6 mmol/l and ECG abnormalities. Thus, this cohort represents a healthier segment of a European population. Baseline examinations began in June 2002 and were completed in July 2005 and included 1,538 participants who received an OGTT. Of these, 1,308 participants also received a euglycaemic hyperinsulinaemic clamp and constituted the baseline cohort; crosssectional data on this cohort have been published [17].

All 1,308 participants in the baseline cohort were recalled 3 years later and 1,048 (80%) participated in the follow-up evaluation. The baseline anthropometric and metabolic characteristics of the 260 participants who were lost to follow up were very similar to those of the individuals who participated (data not shown). The follow-up study included all baseline anthropometric measurements and the OGTT.

Local Ethics Committee approval was obtained by each recruiting centre. Volunteers were given detailed written information on the study as well as a verbal explanation; all provided informed consent.

*Lifestyle and medical history* Information was collected on personal and family medical history of CVD, stroke, hypertension and diabetes in first-degree relatives as well as information on smoking and alcohol habits and physical activity.

*Physical examination* Height was measured on a clinic stadiometer, body weight and fat-free mass (FFM) were evaluated by the TANITA bioimpedance balance (Tanita International Division, UK). Waist, hip and thigh circumferences were measured by tape according to a standardised written protocol.

*Physical activity* Participants were fitted with a CSA Actigraph (MTI: Manufacturing Technology, Fort Walton Beach, FL, USA) attached to a waist belt for 1 week. The Actigraph is a small (43 g) single-channel recording accelerometer capable of continuous data collection for up to 22 days. Data are summated over 1 min periods and processed to evaluate energy expenditure over the entire recording period as well as periods of moderate and intense activity [18].

OGTT Following a 10–12 h overnight fast, blood samples were taken before and 30, 60, 90 and 120 min into a 75 g OGTT. The test was repeated at follow up.

Insulin clamp On a separate day within 1 week of the OGTT, a euglycaemic hyperinsulinaemic clamp was performed in all participants. Exogenous insulin was infused at a rate of 240 pmol min<sup>-1</sup> m<sup>-2</sup> simultaneously with a variable 20% dextrose infusion adjusted every 5–10 min to maintain plasma glucose level within 0.8 mmol/l ( $\pm 15\%$ ) of the target glucose level (4.5–5.5 mmol/l).

*IVGTT* In 761 of the 1,048 participants with follow-up data, at the end of the clamp and while the clamp was continued, a glucose bolus (0.3 mg/kg body weight) was administered over 1 min; plasma glucose and C-peptide concentrations were measured at 2, 4, 6 and 8 min after the bolus.

Analytical procedures Blood samples were separated into plasma and serum, divided into aliquots and stored at  $-80^{\circ}$ C for glucose, insulin, C-peptide and the serum lipid profile determination. Samples were transported on dry ice at prearranged intervals to central laboratories. Plasma glucose was measured by the glucose oxidase technique. Serum insulin was measured by a specific time-resolved immuno-fluorometric assay (TR-IFMA) (AutoDELFIA Insulin kit, Wallac, Turku, Finland), with the following assay characteristics: detection limit >3 pmol/l, intra-assay and inter-assay variation 1.7% and 3.5%, respectively. The intra-assay and inter-assay coefficients of variation were <5% and <10%, respectively. Serum total HDL and LDL-cholesterol were assayed by standard techniques.

*Data analysis* Fat mass was obtained as the difference between body weight and FFM. LDL-cholesterol concentrations were calculated using Friedewald's formula. Glucose tolerance was categorised into normal (NGT, fasting plasma glucose <6.1 mmol/l and 2 h plasma glucose <7.8 mmol/l), IGT (fasting glucose <7.0 mmol/l and 2 h glucose  $\geq$ 7.8 and <11.1 mmol/l) and diabetes (type 2 diabetes diagnosis, fasting glucose  $\geq$ 7.0 mmol/l or 2 h glucose  $\geq$ 11.1 mmol/l or glucose-lowering treatment). IFG was defined as a fasting glucose <7.0 and  $\geq$ 6.1 mmol/l and 2 h glucose  $\geq$ 7.8. Based on the observed changes of glucose tolerance at follow up, participants were classified as 'stable NGT' (i.e. NGT at both baseline and follow up), 'stable non-NGT' (i.e. IFG or IGT at both baseline and follow up), 'progressors' (i.e. those stepping up along the sequences NGT $\rightarrow$ IFG, NGT $\rightarrow$ IGT, NGT $\rightarrow$ type 2 diabetes, IFG $\rightarrow$ IGT, IFG $\rightarrow$ type 2 diabetes, IGT $\rightarrow$ type 2 diabetes, between baseline and follow up) and 'regressors' (i.e. participants stepping down along the same sequences).

Insulin sensitivity was calculated as the ratio of the Mvalue (insulin-mediated whole body glucose disposal) during the final 40 min of the 2 h clamp (normalised to the FFM) to the mean plasma insulin concentration measured during the same interval (M/I) [average glucose infusion rate/steady-state insulin concentration], in units of  $\mu$ mol min<sup>-1</sup> [kg FFM]<sup>-1</sup>[nmol/l]<sup>-1</sup>), as per previous analyses [17]. Using the *M* value not normalised to FFM yielded identical results. To allow comparison of baseline and follow-up values, insulin sensitivity was also estimated from the plasma glucose and insulin levels measured during the OGTT with the use of the oral glucose insulin sensitivity (OGIS) method, which has been validated against the insulin clamp technique [19]. OGIS was optimised for the RISC database by re-estimating the OGIS equation variables based on the RISC database and the original data and by normalising the estimate by lean body mass rather than body surface area [19]. The adjusted OGIS index represents glucose clearance (in ml min<sup>-1</sup> [kg  $FFM]^{-1}$ ) at an insulin concentration typical of the clamp (~600 pmol/l). In the current dataset, M/I and OGIS were correlated with one another, with Spearman correlation coefficients ranging between 0.48 and 0.68 (all p < 0.0001) in the four groups defined above, and the overall correlation between the classical and the adjusted OGIS index was 0.85 (p < 0.0001). Glucose and C-peptide area under the timeconcentration curve were calculated using the trapezium rule. Actigraph readings were summarised as habitual activity (average number of counts per minute worn) [18].

*Beta cell function modelling* The model used to reconstruct insulin secretion and its control by glucose has been previously described [20]. In brief, the model consists of three blocks: (1) a model for fitting the glucose concentration profile, the purpose of which is to smooth and interpolate plasma glucose concentrations; (2) a model describing the dependence of insulin (or C-peptide) secretion on glucose concentration; and (3) a model of Cpeptide kinetics, i.e. the two-exponential model proposed by van Cauter et al. [21], in which the model parameters are individually adjusted to the subject's anthropometric data. In particular, with regard to the insulin secretion block (b), the relationship between insulin release and plasma glucose concentration is modelled as the sum of two components.

The first component represents the dependence of insulin secretion on absolute glucose concentration at any time point, and is characterised by a dose-response function relating the two variables. The characteristic parameter of the dose-response is its mean slope in each individual's glucose range, denoted here as 'glucose sensitivity'. The doseresponse is modulated by both glucose-mediated and nonglucose-mediated factors (i.e. non-glucose substrates, gastrointestinal hormones and neurotransmitters), which are collectively modelled as a potentiation factor. This factor is set to be a positive function of time and to average one during the experiment. The second insulin secretion component represents a dynamic dependence of insulin secretion on the rate of change of glucose concentration. This component is known as the derivative component, and is determined by a single parameter, denoted as 'rate sensitivity'. The model parameters are determined from the glucose and C-peptide data under a smoothness constraint on the potentiation factor. This approach is similar to that usually employed for deconvolution of Cpeptide data, in which a smoothness constraint is imposed on insulin secretion; the smoothness constraint is tuned so that the C-peptide residual error is in the range of the assay precision.

The acute insulin response to the glucose bolus (AIR) was calculated as the ratio of the mean incremental insulin secretion (assessed by using C-peptide deconvolution [21]) during the first 8 min following the glucose bolus to the mean incremental glucose concentration over the same time interval; thus the units of AIR are pmol min<sup>-1</sup>m<sup>-2</sup> (mmol/l)<sup>-1</sup>. Baseline glucose values for calculating the increments were those obtained at the end of the clamp [22].

Statistical analysis Data are presented as median and interguartile range and were transformed into their natural logarithm for use in parametric statistical testing. Participants were grouped according to whether they were stable NGT, regressors to NGT, stable non-NGT or progressors. Group values were compared by the Mann-Whitney or the Kruskal–Wallis test for continuous variables or  $\chi^2$  for nominal variables; paired values were compared using the Wilcoxon test. General linear models were used to adjust group comparisons for potential confounders (centre, sex, age and BMI); when the group factor was significant, individual group comparisons were performed using linear contrasts. Simple associations were tested by Spearman's correlation coefficient. Logistic regression was used to predict progression; using a multistage modelling approach, association of each metabolic variable with progression was first tested with adjustment for covariates, then the metabolic variables were tested simultaneously. Odds ratios were estimated per standard deviation increase in the independent variable. Multiple regression analysis was carried out using a forward stepwise model. A p value <0.05 was considered statistically significant.

#### Results

The general descriptors of the study cohort are given in Table 1. In addition to the typical sex differences, insulin sensitivity was ~30% higher in women than in men. At baseline, 25 participants had IFG (eight women, 17 men, 2.4%) and 94 had IGT (63 women, 31 men, 9.0%); at follow up, 42 had IFG (4.0%), 123 (11.7%) had IGT and 15 (1.4%) had overt type 2 diabetes. Based on these changes, 809 (77%) of the participants were classified as stable NGT and another 49 (5%) were stable non-NGT, while glucose tolerance improved in 61 participants (6%, regressors) and deteriorated in twice as many (129 or 13%, progressors). In the entire cohort, fasting plasma glucose concentrations at follow up were significantly higher than at baseline (5.22 vs 5.09 mmol/l, p < 0.0001, a 1%/year rise) as were glucose levels 2 h following glucose ingestion (5.93 vs 5.72 mmol/l, p < 0.0001, a 2%/ year rise). The plasma glucose responses to oral glucose at baseline and follow up are shown in electronic supplementary material (ESM) Fig. 1 separately for the four groups. The majority of progressors (~90%) originated from the group who were NGT at baseline (which represented 89% of the entire cohort). The baseline fasting and 2 h plasma glucose levels (5.31 and 6.00 mmol/l) of these NGT progressors were significantly higher than those of the stable NGT participants (5.00 and 5.33 mmol/l, respectively, p < 0.0001 for both).

In comparison with the reference group of stable NGT individuals, the other three groups presented a remarkably similar clinical phenotype (higher prevalence of familial diabetes, older age, and higher waist-to-hip ratio, fasting and 2 h plasma glucose, fasting and 2 h plasma insulin, glucagon and proinsulin concentrations) regardless of whether they were progressors, regressors or stable non1511

NGT. Likewise, a metabolic profile characterised by lower insulin sensitivity and reduced beta cell glucose sensitivity with increased fasting secretion rate and total insulin output was common to all these three groups, the differences from the reference group of stable NGT remaining statistically significant after adjustment for centre, sex, age and BMI (Table 2). Accordingly, in the final multivariate logistic model, both insulin sensitivity and glucose sensitivity were independent negative predictors of progression to dysglycaemia (as were also male sex and rate sensitivity), whereas waist-to-hip ratio and fasting glucose levels (and, almost significantly [p=0.06], insulin output) were positively associated with progression (familial diabetes, age, BMI, physical activity, smoking, 2 h plasma glucose, glucagon and proinsulin not being significant independent variables in this model; Fig. 1). When added to this model, the change in BMI between baseline and follow up was also an independent predictor (with an OR of 1.35 [95% CI 1.11-1.63] for each SD change in BMI [=4.4 kg]). Neither insulin sensitivity nor glucose sensitivity showed a significant interaction with sex. A nearly identical pattern was obtained when the group of progressors was contrasted only with the stable NGT group (data not shown). Among people who were non-NGT at baseline (n=119), a positive family history of diabetes was a negative predictor of regression (OR 0.36 [95% CI 0.13-0.95]) while insulin sensitivity was a positive predictor (OR 1.81 [95% CI 1.00-3.28]) after adjusting for centre, sex, age and BMI.

In the whole dataset, the separate impact of baseline insulin sensitivity and glucose sensitivity on fasting and 2 h plasma glucose levels at follow up is depicted in Fig. 2: both contributed significantly to the latter, only glucose insensitivity did to the former. Baseline AIR was lower in the non-NGT groups, although the differences from the

Table 1 Anthropometric and metabolic characteristics of the study	Variable	Men ( <i>n</i> =471)	Women ( <i>n</i> =577)	p value	
	Age (years)	43 (14)	45 (12)	0.01	
	Familial T2DM (%)	27	28	NS	
	BMI (kg/m <sup>2</sup> )	25.9 (4.3)	24.0 (5.0)	< 0.0001	
	Waist:hip ratio	0.92 (0.08)	0.81 (0.10)	< 0.0001	
	Fat mass (%)	22 (8)	33 (10)	< 0.0001	
	Fasting glucose (mmol/l)	5.2 (0.6)	5.0 (0.6)	< 0.0001	
	Fasting insulin (pmol/l)	32 (24)	30 (20)	0.02	
	Fasting glucagon (pmol/l)	9.0 (4.0)	7.0 (3.0)	< 0.0001	
	Proinsulin (pmol/l)	6.0 (5.0)	5.0 (3.0)	< 0.0001	
	HDL-cholesterol (mmol/l)	1.22 (0.37)	1.54 (0.44)	< 0.0001	
	LDL-cholesterol (mmol/l)	3.00 (1.00)	2.80 (1.00)	< 0.0001	
Data are median (interquartile range) for the cohort	Triacylglycerols (mmol/l)	1.07 (0.74)	0.84 (0.47)	< 0.0001	
T2DM. type 2 diabetes mellitus	M/I (µmol min '[kg FFM] <sup>-1</sup> [nmol/1] <sup>-1</sup> )	113 (70)	146 (80)	< 0.0001	

Table 2	Baseline	clinical	and	metabolic	phenotype	according to	subsequent	changes i	n glucose	tolerance
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Variable	Stable NGT	Regressors	Stable non-NGT	Progressors
n (%)	809 (77)	61 (6)	49 (5)	129 (13)
Sex (M/F)	363/446	23/38	22/27	63/66
Familial diabetes (%) <sup>†</sup>	25	34	49	33
Age (years) <sup>‡</sup>	43 (13)	47 (11)	50 (11)	47 (13)
BMI (kg/m <sup>2</sup> ) <sup>‡</sup>	24.5 (4.8)	26.4 (4.6)	26.9 (4.1)	26.1 (5.5)
Waist:hip ratio <sup>‡</sup>	0.85 (0.13)	0.86 (0.15)	0.91 (0.13)	0.90 (0.12)
Fasting glucose (mmol/l) <sup>‡</sup>	5.0 (0.6)	5.3 (1.0) <sup>§</sup>	5.5 (1.1) <sup>§</sup>	5.3 (0.8) <sup>§</sup>
2 h glucose (mmol/l) <sup>‡</sup>	5.3 (1.6)	8.2 (0.9) <sup>§</sup>	8.4 (1.3) <sup>§</sup>	6.1 (1.4) <sup>§</sup>
Fasting insulin (pmol/l) <sup>‡</sup>	29 (20)	39 (28) <sup>§</sup>	42 (29) <sup>§</sup>	34 (24) <sup>§</sup>
Fasting glucagon (pmol/l) <sup>‡</sup>	8.0 (4.0)	8.0 (4.0)	9.0 (5.3)	10 (4.5)
Proinsulin (pmol/l) <sup>‡</sup>	5.0 (4.0)	7.5 (5.0) <sup>§</sup>	7.0 (6.0) <sup>§</sup>	8.0 (5.8) <sup>§</sup>
2 h insulin (pmol/l) <sup>‡</sup>	131 (125)	307 (237) <sup>§</sup>	331 (411) <sup>§</sup>	177 (169) <sup>§</sup>
$M/I \; (\mu mol \; min^{-1} [kg \; FFM] \; ^{-1} \; [nmol/l]^{-1})^{\ddagger}$	137 (84)	97 (88) <sup>§</sup>	92 (62) <sup>§</sup>	106 (79) <sup>§</sup>
Glucose sensitivity (pmol min <sup><math>-1</math></sup> m <sup><math>-2</math></sup> [mmol/l] <sup><math>-1</math></sup> )	121 (81)	69 (60) <sup>§</sup>	68 (44) <sup>§</sup>	95 (50) <sup>§</sup>
OGIS (µmol min <sup>-1</sup> [kg FFM] <sup>-1</sup> ) <sup>‡</sup>	11.8 (3.3)	9.6 (1.9) <sup>§</sup>	8.8 (2.4) <sup>§</sup>	10.3 (3.2) <sup>§</sup>
Rate sensitivity (nmol m <sup>-2</sup> [mmol/l] <sup>-1</sup> )	0.83 (1.33)	0.73 (0.88)	0.72 (0.77)	0.69 (1.12)
Potentiation ratio <sup>‡</sup>	1.79 (1.33)	1.33 (0.90) <sup>§</sup>	1.46 (0.67) <sup>§</sup>	1.51 (0.90) <sup>§</sup>
Fasting insulin secretion (pmol min <sup><math>-1</math></sup> m <sup><math>-2</math></sup> ) <sup>‡</sup>	66 (34)	82 (56) <sup>§</sup>	96 (55) <sup>§</sup>	84 (34) <sup>§</sup>
Total insulin output (nmol/m <sup>2</sup> ) <sup>‡</sup>	38 (16)	45 (19) <sup>§</sup>	50 (22) <sup>§</sup>	44 (15) <sup>§</sup>
AIR ( $n=761$ ) (pmol min <sup>-1</sup> m <sup>-2</sup> [mmol/l] <sup>-1</sup> ) <sup>‡</sup>	59 (43)	47 (42) <sup>§</sup>	48 (36)	52 (50)

Data are median (interquartile range) for the cohort

p=0.001 by  $\chi^2$ ;  $p \le 0.002$  by Kruskal Wallis test; p < 0.001 vs Stable NGT adjusted for centre, sex, age and BMI

M, male; F, female

stable NGT group were less marked than was the case for beta cell glucose sensitivity (Table 2). AIR was inversely related to insulin sensitivity in a log–log fashion in each of the four groups, with correlation coefficients ranging from 0.21 to 0.38 ( $p \le 0.04$ ; ESM Fig. 2). In univariate associa-



Fig. 1 Multiple logistic regression model for deterioration of glucose tolerance over 3 years of follow up. Odds ratio and 95% confidence intervals are calculated for 1 SD change in the predictor variable. The model is adjusted for centre, familial diabetes, age, BMI, physical activity and 2 h plasma glucose levels

tion, AIR did not predict progression significantly (OR 0.74 [95% CI 0.51–1.06]); after including insulin sensitivity it nearly did so (OR 0.70 [95% CI 0.49–1.01]), but failed to add predictivity to the model in Fig. 2.

At follow up, body weight had increased significantly in the whole cohort (by  $1.0\pm0.2$  kg, range -16 kg to +34 kg); across groups, the change was significantly greater in the progressors than in stable NGT participants (Table 3). Metabolic variables were essentially unchanged in the stable NGT and stable non-NGT group, while they had changed in opposite directions in progressors vs regressors. Thus, fasting and 2 h plasma glucose and insulin levels had increased in the progressors and decreased in the regressors. Of the beta cell function variables, insulin sensitivity and glucose sensitivity decreased in the progressors and increased in the regressors, whereas fasting insulin secretion rate and total insulin output were reduced in the regressors and increased in the progressors (Figs 3, 4). By multiple regression analysis (adjusting for centre, sex, familial diabetes, age, BMI, waist-to-hip ratio, physical activity and baseline value of the dependent variable), the change in fasting glucose (as a continuous variable) was predicted by baseline glucose sensitivity (p=0.0003, with a total explained variance of 29%), while the change in 2 h

**Fig. 2** Fasting (**a**) and 2 h (**b**) plasma glucose levels at follow up as predicted by baseline insulin resistance (*M*/*I* from the clamp) and beta cell glucose insensitivity (from modelling of the OGTT), both grouped into sex-specific quartiles. *M*/*I* quartiles are I=207 [75]; II=143 [48]; III=117 [45]; IV=78 [34]. Beta cell glucose sensitivity quartiles are: I=207 [84]; II=131 [22]; III=96 [19]; IV=58 [20]



glucose was predicted by both insulin sensitivity (p < 0.0001) and glucose sensitivity (p=0.04, total explained variance of 32%).

#### Discussion

The RISC cohort is comprised of relatively young, basically healthy European women and men. Over 3 years of observation, the following main findings were obtained. First, progressors, regressors and participants whose dysglycaemia remained stable shared a common clinical and metabolic phenotype broadly indicative of risk of incident diabetes. Thus, not only were they older and heavier, but they also exhibited hyperinsulinaemia and hyperproinsulinaemia, insulin resistance and reduced beta cell glucose sensitivity independently of age and BMI. This result suggests that these individuals (23% of the participants) may derive from a single at-risk pool, whose glucose tolerance oscillates over time between the upper boundary of normality and the diagnostic range (IFG, IGT or overt diabetes): according to the categorisation used here, a single follow-up test may capture them at a peak (progressors) or trough (regressors) while overall their glucose tolerance is deteriorating over time (i.e. there were twice as many progressors as regressors).

Second, weight gain significantly added to the prediction of progression, yielding a separate 35% increase in risk of dysglycaemia for each 4.4 kg of weight gain. This finding is in keeping with the well established notion that weight gain is a potent risk factor for dysglycaemia [23–25].

Third, both baseline insulin sensitivity and beta cell glucose sensitivity protected against progression independently of one another (Fig. 2). Using the dataset including only progressors and stable NGT (n=941) to estimate the effect size, we found a similar odds ratio for individuals in the bottom 25% of insulin sensitivity (median [interquartile

Table 3	Changes	in	clinical	and	metabolic	variables	according	to	changes	in	glucose	tolerance	е
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	Stable NGT	Regressors	Stable non-NGT	Progressors
$\Delta$ Body weight (kg) <sup>†</sup>	0.7 (4.5)	-0.1 (5.6)	1.2 (4.6)	1.7 (4.3) <sup>‡</sup>
$\Delta$ BMI (kg/m <sup>2</sup> ) <sup>†</sup>	0.3 (1.59)	0.1 (2.3)	0.6 (1.7)	0.6 (1.6) <sup>‡</sup>
$\Delta$ Fasting glucose (mmol/l) <sup>†</sup>	0.10 (0.60)	$0.00 \ (0.74)^{\$}$	0.30 (0.62)	0.42 (0.77) <sup>§</sup>
$\Delta$ 2 h glucose (mmol/l) <sup>†</sup>	0.10 (1.60)	-1.74 (2.07) <sup>§</sup>	0.25 (1.55)	$1.90 (1.80)^{\$}$
$\Delta$ Fasting insulin (pmol/l) <sup>†</sup>	1 (16)	-5 (19) <sup>§</sup>	-5 (23)	3 (22)
$\Delta$ 2 h insulin (pmol/l) <sup>†</sup>	3 (103)	-95 (182) <sup>§</sup>	-3 (247)	94 (213) <sup>§</sup>
$\Delta$ Rate sensitivity (nmol m <sup>-2</sup> [mmol/l] <sup>-1</sup> )	0.03 (1.37)	0.14 (1.01)	-0.01 (0.72)	0.04 (0.97)
$\Delta$ Potentiation ratio <sup>†</sup>	-0.06 (1.31)	0.23 (1.16) <sup>§</sup>	-0.01 (0.92)	-0.02 (0.79)
$\Delta$ Fasting insulin secretion (pmol min <sup>-1</sup> m <sup>-2</sup> ) <sup>†</sup>	0 (25)	-3 (30) <sup>§</sup>	-10 (29)	7 (33) <sup>§</sup>
$\Delta$ Total insulin output $(nmol/m^2)^{\dagger}$	2 (13)	$-3 (14)^{\$}$	0 (13)	7 (17) <sup>§</sup>

Data are median (interquartile range) for the cohort

 $p \le 0.02$  by Kruskal Wallis test; p < 0.05 vs Stable NGT adjusted for centre, sex, and age; p < 0.05 vs Stable NGT adjusted for centre, sex, age and BMI



Fig. 3 Insulin sensitivity (as the OGIS index) at baseline and follow up in the four groups. Bars are median and interquartile range. The asterisks denote statistical significance ( $p \le 0.05$ ) for the difference between baseline and follow up (using the Wilcoxon test). Light grey bars, baseline; dark grey bars, follow up

range]: 75 [26]  $\mu$ mol min<sup>-1</sup>[kg FFM]<sup>-1</sup>[nmol/l]<sup>-1</sup>, OR 2.52; 95% CI 1.51-4.21) and for those in the bottom 25% of beta cell glucose sensitivity (61 [20] pmol min<sup>-1</sup>m<sup>-2</sup> [mmol/l]<sup>-1</sup>, OR 2.39; 95% CI 1.6–3.93). When using plasma glucose levels at follow up as the outcome variable, beta cell glucose insensitivity was a determinant of both fasting and post-glucose plasma glucose, whereas insulin resistance was an independent determinant of postglucose but not fasting glycaemia. This finding is consistent with the results obtained using the shift in glucose tolerance category as the outcome, where IFG and IGT were pooled together. Also consistent with expectation is the fact that good insulin sensitivity was a negative predictor of progression and a positive predictor of regression (despite the relatively small number of participants in the regressor group).

Of note is that absolute insulin secretion, whether the fasting rate or the total post-glucose output, trended in the opposite direction with regard to insulin sensitivity and beta



## determinants of glucose tolerance. In contrast, rates of insulin secretion (fasting and total over the 2 h of the

(pmol min<sup>-1</sup> m<sup>-2</sup> [mmol/l]<sup>-1</sup>)

Glucose sensitivity

limit further hyperglycaemia.

insulin secretion (fasting and total over the 2 h of the OGTT) were reciprocally related to insulin sensitivity (data not shown). We have interpreted this finding to indicate that the absolute rate of insulin secretion reflects the set point of beta cell capacity as determined by the level of insulin sensitivity (and, possibly, by some other feature of obesity) [22]. Unlike glucose sensitivity, AIR was reciprocally related to insulin sensitivity (although less strongly than reported in other studies) [27]; this empirical index, however, is a hybrid of true beta cell sensitivity and insulin secretory capacity and shares with the latter the reciprocal relation to insulin sensitivity [22]. Glucose sensitivity and rate sensitivity, on the other hand, express the ability of the beta cell to respond to acute glucose changes by promptly revving up insulin discharge; as such, these modes of beta cell function need not be directly influenced by insulin sensitivity. In fact, when analysing their longitudinal changes across the full spectrum of glucose tolerance,

cell glucose sensitivity, namely, higher insulin secretion was associated with progression and lower insulin secretion

tracked with regression. This finding confirms that changes

in absolute insulin secretion rates are adaptive responses to

changes in glucose levels, which are, in turn, primarily controlled by glucose sensitivity and insulin sensitivity.

Stated otherwise, as beta cell glucose sensitivity deterio-

rates and insulin resistance worsens, plasma glucose rises

and induces enhanced insulin secretion as an attempt to

A further observation is that, while glucose and insulin

sensitivity changed in parallel during progression (or

regression), they were numerically independent of one

another. Neither in the baseline nor in the follow-up dataset

was there a significant relationship between these two



Fig. 4 Beta cell glucose sensitivity at baseline and follow up in the four groups. Bars are median and interquartile range. The asterisks denote statistical significance ( $p \le 0.05$ ) for the difference between baseline and follow up (using the Wilcoxon test). Light grey bars, baseline; dark grey bars, follow up



glucose sensitivity and insulin sensitivity appear to vary consensually (Fig. 5).

Finally, the model in Fig. 1 indicates that glucose sensitivity, rate sensitivity and insulin sensitivity, in that order, are powerful indicators of risk of dysglycaemia and replace the quota of risk conveyed by familial diabetes, age, BMI and physical activity. The waist-to-hip ratio and fasting glycaemia, however, retain a degree of separate predictivity. Possibly, a higher waist-to-hip ratio, which reflects a more central distribution of body fat, carries risk of dysglycaemia through subclinical inflammation [26, 28, 29]; fasting glycaemia, on the other hand, may mark the presence of more marked hepatic insulin resistance [30] and relative hyperglucagonaemia [31, 32].

In summary, this prospective analysis of the RISC cohort demonstrates that glucose homeostasis deteriorates over time even in the relatively young and healthy, with more people progressing to dysglycaemia than regressing from it. Insulin sensitivity and beta cell glucose sensitivity predict and track these changes quantitatively, with roughly equal power. Weight gain, while small on average, carries a significant independent risk of progression. Progressors, regressors and IGT/IFG participants have a common metabolic syndrome-like phenotype, including a degree of insulin resistance and beta cell glucose insensitivity not explained by sex, age and BMI. This 'common soil' of metabolic risk may be the phenotypic expression of enhanced genetic predisposition.

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