Mengozzi, A., Tricò, D., Nesti, L., Petrie, J., Højlund, K., Mitrakou, A., Krebs, M., Mari, A. and Natali, A. (2020) Disruption of fasting and post-load glucose homeostasis are largely independent and sustained by distinct and early major beta-cell function defects: a cross-sectional and longitudinal analysis of the relationship between insulin sensitivity and cardiovascular risk (RISC) study cohort. *Metabolism*, 105, 154185. (doi: 10.1016/j.metabol.2020.154185)

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Deposited on 24 February 2020

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Disruption of fasting and post-load glucose homeostasis are largely independent and sustained by distinct and early major beta-cell function defects: a cross-sectional and longitudinal analysis of the Relationship between Insulin Sensitivity and Cardiovascular risk (RISC) study cohort.

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Word count (main text): 4696

Figures: 7

Tables: 3

References: 32

List of abbreviations

2hPG: Plasma glucose value at 120 min of the OGTT.
βC-GS: beta-cell glucose sensitivity as measured by the slope of the glucose-insulin secretion curve obtained through plasma C-peptide and glucose OGTT data modeling.
δOGTT: post-load glucose homeostasis described as the area of the plasma glucose curve above the fasting level during the OGTT, divided by 120 minutes ($mmol \cdot L^{-1}$).
AIR: Acute Insulin Response as measured through the IVGTT.
AIR/G: ratio of the area under the curve of C-peptide to the maximal plasma glucose concentration gradient achieved with the bolus over 8 minutes.
Clamp M: Glucose disposal at steady state during an euglycemic hyperinsulinemic clamp.
DBP: Diastolic Blood Pressure .
EGP: endogenous glucose production.
FPG: fasting plasma glucose.
FPI: fasting plasma insulin.
GCR_f/I_f: ratio of glucose clearance and insulin at fasting, as measure of whole-body fasting insulin sensitivity.
GCR_c/I_c: ratio of glucose clearance and insulin during clamp, as measure of peripheral insulin sensitivity.
IFG: Impaired Fasting Glucose.
IGT: Impaired Glucose Tolerance.
ISR: Insulin Secretion Rate.
ISR@5: Insulin Secretion Rate at 5 mM glucose (ISR@5), representing the value of insulin secretion that in each individual would occur at 5 mM glucose reflecting the basal (non-stimulated) beta-cell function.
ISR fast: Insulin Secretion Rate at fasting glucose concentrations.
ISR OGTT: Insulin Secretion Rate at OGTT glucose concentrations.
NGT: Normal Glucose Tolerance.
PFR: Potentiation factor ratio, an index of the enhancement of insulin secretion due to a previous exposure to hyperglycemia obtained through OGTT plasma C-peptide and glucose data modeling.
RISC: Relationship between Insulin Sensitivity and Cardiovascular risk.
RS: Rate sensitivity an index of the dynamic response of the beta-cell to the early rise in plasma glucose as derived from OGTT plasma C-peptide and glucose data modeling.
SBP: Systolic Blood Pressure.

Abstract

Background/aims – Uncertainty still exists on the earliest beta-cell defects at the bases of the type 2 diabetes. We assume that this depends on the inaccurate distinction between fasting and post-load glucose homeostasis and aim at providing a description of major beta-cell functions across the full physiologic spectrum of each condition.

Methods – In 1,320 non-diabetic individuals we performed an OGTT with insulin secretion modeling and a euglycemic insulin clamp, coupled in subgroups to glucose tracers and IVGTT; 1,038 subjects underwent another OGTT after 3.5 years. Post-load glucose homeostasis was defined as mean plasma glucose above fasting levels (δOGTT). The analysis was performed by two-way ANCOVA.

Results - Fasting plasma glucose (FPG) and δOGTT were weakly related variables (stβ=0.12) as were their changes over time (r=-0.08). Disruption of FPG control was associated with an isolated and progressive decline (approaching 60%) of the sensitivity of the beta-cell to glucose values within the normal fasting range. Disruption of post-load glucose control was characterized by a progressive decline (approaching 60%) of the slope of the full beta-cell *vs* glucose dose-response curve and an early minor (30%) decline of potentiation. The acute dynamic beta-cell responses, neither per se nor in relation to the degree of insulin resistance appeared to play a relevant role in disruption of fasting or post-load homeostasis. Follow-up data qualitatively and quantitatively confirmed the results of the cross-sectional analysis.

Conclusion - In normal subjects fasting and post-load glucose homeostasis are largely independent, and their disruption is sustained by different and specific beta-cell defects.

Keywords: insulin secretion; beta cell function; glucose tolerance; fasting; post-load; glucose homeostasis;

1. Introduction

Maintenance of normal glucose homeostasis at the whole-body level requires the concerted action of multiple tissue and biologic systems and understanding which ones are responsible for the transition to pre-diabetes is crucial for the design of effective prevention strategies. One major challenge to this aim is that mild hyperglycemia *per se*, if persistent, might significantly affect two of the most important biologic systems: insulin secretion and insulin resistance [1] [2]. To gain insight on the primary mechanisms, therefore, the focus should be placed on the earliest stages of the transition, *i.e.* on normal subjects; a population that has seldom been extensively investigated in terms of all the key determinants of glucose homeostasis. A second challenge is that disruption of glucose homeostasis might involve either fasting or post-load plasma glucose control, each to an extent that is rather variable among individuals; a dissociation justified by the fact that the key processes responsible for the control of fasting and post-load glucose are different. Although the tissues involved are largely the same (liver, β and α-cells, skeletal muscle, adipose tissue, intestine), different can be the specific function/s that in each tissue is/are involved. In the last two decades, a series of studies have been conducted to understand the major defects that characterize the two conditions [3-8]. In synthesis, the loss of post-load glucose control, identified through 2hPG, was associated with variable degrees of whole-body and liver insulin resistance, and with marked impairments of both dynamic and static aspects glucose-stimulated insulin secretion. No alteration has been observed in glucose appearance [9]. Disruption of fasting glucose homeostasis, on the other hand, was found characterized by liver insulin resistance and a selective impairment in the acute/early insulin secretion, while other aspects of glucose-induced insulin secretion (2^{nd}phase and Potentiation) [10] were normal. Whole body insulin sensitivity, in this subgroup, has been found reduced [11], unaltered [12] and even increased [7].

Unfortunately, in addition to some qualitative inconsistencies among studies, there is also uncertainty with regard to the size of each defect in each condition and, therefore, to its relevance

[7]. In particular, whether the defect in insulin secretion is absolute [8] or it emerges only in relationship to the degree of insulin resistance (failure of compensation) [10], and which is/are the earliest detectable defect/s remains unknown. While some of the variability depends on the ethnic background [13, 14] and the prevalence of obesity [15], the relatively small sample size (n=40-664) and the large heterogeneity (in number and quality) of the methods employed must have also contributed. Finally, another factor could be responsible for the lack of accurate and consistent information. All studies have used the canonical fasting and 2hPG cut-off plasma glucose values to categorize and compare the subjects; only few have used continuous values [3, 12, 16], and, most importantly, all somewhat neglected that fasting and 2hPG values are strongly correlated variables [17]. This implies that a substantial overlap exists between the two phenotypes when this strategy is adopted, and it should be carefully taken into consideration. Furthermore, from a physiology perspective, the control of post-load plasma glucose consists in limiting the rise above fasting levels, therefore it *is* better described by considering the whole plasma glucose excursion above basal values. However, to our knowledge, this approach has been used in only one study [18].

The aim of the present study is to accurately describe the whole spectrum of normal fasting and post-load glucose homeostasis, separately, by adopting a novel and more precise definition of post-load glucose tolerance, and to define the specific trajectories of the major metabolic derangements that characterize the transition to the pre-diabetic condition. To this end, we performed a secondary analysis on cross-sectional and longitudinal data from the Relationship between Insulin Sensitivity and Cardiovascular risk (RISC) study, a large cohort of individuals without diabetes extensively characterized in terms of glucose tolerance, insulin sensitivity and insulin secretion.

2. Research design and methods

2.1 Study participants – The RISC study is a multicentre, prospective, observational, European study whose rationale and methodology have previously been described in detail [19]. In brief,

participants were enrolled at 19 clinical centres in 14 European countries, according to the following inclusion criteria: either sex, age 30-60 years (balanced 10-year strata), and clinically healthy allowing for obesity up to class II (BMI<40 kg/m^2). Exclusion criteria were: treatment for any chronic disease, pregnancy, any cardiovascular disease or previous event, cancer, reduced kidney function (eGFR<60 ml/min/1.73m^2) or known liver disease, hypertension, fasting plasma glucose ≥7.0 mmol/l, 2-hour plasma glucose (on OGTT) ≥11.0 mmol/l, total cholesterol ≥7.8 mmol/l, triglycerides ≥4.0 mmol/l.

Of the initial cohort of 1,320 subjects who underwent the OGTT and the euglycemic insulin clamp, hepatic glucose production (tracer dilution) and acute insulin response during an IVGTT were measured in subgroups of 387 and 843 subjects, respectively. Thirty-one subjects were excluded for problems in the baseline biochemical parameter determination (missing values, outliers, poor quality control, internal inconsistencies) therefore the present analysis is based on the baseline data of 1,289 subjects. Local Ethics Committee approval was obtained by each recruiting center and subjects signed an informed consent.

2.3 Euglycemic insulin clamp - Insulin was administered as a primed-continuous infusion at a rate of 240 pmol·min^{-1}·m^{-2}; simultaneously plasma glucose levels were maintained within 4.5-5.5 mM by means of a variable 20% dextrose/water infusion. Insulin sensitivity (M/I, in μmol·min^{-1}·kg_{FFM}^{-1}·pM^{-1}) was calculated as the ratio of the glucose infusion rate (M value), averaged over the final 40 min of the 2-hours clamp and normalized by the fat-free mass (FFM), measured by bioimpedance (TB300, TANITA, Tokyo, Japan), and to the achieved plasma insulin concentration. Then, we then adjusted the M/I value by the clamped glucose level obtaining the ratio of glucose clearance during clamp (GCR_c/I_c), obtaining a more precise index of peripheral insulin sensitivity. At the end of the clamp in the IVGTT subgroup (n=847) a bolus of glucose (0.3 mg/kg of body weight) was injected intravenously over 1 min and blood samples collected every 2 min for 8 min. In another subgroup (n=387) endogenous glucose production (EGP) was measured with [6-6^2H_2] glucose in the fasting state and during the clamp. Fasting glucose clearance (GCR_f) was then

calculated as the ratio of EGP to fasting glucose levels. The ratio of GCR_f to fasting insulin levels (GCR_f/I_f), was adopted as measure of whole-body fasting insulin sensitivity.

2.4 Analytical methods - Plasma and serum aliquots were stored at -80ºC for centralized analytes determination. Serum total cholesterol, HDL-C, LDL-C and triglycerides were measured by enzymatic colorimetric test (Roche Modular systems), NEFA by an immunoenzymatic assay (Randox), plasma insulin and C-peptide by a time resolved fluorimmunoassay (AutoDELFIA Insulin kit, Turku, Finland). The glucagon assay used an in-house assay developed in J. Holst's laboratory in Copenhagen.

2.5 Beta cell function - Parameters of beta-cell function were generated by using the OGTT C-peptide and glucose data. The characteristics of the model used to reconstruct insulin secretion and its control by glucose has previously been described in detail [20]. In brief, the analysis consists of three interacting blocks: a) a model for smoothing and interpolating plasma glucose profile based on the available determinations; b) a model of C-peptide kinetics individually adjusted to the subject's anthropometric data according to Van Cauter et al. [21]; c) a model for describing the dependence of insulin secretion on glucose concentration. With regard to the relationship between insulin release and plasma glucose concentrations (block c), it is modeled as the sum of two components. The first component represents the dependence of insulin secretion on the absolute glucose concentration at any time point and is characterized by a quasi-linear dose-response function whose slope is defined as beta-cell glucose sensitivity (βC-GS). This parameter can be modulated by several factors (i.e., non-glucose substrates, gastrointestinal hormones and neurotransmitters), which are collectively modelled as a potentiation factor whose value is set to be a positive function of time, and to average the value 1 during the duration of the 2-h OGTT. The ratio of the values at 100-120 min *vs* 0-20 min (potentiation factor ratio, PFR) is used to express with a single parameter this component. The second insulin secretion component represents a dynamic dependence of insulin secretion on the rate of change of glucose concentration and is denoted as rate sensitivity (RS). The insulin secretion rate at 5 mM glucose (ISR@5) represents the value of insulin secretion

that in each individual would occur at 5 mM glucose reflecting the basal (non-stimulated) beta-cell function. The fasting insulin secretion rate (ISR fast, $pmol \cdot min^{-1} \cdot m^{-2}$) is the value of insulin secretion measured at time 0 while the total OGTT insulin secretion (ISR OGTT, $nmol \cdot m^{-2}$) is the integral of insulin secretion during the entire 2-hour OGTT. Peripheral insulin clearance was calculated as the ratio of insulin infusion rate to steady state plasma insulin during the clamp. The acute insulin response (AIR/G) during the IVGTT was calculated as the ratio of the area under the curve of C-peptide to the maximal plasma glucose concentration gradient achieved with the bolus over 8 minutes. No significant inhibition of endogenous insulin release during clamp was detected.

2.6 Follow-up - 1,038 subjects underwent a second visit and OGTT after 3.5 years according to the same protocol adopted at baseline; neither the clamp nor the IVGTT in this occasion was performed (249 subjects were lost at follow-up, 2 subjects were excluded for fasting plasma glucose values ≥7 mM). Changes are always calculated as 3.5 years – baseline. FPG and δOGTT progressors were defined as individuals within the fourth quartile of the distribution of the individual changes over the follow-up in either parameter. Subject with a spontaneous decline in either ISR@5 or in βC-GS were defined as those in the lowest tertile of the distribution of the absolute changes in either parameter over 3.5 years.

2.7 Statistical analysis - Statistical analysis was performed using JMP®Pro11.2 software (SAS Institute Inc., Cary, NC). Data are reported as mean ± standard deviation or median [interquartile range], unless otherwise specified.

All continuous variables were tested for normality via Kolmogorov-Smirnoff-Tests and normalized via logarithmic transformation before analysis when appropriate. Differences between means and rates have been evaluated with ANOVA and chi-squared tests, respectively. The subjects were classified according to quintiles of FPG and quintiles of mean plasma glucose increment (above fasting) during the OGTT (δOGTT). Statistics on major variables was tested through two-way ANCOVA always including both classification criteria and major confounders (age, sex, BMI and recruitment center). When both classification factors were statistically significant, their

interaction was also tested. Correlations between variables were tested using Pearson's or Spearman's rank correlations as appropriate. To allow a direct comparison, the estimated multivariable regression coefficients were expressed as standardized coefficients (Stβ).

3. Results

In the whole population, both FPG and δOGTT displayed a quasi-normal distribution and were very weakly correlated (r=0.12, p<0.0001) (**Figure 1a)**. The poor association was confirmed by a minimal, although statistically significant, increment of δOGTT through FPG quintiles (**Figure 1b)**. Similarly, statistically significant, but small was the progressive rise in FPG across quintiles of δOGTT with a maximum gradient of 0.19±0.14 mM between the two extremes (p<0.0001) (**Figure 1b**). Of note, the association between FPG and 2hPG was stronger (r=0.28, p<0.0001) (**Figure 1a**) and 2hPG values showed a progressive and significant rise through FPG quintiles reaching a maximum gradient between extreme quintiles of 1.07±0.12 mM (**Figure 1b**). The association of FPG with 2hPG was stronger than that with δOGTT also after adjusting for sex, BMI, age and center (Stβ=0.29 vs 0.12).

3.1 Clinical phenotypes

Plasma glucose and insulin profiles during the OGTT across quintiles of FPG and δOGTT are presented in **Figure 2**. Both phenotypes (**Table 1**), of worse fasting and post-load glucose control, showed a superimposable profile in terms of age and BMI, with a similar quasi-linear increase across quintiles. A progressive enrichment in male sex was present as FPG increased, while this was only marginally evident across δOGTT quintiles. For these reasons, differences for the other parameters across quintiles of FPG and of δOGTT, were tested with a two-way ANCOVA that included age, sex, BMI and center, in addition to the two classification criteria. Disruption of both fasting and post-load glucose homeostasis was associated with a rising prevalence of family history of diabetes, while neither smoking nor alcohol consumption were related to either phenotype. The

subjects with Impaired Fasting Glucose (IFG) were all in the 5th quintile of FPG representing approximately 60% of the subjects of this subgroup, while individuals with IFG were present in all quintiles of δOGTT with prevalence rates ranging from 7 to 22%. Similarly, subjects with Impaired Glucose Tolerance (IGT) were largely concentrated in the 4th and 5th quintiles of δOGTT (8 and 64%), while they were present in all quintiles of FPG at rates ranging from 6 to 15%.

With regard to the other major metabolic syndrome parameters, we observed that while the individuals with worse post-load glucose homeostasis were characterized by higher triglycerides and lower HDL cholesterol, those with higher FPG had higher blood pressure values and ALT levels.

Plasma glucagon was not affected by the degree of disruption of either fasting or post-load glucose homeostasis, while a progressive rise in plasma NEFA was found through the δOGTT quintiles only.

3.2 Beta-cell function, insulin clearance and insulin sensitivity parameters

Parameters of beta-cell function were associated with specific trends while approaching pre-diabetes in each phenotype (**Table 2, Figure 3**). Subjects with progressively loss of fasting glucose homeostasis displayed decreasing values of ISR@5, which was coupled to a mild (20% max.) increase of PFR and a mild (15% max.) decrease in AIR/G. In contrast, the deterioration of post-load glucose control was characterized by an early and stable reduction (30%) in PFR, a gradual major reduction of βC-GS (60% max.) and a transient increase in RS. A mild (15%) AIR/G reduction was observed also across δOGTT quintiles. Peripheral insulin clearance was similar across quintiles of both FPG and δOGTT. Insulin sensitivity in the stimulated condition, measured through GCR_c/I_c, showed a progressive reduction only across δOGTT quintiles, with a 35% reduction comparing the 5th vs 1st quintile. When GCR_c/I_c was plotted against AIR/G and data fitted with 1/x functions (to assess compensation), no significant difference among the curves (neither in the coefficients, nor in the r values, all <0.001) was present across FPG quintiles, whilst only the

subjects in the 5th quintile of δOGTT showed a 50% reduction in the $1/(GCR_c/I_c)$ coefficient indicating a significant defect in compensation, which was evident in the subset of individuals with severe insulin resistance (**Figure 4**).

In the subgroup of subjects in whom glucose tracer data were available (n= 387) (**Table 2**), fasting endogenous glucose production (EGP) was remarkably stable across quintiles of both fasting or post-load glucose homeostasis. As expected, glucose clearance in the fasting condition progressively declined only across quintiles of FPG, and whole-body fasting insulin sensitivity (GCR_f/I_f) showed an even more marked decline. EGP measured in conditions of high-normal plasma insulin levels (at steady state during the clamp) was similarly suppressed in all study groups (*data not shown*).

In multivariable regression analysis, ISR@5 was the major determinant of FPG (Stβ=-0.42) with minor contributions of male sex (Stβ=0.15, p<0.0001), BMI (Stβ=-0.15, p<0.0001) and age (Stβ=0.09, p<0.0001) together contributing to 48% of its overall variability. βC-GS, GCR_c/I_c, and RS were the major determinants of δOGTT (Stβ=-0.44, -0.23, -0.18, respectively p<0.0001) explaining 45% of its overall variability.

3.3 Follow-up

Six subjects had 2hPG values ≥11.0 mM at OGTT (mean±SD=12.2±1.1 mM) and were left in the analysis. In the population undergoing the 3.5-year follow-up (n=1,038), the changes in FPG and δOGTT were largely independent showing, if anything, an inverse correlation (r=-0.08, p=0.01). Only 25% of the FPG and δOGTT progressors fell in the 4th quartile of the change for both parameters. FPG increased by 0.8 and 0.1 mM in FPG and δOGTT progressors, respectively; while δOGTT doubled in δOGTT progressors and did not change in FPG progressors (**Table 3, Figure 5**). BMI showed a modest increase in both phenotypes (+0.6 units). Fasting insulin secretion (ISR fast) increased by 15% only in FPG progressors and the increase in total OGTT insulin secretion (ISR OGTT) was 3-fold greater in δOGTT progressors (23% vs 7%). At follow-up, the individuals in

whom fasting and post-load glucose control deteriorated exhibited changes in the major glucose homeostatic parameters (**Table 3**) that closely followed the curves built on the bases of the analysis on cross-sectional data (**Figure 3**). In the whole dataset changes in ISR@5 and βC-GS were unrelated (r=0.03, p=ns). Changes in ISR@5, though weakly, were negatively correlated with changes in PFR (r=-0.13, p<0.001), while the changes in βC-GS showed a positive correlation (*i.e.* concomitant disruption) with changes in PFR (r=0.22, p<0.001) and a negative correlation with the changes in RS (r=0.19, p<0.0001).

In order to appreciate the impact on glucose tolerance of a selective decline in ISR@5 or in βC-GS and of concomitant decline of both beta-cell functions, we plotted the baseline and 3.5 years OGTT curves of the subjects who fell in the 1st tertile of the spontaneous changes for each parameter either isolated (n=209 and n=209, respectively) or in combination (n=116) (**Figure 6**). A 50% decline in ISR@5 (from 95±47 to 42±34 pmol·min^{-1}·m^{-2}) resulted in an increase of 0.4±0.5 mM in FPG (p<0.0001) with no change in δOGTT (0.0±1.1 mM), while a 40% decline in βC-GS (from 179±94 to 102±57 pmol·min^{-1}·m^{-2}·mM^{-1}) resulted in an increase of 0.6±1.1mM in δOGTT (p<0.0001) and no change of FPG (0.1±0.5mM). In the subgroup in whom both ISR@5 and βC-GS declined at follow-up (by 50% and 46%, respectively) we observed a worsening of both FPG (0.5±0.7 mM) and δOGTT (0.5±1.1 mM). ISR fast was, if any, reduced in the ISR@5 and ISR@5+βC-GS progressors groups and showed a modest (10%) increase in βC-GS individuals. Total insulin secretion (ISR OGTT) did not differ among study groups.

4. Discussion

4.1 *Pathophysiological findings*

Our findings indicate that in subjects without diabetes fasting and post-load glucose homeostasis are to a large extent, independent and independently regulated. When subjects are

stratified according to quintiles of increasingly worse fasting or post-load glucose control, only a minority (23%) fell in the same quintile (*i.e.* had the same degree of alteration for both criteria), indicating that the derangements in the two systems occur in parallel only in a minority of individuals. This dissociation, coupled to our multivariable adjustment, allowed us to detect and quantify across the whole spectrum of non-diabetes glucose homeostasis, the major clinical and physiologic characteristics that characterize each condition, an information not available in the literature.

In terms of clinical phenotype the closer association of dyslipidemia with post-load glucose is congruent with the known inhibitory effect of triglycerides and HDL-cholesterol on glucose stimulated beta cell function [22, 23], while the association of fasting glucose with waist is likely to be driven by the effect of visceral fat on liver insulin sensitivity [24].

In terms of mechanisms, our data indicate that disruption of fasting glucose control is essentially characterized by the inability of the beta-cell to efficiently increase insulin secretion for glucose concentration values that lie within the fasting normal range. In these subjects, higher plasma glucose levels are required by the beta-cell to secrete the amount of insulin that is necessary to match endogenous glucose production (which appears to be a true homeostatic variable) to whole body glucose utilization. Interestingly, when fasting insulin secretion is plotted *vs* FPG according to quintiles of FPG (**Figure 7a**), the linear fit of this dose-response curve displays a slope of 21, while the slope across quintiles of δOGTT is 98 *i.e.* close to the slope of the full beta-cell dose-response curve in the whole population (median βC-GS=112 $pmol \cdot min^{-1} \cdot m^{-2} \cdot mM^{-1}$, IQR [78-158]). In other words, the same 35% increase in fasting insulin secretion (presumably required to overcome hepatic insulin resistance) is observed across quintiles of both δOGTT and FPG, however for the latter an 8-fold larger FPG gradient is needed (1.6 vs 0.2 mM). This clearly demonstrate a severe beta-cell insensitivity that is restricted to the normal fasting glucose values, the beta-cell response to post-load glucose values being preserved (**Figure 7b**). Notably, these subjects show also a selective and

severe fasting insulin resistance (GCR_f/I_f) *viz* an essentially preserved stimulated insulin sensitivity (GCR_c/I_c). The higher FPG is probably responsible for the relative increase in potentiation [5], which in turn may contribute to the maintenance of post-load glucose homeostasis and justify the dissociation of the two phenotypes. With regard to the mechanisms underlying this distinct beta-cell insensitivity, *i.e.* restricted to the fasting condition, we could not find any clear, strong and plausible signal among candidate variables, including energy substrates, hormones and clinical parameters. The persistence of stable plasma glucagon values *viz* elevated glucose levels might suggest a reduced α-cell sensitivity to glucose in these subjects.

A 50% decline in ISR@5 has been observed also by Kanat *et al* [10] in a small study in Mexican Americans with IFG when compared to NGT/NFG, however no information was given on the trajectory of the defect in relation with FPG. In contrast with other laboratories, we observed only a modest decline in acute/early insulin secretion in subjects with mild elevation in FPG. However, in the study of Bogardus et al [3] the cross-sectional FPG *vs* AIR curve was U-shaped and a true decrease was evident only for fasting glucose values above 6.0 mM; also in a study by De Fronzo et al [10] the association between AIR and FPG was essentially driven by the low AIR of the subjects with FPG above 6.0 mM. The defect, in addition is known to depend on the ethnic background being severe in Hispanic, mild in African American and negligible in White [14]. Finally, our IVGTT test was performed with plasma glucose clamped at approx. 5.0 mM in all subjects. Provided that hyperglycemia, also of mild degree (+2.8 mM), is able to blunt the 1st Phase insulin secretion [25], the severe reduction in AIR observed by other laboratories - with tests performed at the fasting glucose values - could be a secondary phenomenon. A similar increase in potentiation in subjects with isolated IFG has been observed also by Kanat *et al* in a small cohort of Mexican Americans [10], however they also found a major reduction in RS and βC-GS in these subjects. This apparent discrepancy may have arisen because subjects with IFG were compared with subjects with NGT, a group enriched with individuals with optimal stimulated glucose homeostasis,

in whom βC-GS is particularly high; in fact, the NGT group had a βC-GS similar to our 2nd δOGTT quintile.

The deterioration of post-load glucose homeostasis is characterized by the combination of a severe reduction in βC-GS (**Figure 3 and 5**) coupled to a marked reduction of both potentiation and stimulated whole-body insulin sensitivity. The decline in the AIR/G, mild in size, when evaluated with respect to the prevailing insulin sensitivity, demonstrated that the defect in compensation was only present in the subjects of the extreme quintile and with extreme insulin resistance (**Figure 4**). RS, another proxy of acute insulin response, showed an early, transient and modest increase followed by a decline across quintiles of δOGTT confirming that the lack of beta-cell adaptation to the degree of insulin resistance becomes evident only while approaching the diabetic condition. A U-shaped relationship between acute insulin response and post-load glucose was also found by Bogardus et al. [3]. In the prospective data both worsening of post-load glucose homeostasis (**Figure 3**), and also of βC-GS (r=-0.19) were associated with an improvement in RS. We conclude that defective compensation has a minor role being evident only in those with more severely impaired post-load glucose regulation (5th quintile of δGOTT) and with severe insulin resistance (**Figure 4**).

Another novel, and rather unexpected, finding is the early decline of potentiation reaching a 35% reduction already for an intermediated degree of derangement (3rd δOGTT quintile) with no further decline while approaching the pre-diabetes condition. The observed preservation of insulin secretion at low-fasting glucose levels (ISR@5) probably prevents the concomitant loss of fasting plasma glucose control and justifies the dissociation of the two conditions. In quantitative terms, multivariable analysis indicated that βC-GS, insulin sensitivity and RS, in this order, appears to be the major factors characterizing the deterioration of post-load glucose homeostasis. The decline of distinct beta-cell functions therefore appears to be a continuous phenomenon already evident in the earliest stages of glucose homeostasis derangements with no evident threshold while moving toward

diabetes. This is in deep contrast with the consolidated notion that in the stages preceding the onset of type 2 diabetes there is a compensatory beta-cell hyperfunction as the evident hyperinsulinemia might suggest. The only small somewhat compensatory beta-cell responses are PFR and RS for fasting and post load glucose homeostasis, respectively.

4.2 *Clinical relevance*

In terms of clinical relevance, the different metabolic profiles of deterioration in post-load and fasting glucose homeostasis could help in stratifying treatments and in generating more accurate hypotheses to be tested in prospective prevention trials. The availability of heterogeneous classes of pharmacologic glucose lowering agents makes it possible to envisage tailored strategies targeting selectively the major specific defects of each condition. Thus, metformin by acting, already at low doses, on liver insulin sensitivity and fasting glucose clearance [26] may be more effective in targeting fasting hyperglycemia, while exercise, diet and weight loss (or thiazolidinediones), being more effective in restoring systemic insulin sensitivity [27], and incretins, acting mainly on oral glucose stimulated insulin secretion, would be rational choices for preserving post-load glucose homeostasis.

4.3 *Strengths and limitations*

Strengths of this study are: the dimension of the population, the extensive metabolic characterization, the availability of follow-up data, the more physiologic definition of fasting and post-load glucose homeostasis and the robust multivariate approach adopted to detect the specific defects of each condition (adjustments for confounders and cross adjustment of FPG and δOGTT).

A limitation of this study is its large reliance on cross-sectional data, however the follow-up data, though limited in time, are in full agreement with the cross-sectional analysis, strengthening its major results. The dissociation between the deterioration of fasting and post-load glucose

homeostasis was, if anything, even more evident in the follow-up analysis and the changes in the various beta-cell functions were qualitatively and quantitatively superimposable to the ones predicted from cross-sectional trajectories (**Figure 3**). On the other hand, and most importantly, also the impact of the spontaneously occurring declines in ISR@5 and in βC-GS resulted in deterioration of fasting and post-load glucose homeostasis (**Figure 6**), respectively, which were in qualitative and quantitative terms very close to what predicted from the cross-sectional analysis. Another limitation is that δOGTT does not represent a clinical meaningful variable in itself, in this respect both 1h and 2h OGTT glucose values are undoubtedly more relevant since they are established predictors of diabetes, however an elegant study by Abdul Ghani et al [28] has clearly shown that among normal and IGT subjects with identical fasting and 2h glucose the risk to develop diabetes is proportional to the incremental area under the plasma glucose curve (i.e. δOGTT·120). The characteristics of our study population, with a large fraction of subjects with normal glucose tolerance, might have led to an overestimation of the defects underlying impaired glucose homeostasis, however as evident also from our results the deterioration of glucose homeostasis is a continuous phenomenon with no clear threshold and the description of the trajectories contains information with regard to the dynamics and the relevance of the defects at the bases this phenomenon. We also acknowledge that the IVGTT data, used to determine first-phase insulin secretion, might be influenced by the antecedent exogenous insulin administration, which is known to inhibit unstimulated endogenous insulin secretion especially in subject with insulin resistance [29-32]. Indeed, we observed a correlation between insulin resistance and the decline in plasma C-peptide during the clamp ($r=0.24$, $p<0.0001$), however the latter showed, if any, a positive correlation with the C-peptide AUC ($r=0.15$, $p=0.0001$).

5. Conclusions

In normal subjects the deterioration of fasting and post-load glucose homeostasis are largely independent phenomena and are characterized by the decline of distinct beta-cell functions, which are progressive and appear already at the earliest stages of metabolic derangement in absence of relevant compensatory responses. These novel notions could be exploited for a direct clinical application of tailored preventive medicine.

Figure legend

Figure 1 – (a) Scatterplot and frequency distribution of fasting plasma glucose (FPG) *vs* mean plasma glucose above fasting levels (δOGTT) and 2h plasma glucose (2hPG) in the 1,289 subjects at baseline. R^2 values represent the strength of the correlation between FPG and δOGTT and FPG and 2hPG; (b) Histogram bars of mean and SE values of FPG (black), δOGTT (dark grey) and 2hPG (light grey) according to quintiles of FPG and δOGTT.

Figure 2 – OGTT plasma glucose (upper panels) and insulin (lower panels) mean and SEM values in each quintile (1^{st} to 5^{th}, from the left to the right) of fasting (FPG) (left panels) and post-load plasma glucose (δOGTT) (right panels) in the 1,289 subjects at baseline.

Figure 3 - Plot of insulin secretion, endogenous glucose production (EGP) and insulin sensitivity parameters expressed as percent of first quintile of fasting (FPG) and post-load plasma glucose (δOGTT) across quintiles of FPG and δOGTT. Values are adjusted for age, BMI, sex, recruitment centre and also the cognate classification criteria in the 1,289 subjects at baseline. Arrows indicate the changes of the beta cell function parameters that showed a significant variation at the 3.5 years follow-up in the 25% of subjects (of the 1,038 at follow-up; data are available only for insulin secretion) who displayed the largest spontaneous increase in either FPG or δOGTT. Continuous lines indicate that the means were found statistically different, dotted lines were not.
βC-GS: beta-cell glucose sensitivity; AIR/G: ratio of the area under the curve of C-peptide to the maximal plasma glucose concentration gradient achieved with the bolus over 8 minutes; GCR_f/I_c: ratio of glucose clearance at fasting and insulin;: ratio of glucose clearance during clamp; ISR@5: Insulin Secretion Rate at glucose 5 mM; ISR fast: Insulin Secretion Rate at fasting glucose concentrations; ISR OGTT: Insulin Secretion Rate at OGTT glucose concentrations; PFR: Potentiation Factor Ratio; RS: Rate sensitivity.

Figure 4 – Best (y=a+b*1/x) fit of AIR/G (on y axis) and glucose clearance during clamp ($GCR_s/I_{c)}$ in each fasting plasma glucose (FPG) quintile (upper panel) and each post load glucose control (δOGTT) quintile (lower panel). Only the b coefficient for the 5^{th} δOGTT quintile was statistically significant different from the others (Dunnet tests).

Figure 5 – OGTT plasma glucose at baseline and after 3.5 years of follow-up values in the subjects who displayed the greater deterioration (>75% percentile of the 1,038 subjects at follow-up) of fasting (FPG; left panel) and post load (δOGTT; right panel) glucose control across 3.5 years of follow-up.

Figure 6 – OGTT plasma profiles at baseline (gray) and at 3.5 follow-up (black) in the individuals in whom a spontaneous and selective decline (>67% percentile) of ISR@5 (ISR@5↓ βC-GS→; n=209), or βC-GS (ISR@5→ βC-GS↓; n=209) or both (ISR@5↓ βC-GS↓; n=116) has occurred. βC-GS: beta-cell glucose sensitivity; ISR@5: Insulin Secretion Rate at glucose 5 mM.

Figure 7 – a) Scatterplot of fasting plasma glucose *vs* fasting insulin secretion in quintiles of fasting plasma glucose (FPG) (gray) and post load glucose control (δOGTT) (black) with regression dose-response lines calculated on mean values. **b)** Insulin secretion and plasma glucose dose-response curves in quintiles of FPG and δOGTT.

Acknowledgements: Preliminary study findings were presented as an abstract at the 55th EASD Annual Meeting in Barcelona, Spain, 2019.

Data availability: The data that support the findings of this study are available from the corresponding author on reasonable request.

Funding: The RISC Study was partly supported by EU grant QLG1-CT-2001-01252. DT is funded by the European Foundation for the Study of Diabetes (EFSD) Mentorship Programme.

Duality of interests: The authors declare that there is no duality of interest associated with this manuscript.

Author contributions: AMe: study design, data analysis and interpretation, manuscript write-up; DT, LN, JP, KH, AMi, MK: data collection, manuscript editing; AMa: mathematical modeling of insulin secretion and beta-cell function parameters, data interpretation, manuscript editing; AN: study design and supervision, funding, data collection, analysis and interpretation, manuscript editing and critical revision. All authors read and approved the final submitted version of the manuscript. AMe and AN are the guarantors of this work and, as such, have full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Table 1 - Clinical characteristics of the study population stratified according to quintiles of fasting plasma glucose (FPG) and OGTT mean plasma glucose (δOGTT). The EGIR-RISC study

		Quintile Groups					
		I	**II**	**III**	**IV**	**V**	*p-values**
n	*FPG*	265	256	211	273	286	
	δOGTT	259	258	260	259	255	
Age	*FPG*	41±8	43±8	43±9	45±8	46±8	*<0.0001*
(years)	*δOGTT*	42±8	43±9	45±9	45±8	46±8	*<0.0001*
Male	*FPG*	25	35	46	51	64	*<0.0001*
(%)	*δOGTT*	38	37	48	47	53	*ns*
BMI	*FPG*	24.2±4.2	24.7±4.0	25.1±3.6	25.9±3.7	27.1±3.9	*<0.0001*
($kg \cdot m^{-2}$)	*δOGTT*	24.2±3.2	25.0±4.0	26.0±4.1	25.7±3.9	26.9±4.3	*<0.0001*
Alcohol	*FPG*	30[11-63]	41[11-85]	25[23-28]	50[15-114]	66[15-135]	*ns*
($g \cdot week^{-1}$)	*δOGTT*	42 [15-105]	39 [11-90]	46 [15-91]	48 [15-106]	51 [15-131]	*ns*
Smokers	*FPG*	49/31/20	47/24/29	44/30/26	46/25/29	45/25/30	*ns*
(nev./ex/curr.)	*δOGTT*	48/25/27	46/23/31	47/25/28	46/25/29	45/35/20	*ns*
FHD	*FPG*	22	26	22	30	36	*0.0024*
(%)	*δOGTT*	19	22	25	30	39	*0.0057*
IFG	*FPG*	0	0	0	0	58.7	*ns*
(%)	*δOGTT*	7	8	13	14	22	*<0.0001*
IGT	*FPG*	5.7	5.5	9.5	9.5	15.0	*ns*
(%)	*δOGTT*	0	0	0	8	64	*<0.0001*
SBP	*FPG*	113±12	115±12	118±12	118±12	123±12	*0.0050*
(mmHg)	*δOGTT*	115±12	117±12	118±12	118±14	120±11	*ns*
DBP	*FPG*	72±8	73±8	75±8	75±8	77±7	*0.0169*
(mmHg)	*δOGTT*	73±8	74±8	74±8	75±8	76±8	*ns*
T. Chol	*FPG*	4.6±0.9	4.7±0.9	4.8±0.8	4.9±0.9	5.1±0.8	*ns*
($mmol \cdot L^{-1}$)	*δOGTT*	4.8±0.9	4.8±0.9	4.8±0.9	4.9±0.9	5.0±0.8	*ns*
LDL Chol	*FPG*	2.7±0.8	2.8±0.8	2.9±0.8	3.0±0.8	3.1±0.8	*ns*
($mmol \cdot L^{-1}$)	*δOGTT*	2.8±0.9	2.9±0.8	2.9±0.8	2.9±0.8	3.1±0.8	*ns*
HDL Chol	*FPG*	1.5±0.4	1.5±0.4	1.4±0.4	1.4±0.4	1.3±0.4	*ns*
($mmol \cdot L^{-1}$)	*δOGTT*	1.5±0.4	1.5±0.4	1.4±0.3	1.4±0.4	1.3±0.4	*0.0089*
Triglycerides	*FPG*	0.9±0.5	1.0±0.5	1.1±0.7	1.2±0.7	1.4±1.0	*ns*
($mmol \cdot l^{-1}$)	*δOGTT*	1.0±0.5	1.0±0.5	1.0±0.5	1.1±0.8	1.4±1.1	*<0.0001*
ALT	*FPG*	17±10	18±10	19±9	21±10	24±15	*0.0199*
($U \cdot L^{-1}$)	*δOGTT*	18±8	19±9	20±14	20±13	23±11	*ns*
AST	*FPG*	21±8	21±9	21±9	22±8	23±11	*ns*
($U \cdot L^{-1}$)	*δOGTT*	21±8	21±8	21±10	22±8	23±10	*ns*
Glucagon	*FPG*	8.5±4.1	8.8±3.5	9.3±4.7	9.1±4.2	9.4±4.1	*ns*
($pmol \cdot L^{-1}$)	*δOGTT*	8.3±4.0	8.4±3.4	9.2±4.5	9.7±4.3	9.5±4.1	*ns*
NEFA	*FPG*	575±283	541±225	535±228	539±209	512±206	*ns*
($umol \cdot L^{-1}$)	*δOGTT*	497±196	549±254	527±216	556±241	582±286	*<0.0001*

Data shown are n, %, mean±SD, FHD= family history of diabetes.

* p-values from two-way ANOVA for age, BMI, sex with FPG and δOGTT as independent variables, both in the model; for the other variables; two-way ANCOVA with independent variables FPG, δOGTT, and covariates age, BMI, sex and recruitment center; interactions between FPG and δOGTT were never statistically significant.

Table 2 - Insulin secretion and insulin sensitivity indices of the study population stratified according to quintiles of fasting plasma glucose (FPG) and OGTT mean plasma glucose (δOGTT). The EGIR-RISC study

		Quintile Groups					*p-values**
		I	**II**	**III**	**IV**	**V**	
ISR fast ($pmol \cdot min^{-1} \cdot m^{-2}$)	*FPG*	65.2±26.4	65.3±26.9	75.2±35.0	78.8±33.1	94.2±38.6	*<0.0001*
	δOGTT	67.9±31.5	70.9±29.2	73.6±32.4	78.9±34.7	90.2±37.5	*0.0005*
ISR OGTT ($nmol \cdot m^{-2}$)	*FPG*	38.7±12.7	39.2±13.2	40.4±15.4	43.0±14.4	44.2±14.2	*0.0297*
	δOGTT	32.9±10.4	37.4±11.8	40.0±12.1	44.2±11.8	51.7±16.2	*<0.0001*
ISR@5 ($pmol \cdot min^{-1} \cdot m^{-2}$)	*FPG*	131±81	81±36	72±35	61±32	55±36	*<0.0001*
	δOGTT	80.6±63.7	83.3±67.5	74.4±49.4	76.2±44.1	84.2±47.7	*ns*-
βC-GS ($pmol \cdot min^{-1} \cdot m^{-2} \cdot mM^{-1}$)	*FPG*	147.9±106.4	143.1±88.2	133.8±95	128.3±83.2	110.6±64.7	*ns*
	δOGTT	201.2±136.1	158.7±82.3	120.4±50.4	101.9±36.2	78.4±30.8	*<0.0001*
RS ($pmol \cdot m^{-2} \cdot mM^{-1}$)	*FPG*	801 [0-1478]	782 [122-1441]	862 [121-1349]	885 [274-1572]	637 [230-1196]	*ns*
	δOGTT	833 [0-2382]	848 [0-1678]	844 [245-1390]	807 [351-1228]	691 [287-1061]	*<0.0001*
PFR	*FPG*	1.5 [1.1-2.3]	1.7 [1.2-2.5]	1.6 [1.1-2.5]	1.7 [1.2-2.4]	1.8 [1.4-2.4]	*0.0015*
	δOGTT	2.4 [1.6-2.5]	1.8 [1.2-2.7]	1.6 [1.2-2.3]	1.4 [1.1-1.9]	1.5 [1.1-1.9]	*<0.0001*
AIR/G ($pM \cdot min \cdot mM^{-1}$)	*FPG*	68±36	67±38	63±34	58±35	58±30	*0.0009*
	δOGTT	66±35	63±35	63±34	63±36	56±33	*0.0013*
Insulin clearance ($L \cdot min^{-1} \cdot m^{-2}$)	*FPG*	0.64±0.35	0.62±0.15	0.62±0.15	0.61±0.17	0.63±0.26	*ns*
	δOGTT	0.65±0.24	0.64±0.34	0.62±0.16	0.61±0.15	0.61±0.22	*ns*
GCR_s/I_c ($L \cdot kg_{FFM}^{-1} \cdot min^{-1} \cdot pM^{-1}$)	*FPG*	0.031±0.019	0.030±0.014	0.030±0.015	0.027±0.014	0.025±0.014	*ns*
	δOGTT	0.034 ±0.017	0.032±0.019	0.028±0.014	0.025±0.012	0.022±0.013	*<0.0001*
EGP°° ($\mu mol \cdot min^{-1} \cdot kg^{-1}$)	*FPG*	15.9±3.5	17.5±6.0	15.8±5.2	16.1±4.6	16.1±4.5	*ns*
	δOGTT	16.8±5.0	16.5±4.3	15.9±4.6	16.1±5.1	15.9±4.6	*ns*
GCR_f/I_c ($L \cdot kg_{FFM}^{-1} \cdot min^{-1} \cdot pM^{-1}$)	*FPG*	0.14±0.07	0.14±0.10	0.12±0.10	0.10±0.10	0.08±0.05	*0.0002*
	δOGTT	0.14±0.11	0.12±0.07	0.11±0.06	0.11±0.06	0.09±0.06	*ns*

Data shown are n, %, mean±SD

° AIR-∂Cp/∂G number of subjects n= 848

°° EGP number of subjects n= 387

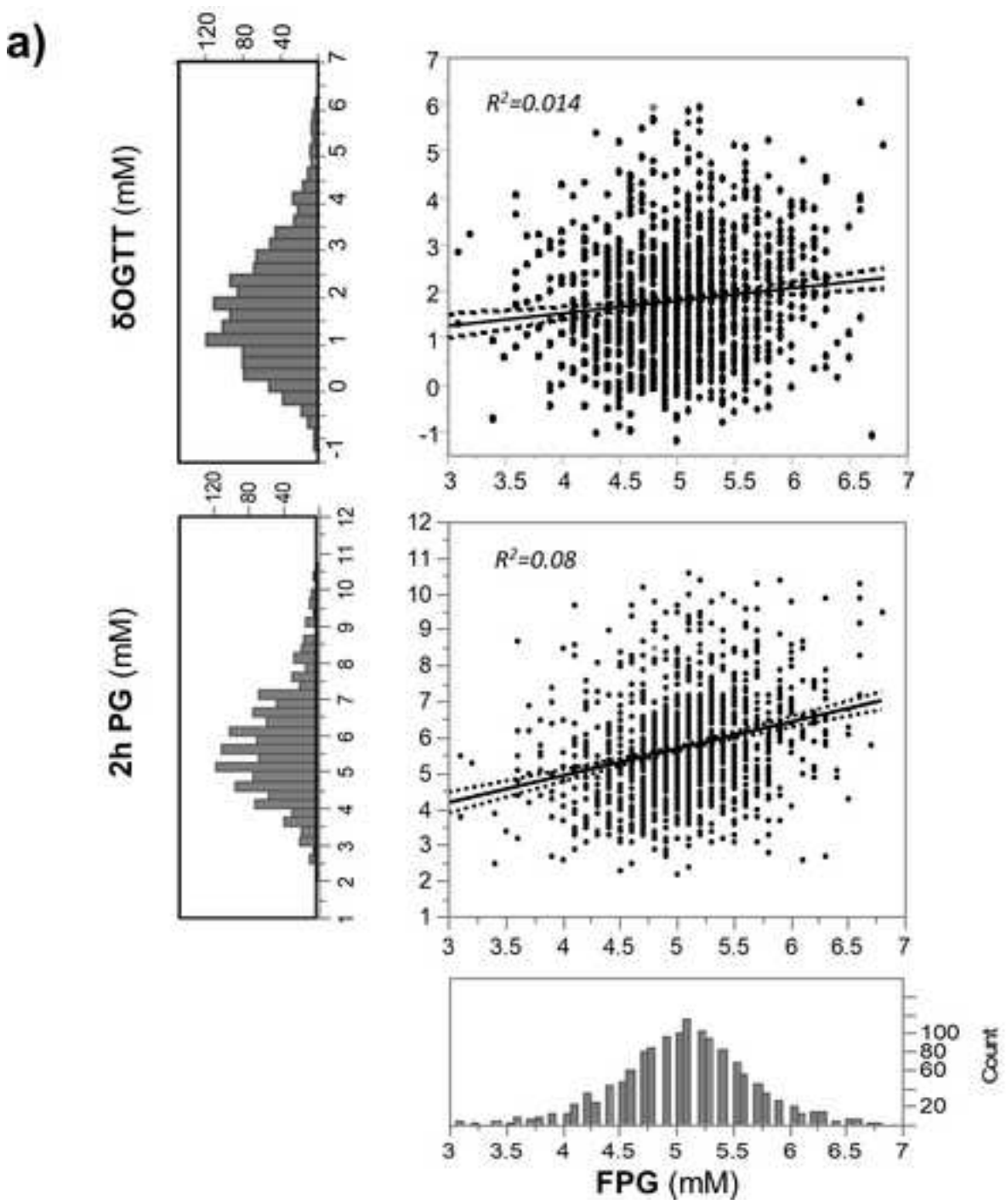
* p-values from two-way ANCOVA on FPG and δOGTT, adjusted for covariates age, BMI, sex and recruitment center; interactions between FPG and ∂OGTT were never statistically significant

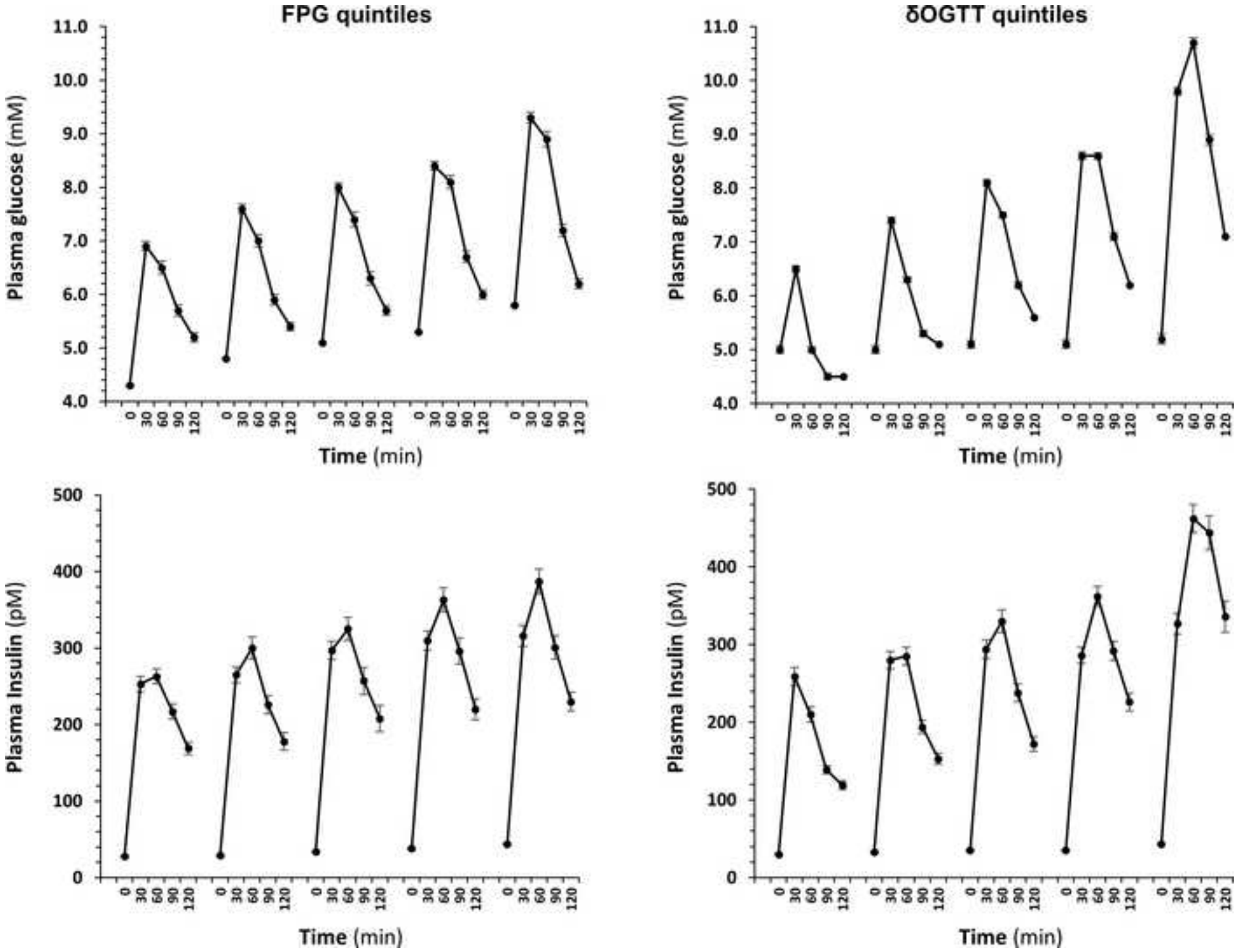
Table 3 – Baseline and 3-year follow-up data of progressors (4th quartile of change in FPG or δOGTT). The EGIR-RISC study

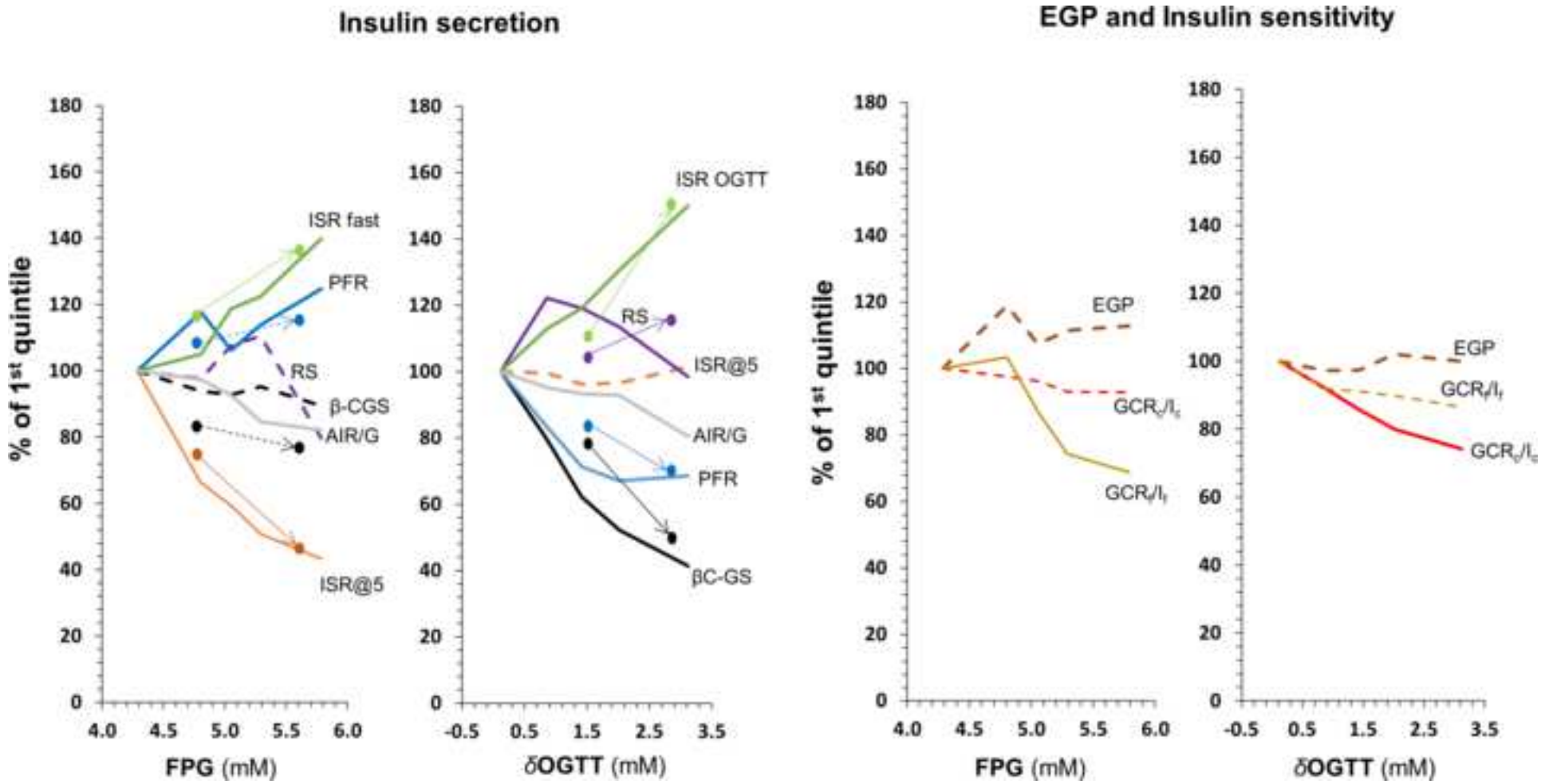
	Progressor	Baseline	3.5-year	*p-value**
FPG ($mmol \cdot L^{-1}$)	*FPG*	4.8±0.5	5.6±0.6	*<0.0001*
	δOGTT	5.1±0.5	5.2±0.7	*0.0006*
δOGTT ($mmol \cdot L^{-1}$)	*FPG*	2.2±1.3	2.2±1.5	*ns*
	δOGTT	1.3±1.2	2.8±1.3	*<0.0001*
BMI ($kg \cdot m^{-2}$)	*FPG*	25.7±3.9	26.3±4.4	*<0.0001*
	δOGTT	25.3±3.9	25.9±4.6	*<0.0001*
ISR fast ($pmol \cdot min^{-1} \cdot m^{-2}$)	*FPG*	75±30	87±35	*<0.0001*
	δOGTT	76±30	78±35	*ns*
ISR OGTT ($nmol \cdot m^{-2}$)	*FPG*	42±14	45±16	*<0.0001*
	δOGTT	39±13	48±16	*<0.0001*
ISR@5 ($pmol \cdot min^{-1} \cdot m^{-2}$)	*FPG*	95±64	57±37	*<0.0001*
	δOGTT	85±75	77±98	*ns*
PFR	*FPG*	1.6 [1.1-2.3]	1.7 [1.2-2.5]	*ns*
	δOGTT	1.7 [1.2-2.5]	1.4 [1.1-2.0]	*<0.0001*
RS ($pmol \cdot m^{-2} \cdot mM^{-1}$)	*FPG*	851 [201-1404]	706 [276-1287]	*ns*
	δOGTT	709 [0-1554]	783 [405-1289]	*ns*
ßC-GS ($pmol \cdot min^{-1} \cdot m^{-2} \cdot mM^{-1}$)	*FPG*	123±84	113±69	*ns*
	δOGTT	150±109	111±58	*<0.0001*

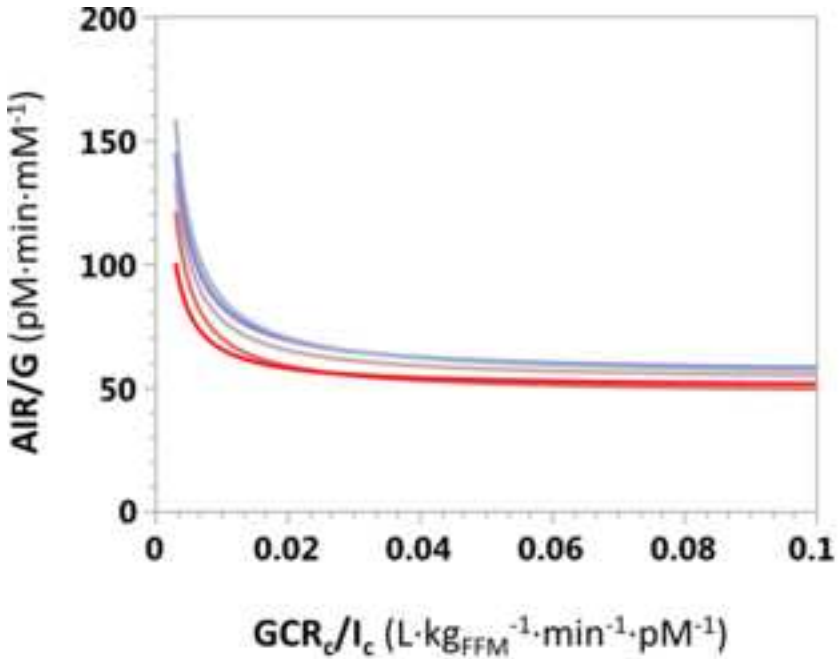
Data shown are mean±SD, or median [interquartile range]

*p-values from unadjusted paired t-tests


a)
δOGTT (mM)
2h PG (mM)
R²=0.014
R²=0.08
FPG (mM)
Count
b)
Plasma Glucose (mM)
FPG
2hPG
δOGTT
I
II
III
IV
V
FPG quintiles
δOGTT quintiles


FPG quintiles
δOGTT quintiles
Plasma glucose (mM)
Plasma Insulin (pM)
Time (min)
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
500
400
300
200
100
0
0
30
60
90
120


Insulin secretion
EGP and Insulin sensitivity
% of 1st quintile
FPG (mM)
δOGTT (mM)
ISR fast
PFR
RS
β-CGS
AIR/G
ISR@5
ISR OGTT
RS
ISR@5
AIR/G
PFR
βC-GS
EGP
GCR$_f$/I$_f$
GCR$_o$/I$_o$
EGP
GCR$_f$/I$_f$
GCR$_o$/I$_o$



FPG quintiles fit

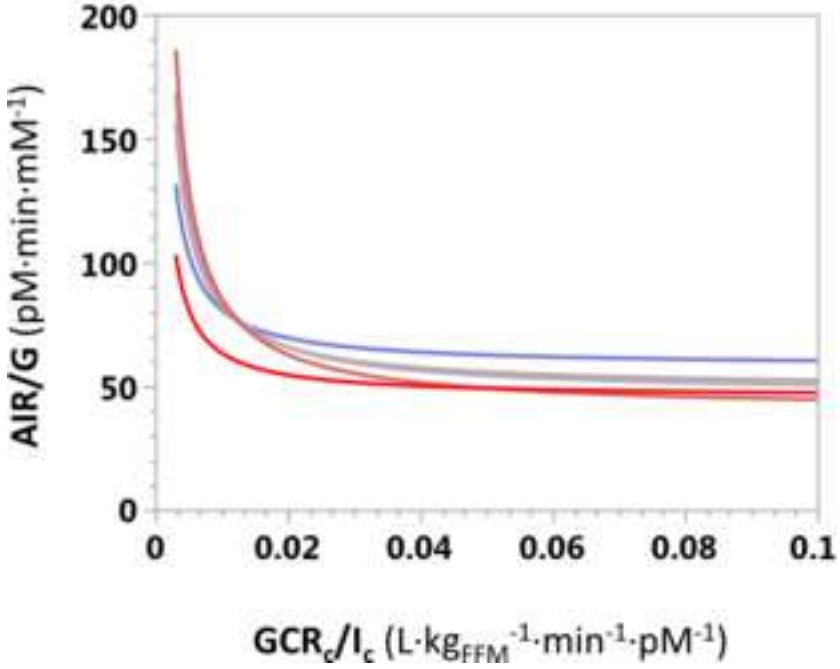
I) AIR = 55±6 + 0.280±0.111 * $1/(GCR_c/I_c)$

II) AIR = 54±5 + 0.326±0.094 * $1/(GCR_c/I_c)$

III) AIR = 52±6 + 0.253±0.116 * $1/(GCR_c/I_c)$

IV) AIR = 47±5 + 0.230±0.093 * $1/(GCR_c/I_c)$

V) AIR = 50±5 + 0.157±0.077 * $1/(GCR_c/I_c)$



δOGTT quintiles fit

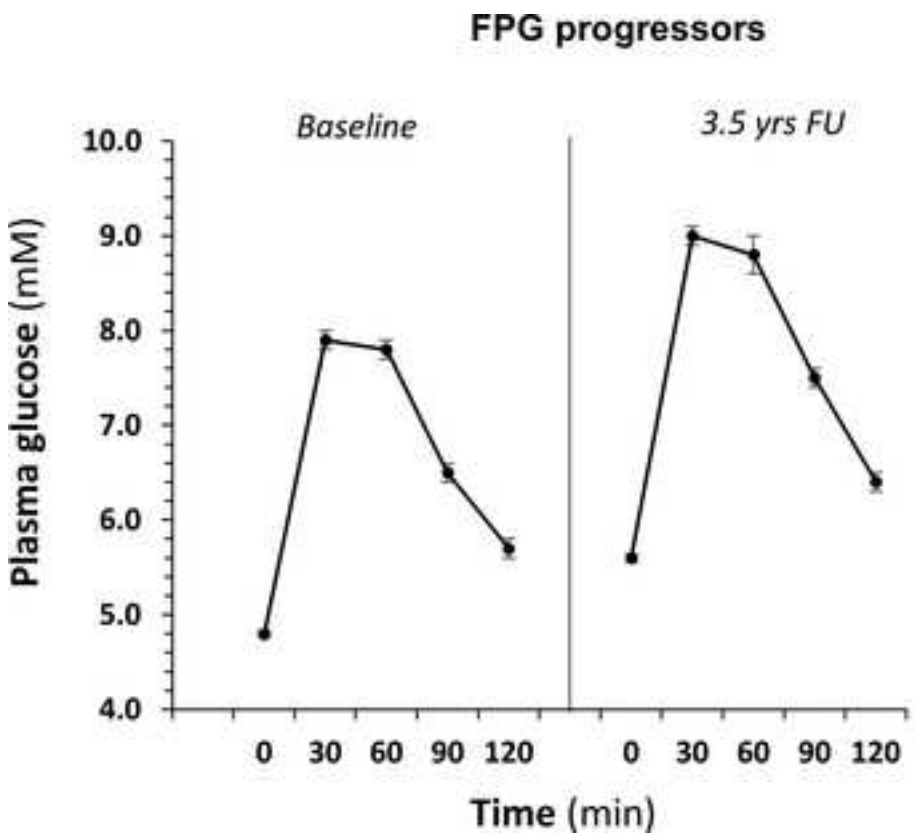
I) AIR = 57±5 + 0.229±0.136* $1/(GCR_c/I_c)$

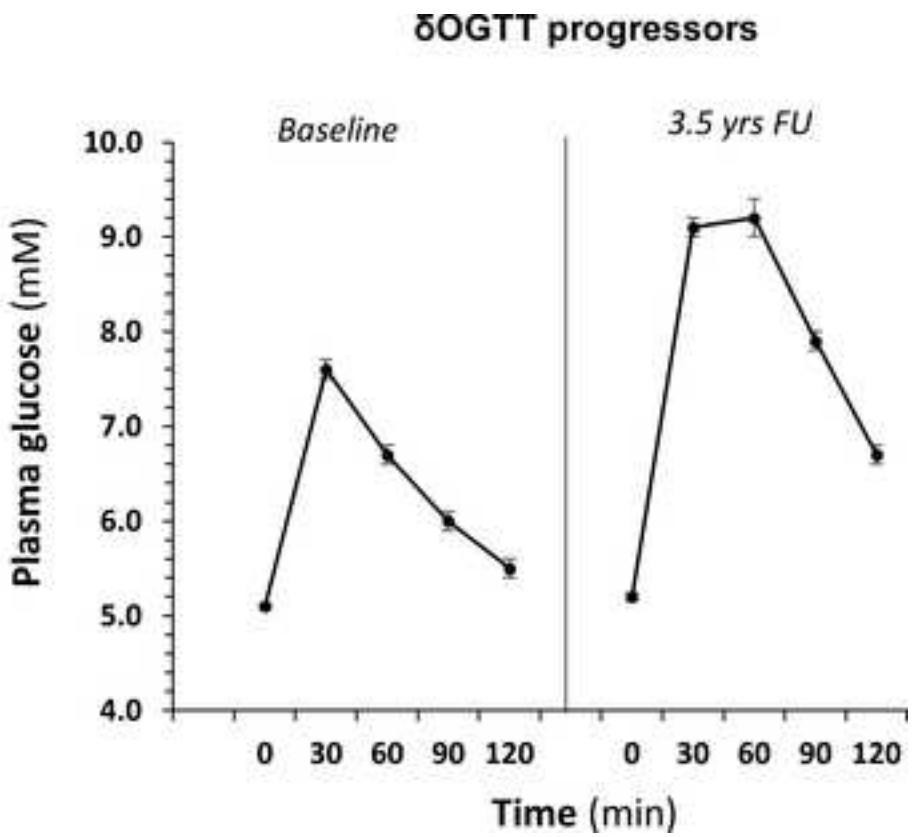
II) AIR = 47±5 + 0.379±0.115 * $1/(GCR_c/I_c)$

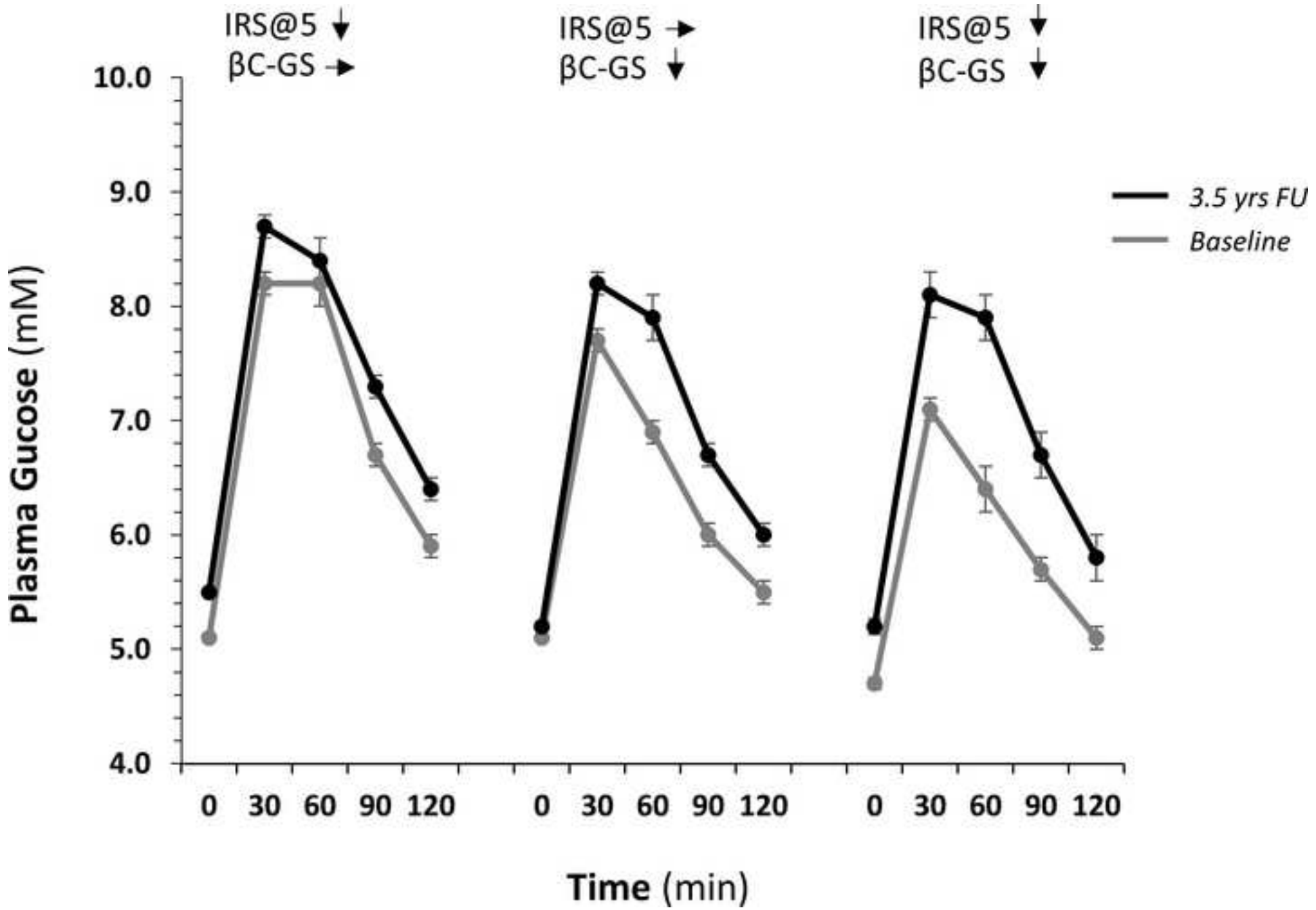
III) AIR = 49±6 + 0.335±0.137 * $1/(GCR_c/I_c)$

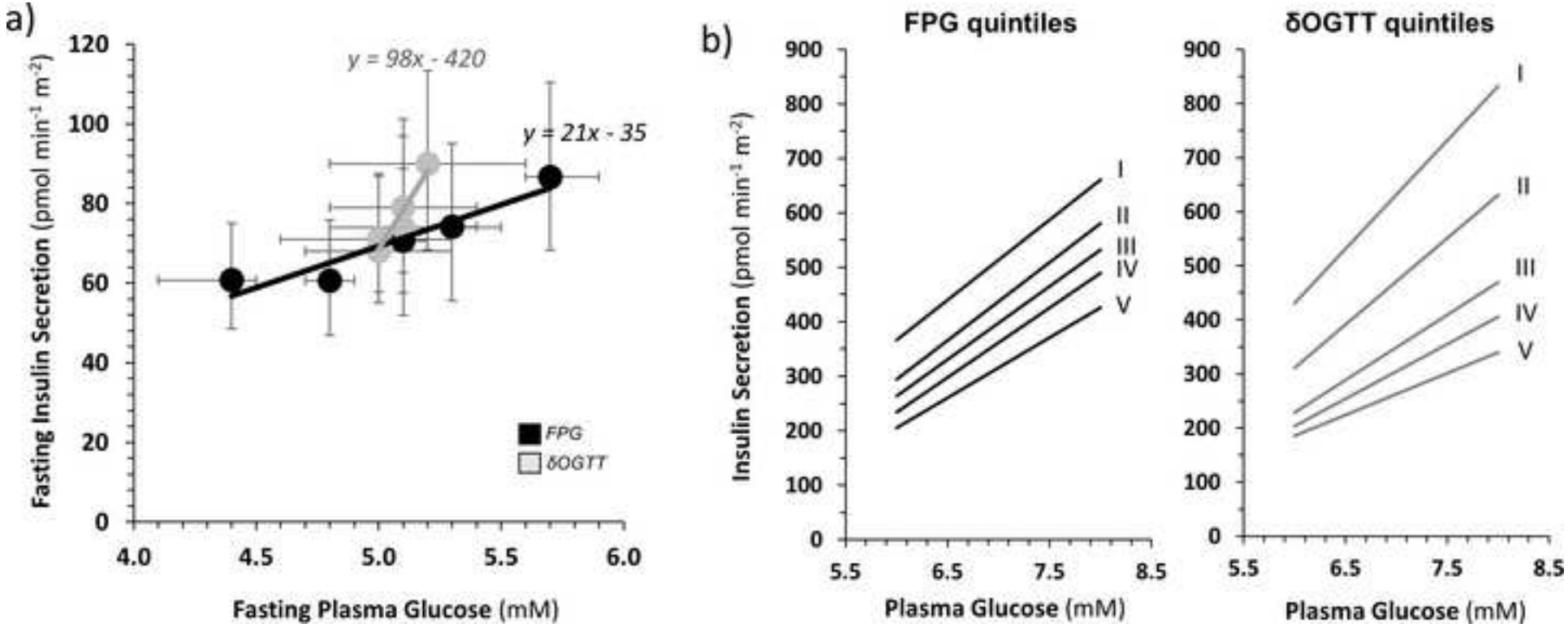
IV) AIR = 40±6 + 0.455±0.096 * $1/(GCR_c/I_c)$

V) AIR = 45±5 + 0.179±0.067 * $1/(GCR_c/I_c)$


FPG progressors
Baseline
3.5 yrs FU
Plasma glucose (mM)
10.0
9.0
8.0
7.0
6.0
5.0
4.0
0 30 60 90 120
0 30 60 90 120
Time (min)


δOGTT progressors
Baseline
3.5 yrs FU
Plasma glucose (mM)
10.0
9.0
8.0
7.0
6.0
5.0
4.0
0 30 60 90 120
0 30 60 90 120
Time (min)


IRS@5 ↓
βC-GS →
IRS@5 →
βC-GS ↓
IRS@5 ↓
βC-GS ↓
3.5 yrs FU
Baseline
Plasma Gucose (mM)
10.0
9.0
8.0
7.0
6.0
5.0
4.0
0 30 60 90 120
0 30 60 90 120
0 30 60 90 120
Time (min)


a)
y = 98x - 420
y = 21x - 35
FPG
δOGTT
Fasting Insulin Secretion (pmol min-1 m-2)
Fasting Plasma Glucose (mM)
b)
FPG quintiles
δOGTT quintiles
Insulin Secretion (pmol min-1 m-2)
Plasma Glucose (mM)
I
II
III
IV
V