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Profiles of glucose metabolism in different prediabetes phenotypes, classified by fasting glycemia, 2-hour OGTT, glycated hemoglobin, and 1-hour OGTT: An IMI DIRECT study

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Best summarizing figure: Figure 1.

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Abstract

Differences in glucose metabolism among categories of prediabetes have not been systematically investigated. In this longitudinal study, participants (*N*=2111) underwent 2h-75g OGTT at baseline and 48 months. HbA1c was also measured. We classified participants as having isolated prediabetes defect (impaired fasting glucose, IFG; impaired glucose tolerance, IGT; HbA1c-prediabetes, IA1c), two defects (IFG+IGT, IFG+IA1c, IGT+IA1c), or all defects (IFG+IGT+IA1c). Beta-cell function (BCF) and insulin sensitivity (IS) were assessed from OGTT. At baseline, when pooling participants with isolated defects, they showed impairment in both BCF and IS compared to healthy controls. Pooled groups with two or three defects showed progressive further deterioration. Among groups with isolated defect, IGT showed lower IS, insulin secretion at reference glucose (ISR_r), and insulin secretion potentiation ($p<0.002$). Conversely, IA1c showed higher IS and ISR_r ($p<0.0001$). Among groups with two defects, we similarly found differences in both BCF and IS. At 48 months, we found higher type 2 diabetes incidence for progressively increasing number of prediabetes defects (odds ratio >2, $p<0.008$). In conclusion, the prediabetes groups showed differences in type/degree of glucometabolic impairment. Compared to the pooled group with isolated defects, those with double or triple defect showed progressive differences in diabetes incidence.

The current prediabetes conditions include impaired fasting glucose (IFG), impaired glucose tolerance (IGT) and HbA1c prediabetes (1). Some studies found that IFG and IGT differ in the mechanisms involved in glucose homeostasis (2). The study (2) summarized that, though both individuals with IFG and IGT show reduction in early-phase insulin secretion, those with IGT also have impaired late-phase insulin secretion. Furthermore, individuals with IGT have marked peripheral insulin resistance with only mild hepatic insulin resistance, whereas those with IFG show the opposite condition. However, few studies considered all the prediabetes groups and their combinations, and they suffered from several limitations, especially lack of longitudinal data (3–6).

In this study, we investigated the differences in the main parameters of glucose metabolism in all categories of prediabetes, *i.e.*, IFG, IGT, HbA1c prediabetes, and their combinations. Furthermore, we considered 1h OGTT glycemia, since increasing consensus is emerging for this criterion to characterize glucose tolerance (7). We also investigated differences between groups in the incidence of type 2 diabetes onset after 48 months. Finally, we briefly investigated reversal to normal glucose tolerance.

Research Design and Methods

Study design and participants

We used data from the IMI-DIRECT (Diabetes Research on Patient Stratification) European multicentre project, aimed to validate biomarkers of glycemic deterioration before and after type 2 diabetes onset (ClinicalTrials.gov identifier NCT03814915) (8) The present analysis considers a cohort of European adults without diabetes, with focus on data collected at baseline (month 0) and month 48. A screening tool was used to identify from previous cohort studies at-risk participants to be recruited into the new study (8). Inclusion criteria were: *a)* No

treatment with insulin-sensitising, glucose-lowering or other antidiabetic drugs; *b)* Fasting capillary blood glucose <10 mmol/l at baseline; *c)* White European ethnicity; *d)* Age ≥35 and <75 years. Exclusion criteria were: *a)* Diagnosed diabetes of any type; *b)* For women: pregnancy, lactation or plans to conceive within the study period; *c)* Any significant medical reason for exclusion as determined by the investigators. All participants provided written informed consent and the study protocol was approved by the regional research ethics review boards. The research conformed to the ethical principles for medical research involving human participants outlined in the Declaration of Helsinki.

Data collection

Participants underwent 75 g OGTT at baseline and at 48 months, with measurement of glucose, insulin and C-peptide at 0, 15, 30, 45, 60, 90, and 120 min. Plasma glucose was analyzed using enzymatic method and photometric measurement. Plasma insulin and C-peptide were analyzed using chemiluminometric immunoassay. HbA1c was measured by liquid chromatography. Assays were carried out centrally, at the University of Eastern Finland (Kuopio, Finland) for glucose, insulin and C-peptide (within and between run coefficients of variation ≤6.6%), and the University of Exeter (Exeter, UK) for HbA1c (coefficients of variation ≤3%). Further details were reported previously (8).

Stratification according to the prediabetes criteria

Based on the definition of prediabetes of the American Diabetes Association (ADA) (1) we classified the participants as having impaired fasting glucose (IFG, fasting glucose ≥5.6 and ≤6.9 mmol/l), impaired glucose tolerance (IGT, 2h glucose ≥7.8 and ≤11.0 mmol/l), or HbA1c prediabetes (IA1c, HbA1c ≥39 and ≤47 mmol/mol). We stratified the participants in groups having a single defect (isolated IFG, IGT, or IA1c), two defects (IFG+IGT, IFG+IA1c, or

IGT+IA1c), or all three defects (IFG+IGT+IA1c). We also considered participants with normal glucose tolerance (NGT, fasting glucose <5.6 and 2h glucose <7.8 mmol/l, and HbA1c <39 mmol/mol). In a separate analysis, we also considered participants having the single defect of 1h hyperglycemia (7) (I1hG, 1h glycemia ≥8.6 mmol/l), thus without the defects of fasting and 2h glucose, and HbA1c (*i.e.*, < 5.6 and < 7.8 mmol/l, and <39 mmol/mol, respectively). In this analysis, the IFG, IGT, IA1c and NGT groups were redefined by excluding from each group the participants with 1h hyperglycemia.

From the 2127 participants initially included, 16 were excluded due to lack of data relevant for the analyses; thus, 2111 participants were studied. The 1691 participants that completed the final examination at 48 months were analyzed to determine the incidence of type 2 diabetes, diagnosed according to at least one of ADA criteria (1), or based on records of clinical diagnosis or use of antidiabetic medications.

Parameters of glucose metabolism

Beta-cell function was assessed by mathematical modeling and quantified by glucose sensitivity, G_{SENS} (slope of relationship between insulin secretion and glucose concentration), rate sensitivity, R_{SENS} (index of early secretion), insulin secretion at reference glucose of 6 mmol/l (rounded mean basal glucose in all participants), ISR_r, potentiation factor ratio, PFR (index of OGTT insulin secretion potentiation), and basal and total insulin secretion, ISR_b and ISR_t (9). Insulin resistance was estimated at fasting by the homeostasis model assessment index, HOMA-IR (10), and insulin sensitivity from the OGTT by PREDIM (11), a surrogate of the clamp M-value. Insulin clearance, CL_{ins}, was obtained as ratio between the area-under-the-curve of total insulin secretion and that of plasma insulin (12).

Statistical analyses

Normality of parameter distributions was tested with Shapiro-Wilk test. In case of skewed distributions, values were logarithmically transformed. Differences among groups in parameter means were assessed with one-way analysis of covariance (ANCOVA), adjusting for sex, age and BMI. Pairwise comparisons were also performed. In case of inhomogeneity of parameter variances, assessed with Levene test, heteroscedasticity was addressed by generalized least squares allowing separate variances per group. Moreover, we used Tukey's HSD to adjust for multiple statistical testing. Logistic regression analysis was used to assess differences and odds ratios for type 2 diabetes incidence in the studied groups (or similar analyses), with adjustment for sex, age and BMI. Parameters changes between baseline and follow-up were assessed by paired *t*-test, following logarithmic transformation for skewed distributions and assuming inequality of variances if appropriate. Difference in sex distribution among groups was assessed by χ^2 test. Values are presented as mean±standard deviation (SD), unless otherwise specified. Two-sided $p<0.05$ was considered statistically significant.

Data and Resource Availability

Due to the type of consent provided by study participants and the ethical approvals for this study, individual-level data from IMI-DIRECT cohorts cannot be transferred from the centralized IMI-DIRECT repository. Requests for access to IMI-DIRECT data, including those presented here, can be made to DIRECTdataaccess@Dundee.ac.uk. Requestors will be provided with information and assistance on how data can be accessed via DIRECT Computerome secure analysis platform following submission of appropriate documentation.

Results

Participants with single, double and triple defect

Single defect *vs.* NGT: *I)* Basic characteristics. We first compared participants with single defect pooled (1DEF) to the NGT group (Table 1). 1DEF were slightly younger ($p<0.04$), but had higher BMI ($p<0.0001$). As expected, glycemia and HbA1c were higher ($p<0.0001$).

II) Metabolic parameters. 1DEF had worse insulin sensitivity both at fasting and during the OGTT, as assessed by HOMA-IR and PREDIM ($p<0.0001$). 1DEF also showed higher insulin secretion, both at fasting, ISR_b, and total during the OGTT, ISR_t ($p<0.0001$). In contrast, insulin secretion at reference glucose, ISR_r, was lower ($p<0.0001$). Beta-cell glucose sensitivity, G_{SENS}, was impaired ($p<0.002$). Insulin clearance, CL_{ins}, was lower ($p<0.0001$). In Online-Only Supplemental Material, Figure S1 (panel A) reports the model-determined relationship between insulin secretion and glucose concentration (*i.e.*, the dose-response, whose average slope is G_{SENS}) for NGT and 1DEF, as well as for other groups, as outlined below.

Double *vs.* single defect: *I)* Basic characteristics. When comparing double defect participants pooled (2DEF) to 1DEF (Table 1), 2DEF were slightly older ($p<0.05$). Both glycemia and HbA1c were higher ($p<0.0001$).

II) Metabolic parameters. 2DEF had worse insulin sensitivity, both HOMA-IR and PREDIM ($p<0.003$). 2DEF also showed higher ISR_b and ISR_t, but lower ISR_r ($p<0.003$). G_{SENS} was impaired ($p<0.0001$). Slight impairment was found for the potentiation factor ratio, PFR ($p<0.02$). Beta-cell dose-response for 2DEF is reported in Figure S1 (panel A).

Triple *vs.* double defect: *I)* Basic characteristics. When comparing the group with all three defects (3DEF, *i.e.*, IFG+IGT+IA1c) to 2DEF (Table 1), 3DEF were slightly older ($p<0.05$), and had higher BMI ($p<0.0006$). Glycemia and HbA1c were higher ($p<0.0002$).

II) Metabolic parameters. 3DEF had both worse HOMA-IR and PREDIM ($p<0.002$). 3DEF also showed higher ISR_b and ISR_t, and lower ISR_r ($p<0.02$). G_{SENS} and PFR were lower ($p<0.03$). Beta-cell dose-response for 3DEF is reported in Figure S1 (panel A).

The groups with single defect

IFG, IGT, IA1c: *I)* Basic characteristics. The percentage of participants with single defect, compared to that of participants with double or triple defect, is displayed in Figure S2 (panel A). When comparing the single defect groups (Table 2), IFG were slightly younger ($p<0.02$) than IGT and IA1c. Glycemia was typically lower in IA1c, intermediate in IFG, and higher in IGT ($p<0.0001$).

II) Metabolic parameters. IFG showed worse HOMA-IR ($p<0.0001$) compared to IGT and IA1c, whereas PREDIM was lower in IGT, intermediate in IFG, and higher in IA1c ($p<0.0001$). IFG had higher ISR_b compared to IA1c ($p<0.002$), whereas ISR_t was higher in IGT ($p<0.001$). IGT also had lower ISR_r compared to IFG and IA1c ($p<0.002$). In contrast, in IA1c, ISR_r was higher than in both IGT and IFG ($p<0.0001$). PFR was different in the three groups, being lower in IGT, intermediate in IA1c, and higher in IFG ($p<0.0003$). A summary of the differences among groups for the main parameters of insulin sensitivity/resistance and insulin secretion/beta-cell function is reported in Figure 1 (panel A).

The groups with double defect

IFG+IGT, IFG+IA1c, IGT+IA1c: *I)* Basic characteristics. The percentage of participants with double defect is displayed in Figure S2 (panel A). When comparing the double defect groups (Table 2), IGT+IA1c were somehow older ($p<0.008$) than the other two groups. Expectedly, the two groups including IFG had higher fasting glycemia than IGT+IA1c ($p<0.0001$), whereas the two groups including IGT had higher 2h glycemia than IFG+IA1c ($p<0.0001$); 1h glycemia

was lower in IFG+IA1c than in the other two groups ($p<0.003$). Mean glycemia was lower in IFG+IA1c, intermediate in IGT+IA1c, and higher in IFG+IGT ($p<0.004$), whereas HbA1c was higher in the two groups including IA1c ($p<0.0001$).

II) Metabolic parameters. Insulin sensitivity during the OGTT was lower in IFG+IGT, intermediate in IGT+IA1c, and higher in IFG+IA1c (PREDIM, $p<0.03$), whereas HOMA-IR was only slightly higher in IFG+IGT than in IGT+IA1c ($p<0.05$). ISR_t was higher in IFG+IGT compared to IFG+IA1c ($p<0.0001$). ISR_r was lower in IFG+IGT than the other two groups ($p<0.005$). PFR was different in the three groups, being lower in IGT+IA1c, intermediate in IFG+IGT, and higher in IFG+IA1c ($p<0.0001$ for the difference between IFG+IA1c and the other two groups, $p<0.05$ for the difference between IFG+IGT and IGT+IA1c). A summary of the differences among groups for the main parameters of insulin sensitivity/resistance and insulin secretion/beta-cell function is reported in Figure 1 (panel B).

Adding 1h hyperglycemia

I1hG *vs.* NGT: *I)* Basic characteristics. The percentage of participants with 1h hyperglycemia, compared to that of participants with a traditional single defect, is displayed in Figure S2 (panel B). Of note, the number of participants in the groups is different from the previous analyses, as we have now considered one additional criterion. We first compared I1hG with NGT (Table 3). Expectedly, glycemia was higher in I1hG ($p<0.0001$).

II) Metabolic parameters. I1hG had worse PREDIM ($p<0.0001$). I1hG also showed higher ISR_t and lower ISR_r ($p<0.0001$). G_{SENS} was impaired in I1hG ($p<0.0001$). CL_{ins} was lower ($p<0.02$).

I1hG, IFG, IA1c: *I)* Basic characteristics. We then compared I1hG to the other single defect groups (Table 3) excluding IGT, due to low number of participants ($N=5$). I1hG were younger than IA1c ($p<0.005$). Glycemia was higher in I1hG ($p<0.0002$) than in the other two groups,

except for fasting glycemia compared to IFG, as expected; HbA1c was obviously lower than in IA1c ($p<0.0001$), but similar to IFG.

II) Metabolic parameters. I1hG had better HOMA-IR compared to IFG ($p<0.0001$), but lower PREDIM compared to IA1c ($p<0.0009$). I1hG also showed higher ISR_t ($p<0.0001$), but lower ISR_b compared to IFG ($p<0.0001$). Also, in I1hG, ISR_r was lower ($p<0.0001$). G_{SENS} was impaired ($p<0.0008$) in I1hG, which also showed lower PFR compared to IFG ($p<0.005$). The beta-cell dose-response for the groups of this analysis is reported in Figure S1 (panel B). A summary of the differences among groups for the main parameters of insulin sensitivity/resistance and insulin secretion/beta-cell function is reported in Figure 2.

Incidence of type 2 diabetes and parameters at follow-up

We evaluated how many participants developed type 2 diabetes by the 48 months follow-up visit. Among the 1691 participants studied, the percentage that developed diabetes in each group is shown in Figure 3. According to logistic regression analysis, 1DEF showed higher percentage of diabetes compared to NGT ($p<0.0002$), with odds ratio much higher than 1 (OR=6.23, 95% Confidence Interval, CI: 2.45-15.87). However, among the three groups with single defect there were no differences in the percentage of participants developing diabetes ($p\geq0.64$).

Similarly, when comparing 2DEF to 1DEF, 2DEF showed higher percentage of participants with diabetes ($p<0.0001$, OR=3.03, 95% CI: 1.99-4.61). However, there were no differences in the percentage of participants with diabetes among the three groups with double defect ($p\geq0.59$). When comparing the participants with triple defect to 2DEF, the former had higher percentage of participants developing diabetes ($p<0.008$, OR=2.18, 95% CI: 1.23-3.88).

Of note, when analyzing progression to the triple prediabetes defect rather than to overt diabetes, we found higher incidence in IGT than in IFG and IA1c (9 of 43 *vs*. 42 of 538 and 11

of 157 participants; p<0.007, OR=3.10, 95% CI: 1.37-7.04, and p<0.008, OR=3.84, 95% CI: 1.43-10.31, respectively), as well as in IGT+IA1c than in IFG+IA1c (p<0.02, OR=4.46, 95% CI: 1.31-15.12).

In the analysis including I1hG, 2 of 381 participants developed diabetes in NGT, 7 of 231 in IFG, 4 of 86 in IA1c, and 3 of 150 in I1hG. There were no differences in the percentage of participants developing diabetes in I1hG compared to the other two groups with single defect, *i.e.*, IFG and IA1c (p>0.13). However, single defect groups pooled (*i.e.*, IFG, IA1c, I1hG grouped) showed higher percentage of diabetes onset compared to NGT (p<0.03, OR=5.71, 95% CI: 1.29-25.29), consistently with previous findings.

For participants that completed the follow-up, we also compared the parameters value at baseline and 48 months. All groups, including NGT, showed deterioration of several metabolic parameters (Table 4 and Table 5).

Reverting to normal glucose tolerance

We also investigated the incidence of reversal to normal glucose tolerance at follow-up. In the groups with single defect, the fraction of participants reverting to normal glucose tolerance was 26/538 in IFG, 4/43 in IGT and 2/157 in IA1c. In the latter, the percentage was lower than in IFG and IGT (p<0.03, OR=0.18, 95% CI: 0.04-0.80 *vs*. IFG, and p<0.01, OR=0.08, 95% CI: 0.01-0.54 *vs*. IGT), whereas there was no difference between IFG and IGT (p>0.34). In groups with double or triple defect, reversal was negligible (2/421 participants in total). In the analysis with 1h hyperglycemia, there was no difference between I1hG and the other groups with single defect (p>0.08).

Discussion

In this study, we investigated the differences in the main parameters of glucose metabolism in the prediabetes categories according to all established criteria, *i.e.*, defect in fasting glycemia, 2h OGTT glycemia and glycated hemoglobin (1). We also investigated the differences among groups in the incidence of type 2 diabetes. To our knowledge, this is the first study presenting the analysis of glucose metabolism profiles in groups identified by all prediabetes criteria, in isolation and combination. Thus, even for the widely studied IFG and IGT populations, previous investigations typically did not analyze the "pure", single metabolic defects (at basal or at 2h), since they rarely accounted for the third possible defect, *i.e.*, HbA1c-based prediabetes. Furthermore, none of the previous studies comparing the different prediabetes defects reported longitudinal data.

Among the groups with isolated prediabetes defect, we found differences in the type or degree of impairment for both insulin sensitivity and beta-cell function. Similar results were found for the groups with double defect. In line with the concepts of precision medicine in diabetes (13), our findings indicate the potential clinical benefit of treating each category of prediabetes with optimized strategies, the success of which in preventing or delaying type 2 diabetes appears enhanced with interventions designed to correct the underlying pathophysiological disturbances (14).

When comparing the groups with isolated defect, in IFG and IGT our findings confirmed the known notions on fasting insulin resistance in IFG (partly reflecting hepatic insulin resistance (15)) and OGTT insulin resistance in IGT (2). In IGT we also found lower insulin secretion at reference glucose, ISR_r, and lower potentiation factor, PFR, which is related to the enhancing incretin effect on insulin secretion (16), though this cannot be investigated in detail in this analysis. In fact, incretin effect alterations were observed in IGT (16,17). In summary, IGT appears the worst phenotype among the three phenotypes with isolated defect.

In IA1c, we found fasting insulin resistance similar to that of IGT, but lower than that of IFG, whereas OGTT insulin sensitivity was higher compared to both IFG and IGT. IA1c also had ISR_r higher than both IFG and IGT. Glucose and rate sensitivities were similar to those of IFG and IGT, whereas PFR was somehow lower than in IFG, but higher than in IGT. Thus, among the three groups with isolated defect, IA1c showed overall less severe impairment in glucose metabolism. Notably, to our knowledge only one study reported detailed (*i.e.*, model-derived) information on beta-cell function in isolated HbA1c-based prediabetes (18), and comparison with other prediabetic groups was limited.

When comparing the groups with two defects, our findings are consistent with those in the groups with isolated defect, where the metabolic impairment appears more severe in IGT and, in contrast, less severe in IA1c. Thus, IFG+IGT was the worst phenotype, with more severe impairment in both fasting and OGTT insulin sensitivity, and partly in beta-cell function.

One-hour hyperglycemia has been proposed as possible further marker indicating abnormal glucose metabolism (7,19–22). In our analysis, I1hG showed differences in both insulin sensitivity and beta-cell function compared to the other groups with isolated defect, though comparison with IGT was not possible. Interestingly, I1hG showed impairment in both glucose sensitivity (typically the most important beta-cell function parameter) and potentiation factor, and in ISR_r. Thus, 1h hyperglycemia may identify a prediabetes phenotype with specific metabolic defects (especially, possibly greater beta-cell dysfunction). To our knowledge, no previous studies compared isolated 1h hyperglycemia to isolated IFG and isolated HbA1c-based prediabetes.

We also studied the incidence of type 2 diabetes within four years from baseline. We did not find differences in the diabetes incidence among the groups with isolated defect (despite our findings suggesting more severe metabolic impairment in IGT), and similarly among the double defect groups. These findings, which partly differ from those of some previous studies

in prediabetes (23), may be due to the follow-up duration, which could have been insufficient to disclose possible differences in diabetes incidence among groups with equal number of defects. This is in fact suggested by the observation that, when comparing IFG, IGT and IA1c in terms of progression to the triple prediabetes defect rather than to overt diabetes, we found higher incidence in IGT compared to both IFG and IA1c, and similarly for IGT+IA1c compared to IFG+IA1c. Thus, it can be hypothesized that with longer follow-up duration some differences may emerge in the diabetes incidence, among both the groups with isolated defect and those with double defect (with IGT phenotype possibly more prone to develop diabetes, both as isolated defect and in combination with a second defect). On the other hand, four years follow-up was sufficient to reveal differences in diabetes incidence for progressively higher number of prediabetes defects, *i.e.*, from NGT to triple defect. In the context of precision diagnostics (13), this suggests that people known to be at risk for type 2 diabetes should be ideally tested to disclose the possible presence of all three prediabetes defects, as this appears relevant for determination of the actual risk to develop diabetes.

Recent studies suggest that insulin clearance is an independent process that can adapt to the metabolic demand to maintain glucose homeostasis (24). Of note, one study reported that both increase in insulin secretion and decrease in insulin clearance may compensate for insulin resistance, but insulin clearance decrease may be the first mechanism providing adaptation to insulin resistance (25). In our study, we found that insulin clearance was similar among groups with equal number of defects, but it showed a tendency to progressive decline, ranging from NGT to triple defect. This appears consistent with what reported in some studies (24, 26), though other studies reported different findings (27, 28). Study (27) reported no difference in insulin clearance between participants with and without diabetes. Study (28) found increased hepatic component in type 2 diabetes, but decreased extrahepatic component. In a previous study, we found increased insulin clearance in women with former gestational diabetes

progressing to type 2 diabetes, compared to women remaining diabetes-free (29), possibly explained with the role of the SLC30A8 gene (30, 31).

Few previous studies analyzed the glucose metabolism profile, including both insulin sensitivity and beta-cell function, in several different groups with prediabetes. In study (3) participants were stratified into isolated IFG, IGT, HbA1c-based prediabetes, and in further two groups combining HbA1c with IFG and IGT, but IFG+IGT and the triple defect were not considered. Nonetheless, similar to our findings, fasting insulin resistance was higher in IFG and in IFG plus HbA1c-based prediabetes. Beta-cell function results of study (3) were limited by the lack of C-peptide. This may explain the partial disagreement with our findings, as the worse IGT condition in beta-cell function was observed compared to IA1c, but not to IFG. Furthermore, 1h glycemia defect was not considered, and longitudinal data were not provided. Similar limitations hold for studies (4,5). In our previous study (6), due to limitations in the dataset, we analyzed only IFG and IGT combined, plus one group with added 1h hyperglycemia, and another group with further HbA1c defect. In agreement with the present findings, we found progressive deterioration for both insulin sensitivity and beta-cell function for increasing number of defects. However, similarly to studies (3–5), C-peptide was not measured, and longitudinal data were not available. Other studies reported separate analyses for some prediabetes categories (32–37). However, the focus was typically not on the assessment of glucose metabolism, thus the analysis of insulin sensitivity and beta-cell function was limited or absent. Another study focused on the definition of prediabetes phenotypes with different metabolic abnormalities and risk for type 2 diabetes (38). Specifically, several variables were considered (glycemia, insulin sensitivity and secretion, lipids, tissue fat content, anthropometry, polygenic diabetes risk score), yielding the definition of six prediabetes phenotypes at different risk for type 2 diabetes. However, direct comparison with our findings

is difficult, due to the peculiar definition of the different prediabetes phenotypes of such study (38).

Some studies, such as (39-44), focused on reversal from prediabetes to normal glucose tolerance. Interestingly, in our study we found somehow lower reversal in IA1c compared to IFG and IGT, which may be partly due to greater stability of HbA1c compared to glycemia. This however requires further investigations.

Our study has some limitations. The prevalence of the different prediabetes conditions is likely not representative of the general population, as it appears from the disparity in IFG and IGT proportions. This is due to the recruitment process (see the study inclusion criteria). However, the size of the study allowed recruitment of sufficient number of participants even in the less represented prediabetes categories, thus allowing appropriate analyses. Another limitation concerning diabetes incidence may be the duration of the follow-up.

In conclusion, we investigated the differences in several parameters of glucose metabolism in all groups with prediabetes, and we assessed the incidence at 48 months of type 2 diabetes onset in each group. Heterogeneity in the level of impairment of the metabolic parameters suggests that the different prediabetes phenotypes may benefit from specific treatment approaches. Furthermore, our findings indicate that people known to be at risk for diabetes should be tested to disclose the possible presence of all three prediabetes defects, as this appears relevant for determination of the actual risk to develop the disease.

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Author Contributions

A.T. designed the analysis and analyzed the data. E.G. reviewed the modeling analysis. C.G. supervised the statistical analysis. A.M. (Andrea Mari) supervised the whole analysis. A.T., E.G., C.G. and A.M. interpreted the results. A.T. wrote the manuscript. E.G., C.G. and A.M. reviewed the manuscript. All authors were involved in the DIRECT study at different levels, and were essential for the production, release and management of the data analyzed here. All authors approved the final version of the manuscript. A.T. and A.M. are the guarantors of this work and, as such, had 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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Tables

Table 1 - Basic characteristics and metabolic parameters (mean±SD) for NGT participants and for the participants grouped into single defect (1DEF) and double defect (2DEF) groups, as well as participants with all three defects (3DEF).

	NGT	1DEF	2DEF	3DEF
N (Male/Female)	665 (477/188)	898 (695/203) *	447 (351/96)	101 (73/28)
Basic characteristics				
Age (years)	62.4±6.2	61.7±6.3 *	62.3±5.9 †	63.8±6.2 ‡
BMI (kg/m^2)	27.1±3.7	28.0±3.8 *	28.5±4.3	30.0±4.1 ‡
HbA1c (mmol/mol)	35.3±2.2	36.8±2.4 *	39.4±2.7 †	40.6±1.6 ‡
Glucose, insulin, C-peptide plasma concentrations				
G_b (mmol/l)	5.21±0.32	5.74±0.45 *	6.00±0.38 †	6.15±0.35 ‡
G_{60} (mmol/l)	7.49±1.86	8.91±2.11 *	10.19±2.17 †	11.58±1.72 ‡
G_{120} (mmol/l)	5.23±1.17	5.73±1.40 *	6.64±1.83 †	8.94±0.78 ‡
G_m (mmol/l)	6.77±1.09	7.74±1.26 *	8.68±1.33 †	9.92±1.03 ‡
I_b (pmol/l)	48.5±29.4	62.4±42.5 *	66.9±39.4	86.2±51.3 ‡
I_m (pmol/l)	305.6±195.6	392.1±250.1 *	444.1±305.5 †	552.2±334.4 ‡
CP_b (pmol/l)	723±253	862±307 *	934±338 †	1100±415 ‡
CP_m (pmol/l)	2503±762	2840±877 *	3004±970 †	3321±986
Insulin sensitivity/resistance				
HOMA-IR (non-dim.)	1.88±1.14	2.68±1.9 *	2.99±1.82 †	3.96±2.48 ‡
PREDIM (mg kg^{-1} min^{-1})	5.93±1.77	4.94±1.5 *	4.39±1.51 †	3.17±0.88 ‡
Beta-cell function and insulin secretion				
G_{SENS} (pmol min^{-1} m^{-2} mM^{-1})	124.0±62.4	113.6±53.5 *	99.7±48.1 †	90.6±28.9 ‡
R_{SENS} (pmol m^{-2} mM^{-1})	1021±778	918±706	787±543	869±561
PFR (non-dim.)	1.74±0.63	1.80±0.66	1.70±0.58 †	1.41±0.39 ‡
ISR_r (pmol min^{-1} m^{-2})	277.0±113.4	231.4±98.7 *	194.5±85.8 †	152.7±62.4 ‡
ISR_b (pmol min^{-1} m^{-2})	90.4±31.2	108.5±38.9 *	116.8±42.0 †	136.8±51.5 ‡
ISR_t (nmol m^{-2})	46.1±14.7	52.7±17.3 *	56.7±18.8 †	64.4±19.4 ‡
Other parameters				
CL_{ins} (l min^{-1} m^{-2})	1.48±0.47	1.32±0.40 *	1.28±0.41	1.15±0.38

BMI: body mass index; G_b, G_{60}, G_{120}, G_m: glucose at basal (fasting), 60 min, 120 min and mean glucose during OGTT; I_b, I_m: basal and mean insulin; CP_b, CP_m: basal and mean C-peptide; HOMA-IR: homeostasis model assessment - insulin resistance; PREDIM: predicted M; G_{SENS}: glucose sensitivity; R_{SENS}: rate sensitivity; PFR: potentiation factor ratio; ISR_r: insulin secretion at reference glucose (6 mmol/l); ISR_b: basal insulin secretion; ISR_t: total insulin secretion; CL_{ins}: insulin clearance; *: significant difference in 1DEF vs. NGT; †: 2DEF vs. 1DEF; ‡: 3DEF vs. 2DEF. HbA1c (%): 5.4±0.20 (NGT); 5.5±0.22 (1DEF); 5.8±0.25 (2DEF); 5.9±0.15 (3DEF) (from http://www.ngsp.org/convert1.asp).

Table 2 - Basic characteristics and metabolic parameters (mean±SD) for the different groups of participants with single and with double defect.

	IFG	**IGT**	**IA1c**	**IFG+IGT**	**IFG+IA1c**	**IGT+IA1c**
N (Males/Females)	643 (540/103)	57 (39/18) *	198 (116/82) †	96 (79/17)	327 (262/65)	24 (10/14) $^{\|, \P}$
Basic characteristics						
Age (years)	60.9±6.0	63.1±7.1 *	63.8±6.5 †	62.5±6.4	62.0±5.6	66.2±5.9 $^{\|, \P}$
BMI (kg/m^2)	28.1±3.6	28.8±3.7	27.7±4.5 ‡	28.8±4.0	28.3±4.5	28.8±3.4
HbA1c (mmol/mol)	35.9±1.9	35.8±1.8	39.9±1.2 $^{\dagger, \ddagger}$	35.6±2.3	40.5±1.6 §	40.4±1.4 $^{\|}$
Glucose, insulin, C-peptide plasma concentrations						
G_b (mmol/l)	5.95±0.29	5.22±0.37 *	5.21±0.33 †	6.07±0.32	6.04±0.32	5.16±0.30 $^{\|, \P}$
G_{60} (mmol/l)	8.99±2.11	10.44±1.63 *	8.21±1.93 ‡	11.51±1.92	9.79±2.13 §	10.35±1.65 ¶
G_{120} (mmol/l)	5.56±1.17	8.72±0.73 *	5.44±1.26 ‡	9.09±0.87	5.75±1.17 §	8.91±0.70 ¶
G_m (mmol/l)	7.81±1.2	8.99±0.98 *	7.16±1.17 $^{\dagger, \ddagger}$	9.96±1.14	8.28±1.16 §	8.91±0.94 $^{\|, \P}$
I_b (pmol/l)	65.3±45.6	59.5±34.5 *	53.7±31.7 †	73.1±40.1	65.2±39.5	64.4±32.5
I_m (pmol/l)	393.6±245.6	487.3±328.9	359.5±231.8 ‡	524.9±316.4	419.7±303.6 §	452±234
CP_b (pmol/l)	880±306	844±295	810±311 †	992±359	919±332	908±310
CP_m (pmol/l)	2849±848	3128±1033	2730±903 ‡	3271±968	2920±955 §	3079±1018
Insulin sensitivity/resistance						
HOMA-IR (non-dim.)	2.89±2.05	2.32±1.37 *	2.08±1.26 †	3.30±1.84	2.94±1.84	2.48±1.31 $^{\|}$
PREDIM (mg kg^{-1} min^{-1})	4.84±1.39	3.93±1.11 *	5.55±1.71 $^{\dagger, \ddagger}$	3.47±1.14	4.71±1.52 §	3.78±0.90 $^{\|, \P}$
Beta-cell function and insulin secretion						
G_{SENS} (pmol min^{-1} m^{-2} mM^{-1})	114.1±53.6	98.4±37.4	116.2±56.2	94.2±44.6	101.7±49.8	94.5±36.5
R_{SENS} (pmol m^{-2} mM^{-1})	917±688	967±554	906±798	907±504	744±551	893±516
PFR (non-dim.)	1.88±0.63	1.23±0.30 *	1.71±0.73 $^{\dagger, \ddagger}$	1.37±0.35	1.83±0.59 §	1.21±0.31 $^{\|, \P}$
ISR_r (pmol min^{-1} m^{-2})	222.3±90.1	183.6±67.0 *	274.8±117.6 $^{\dagger, \ddagger}$	143.7±62.7	208.8±87.2 §	202.4±70.3 $^{\|}$
ISR_b (pmol min^{-1} m^{-2})	111.3±38.6	104.9±37.7	100.7±39.1 †	123.7±45.3	115.1±41.0	112.3±40.0
ISR_t (nmol m^{-2})	52.5±16.4	62±21 *	50.8±18.1 ‡	64.5±19.3	54.1±17.9 §	60.7±20.8
Other parameters						
CL_{ins} (l min^{-1} m^{-2})	1.30±0.39	1.29±0.43	1.39±0.42 †	1.23±0.41	1.29±0.41	1.30±0.44

BMI: body mass index; G_b, G_{60}, G_{120}, G_m: glucose at basal (fasting), 60 min, 120 min and mean glucose during OGTT; I_b, I_m: basal and mean insulin; CP_b, CP_m: basal and mean C-peptide; HOMA-IR: homeostasis model assessment - insulin resistance; PREDIM: predicted M; G_{SENS}: glucose sensitivity; R_{SENS}: rate sensitivity; PFR: potentiation factor ratio; ISR_r: insulin secretion at reference glucose (6 mmol/l); ISR_b: basal insulin secretion; ISR_t: total insulin secretion; CL_{ins}: insulin clearance; *: significant difference in IGT vs. IFG; †: IA1c vs. IFG; ‡: IA1c vs. IGT; §: IFG+IA1c vs. IFG+IGT; $^{\|}$: IGT+IA1c vs. IFG+IGT; ¶: IGT+IA1c vs. IFG+IA1c. HbA1c (%): 5.4±0.17 (IFG); 5.4±0.16 (IGT); 5.8±0.11 (IA1c); 5.4±0.21 (IFG+IGT); 5.9±0.15 (IFG+IA1c); 5.8±0.13 (IGT+IA1c) (from http://www.ngsp.org/convert1.asp).

Table 3 - Basic characteristics and metabolic parameters (mean±SD) in the analysis including 1h hyperglycemia for the different groups of participants with a single defect and for NGT. IGT was excluded because of low sample size.

	NGT	IFG	IA1c	I1hG
N (Males/Females)	491 (324/167)	275 (210/65)	112 (53/59)	173 (152/21) *,†,‡
Basic characteristics				
Age (years)	62.6±6.2	60.8±6.2	64.1±6.8	61.8±6.0 ‡
BMI (kg/m^2)	27.1±3.8	27.7±3.3	27.2±3.9	27.2±3.4
HbA1c (mmol/mol)	35.2±2.2	35.9±1.8	39.9±1.2	35.5±2.2 ‡
Glucose, insulin, C-peptide plasma concentrations				
G_b (mmol/l)	5.17±0.34	5.86±0.22	5.14±0.34	5.31±0.22 *,†,‡
G_{60} (mmol/l)	6.62±1.14	7.04±1.07	6.87±1.12	9.94±1.17 *,†,‡
G_{120} (mmol/l)	5.01±1.08	5.14±1.15	5.10±1.15	5.85±1.21 *,†,‡
G_m (mmol/l)	6.30±0.76	6.78±0.67	6.41±0.77	8.09±0.74 *,†,‡
I_b (pmol/l)	48.0±29.6	64.7±44.2	49.9±24.6	49.8±28.7 †
I_m (pmol/l)	280.3±167.4	329.9±177.0	294.2±178.9	375.7±246.1 *,‡
CP_b (pmol/l)	715±252	853±309	757±245	743±255 †
CP_m (pmol/l)	2408±708	2608±750	2470±790	2766±845 *,‡
Insulin sensitivity/resistance				
HOMA-IR (non-dim.)	1.85±1.14	2.82±1.96	1.91±0.97	1.96±1.13 †
PREDIM (mg kg^{-1} min^{-1})	6.11±1.79	5.21±1.39	5.86±1.53	5.42±1.6 *,‡
Beta-cell function and insulin secretion				
G_{SENS} (pmol min^{-1} m^{-2} mM^{-1})	133.8±66.4	131.2±63.4	124.3±60.1	95.9±37.1 *,†,‡
R_{SENS} (pmol m^{-2} mM^{-1})	1080±823	1064±804	1006±941	853±609
PFR (non-dim.)	1.83±0.59	1.86±0.66	1.76±0.84	1.66±0.51 †
ISR_r (pmol min^{-1} m^{-2})	301.9±115.5	260.4±93.3	304.5±126.4	206.4±69.1 *,†,‡
ISR_b (pmol min^{-1} m^{-2})	89.4±30.9	107.9±38.4	93.9±30.6	92.9±32.0 †
ISR_t (nmol m^{-2})	43.8±13.4	46.4±14.0	45.1±15.8	52.4±16.4 *,†,‡
Other parameters				
CL_{ins} (l min^{-1} m^{-2})	0.09±0.03	0.08±0.02	0.09±0.02	0.08±0.03 *

BMI: body mass index; G_b, G_{60}, G_{120}, G_m: glucose at basal (fasting), 60 min, 120 min and mean glucose during OGTT; I_b, I_m: basal and mean insulin; CP_b, CP_m: basal and mean C-peptide; HOMA-IR: homeostasis model assessment - insulin resistance; PREDIM: predicted M; G_{SENS}: glucose sensitivity; R_{SENS}: rate sensitivity; PFR: potentiation factor ratio; ISR_r: insulin secretion at reference glucose (6 mmol/l); ISR_b: basal insulin secretion; ISR_t: total insulin secretion; CL_{ins}: insulin clearance; *: significant difference in I1hG vs. NGT; †: I1hG vs. IFG; ‡: I1hG vs. IA1c. HbA1c (%): 5.4±0.20 (NGT); 5.4±0.16 (IFG); 5.8±0.11 (IA1c); 5.4±0.20 (I1hG) (from http://www.ngsp.org/convert1.asp).

Table 4 - Basic characteristics and metabolic parameters (mean±SD) for NGT participants and for the different groups of participants with single, double and triple defect (*i.e.*, IFG+IGT+IA1c, also named 3DEF), which completed the follow-up. For each parameter, we reported baseline value, 48 months value, and indication of significant variation (↑ or ↓ for significant increase or decrease, respectively; n. s. (not significant) otherwise).

	NGT	**IFG**	**IGT**	**IA1c**	**IFG+IGT**	**IFG+IA1c**	**IGT+IA1c**	**IFG+IGT+IA1c**
N (Males/Females)	532 (400/132)	538 (471/77)	43 (32/11)	157 (97/60)	72 (64/8)	253 (215/38)	17 (10/7)	79 (56/23)
Basic characteristics								
Age (years)	62.0±6.1 66.1±6.1 ↑	60.5±5.9 64.6±5.9 ↑	63.0±6.6 67.1±6.6 ↑	63.3±6.5 67.5±6.5 ↑	62.1±6.0 66.2±5.9 ↑	61.6±5.6 65.8±5.5 ↑	64.8±6.0 68.9±5.9 ↑	63.6±6.3 67.7±6.3 ↑
BMI (kg/m^2)	27.1±3.6 27.4±3.9 ↑	28.1±3.5 28.1±3.8 n. s.	28.6±3.6 28.5±4.1 n. s.	27.5±4.4 27.8±4.6 n. s.	28.8±3.9 28.3±3.9 ↓	28.1±4.0 28.1±4.3 n. s.	28.2±3.2 27.9±3.2 n. s.	29.9±3.8 29.8±3.6 n. s.
HbA1c (mmol/mol)	35.2±2.2 38.7±2.8 ↑	35.9±1.9 39.3±2.5 ↑	35.9±1.9 39.0±3.1 ↑	39.8±1.2 42.6±2.3 ↑	35.7±2.2 39.1±2.4 ↑	40.5±1.6 43.3±2.5 ↑	40.2±1.1 43.5±1.8 ↑	40.5±1.5 43.6±2.9 ↑
Glucose, insulin, C-peptide plasma concentrations								
G_b (mmol/l)	5.23±0.30 5.62±0.48 ↑	5.95±0.29 6.12±0.49 ↑	5.30±0.24 5.69±0.53 ↑	5.24±0.30 5.71±0.51 ↑	6.08±0.32 6.21±0.46 ↑	6.06±0.33 6.32±0.60 ↑	5.27±0.23 5.64±0.61 ↑	6.11±0.32 6.44±0.65 ↑
G_{60} (mmol/l)	7.57±1.88 8.28±2.26 ↑	9.00±2.13 9.52±2.49 ↑	10.61±1.67 10.32±2.36 n. s.	8.32±1.91 9.11±2.26 ↑	11.72±1.87 11.36±2.11 n. s.	9.83±2.06 10.63±2.56 ↑	10.76±1.61 11.08±1.79 n. s.	11.58±1.59 11.86±2.03 n. s.
G_{120} (mmol/l)	5.21±1.17 5.88±1.57 ↑	5.56±1.15 6.23±1.64 ↑	8.72±0.73 7.89±2.54 ↓	5.44±1.26 6.17±1.73 ↑	9.05±0.89 7.93±2.24 ↓	5.72±1.22 6.79±1.97 ↑	8.97±0.80 8.51±2.36 n. s.	8.89±0.74 9.03±2.26 n. s.
G_m (mmol/l)	6.82±1.11 7.45±1.40 ↑	7.81±1.21 8.27±1.56 ↑	9.07±1.04 8.87±1.73 n. s.	7.24±1.12 7.98±1.52 ↑	10.08±1.09 9.61±1.47 ↓	8.29±1.15 9.00±1.67 ↑	9.14±0.98 9.37±1.29 n. s.	9.90±0.94 10.16±1.52 n. s.
I_b (pmol/l)	47.2±26.9 59.5±37.1 ↑	64.9±41.8 68.1±45.9 n. s.	58.9±35.5 71.8±34.3 ↑	53.2±32.1 65.6±37.4 ↑	72.5±38.2 72.0±39.2 n. s.	63.9±39.0 67.7±46.3 n. s.	61.4±34.9 71.4±35.7 n. s.	84.1±40.5 91.9±46.0 ↑
I_m (pmol/l)	302.0±190.7 377.0±283.7 ↑	396.6±245.1 450.1±319.8 ↑	498.4±351.2 485.7±330.7 n. s.	352.6±220.7 430.1±261.3 ↑	528.4±309.9 516.5±285.7 n. s.	416.8±305.1 454.4±288.7 ↑	427.1±257.7 485.3±269.4 n. s.	535.9±270.5 554.5±281.0 n. s.
CP_b (pmol/l)	715±239 826±326 ↑	873±305 921±385 ↑	824±276 962±302 ↑	800±301 919±348 ↑	1001±346 1008±319 n. s.	906±317 971±409 ↑	868±329 987±343 ↑	1089±374 1162±364 ↑
CP_m (pmol/l)	2489±742	2836±855	3151±1079	2700±838	3318±976	2910±972	2898±1074	3297±896

	2755±930 ↑	2984±997 ↑	3177±1064 n. s.	2969±935 ↑	3264±838 n. s.	3084±1011 ↑	3199±1014 ↑	3393±876 n. s.
Insulin sensitivity/resistance								
HOMA-IR (non-dim.)	1.84±1.07 2.51±1.63 ↑	2.87±1.88 3.15±2.30 ↑	2.32±1.42 3.06±1.53 ↑	2.08±1.31 2.81±1.67 ↑	3.28±1.74 3.36±1.98 n. s.	2.89±1.84 3.26±2.46 ↑	2.42±1.45 3.04±1.67 n. s.	3.83±1.96 4.47±2.49 ↑
PREDIM (mg kg^{-1} min^{-1})	5.94±1.72 5.19±1.72 ↓	4.82±1.39 4.55±1.59 ↓	3.91±1.01 3.92±1.18 n. s.	5.58±1.68 4.71±1.58 ↓	3.45±1.06 3.87±1.46 ↑	4.78±1.55 4.39±1.71 ↓	3.90±0.95 3.80±1.10 n. s.	3.16±0.77 3.12±0.93 n. s.
Beta-cell function and insulin secretion								
G_{SENS} (pmol min^{-1} m^{-2} mM^{-1})	122.5±63.5 123.2±59.8 n. s.	113.7±53.8 115.8±58.9 n. s.	99.3±38.5 107.9±40.4 n. s.	112.3±48.0 116.3±59.0 n. s.	91.8±44.2 98.8±48.5 n. s.	101.8±51.1 101.6±47.5 n. s.	86.6±38.5 83.5±19.8 n. s.	87.6±25.6 92.9±35.6 n. s.
R_{SENS} (pmol m^{-2} mM^{-1})	987±720 932±634 n. s.	911±690 865±677 n. s.	1030±544 1040±632 n. s.	876±753 812±565 n. s.	947±530 865±479 n. s.	739±553 700±512 n. s.	832±509 1000±652 n. s.	887±555 748±448 n. s.
PFR (non-dim.)	1.75±0.64 1.83±0.74 ↑	1.86±0.60 1.94±0.70 ↑	1.25±0.27 1.56±0.46 ↑	1.71±0.77 1.90±0.69 ↑	1.38±0.35 1.78±0.66 ↑	1.85±0.61 2.05±0.86 ↑	1.24±0.34 1.65±0.81 ↑	1.46±0.38 1.54±0.49 n. s.
ISR_r (pmol min^{-1} m^{-2})	271.2±109.2 240.8±106.9 ↓	221.9±92.0 202.2±97.1 ↓	178.1±63.2 193.7±85.3 n. s.	264.3±106.2 236.0±101.1 ↓	144.5±64.6 167.5±84.9 ↑	206.1±85.8 193.4±111.0 ↓	178.6±55.4 191.8±80.0 n. s.	157.4±61.4 159.8±77.9 n. s.
ISR_b (pmol min^{-1} m^{-2})	89.6±29.7 102.2±39.8 ↑	110.7±38.7 114.5±47.3 n. s.	102.6±35.5 118.7±37.9 ↑	99.4±37.7 112.7±43.0 ↑	125.3±44.4 124.6±40.4 n. s.	113.9±39.6 120.3±50.4 ↑	108.9±44.2 121.3±44.4 ↑	135.5±46.1 143.2±44.5 ↑
ISR_t (nmol m^{-2})	45.8±14.3 50.8±18.0 ↑	52.3±16.6 55.3±19.4 ↑	62.8±21.9 61.3±21.3 n. s.	50.3±16.7 55.3±18.7 ↑	65.5±19.2 62.5±16.2 n. s.	54.1±18.4 57.8±19.5 ↑	57.5±22.2 62.9±20.8 ↑	64.0±17.9 65.4±18.1 n. s.
Other parameters								
CL_{ins} (l min^{-1} m^{-2})	1.48±0.47 1.35±0.41 ↓	1.29±0.39 1.25±0.41 ↓	1.31±0.46 1.24±0.39 n. s.	1.39±0.41 1.26±0.40 ↓	1.23±0.42 1.20±0.40 n. s.	1.29±0.41 1.25±0.39 ↓	1.33±0.46 1.25±0.42 n. s.	1.12±0.34 1.10±0.32 n. s.

BMI: body mass index; G_b, G_{60}, G_{120}, G_m: glucose at basal (fasting), 60 min, 120 min and mean glucose during OGTT; I_b, I_m: basal and mean insulin; CP_b, CP_m: basal and mean C-peptide; HOMA-IR: homeostasis model assessment - insulin resistance; PREDIM: predicted M; G_{SENS}: glucose sensitivity; R_{SENS}: rate sensitivity; PFR: potentiation factor ratio; ISR_r: insulin secretion at reference glucose (6 mmol/l); ISR_b: basal insulin secretion; ISR_t: total insulin secretion; CL_{ins}: insulin clearance. HbA1c (%): 5.4±0.20, 5.7±0.26 (NGT); 5.4±0.17, 5.7±0.23 (IFG); 5.4±0.17, 5.7±0.28 (IGT); 5.8±0.11, 6.0±0.21 (IA1c); 5.4±0.20, 5.7±0.22 (IFG+IGT); 5.9±0.15, 6.1±0.23 (IFG+IA1c); 5.8±0.10, 6.1±0.16 (IGT+IA1c); 5.9±0.14, 6.1±0.27 (IFG+IGT+IA1c) (from http://www.ngsp.org/convert1.asp).

Table 5 - Basic characteristics and metabolic parameters (mean±SD) in the analysis including 1h hyperglycemia for participants that completed the follow-up in the groups with a single defect and in NGT. IGT was excluded because of low sample size. For each parameter, we reported baseline value, 48 months value, and indication of significant variation (↑ or ↓ for significant increase or decrease, respectively; n. s. (not significant) otherwise).

	NGT	**IFG**	**IA1c**	**I1hG**
N (Males/Females)	381 (267/114)	231 (181/50)	86 (43/43)	150 (132/18)
Basic characteristics				
Age (years)	62.2±6.3 66.3±6.3 ↑	60.5±6.0 64.6±5.9 ↑	63.6±7.0 67.7±7.0 ↑	61.7±5.6 65.9±5.6 ↑
BMI (kg/m^2)	27.1±3.7 27.5±4.1 ↑	27.6±3.3 27.7±3.6 n. s.	27.3±3.8 27.6±3.9 n. s.	27.2±3.4 27.1±3.3 n. s.
HbA1c (mmol/mol)	35.1±2.1 38.7±2.7 ↑	35.8±1.9 39.0±2.4 ↑	39.8±1.3 42.6±2.3 ↑	35.5±2.3 38.7±3.0 ↑
Glucose, insulin, C-peptide plasma concentrations				
G_b (mmol/l)	5.19±0.32 5.60±0.50 ↑	5.85±0.22 6.00±0.43 ↑	5.17±0.29 5.68±0.54 ↑	5.32±0.21 5.67±0.42 ↑
G_{60} (mmol/l)	6.64±1.16 7.64±1.88 ↑	7.04±1.09 8.04±1.95 ↑	6.96±1.06 8.26±1.99 ↑	9.94±1.14 9.89±2.33 n. s.
G_{120} (mmol/l)	4.97±1.06 5.68±1.38 ↑	5.11±1.11 5.85±1.34 ↑	5.16±1.17 5.78±1.35 ↑	5.82±1.22 6.39±1.88 ↑
G_m (mmol/l)	6.31±0.77 7.08±1.16 ↑	6.77±0.67 7.44±1.18 ↑	6.50±0.75 7.43±1.21 ↑	8.10±0.76 8.38±1.54 n. s.
I_b (pmol/l)	45.9±26.2 60.5±38.3 ↑	64.9±46.4 65.7±44.9 n. s.	50.0±24.6 61.9±27.4 ↑	50.2±28.5 56.8±33.5 ↑
I_m (pmol/l)	273.1±159.3 363.1±280.9 ↑	334.4±185.5 404.8±268.9 ↑	294.1±168.3 391.6±217.8 ↑	373.4±238.7 410.9±289.1 ↑
CP_b (pmol/l)	704±234 827±336	844±312 877±399	751±233 882±287	744±249 825±300

	↑	n. s.	↑	↑
CP_m (pmol/l)	2385±695 2711±947 ↑	2599±778 2848±986 ↑	2453±752 2866±896 ↑	2749±792 2864±881 ↑
Insulin sensitivity/resistance				
HOMA-IR (non-dim.)	1.78±1.05 2.55±1.69 ↑	2.83±2.07 2.97±2.14 n. s.	1.92±0.99 2.64±1.26 ↑	1.98±1.12 2.41±1.47 ↑
PREDIM (mg kg^{-1} min^{-1})	6.15±1.73 5.27±1.76 ↓	5.21±1.39 4.85±1.60 ↓	5.81±1.47 4.91±1.45 ↓	5.42±1.59 5.01±1.61 ↓
Beta-cell function and insulin secretion				
G_{SENS} (pmol min^{-1} m^{-2} mM^{-1})	133.1±68.9 131.9±63.4 n. s.	130.4±64.9 134.9±69.0 n. s.	121.7±53.4 125.9±64.6 n. s.	95.2±35.0 101.4±42.6 n. s.
R_{SENS} (pmol m^{-2} mM^{-1})	1039±748 974±643 n. s.	1058±821 1006±788 n. s.	975±883 892±625 n. s.	854±628 820±600 n. s.
PFR (non-dim.)	1.79±0.67 1.85±0.72 n. s.	1.84±0.63 1.89±0.60 n. s.	1.74±0.90 2.01±0.68 ↑	1.65±0.53 1.78±0.78 n. s.
ISR_r (pmol min^{-1} m^{-2})	297.8±111.7 256.9±110.1 ↓	261.7±96.6 224.5±107.2 ↓	290.3±111.5 254.7±106.2 ↓	203.8±65.4 200.0±86.1 n. s.
ISR_b (pmol min^{-1} m^{-2})	88.2±28.9 102.1±40.7 ↑	107.0±39.0 109.1±48.4 n. s.	93.0±29.2 107.7±35.2 ↑	93.0±31.4 102.5±37.5 ↑
ISR_t (nmol m^{-2})	43.3±13.2 49.6±18.2 ↑	46.3±14.6 51.5±18.6 ↑	44.9±15.2 52.8±18.1 ↑	52.0±15.2 53.9±17.4 n. s.
Other parameters				
CL_{ins} (l min^{-1} m^{-2})	1.52±0.45 1.36±0.40 ↓	1.32±0.37 1.27±0.40 ↓	1.45±0.41 1.29±0.35 ↓	1.40±0.52 1.32±0.45 ↓

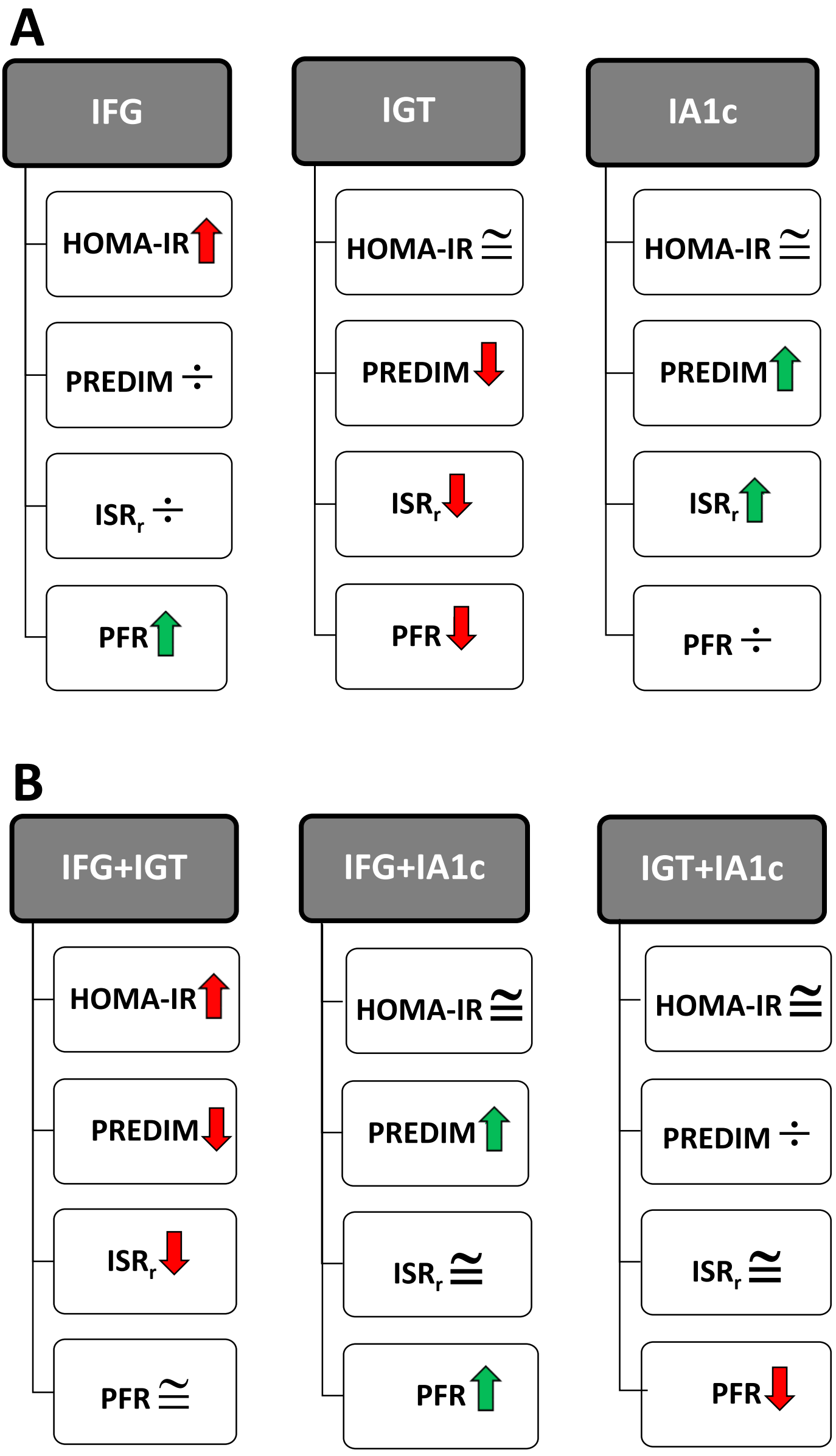
BMI: body mass index; G_b, G_{60}, G_{120}, G_m: glucose at basal (fasting), 60 min, 120 min and mean glucose during OGTT; I_b, I_m: basal and mean insulin; CP_b, CP_m: basal and mean C-peptide; HOMA-IR: homeostasis model assessment - insulin resistance; PREDIM: predicted M; G_{SENS}: glucose sensitivity; R_{SENS}: rate sensitivity; PFR: potentiation factor ratio; ISR_r: insulin secretion at reference glucose (6 mmol/l); ISR_b: basal insulin secretion; ISR_t: total insulin secretion; CL_{ins}: insulin clearance. HbA1c (%): 5.4±0.19, 5.7±0.25 (NGT); 5.4±0.17, 5.7±0.22 (IFG); 5.8±0.12, 6.0±0.21 (IA1c); 5.4±0.21, 5.7±0.27 (I1hG) (from http://www.ngsp.org/convert1.asp).

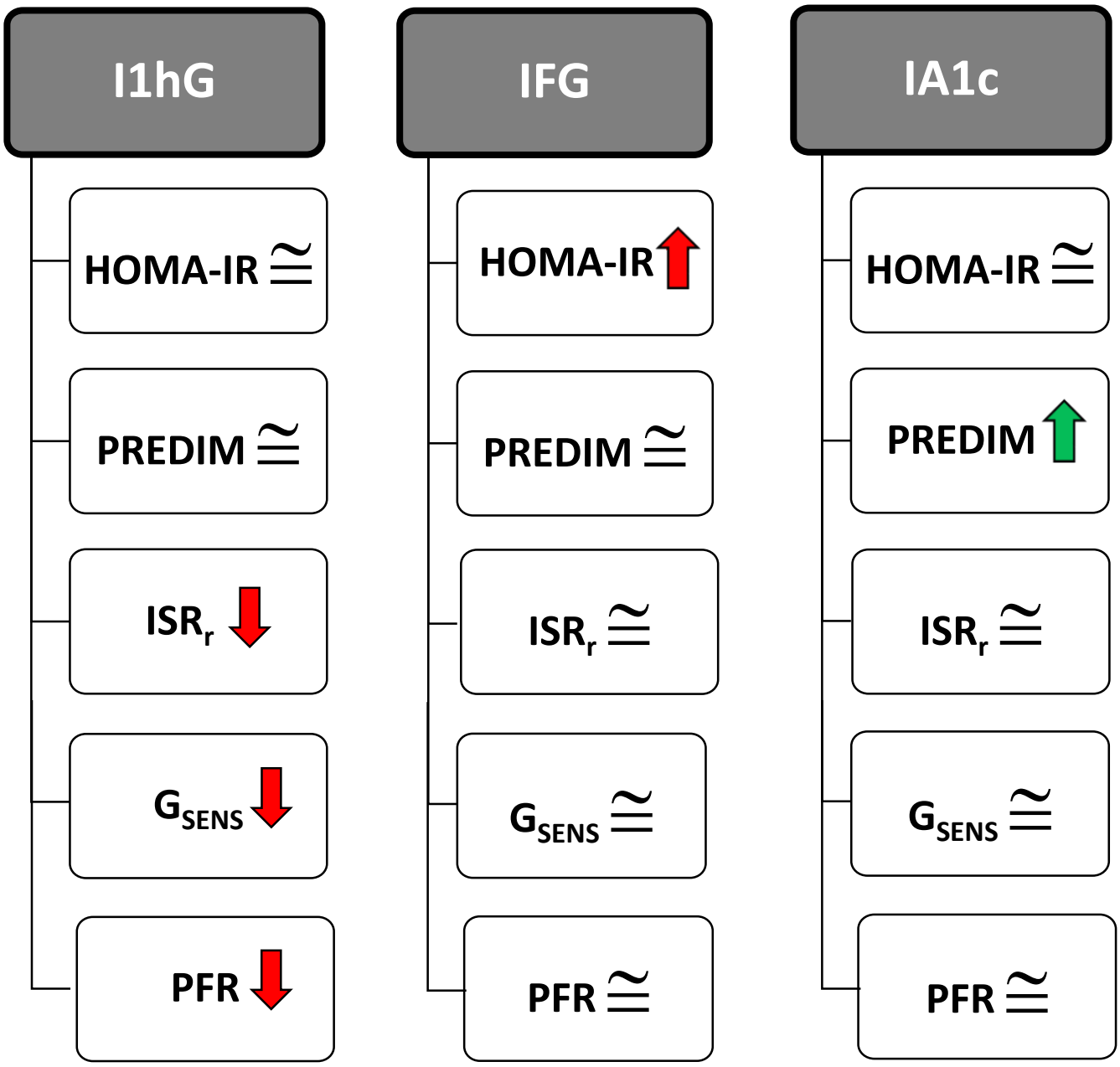
Figure Legends

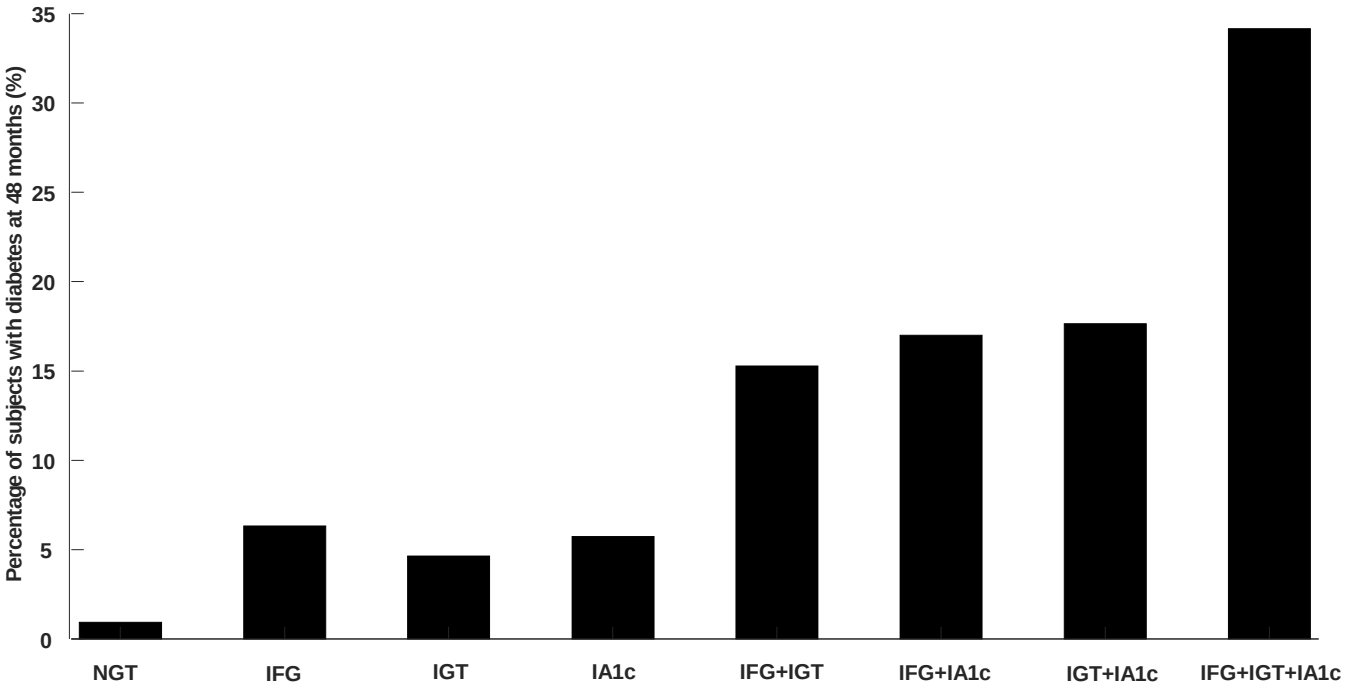
Figure 1 - Summary of the main differences among groups of prediabetes with an isolated defect (panel A) and with a double defect (panel B) for the parameters of insulin sensitivity/resistance and insulin secretion/beta-cell function. HOMA-IR: homeostasis model assessment - insulin resistance; PREDIM: predicted M; ISR_r: insulin secretion at reference glucose (6 mmol/l); PFR: potentiation factor; ⇑: higher parameter value; ⇓: lower parameter value; ÷: intermediate parameter value; ≅: similar parameter values (in two groups); green: better condition; red: worse condition.

Figure 2 - Summary of the main differences among I1hG, IFG and IA1c for the parameters of insulin sensitivity/resistance and insulin secretion/beta-cell function. HOMA-IR: homeostasis model assessment - insulin resistance; PREDIM: predicted M; ISR_r: insulin secretion at reference glucose (6 mmol/l); PFR: potentiation factor; R_{SENS}: rate sensitivity; ⇑: higher parameter value; ⇓: lower parameter value; ÷: intermediate parameter value; ≅: similar parameter values (in two groups); green: better condition; red: worse condition.

Figure 3 - Percentage of participants with type 2 diabetes at 48 months in the different groups. The number of participants developing diabetes on the total of each glucose tolerance group was 5/532 in NGT, 34/538 in IFG, 2/43 in IGT, 9/157 in IA1c, 11/72 in IFG+IGT, 43/253 in IFG+IA1c, 3/17 in IGT+IA1c, 27/79 in IFG+IGT+IA1c (*i.e.*, 3DEF), respectively.


A
IFG
HOMA-IR ↑
PREDIM ÷
ISR$_r$ ÷
PFR ↑
IGT
HOMA-IR ≅
PREDIM ↓
ISR$_r$ ↓
PFR ↓
IA1c
HOMA-IR ≅
PREDIM ↑
ISR$_r$ ↑
PFR ÷
B
IFG+IGT
HOMA-IR ↑
PREDIM ↓
ISR$_r$ ↓
PFR ≅
IFG+IA1c
HOMA-IR ≅
PREDIM ↑
ISR$_r$ ≅
PFR ↑
IGT+IA1c
HOMA-IR ≅
PREDIM ÷
ISR$_r$ ≅
PFR ↓


I1hG
HOMA-IR ≅
PREDIM ≅
ISR$_r$ ↓
G$_{SENS}$ ↓
PFR ↓
IFG
HOMA-IR ↑
PREDIM ≅
ISR$_r$ ≅
G$_{SENS}$ ≅
PFR ≅
IA1c
HOMA-IR ≅
PREDIM ↑
ISR$_r$ ≅
G$_{SENS}$ ≅
PFR ≅


Percentage of subjects with diabetes at 48 months (%)
35
30
25
20
15
10
5
0
NGT
IFG
IGT
IA1c
IFG+IGT
IFG+IA1c
IGT+IA1c
IFG+IGT+IA1c

Online-Only Supplemental Material

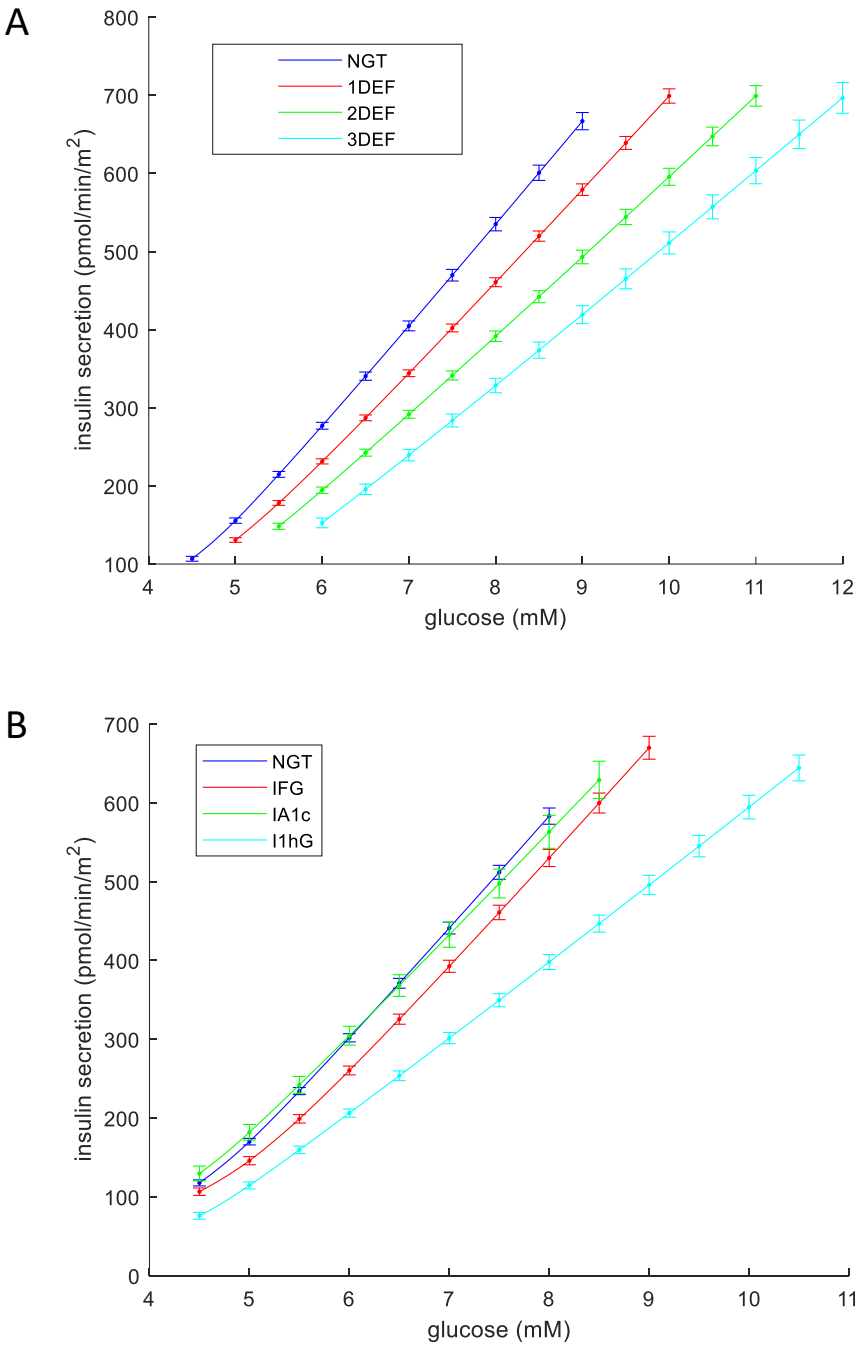


Figure S1 - Model-determined relationship between insulin secretion and glucose concentration in NGT, 1DEF, 2DEF and 3DEF (panel A), and in NGT, IFG, IA1c and I1hG (IGT excluded for low sample size), in the analysis with added 1h glycemia criterion (panel B) (mean±standard error). The average slope of the curves is the beta-cell glucose sensitivity, G_{SENS}.

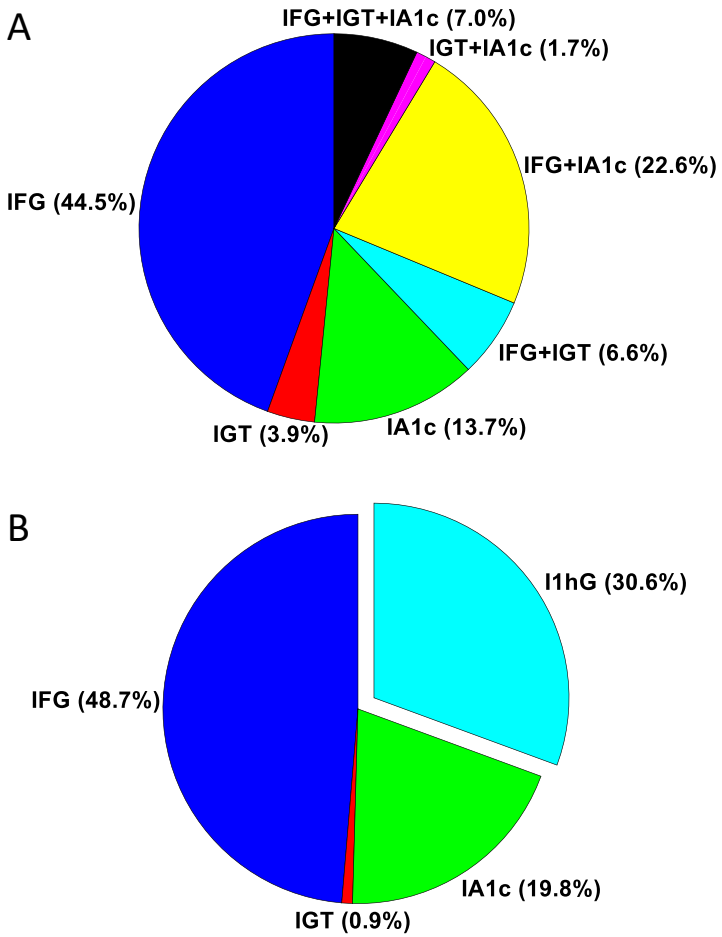


Figure S2 - Percentage of participants in the different prediabetes groups. Percentages in the groups pooling a single or a double defect were 62.1% and 30.9%, respectively (panel A); percentage of participants with a single defect, including 1h glycemia criterion (panel B).