Relationship Between Insulin Resistance and β-Cell Dysfunction in Subphenotypes of Prediabetes and Type 2 Diabetes

Kristine Færch, Nanna B. Johansen, Daniel R. Witte, Torsten Lauritzen, Marit E. Jørgensen, and Dorte Vistisen

Steno Diabetes Center (K.F., N.B.J., M.E.J., D.V.), 2820 Gentofte, Denmark; Danish Diabetes Academy (N.B.J.), 5000 Odense, Denmark; Centre de Recherche Public de la Santé (D.R.W.), 1445 Strassen, Luxembourg; and Institute of Public Health (T.L.), Section of General Practice, University of Aarhus, 8000 Aarhus, Denmark

Context: There is little overlap between diabetes diagnosed by glycated hemoglobin (HbA1c) and blood glucose, and it is unclear which pathophysiological defects are captured when using HbA1c for diagnosis.

Objective: We examined and compared the relationship between insulin sensitivity and β-cell function in different subphenotypes of prediabetes and type 2 diabetes (T2D).

Design, Setting, and Participants: A cross-sectional analysis of the Danish ADDITION-PRO study was performed (n = 1713). Participants without known diabetes were classified into subgroups of prediabetes and T2D based on fasting or 2-hour glucose criteria or HbA1c. Insulin sensitivity and insulin release were determined from glucose and insulin concentrations during the oral glucose tolerance test, and disposition indices were calculated.

Results: Individuals with prediabetes or T2D diagnosed by fasting glucose had lower absolute insulin release (P < .01) and higher insulin sensitivity in response to glucose intake (P < .01) but a similar disposition index (P = .36), compared with individuals with elevated 2-hour glucose concentrations. Individuals with HbA1c-defined T2D or prediabetes had a mixture of the pathophysiological defects observed in the glucose-defined subgroups, and individuals with normoglycemia by HbA1c had worse pathophysiological abnormalities than individuals with normoglycemia by the glucose criteria.

Conclusions: On average, the diagnostic HbA1c criteria for diabetes and prediabetes identified individuals with a mixture of the pathophysiological characteristics found when using the glucose criteria, but the diversity and pathophysiology captured by the oral glucose tolerance test cannot be captured when applying the more simple HbA1c criteria. Whether the disease progression and prognosis will differ in individuals diagnosed by fasting glucose, 2-hour glucose, or HbA1c should be examined in longitudinal studies. (J Clin Endocrinol Metab 100: 707–716, 2015)
(i-IFG or i-IGT) or in combination (IFG+IGT). These phenotypes are identified by measuring plasma glucose concentrations in the fasting state and after an oral glucose tolerance test (OGTT).

In some individuals, T2D develops as a consequence of early β-cell dysfunction; in others, the development of insulin resistance precedes defects in the pancreatic β-cells (3–5). These findings underscore that T2D is not a single disease entity but rather multiple diseases or phenotypes with different origins and disease developments. Thus, it is likely that the heterogeneity observed in the prediabetic stages is still present when fasting and 2-hour glucose levels increase into the diabetic range. Part of this heterogeneity may be explained by differences in overall obesity or body fat distribution because obesity and especially the visceral abdominal adipose tissue (VAT) are associated with insulin resistance (6, 7).

In addition to fasting and 2-hour glucose concentrations, glycated hemoglobin (HbA1c) is now recommended for diagnosis of prediabetes and T2D (8, 9). HbA1c in the prediabetic and diabetic range is associated with increased CVD morbidity and mortality (10–12), and because HbA1c does not require a fasting sample and has considerably lower variability than the glucose measures, it is often the preferred test. However, it is not clear whether the pathophysiological defects present in the distinct fasting and 2-hour glucose-derived prediabetic and diabetic subphenotypes are captured in the new HbA1c-based prediabetic and diabetic phenotypes. More epidemiological studies focusing on the heterogeneity of prediabetes and T2D will increase our understanding of the complexity of T2D as well as the implications of changing diagnostic criteria. In combination with smaller metabolic studies, large-scale epidemiological studies can also contribute to developing targeted strategies for prevention and treatment of T2D.

The aim of this study was to examine and compare the relationship between insulin sensitivity and β-cell function in different subphenotypes of prediabetes and screen-detected T2D diagnosed by fasting glucose, 2-hour glucose, and HbA1c criteria. Moreover, we aimed to examine whether the pathophysiologic differences in subphenotypes of prediabetes and T2D could be explained by differences in overall and abdominal obesity.

**Research Design and Methods**

**Study population**

The ADDITION-PRO study is a longitudinal risk-stratified cohort study of individuals at high risk for developing T2D. After participation in a population-based step-wise screening program in Danish general practice between 2001 and 2006 (13), 16 136 eligible individuals were identified. All individuals with impaired glucose regulation at screening and a random subsample of individuals at lower diabetes risk were invited to attend a follow-up health examination in 2009–2011. Of these participants, 2082 (50% of invited), mainly of white ethnicity (98%), attended (14).

The study was approved by the Ethical Committee of the Central Denmark Region (journal number 20080229) and was conducted in accordance with the Helsinki Declaration. All participants provided written informed consent before participating in the study.

**Study procedures 2009–2011**

After an overnight fast of 8 or more hours, a standard 75-g OGTT was given to all participants without known diabetes, and a physical examination was performed. Blood samples were drawn at 0, 30, and 120 minutes for the assessment of plasma glucose and serum insulin concentrations.

Information on age and sex was obtained from the unique Danish civil registration number. Body weight was measured in light indoor clothing without shoes to the nearest 0.1 kg with a Tanita body composition analyzer, and height was measured to the nearest millimeter using a fixed rigid stadiometer (Seca; Medical Scales and Measuring Systems). Clothes were estimated to weigh 0.5 kg, which was deducted from the total body weight, and body mass index (BMI) was calculated. Waist circumference was measured with an unstretchable tape measure to the nearest millimeter at the midpoint between the lower costal margin and the anterior superior iliac crest. Abdominal fat distribution was assessed by ultrasonography according to a validated protocol on a subset of participants (n = 1463 with complete data) with the participant in the supine position (Logiq9 ultrasound machine; GE Healthcare). The transducer was placed on the abdomen at the point at which the xiphoid line crosses the waist circumference. The measurement was performed at the end of a quiet expiration with minimum pressure of the transducer on the skin. The VAT was assessed by measuring the intraperitoneal distance with a 4°C (1.5–4.5 MHz) transducer placed in the longitudinal position. The distance from the peritoneum to the spine was measured twice, and the average of the two measurements was used (15). The within- and between-sonographer coefficients of variation for the VAT were 4.0% or less. The ADDITION-PRO study is described in detail elsewhere (14).

**Classification of subphenotypes of prediabetes and T2D**

We classified all study participants according to the OGTT [World Health Organization 2006 criteria (16)] as having normal glucose tolerance (NGT), intermediate hyperglycemia (i-IFG, i-IGT, IFG+IGT) or screen-detected T2D by fasting glucose only (F-DM), 2-hour glucose only (2h-DM) or both fasting and 2-hour glucose (F-2h-DM). In addition, all participants were classified according to their HbA1c levels as having normal HbA1c, high-risk (prediabetic) HbA1c, or screen-detected T2D (9 (Supplemental Figure 1)). The overlap between the different diagnostic criteria for prediabetes and T2D is shown in Supplemental Figure 2.

**Biochemical measures**

All biochemical measures were performed at the Steno Diabetes Center (Gentofte, Denmark). Plasma glucose was determined using the Hitachi 912 system (Roche Diagnostics) or the
Calculations

Using plasma glucose and serum insulin levels from the OGTT, we calculated the insulin sensitivity index (ISI0–120) (17), reflecting insulin sensitivity after oral glucose intake, and the homeostasis model assessment of insulin sensitivity (HOMA-IS) (18), reflecting insulin sensitivity in the fasting state. As a measure of absolute β-cell function, the index of acute insulin response (BIGTT-AIR) was calculated (19). BIGTT-AIR reasonably well reflects first-phase insulin release as measured by an iv glucose tolerance test in individuals with prediabetes (20). Because the amount of insulin secreted from the β-cells depends on the plasma glucose levels and the degree of insulin resistance, we also estimated two different oral disposition indices (DI): one multiplying BIGTT-AIR with ISI0–120 (DIogtt) and one multiplying BIGTT-AIR with HOMA-IS (DI fasting). The rationale for calculating both of these indices is that insulin resistance measured in the fasting state and after glucose intake reflects different mechanisms. Insulin resistance in the fasting state is mainly related to the liver, whereas the peripheral tissues play a more predominant role for insulin resistance measured during an OGTT; both features are relevant when estimating the DI (21).

Statistical analysis

The present analysis is based on data collected at the follow-up examination (cross-sectional analysis). Participants with known diabetes (n = 336), those fasting less than 8 hours prior to the health examination (n = 19), and those who could not be classified due to missing information were excluded (n = 14), leaving 1713 individuals for analysis (only 1463 for analyses including VAT).

To examine differences in characteristics between glycemic groups, we performed an overall ANOVA, and if significant, post hoc t tests were used to study pairwise differences. The first model was adjusted for age and sex; the next included further adjustment for BMI or VAT, height, and waist circumference. The ISI0–120, HOMA-IS, BIGTT-AIR, and disposition indices were logarithmically transformed before the analysis to fulfill the assumption of normality of the residuals. Statistical analyses were performed in SAS version 9.2 (SAS Institute). A two-sided 5% level of significance was adjusted for multiple testing with the method of Benjamini and Hochberg (22) in all pair-wise comparisons regarding insulin sensitivity and β-cell function.

Results

Characteristics of the study population

Using the OGTT criteria, more men than women had i-IFG and T2D (P ≤ .047 for pair-wise differences; Table 1). Individuals with i-IGT were slightly older than the other groups (P ≤ .043) except the 2h-DM group (P = .179). BMI, VAT, and waist circumference were higher in individuals with T2D and IFG+IGT than in those with NGT (P < .001), and the F-2h-DM group had the highest BMI (P ≤ .003), waist circumference (P ≤ .009), and VAT (P ≤ .028) among all groups.

| Characteristics of the study population | Characteristics of the study population |
|----------------------------------------|----------------------------------------|
| OGTT Criteria                          |                                       |
| NGT                                    | n = 336                               |
| IFG                                    | 66.3 (6.7)                             |
| IGT                                    | 63.3 (6.0)                             |
| FDM                                    | 64.8 (6.2)                             |
| 2hDM                                   | 66.7 (6.4)                             |
| F-2hDM                                 | 64.8 (6.2)                             |
| Overall P value                        | <.001                                  |
| HbA1c < 6.0% (42–46 mmol/mol)          | 32.2 (5.6)                             |
| HbA1c 6.0%–6.4% (42–46 mmol/mol)       | 32.2 (5.6)                             |
| HbA1c ≥ 6.5% (42–46 mmol/mol)          | 32.2 (5.6)                             |
| Overall P value                        | <.001                                  |

Data are percentages (95% confidence interval) or means (SD).

a OGTT criteria, P < .05 vs NGT. b OGTT criteria, P < .05 vs i-IFG. c OGTT criteria, P < .05 vs i-IGT. d OGTT criteria, P < .05 vs IFG+IGT. e OGTT criteria, P < .05 vs F-DM. f OGTT criteria, P < .05 vs 2 h-DM. g HbA1c criteria, P < .05 vs HbA1c, less than 6.0%.

HbA1c criteria, P < .05 vs HbA1c, 6.0%–6.4%.
By the use of the HbA1c criteria, the proportion of men and women did not differ between groups, but individuals with prediabetes were significantly older than those with T2D or normal HbA1c ($P \leq .002$; Table 1). BMI, VAT, and waist circumference were highest in individuals with T2D ($P < .001$ for all) but also were higher in individuals with prediabetic HbA1c compared with the group with normal HbA1c ($P < .001$).

### Insulin sensitivity and absolute insulin secretion

After adjustment for age, sex, and multiple testing, insulin sensitivity after oral glucose intake (ISI0–120) was not statistically significantly different between the i-IGT and F-DM group ($P = .093$) but differed between all other groups with the i-IGT and 2h-DM groups having lower ISI0–120 than the i-IFG and F-DM groups, respectively ($P \leq .012$ for all; Figure 1A). The 2h-DM, IFG+IGT, and F-DM groups did not differ with regard to insulin sensitivity in the fasting state (HOMA-IS, $P \geq .103$; Figure 1B), but there was a tendency of a difference between the 2h-DM and F-DM groups ($P = .057$). All other groups differed from each other ($P \leq .019$) with the F-2h-DM group having the lowest HOMA-IS (Figure 1B). In terms of absolute $\beta$-cell function, BIGTT-AIR was significantly lower in individuals with i-IFG ($P < .001$) and IFG+IGT ($P < .001$) but was higher in the i-IGT group ($P < .001$) than in the NGT group (Figure 1, A and B). The diabetic groups with F-DM and F-2h-DM did not differ with regard to BIGTT-AIR ($P = .505$), and those with 2h-DM had BIGTT-AIR levels comparable with the NGT ($P = .164$) and i-IGT ($P = .497$) groups (Figure 1, A and B). All other comparisons of BIGTT-AIR were statistically significant ($P \leq .002$ for all).

In general, the group with normal HbA1c seemed to have lower levels of absolute insulin secretion and insulin sensitivity compared with those with NGT (Figure 1, C and D). Within the HbA1c groups, those with prediabetes had lower BIGTT-AIR than those with normal HbA1c ($P < .001$) but higher than those with T2D ($P = .007$).

### Disposition indices

There was a step-wise decline in DI0GTT from NGT to prediabetes and T2D ($P < .001$ for all; Figure 2A). However, DI0GTT was not significantly different when comparing i-IFG with i-IGT ($P = .599$) and F-DM with 2h-DM ($P = .361$). All other pair-wise differences in DI0GTT were statistically significant ($P \leq .003$ for all). DI2hDM was lower in i-IFG than in i-IGT ($P < .001$) and in F-DM than in 2h-DM ($P < .001$). In contrast, DI2hDM was not lower in individuals with IFG+IGT ($P = .102$) or 2h-DM ($P = .214$) than in those with i-IFG; but all other differences in DI2hDM were statistically significant ($P \leq .034$; Figure 2B).

Compared with individuals with NGT, people with HbA1c $< 6.0\%$ tended to have lower disposition indices (Figure 2, A and B). Both DI0GTT and DI2hDM differed significantly among all three HbA1c groups ($P \leq .001$).

### Role of obesity

Figure 3 shows percentage differences in the above pathophysiological features in the different OGTT and HbA1c defined subphenotypes of prediabetes and T2D in relation to those with normal glucose regulation before and after adjustment for BMI, age, and sex. With the adjustment for BMI, the following differences became insignificant: BIGTT-AIR between i-IGT and NGT ($P = .669$), HOMA-IS between F-2h-DM and F-DM ($P = .191$), ISI0–120 between IFG+IGT and i-IGT ($P = .124$) and between F-2h-DM and 2h-DM ($P = .111$). All other differences remained statistically significant after adjustment for BMI (and multiple comparisons). By adjustment for VAT, height, and waist circumference the same differences as adjustment for BMI became insignificant. In addition, the differences in HOMA-IS between IFG+IGT and i-IFG ($P = .177$) and between 2h-DM and i-IGT ($P = .068$) disappeared.

A schematic overview of the differences in absolute early insulin release, insulin sensitivity, and disposition indices between groups is presented in Table 2.

### Discussion

This study highlights that T2D is not a single disease entity but rather multiple subdiseases or subphenotypes characterized by different underlying pathophysiological mechanisms, starting in the early prediabetic states. Our findings also suggest that the differences in $\beta$-cell function and overall insulin sensitivity between subgroups of individuals with prediabetes and type 2 diabetes are not explained by overall and visceral fat. In addition, we showed that the newly implemented diagnostic HbA1c criteria on average identify prediabetic and diabetic individuals with a mixture of pathophysiological characteristics compared with those found by the glucose criteria. However, the normoglycemic individuals defined by HbA1c have slightly worse insulin resistance and $\beta$-cell function than those with normoglycaemia by the OGTT.

### Subphenotypes of prediabetes

Based on the OGTT criteria, we identified three subgroups of prediabetes and three subgroups of T2D, all with different underlying pathophysiology. Although we found large variation within each group, the mean differences between subphenotypes of prediabetes are in alignment with previous observations. By use of detailed gold
standard methods, it has been shown that insulin resistance in the liver is a characteristic of people with i-IFG, whereas insulin resistance in the peripheral tissues is an important feature of i-IGT (23–26). Our surrogate markers of insulin sensitivity in the fasting state (mainly liver) and during the OGTT (mainly peripheral tissues) support this notion.

Individuals with i-IFG had reduced early-phase insulin release compared with individuals with i-IGT, which is also in accordance with previous findings (23, 24). It has previously been demonstrated that obese individuals with i-IFG have a 40% deficit in relative β-cell volume compared with obese individuals with normoglycemia (27). This observation suggests that the β-cell failure observed in individuals with i-IFG represents an early process in the development of T2D, which is not likely to be secondary to hyperglycemia or insulin resistance. Whether the reduction in β-cell function in i-IFG is caused by genetic factors, chronic low-grade inflammation, amyloid deposition, or other factors (28–30) warrant further studies.

In terms of relative β-cell function, DI_{OGTT} was comparable between the i-IFG and i-IGT groups, whereas DI_{fasting} was lower in i-IFG than in i-IGT, potentially demonstrating different relative contributions of the liver and the skeletal muscles in the control of fasting vs post-OGTT glucose regulation (21, 31, 32). The IFG+IGT group had a combination of the defects observed in the isolated IFG and IGT groups but with disposition indices close to the levels observed in F-DM and 2h-DM, sup-

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**Figure 1.** BIGTT-AIR as a function of ISI_{0–120} (panels A and C) and HOMA-IS (panels B and D) in individuals with different subphenotypes of prediabetes and type 2 diabetes based on the glucose (panels A and B) or HbA1c criteria (panels C and D). The thin gray lines show different levels of the disposition index. Data are medians (interquartile range).
porting progressive β-cell failure when moving from i-IFG to IFG+IGT (33).

Prediabetic individuals defined by HbA1c had pathophysiological defects resembling a mixture of the three prediabetic groups defined by the OGTT. That HbA1c reflects an average of the pathophysiological defects captured by the OGTT-defined groups of prediabetes has also been found in two Italian studies (34, 35).

Subphenotypes of type 2 diabetes

We hypothesized that the pathophysiological drivers of elevated fasting and 2-hour glucose concentrations in the prediabetic states may continue to operate in the diabetic range. Therefore, we subdivided patients with screen-detected T2D into subgroups based on the fasting and 2-hour plasma glucose levels.

Compared with individuals with i-IGT, diabetic individuals with isolated elevated 2-hour glucose concentrations (ie, 2h-DM) had the same level of absolute early insulin release but significantly lower insulin sensitivity in both the fasting and glucose-stimulated state, resulting in lower disposition indices. Despite the cross-sectional nature of these results, they indicate that progression from i-IGT to 2h-DM is characterized by development of insu-
lin resistance and a lack of ability to sufficiently compensate by increasing insulin secretion. Recently we showed in a longitudinal cohort of British individuals that the 2h-DM phenotype was preceded by a steep increase in 2-hour insulin concentration, a decline in insulin sensitivity, and a stable relative β-cell function (3), supporting our observations from the present study.

In contrast to the 2h-DM phenotype, F-DM was characterized by significantly reduced β-cell function as compared with i-IFG individuals, both in absolute terms and in relation to insulin resistance (DIOGTT and DIfasting). Individuals with F-DM also had a significant reduction in insulin sensitivity as compared with individuals with i-IFG. Chinese individuals with F-DM were also characterized by reduced β-cell function as compared with individuals with i-IFG, whereas whole-body insulin sensitivity did not differ between people with i-IFG and F-DM (5). In the longitudinal Whitehall II study, individuals diagnosed with F-DM had impaired β-cell function up to 18 years before their T2D diagnosis (3), suggesting that a reduction in the insulin secretory capacity precedes the development of peripheral insulin resistance in individuals with isolated hyperglycaemia. However, more prospective studies are needed to confirm this hypothesis.

As expected, the F-2h-DM phenotype was characterized by worse pathophysiological defects than the groups diagnosed with isolated fasting or 2-hour hyperglycaemia. These results are in accordance with other studies (3, 5). However, most people will progress to F-2h-DM from either F-DM, 2h-DM, or IFG+IGT and the underlying pathophysiological defects leading to F-2h-DM may...
therefore differ, depending on how far in the diabetes progression the individual is.

The diversity found within the diabetes subgroups captured by the glucose criteria cannot be reflected when using the HbA1c definition of diabetes because this is only one category. In general, patients with HbA1c-diagnosed diabetes had insulin resistance and β-cell dysfunction in the same low range as patients diagnosed by F-DM and F-2h-DM, suggesting that the cut point for HbA1c of 6.5% identifies individuals in direct need for therapies to correct disturbances in beta cell function.

Role of obesity

Because obesity and mainly abdominal fat is an important driver of insulin resistance (6, 7), we hypothesized that part of the differences in insulin sensitivity observed between the prediabetic and diabetic subphenotypes were attributed differences in overall and/or abdominal obesity. BMI or VAT did not explain the differences in ISI0–120 between the i-IFG vs i-IGT or F-DM vs 2h-DM groups, but differences in HOMA-IS between groups were largely explained by differences in overall and particularly abdominal visceral obesity. This observation indicates a close link between fat depots in the abdomen and glucose regulation in the fasting state, potentially mediated by adipokines (36). Of interest, adjustment for overall or abdominal visceral obesity also explained the excessive early insulin release in the i-IGT group compared with i-IFG. However, it did not change the differences in disposition indices between groups, indicating that the effect of obesity is accounted for by taking insulin resistance into account when estimating in vivo β-cell function.

Fasting glucose, OGTT, or HbA1c for diagnosis?

The availability and practical feasibility of the different diagnostic tests should drive the decision of which test to use. Yet our findings show that diagnosis of prediabetes or T2D based on fasting glucose, 2-hour glucose, or HbA1c will identify people with a different underlying pathophysiology. On average, HbA1c identifies individuals with both insulin resistance and β-cell function, but HbA1c does not reflect the heterogeneity of prediabetes and T2D captured by the OGTT and therefore cannot stand alone if reversal of the underlying pathophysiology is a treatment goal (32).

Despite the normal absolute insulin secretion in individuals with 2h-DM, they have the same excess risk of all-cause and CVD mortality as individuals diagnosed with T2D based on fasting glucose or HbA1c (37). Only part of the individuals with 2h-DM (~42%) will be captured by the new HbA1c criterion for diabetes (38), and because of the limited use of the OGTT in clinical practice, many of these high-risk individuals will remain undiagnosed.

Strengths and limitations

The main strength of this cross-sectional study is the large number of study participants from whom we have measurements of circulating glucose and insulin concentrations during OGTTs as well as detailed measures of anthropometry. Because of the large study population, we used surrogate measures of insulin sensitivity, early insulin release, and β-cell function. Because fasting and 2-hour glucose concentrations were included in the calculations of insulin sensitivity and β-cell function, they partly overlap with the classification of prediabetes and T2D by the glucose criteria. However, in the calculations of ISI0–120 and BIGTT-AIR, also fasting and 2-hour insulin concentrations as well as information on body weight was included, limiting the risk of circular conclusions. Moreover, the pathophysiological defects in prediabetic individuals observed using surrogate measures of insulin sensitivity and early insulin release correspond well with

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**Table 2. Overview of the Defects in Insulin Sensitivity and β-Cell Function Observed in the Different Subgroups of Prediabetes and T2D**

| OGTT definition | Absolute Early Insulin Release | Insulin Sensitivity (Glucose-Stimulated State) | Insulin Sensitivity (Fasting State) | Relative β-Cell Function (Glucose Stimulated State) | Relative β-Cell Function (Fasting State) |
|-----------------|-------------------------------|-----------------------------------------------|-----------------------------------|-----------------------------------------------|-----------------------------------------------|
| NGT             | Ref.                          | Ref.                                          | Ref.                              | Ref.                                          | Ref.                                          |
| i-IFG           | ↓                             | ↑                                             | ↓                                 | ↓                                             | ↓                                             |
| i-IGT           | ↓                             | ↑                                             | ↓                                 | ↓                                             | ↓                                             |
| IFG+IGT         | ↓                             | ↓                                             | ↓                                 | ↓                                             | ↓                                             |
| F-DM            | ↑                             | ↑                                             | ↑                                 | ↑                                             | ↑                                             |
| 2h-DM           | ↓                             | ↑                                             | ↓                                 | ↓                                             | ↓                                             |
| F-2h-DM         | ↑                             | ↑                                             | ↑                                 | ↑                                             | ↑                                             |
| HbA1c definition | HbA1c < 6.0%                  | ↑                                             | ↓                                 | ↓                                             | ↓                                             |
| HbA1c 6.0%–6.4% | ↓                             | ↓                                             | ↓                                 | ↓                                             | ↓                                             |
| HbA1c ≥ 6.5%    | ↓                             | ↓                                             | ↓                                 | ↓                                             | ↓                                             |

+++ unchanged; ↓↓ mildly decreased; ↓↓↓ moderately decreased; ↓↓↓↓ highly decreased; ↑↑ mildly increased; Ref, Reference.
previous findings in smaller studies using the gold standard clamp technique as well as an iv glucose tolerance test (24). This suggests that relatively simple surrogate measures can be used to estimate pathophysiological defects in high-risk individuals. How to implement knowledge on individual pathophysiology in the prevention and treatment of T2D still needs to be determined. Because of the relatively large day-to-day variation in fasting and especially 2-hour plasma glucose levels (39) and thereby a potential risk of misclassification, repeated measurements are necessary before universal or population-specific cut points for determining, for example, insulin resistance should be used on an individual level.

The sampling of participants for the ADDITION-PRO study was predominantly based on glucose tolerance status as measured by an OGTT, and not by HbA1c, at a step-wise screening 5–7 years before the ADDITION-PRO examination (14). This selection of participants means that the likelihood of being classified with prediabetes or diabetes by the OGTT at follow-up was larger than the likelihood of being classified by HbA1c. Therefore, the distribution of participants according to the different diagnostic criteria is not representative for the general Danish population. There is no reason to believe that the differences found between groups should not apply to the general population in Denmark, but our findings need confirmation in other European and non-European cohorts and ultimately in longitudinal studies in which the pathophysiology underlying progression from one glucose tolerance state to another can be determined.

Conclusion and perspectives
In conclusion, the relative contributions of insulin resistance and defective insulin release differ widely between the prediabetic and diabetic subgroups diagnosed by fasting vs 2-hour glucose concentrations. Individuals diagnosed with prediabetes or T2D by elevated fasting plasma glucose levels had lower absolute insulin release and higher overall insulin sensitivity than those diagnosed by 2-hour glucose concentrations, although their overall β-cell function (ie, DI) was comparable. Overall and abdominal obesity partly explained this diversity.

On average, the diagnostic HbA1c criteria for diabetes and prediabetes identified individuals with a mixture of the pathophysiological characteristics found when using plasma glucose criteria, but the diversity identified by the glucose criteria is not captured when applying the more simple HbA1c criteria. Our findings confirm that T2D is not a single disease entity but rather multiple subdiseases with different characteristics. There is a need for longitudinal studies examining whether disease progression and prognosis will differ in individuals diagnosed by fasting glucose, 2-hour glucose, or HbA1c. Moreover, randomized controlled trials should clarify whether a treatment targeting the different phenotypes will prevent the progression of prediabetes or T2D and reverse β-cell dysfunction in individuals with early insulin-secretory defects.

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Author contributions included the following: K.F. conceived the idea, researched and interpreted the data, and wrote the manuscript. D.V. researched and interpreted the data, contributed to the discussion, and reviewed and edited the manuscript. N.B.J., D.R.W., and T.L. designed the ADDITION-PRO study, contributed to the discussion, and reviewed and edited the manuscript. M.E.J. contributed to the discussion and reviewed and edited the manuscript. All authors approved the final version of the manuscript. K.F. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Address all correspondence and requests for reprints to: Kristine Færch, PhD, Steno Diabetes Center A/S, Niels Steensens Vej 2, DK-2820 Gentofte, Denmark. E-mail: krif@steno.dk.

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