Pathophysiological Characteristics Underlying Different Glucose Response Curves: A Latent Class Trajectory Analysis From the Prospective EGIR-RISC Study

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OBJECTIVE

Glucose measurements during an oral glucose tolerance test (OGTT) are useful in predicting diabetes and its complications. However, knowledge of the pathophysiology underlying differences in glucose curve shapes is sparse. We examined the pathophysiological characteristics that create different glucose curve patterns and studied their stability and reproducibility over 3 years of follow-up.

RESEARCH DESIGN AND METHODS

We analyzed data from participants without diabetes from the observational cohort from the European Group for the Study of Insulin Resistance: Relationship between Insulin Sensitivity and Cardiovascular Disease study; participants had a five–time point OGTT at baseline (n = 1,443) and after 3 years (n = 1,045). Measures of insulin sensitivity and secretion were assessed at baseline with a euglycemic-hyperinsulinemic clamp and intravenous glucose tolerance test. Heterogeneous glucose response patterns during the OGTT were identified using latent class trajectory analysis at baseline and at follow-up. Transitions between classes were analyzed with multinomial logistic regression models.

RESULTS

We identified four different glucose response patterns, which differed with regard to insulin sensitivity and acute insulin response, obesity, and plasma levels of lipids and inflammatory markers. Some of these associations were confirmed prospectively. Time to glucose peak was driven mainly by insulin sensitivity, whereas glucose peak size was related to both insulin sensitivity and secretion. The glucose patterns identified at follow-up were similar to those at baseline, suggesting that the latent class method is robust. We integrated our classification model into an easy-to-use online application that facilitates the assessment of glucose curve patterns for other studies.

CONCLUSIONS

The latent class analysis approach is a pathophysiologically insightful way to classify individuals without diabetes based on their response to glucose during an OGTT.

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The term "prediabetes" covers a range of heterogeneous metabolic states with varying degrees of insulin resistance and β-cell dysfunction (1). Fasting plasma glucose (FPG) and 2-h postload plasma glucose (2hPG) measurements during an oral glucose tolerance test (OGTT) are the most common ways to classify individuals with prediabetes. The OGTT is seldom used in clinical practice, where FPG or HbA_{1c} are preferred for practical reasons. However, a recent study showed that HbA_{1c} may not be able to capture the pathophysiological diversity of impaired fasting glycemia (IFG) and impaired glucose tolerance (IGT) (2). Also, individuals identified as having prediabetes on the basis of the HbA_{1c} criterion seem to have a different response to physical activity and lower probability of reverting to normoglycemia than those identified through the use of the glucose criteria (3). Thus, understanding the pathophysiological differences between subgroups of individuals with prediabetes is crucial to stratify risk and to facilitate more targeted prevention-focused interventions. In line with this, it has recently been suggested that insulin sensitivity and secretion should be assessed in detail from glucose and insulin measures at 0, 30, and 120 min during an OGTT in all individuals with IFG or IGT, despite the increased cost and effort (4).

While 30-min plasma glucose and insulin concentrations are necessary to evaluate first-phase insulin secretion, intermediate time points during an OGTT also have been shown to be useful in predicting diabetes. For instance, 1-h postload plasma glucose (1hPG) concentration has consistently been shown to have a stronger association with incidence of type 2 diabetes than 2hPG, and it has been associated with both cardiovascular disease and mortality (5–7). Likewise, individuals without diabetes but who have elevated 30-min plasma glucose levels have an increased risk of future diabetes and all-cause mortality, independently of FPG and 2hPG levels (8). However, most previous studies considered the different time points during an OGTT separately and thereby missed the opportunity to consider different OGTT curve features in relation to future end points. We recently demonstrated how assessing time to glucose peak through the use of a simple longitudinal analysis technique, rather than just selecting the time point with the highest value, can improve the predictive power of this variable (9). When considering the shape of glucose curves during OGTTs, the most common approach is to classify them as mono- or biphasic shapes (10). This method does not, however, take into account measurement error; it fails to categorize some individuals, and, most importantly, it may be too crude to capture subtle differences between groups. We have taken a different approach and used a data-driven method—latent class trajectory analysis—to classify individuals based only on their glucose response during an OGTT (11,12). A major advantage of this approach over the mono-/biphasic classification is that it does not necessarily require a five-point OGTT to classify individuals. Also, it returns class membership probabilities indicating the certainty of the classification. Using this method, we identified heterogeneous patterns of glucose response that cannot be captured by the mono-/biphasic classification (13). However, previous studies have not addressed how pathophysiological characteristics and biomarkers of metabolic functions are linked to different glucose curve patterns. Moreover, the stability and reproducibility of the different glucose curve patterns over time have never been examined.

Thus, the aims of this study were threefold: *1*) to examine the association of pathophysiological characteristics and biomarkers of metabolic functions with different glucose curve patterns, *2*) to study the stability and reproducibility of glucose curve patterns over time, and *3*) to develop an easy-to-use online application that facilitates the assessment of glucose curve patterns for individuals in other populations and settings.

RESEARCH DESIGN AND METHODS

Study Design and Participants

The Relationship between Insulin Sensitivity and Cardiovascular Disease (RISC) study is an observational prospective study with follow-up examinations at baseline (n = 1,566) and at 3 years (n = 1,059). At baseline the cohort was clinically healthy; individuals who were receiving treatment or who had a serious medical condition were not eligible to participate (14). The process used to select participants for our analyses is displayed in Supplementary Fig. 1. For the cross-sectional analysis, we excluded individuals with missing information on age or sex (n = 53), those who did not have plasma glucose measured at all five time points during the OGTT (n = 42), and all participants with diabetes detected through screening (n = 28). This resulted in a study population of 1,443 individuals for the cross-sectional analysis. Among those, 384 individuals did not attend follow-up, 3 were receiving glucose-lowering treatment, and 11 did not have glucose measurements at all five OGTT time points at follow-up, resulting in 1,045 participants for the prospective analysis. Baseline characteristics were compared between participants and nonparticipants at follow-up.

Procedures and Measurements

At the baseline and follow-up examinations, participants underwent a 75-g OGTT after an overnight fast. Plasma glucose, serum insulin, and C-peptide concentrations were assessed at five time points during the OGTT (0, 30, 60, 90, 120 min). A euglycemic-hyperinsulinemic clamp and an intravenous glucose tolerance test (IVGTT) were performed at baseline. A detailed description of the clamp protocol was published previously (15). Insulin sensitivity was described by M/I, where M is the mean glucose concentration infused over the last 40 min of the clamp and I is the steady-state serum insulin concentration measured over the same time period. Glucose-induced secretory response (GISR) was expressed as the integral of incremental insulin secretion divided by the mean incremental glucose following administration of an intravenous glucose bolus (15). The disposition index was calculated as GISR $\times$ M/I.

Plasma concentrations of adiponectin and leptin were measured at baseline after an overnight fast. C-reactive protein (CRP) and interleukin-6 (IL-6) were measured in those participating at both baseline and follow-up, and samples were analyzed at the same time. Body composition (fat mass) was determined with a bioimpedance TBF-300 Total Body Composition Analyzer (Tanita, Tokyo, Japan). Data on physical activity, alcohol consumption, smoking habits, and family history of diabetes were assessed using standard questionnaires.

Type 2 diabetes was defined as FPG $\geq$7.0 mmol/L, 2hPG $\geq$11.1 mmol/L, having diabetes diagnosed by a doctor, or using glucose-lowering treatment.

Detailed descriptions of the study protocol were published previously (14,16).

Statistical Analysis

Assessment of Glucose Patterns During the OGTT

We applied latent class trajectory analysis to identify glucose patterns during the OGTT based on measurements taken at all five time points. This approach offers a data-driven way to classify individuals into subgroups that differ with regard to specific parameters—in our case, change over time (11,12). We included linear, quadratic, and cubic time terms to model the change in plasma glucose over the 2 h. These time effects could vary between the latent classes. We included an individual-specific random intercept in the model to account for the repeated nature of the data. The optimal number of classes was determined by considering model fit (Bayesian Information Criterion, a measure based on the log-likelihood penalizing complex models), distribution of class membership probabilities, class sizes, and interpretability of the identified patterns (12). The model returns not only the identified global glucose response patterns but also class membership probabilities for each participant, and these can be used to assign individuals to the most likely latent glucose response pattern class (hard assignment based on the highest class membership probability). It can also classify new observations into the previously derived glucose response pattern classes. This feature was used to determine class memberships at follow-up. A detailed, more technical description of the latent class analysis and the development of the online application is included in the Supplementary Data.

Cross-sectional Associations

Categorical and continuous variables across latent classes are described as frequencies (percentages) and the median (interquartile range), respectively. Adjusted differences were estimated with multinomial logistic and quantile regression models using two nested sets of variables: age and sex (model 1), and the model 1 variables plus smoking status, physical activity, alcohol consumption, and BMI (model 2).

We also investigated the associations of insulin sensitivity and secretion with glucose peak characteristics (time and size). To assess glucose peak time and size, we used mixed-effects models to fit cubic glucose trajectories. We included random effects corresponding to all fixed terms to get individual-specific model coefficients. Then we used these estimates to extract the peak time and size, as described previously (9). We applied relative importance analyses, with continuous peak size and time as outcomes and with insulin sensitivity (M/I) and secretion (GISR) as determinants, using the relaimpo R package (17). We used a metric for importance based on the decomposition of R^2 in the model.

Stability and Reproducibility of Patterns

First, we investigated how the glucose curves looked at follow-up based on the individuals' classification at baseline (irrespective of their classification at follow-up). We fitted cubic trajectories using mixed-effects models that included the interaction between time terms and baseline latent classes. Second, we repeated the latent class trajectory analysis at follow-up to reclassify each individual based on their OGTT at the follow-up examination. We present the curves of the four classes we identified at follow-up.

We used a Sankey diagram to visualize the transition between different classes from baseline to follow-up. As a sensitivity analysis, we also examined the proportion of participants who stayed in the same class among those who had a class membership probability above 0.90 at baseline (n = 643). Then we selected the largest class at baseline and characterized transitions from this class. We used age- and sex-adjusted multinomial logistic regression models with class at follow-up as the outcome and baseline values of the different cardiometabolic risk factors and change during follow-up as exposures. This analysis was restricted to those exposures that exhibited a strong association with glucose pattern class membership at baseline.

RESULTS

Participants

The study population for the cross-sectional analyses included 1,443 individuals (644 men and 799 women) without diabetes (Supplementary Fig. 1). The cohort had a median (IQR) age of 44 years (37–50) and BMI of 25.1 kg/m^2 (22.8–27.9). One in four participants was a current smoker, and one in five reported being physically inactive at baseline. Baseline characteristics did not differ between participants and nonparticipants at the follow-up examination (16).

Latent Classes of Glucose Patterns

We considered a four- and a five-class solution because while the latter had the lowest Bayesian Information Criterion, class membership probabilities were higher in the four-class solution (Supplementary Figs. 2 and 3). We found this last feature more important and therefore we used the four-class solution here. We numbered the classes from one to four based on the increasing areas under the glucose curves.

Glucose, Insulin, and C-peptide Responses

The identified glucose pattern classes are plotted in Fig. 1*A* alongside their corresponding insulin and C-peptide trajectories (Fig. 1*B* and *C*). Glucose curves varied greatly between classes, with peaks occurring after 32–61 min at plasma glucose concentrations between 6.5 and 11.5 mmol/L (Fig. 1*A*). The highest peak was observed in class 3, which was characterized by the most rapid increase up to 44 min and then a similarly rapid decrease to the second-lowest 2hPG level. Insulin levels peaked consistently later than glucose levels, with the largest difference (16 min) observed in class 3. C-peptide curves peaked later than insulin curves: in classes 1, 2, and 3, ~25 min after reaching the glucose peak; in class 4, the serum C-peptide concentration was still on the rise after 2 h.

Cross-sectional Associations

Demographic and lifestyle factors by glucose pattern classes are reported in Table 1. Individuals in class 1 had the most favorable risk profile. They were youngest, most physically active, and most likely to be women, and only one in five reported a first-degree relative with diabetes. Men had more than threefold higher odds than women of being in class 3 than in class 1. Class 3 had the highest proportion of current smokers and its members had the highest degree of alcohol consumption. However, class 4 had the highest proportion of individuals with a family history of diabetes and the most physically inactive individuals.

Clinical characteristics by glucose pattern classes are also reported in Table 1. BMI and fat mass were gradually higher from class 1 to class 4, and the differences

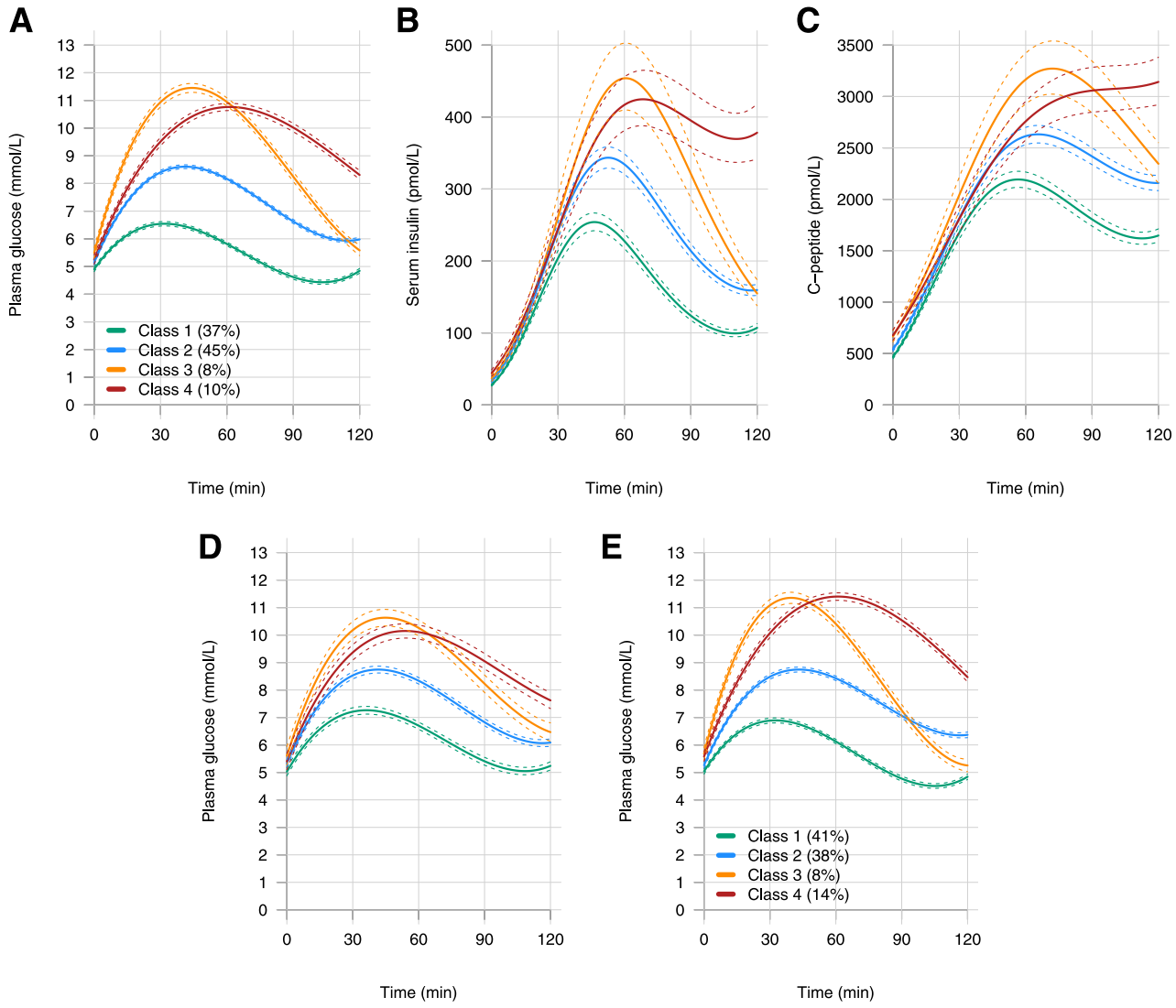

Figure 1—Plasma glucose patterns identified at baseline (*A*), corresponding serum insulin (*B*) and C-peptide (*C*) curves, and plasma glucose trajectories at follow-up for the groups identified at baseline (*D*) and independently at follow-up (*E*). Curves are estimated mean trajectories (solid lines) with 95% CIs (dashed lines).

were not explained by lifestyle factors. In the adjusted models, LDL cholesterol did not differ between classes, but HDL cholesterol was lower, whereas triglycerides were higher, from class 1 to class 4, regardless of adjustment. The inflammatory markers CRP and IL-6 both showed associations with class membership in the age- and sex-adjusted models. The association with CRP levels remained statistically significant after adjustment for lifestyle factors and BMI. Levels of adiponectin were lower from class 1 to class 4, regardless of adjustment. Although we observed large differences in absolute leptin levels by glucose pattern class, they were markedly attenuated by adjustment for lifestyle factors and BMI.

Pathophysiological Measures From the Clamp and IVGTT

Figure 2 shows clamp-based measures of insulin secretion as a function of insulin sensitivity stratified by glucose pattern class, glucose peak time, and the size of the glucose peak. A position closer to the origin, meaning also a lower disposition index, indicates higher risk for diabetes (18). The disposition index was consistently lower from class 1 to class 4, but impaired insulin sensitivity and secretion had different relative contributions (Fig. 2*A*). Individuals in classes 1 and 2 had similar insulin secretion, but those in class 2 had lower insulin sensitivity than those in class 1, also after adjustment for age and sex (median difference −27 [95% CI −33 to −21]). Classes 2 and 3 were similar with regard to insulin sensitivity, but individuals in class 3 had lower insulin secretion (median difference −13 [95% CI −21 to −4]). Last, individuals in classes 3 and 4 had similar insulin secretion, but those in class 4 had lower insulin sensitivity (median difference −33 [95% CI −42 to −19]).

Time of glucose peak was almost exclusively determined by insulin sensitivity, indicated by a 96% contribution in the relative importance analysis. The size of the glucose peak was also, and to a larger extent, driven by insulin sensitivity; however, for this characteristic insulin secretion also made a substantial contribution (32%). Both later and higher glucose peaks were associated with lower disposition indices (Fig. 2*B* and *C*).

Table 1—Demographics, lifestyle factors, and clinical characteristics of participants by latent glucose pattern classes at baseline

	Class 1	Class 2	Class 3	Class 4
Demographic and lifestyle factors				
Individuals	534 (37)	651 (45)	112 (8)	146 (10)
Age (years)	41 (35–48)	45 (38–51)	47 (41–54)	47 (41–53)
Male sex (%)	178 (33)	329 (50)	76 (69)	61 (45)
Currently smoking (%)	131 (25)	154 (24)	49 (45)	38 (29)
Physical activity				
Inactive	99 (19)	127 (21)	20 (19)	32 (25)
Minimally active	209 (41)	273 (44)	47 (44)	54 (43)
Active	205 (40)	214 (35)	39 (37)	41 (32)
Alcohol consumption per week				
<30 g	210 (40)	222 (35)	24 (22)	59 (44)
30–80 g	170 (32)	204 (32)	24 (22)	33 (25)
>80 g	145 (28)	214 (33)	61 (56)	42 (31)
Family history of diabetes (%)	106 (20)	177 (28)	45 (41)	64 (47)
Clinical characteristics				
Glucose tolerance status				
Normal	494 (93)	503 (77)	59 (53)	36 (25)
IFG	39 (7)	116 (18)	52 (46)	6 (4)
IGT	1 (0)	26 (4)	0 (0)	68 (47)
IFG and IGT	0 (0)	6 (1)	1 (1)	36 (25)
BMI (kg/m^2)	23.8 (21.7–26.3)	25.4 (23.2–28.0)	27.0 (23.9–29.7)	27.0 (25.0–29.7)
Difference[1]	Reference	1.0 (0.4–1.3)	2.2 (1.0–3.4)	2.7 (1.9–3.4)
Difference[2]	Reference	0.8 (0.3–1.3)	2.0 (1.1–3.0)	2.2 (1.5–3.0)
Fat mass (%)	27.0 (20.0–33.5)	27.1 (20.9–33.7)	28.6 (23.0–33.2)	31.2 (25.9–36.6)
Difference[1]	Reference	1.4 (0.7–2.3)	4.2 (2.2–5.3)	5.4 (4.0–6.4)
Difference[2]	Reference	1.3 (0.5–2.4)	3.6 (2.6–5.3)	4.5 (3.4–5.9)
LDL cholesterol (mmol/L)	2.7 (2.2–3.3)	2.9 (2.4–3.4)	3.2 (2.5–3.6)	3.1 (2.6–3.5)
Difference[1]	Reference	0.08 (−0.01 to 0.18)	0.23 (0.02–0.34)	0.28 (0.12–0.43)
Difference[3]	Reference	0.11 (−0.03 to 0.19)	0.14 (−0.11 to 0.26)	0.16 (−0.06 to 0.38)
HDL cholesterol (mmol/L)	1.49 (1.26–1.74)	1.33 (1.14–1.60)	1.30 (1.07–1.53)	1.27 (1.04–1.54)
Difference[1]	Reference	−0.09 (−0.14 to −0.05)	−0.12 (−0.21 to −0.03)	−0.22 (−0.25 to −0.16)
Difference[3]	Reference	−0.05 (−0.10 to −0.01)	−0.04 (−0.15 to 0.01)	−0.10 (−0.16 to −0.03)
Triglycerides (mmol/L)	0.82 (0.61–1.10)	0.96 (0.70–1.27)	1.25 (0.78–1.66)	1.13 (0.86–1.63)
Difference[1]	Reference	0.09 (0.04–0.14)	0.25 (0.14–0.43)	0.24 (0.18–0.36)
Difference[3]	Reference	0.06 (0.01–0.09)	0.19 (0.02–0.35)	0.21 (0.12–0.29)
CRP (mg/L)	0.48 (0.21–1.02)	0.71 (0.32–1.57)	0.67 (0.35–1.49)	1.33 (0.60–3.09)
Difference[1]	Reference	0.20 (0.07–0.33)	0.17 (0.04–0.32)	0.88 (0.47–1.41)
Difference[3]	Reference	0.11 (0.02–0.20)	0.10 (−0.08 to 0.23)	0.50 (0.26–1.15)
IL-6 (pg/mL)	0.63 (0.47–1.02)	0.73 (0.49–1.15)	0.86 (0.64–1.56)	0.93 (0.58–1.42)
Difference[1]	Reference	0.06 (0.01–0.12)	0.16 (0.04–0.36)	0.23 (0.12–0.44)
Difference[3]	Reference	−0.01 (−0.06 to 0.05)	0.03 (−0.06 to 0.22)	0.18 (−0.03 to 0.34)
Adiponectin (mg/L)	8.5 (6.4–11.5)	7.5 (5.5–10.0)	6.3 (4.9–9.2)	6.3 (5.0–9.0)
Difference[1]	Reference	−0.55 (−0.90 to −0.24)	−1.17 (−1.61 to −0.38)	−1.60 (−2.33 to −0.78)
Difference[3]	Reference	−0.47 (−0.84 to 0.06)	−0.89 (−1.43 to −0.17)	−1.03 (−1.38 to −0.38)
Leptin (ng/mL)	9.4 (4.3–16.4)	9.2 (4.2–18.1)	8.6 (4.1–18.4)	13.6 (7.9–25.3)
Difference[1]	Reference	1.7 (0.9–2.3)	3.3 (2.2–4.6)	5.9 (4.1–7.5)
Difference[2]	Reference	0.0 (−0.6 to 0.5)	0.5 (−0.5 to 1.6)	0.7 (−0.8 to 2.4)

For the continuous clinical characteristics, the median (Q1–Q3) is presented in the first row followed by the median (95% CI) for adjusted differences (different levels of adjustment from quantile regression models) in the second and third rows. Other data are *n* (%), except for age, which is presented as median (Q1–Q3). [1]Adjusted for age and sex. [2]Adjusted for age, sex, smoking status, physical activity, and alcohol consumption. [3]Adjusted for age, sex, smoking status, physical activity, alcohol consumption, and BMI.

Stability and Transitions

Glucose curves for each baseline latent class at the 3-year follow-up are shown in Fig. 1*D*. We observed regression to the mean; however, differences in shape and peak rankings between classes were still clear. When we conducted the latent class trajectory analysis using measurements from follow-up, we found patterns similar to those identified at baseline (Fig. 1*E*).

In the transition analysis we determined class memberships at follow-up using the baseline model and data from the OGTT at follow-up. Half of the cohort (55%) was in the same class at follow-up as at baseline, whereas 19% moved to a class with a more favorable glucose profile (lower area under the curve [AUC]) and 27% moved to a class with a less favorable profile (higher AUC) (Supplementary Fig. 4). We found that younger age and both lower baseline levels of and declines from baseline to follow-up in BMI, fat mass, triglycerides, and CRP were associated with transition from class 2 to class 1 compared with staying in class 2 at the follow-up examination (Table 2).

We implemented our latent class model in an online application, the

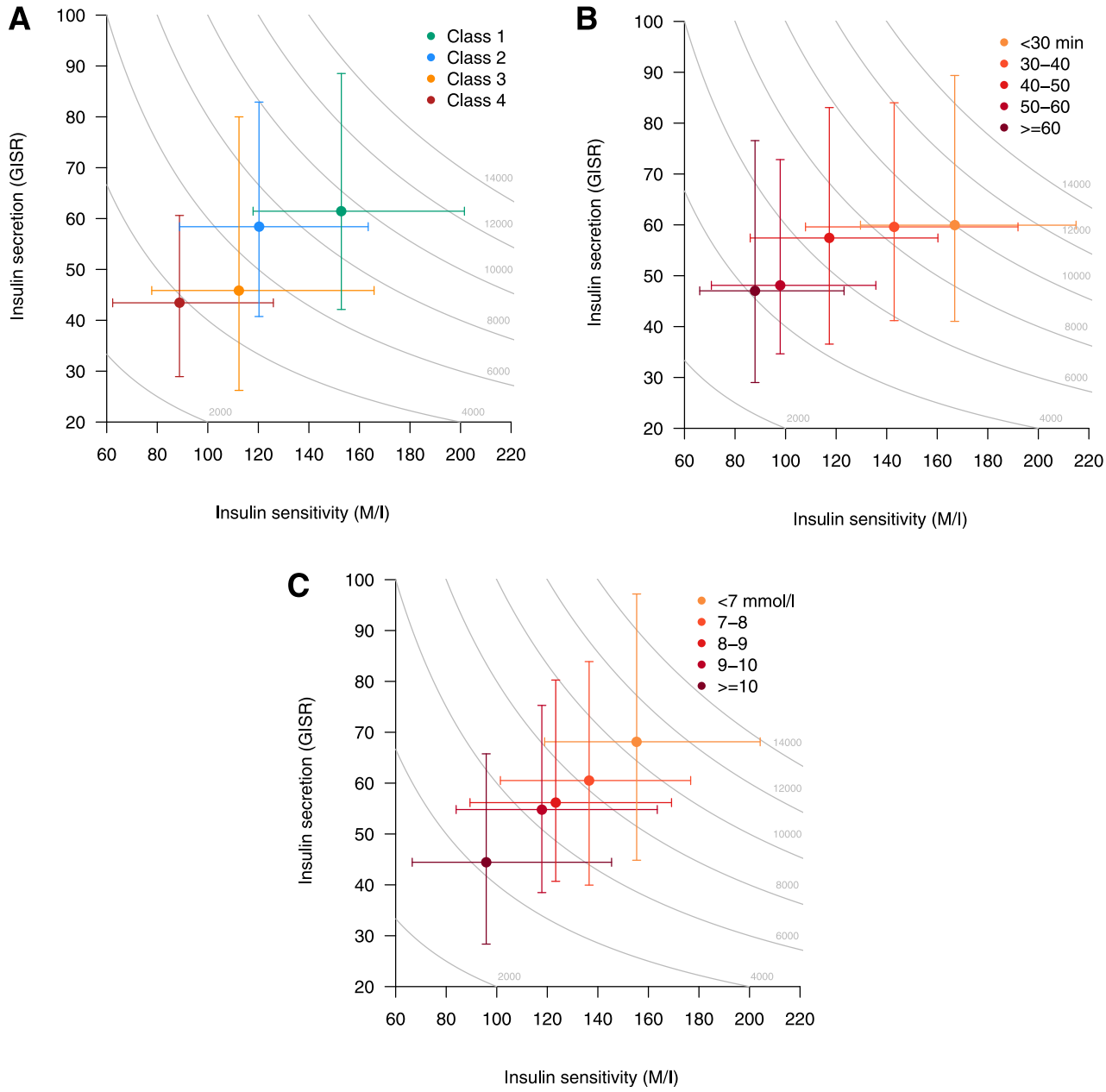

Figure 2—Insulin sensitivity (*M*/*I*) and acute insulin secretion (GISR) by latent glucose pattern classes (*A*) and the time (*B*) and size (*C*) of the glucose peak. Thin gray hyperbolas represent different levels of the disposition index, calculated as the product of insulin sensitivity (*M*/*I*) and acute insulin secretion (GISR) during the clamp. Data are the median and quartiles (Q1–Q3) of *M*/*I* (horizontally) and GISR (vertically).

Glucose Response Classifier, which is now available at https://steno.shinyapps.io/grc2h. The application calculates glucose response class membership probabilities for a person based on glucose concentrations during a frequently sampled OGTT. Technical details of the model are described in the Supplementary Data.

CONCLUSIONS

This study highlights a novel way of analyzing glucose responses during the OGTT and provides new information on the contributions of insulin sensitivity and first-phase insulin secretion to differences in glucose curve shapes. The identified glucose curve patterns differed from each other with regard to insulin sensitivity and acute insulin response, obesity, lipid levels, and inflammatory markers. In prospective analyses, changes in obesity, triglycerides, and inflammatory markers were associated with transition from one glucose curve pattern to another. Together, these findings suggest that the glucose curve patterns identified by latent class trajectory analysis are clinically relevant and modifiable by lifestyle factors. An application was published online to provide a tool to classify individuals outside this study.

The detailed assessment of first-phase insulin secretion and insulin sensitivity in the RISC cohort allowed us to investigate further the pathophysiological features responsible for the different curve shapes. Individuals with an early but relatively low glucose peak had the

Table 2—Risk factors associated with transitioning from class 2 at baseline to another class at the 3-year follow-up

	Class at follow-up			
	Class 1	Class 2	Class 3	Class 4
Individuals, *n* (%)	112 (23)	258 (54)	45 (9)	63 (13)
Age (years)	0.96 (0.94–0.99)	1.00	1.02 (0.98–1.06)	1.01 (0.98–1.04)
Sex (men vs. women)	0.74 (0.47–1.17)	1.00	0.95 (0.50–1.80)	0.86 (0.49–1.50)
Family history of diabetes	0.65 (0.38–1.12)	1.00	1.36 (0.69–2.71)	1.39 (0.77–2.50)
Insulin sensitivity (per 20 *M/I* units)	1.08 (1.01–1.16)	1.00	1.02 (0.91–1.13)	0.86 (0.76–0.96)
Insulin secretion (per 10 GISR units)	0.99 (0.92–1.07)	1.00	0.88 (0.77–0.998)	0.99 (0.89–1.09)
Risk factors measured at both baseline and follow-up				
BMI (kg/m^2)	0.92 (0.86–0.99)	1.00	1.06 (0.97–1.14)	0.99 (0.92–1.08)
ΔBMI (kg/m^2)	0.76 (0.64–0.91)	1.00	1.25 (1.04–1.50)	1.05 (0.87–1.27)
Fat mass (%)	0.95 (0.91–0.98)	1.00	1.03 (0.98–1.09)	1.00 (0.95–1.06)
ΔFat mass (%)	0.93 (0.88–0.99)	1.00	1.00 (0.93–1.09)	1.03 (0.97–1.10)
HDL cholesterol (0.5 mmol/L)	0.99 (0.70–1.42)	1.00	0.79 (0.46–1.37)	0.62 (0.38–1.01)
ΔHDL cholesterol (0.5 mmol/L)	1.21 (0.74–1.97)	1.00	0.71 (0.33–1.52)	0.65 (0.33–1.28)
Triglycerides ($\log_2$ transformed)	0.56 (0.36–0.87)	1.00	1.56 (0.89–2.75)	1.28 (0.77–2.12)
ΔTriglycerides (0.5 mmol/L)	0.65 (0.49–0.85)	1.00	1.08 (0.80–1.45)	0.87 (0.65–1.15)
CRP ($\log_2$ transformed)	0.79 (0.69–0.91)	1.00	1.33 (1.07–1.65)	0.98 (0.82–1.17)
ΔCRP (mg/L)	0.81 (0.67–0.98)	1.00	1.09 (0.98–1.21)	1.10 (0.99–1.21)

Data are odds ratios (95% CI) unless otherwise indicated. Age- and sex-adjusted odds ratios were calculated using multinomial logistic regression models with follow-up class membership as the outcome (reference outcome: staying in class 2). Odds ratios represent the association between moving to a specific class rather than staying in class 2 and a unit difference in the risk factors listed in the first column. Values are baseline measurements or are changes (Δ) between baseline and follow-up (e.g., ΔBMI = BMI at follow-up − BMI at baseline). Odds ratios for $\log_2$-transformed variables should be interpreted as the effect of doubling the predictor's value. Estimates for a variable and the change in the same variable are from the same model including both variables at the same time.

most favorable pathophysiological profile (class 1), whereas participants belonging to class 2 had lower insulin sensitivity but normal insulin secretion. This finding indirectly suggests that individuals with this risk profile are likely to benefit from interventions aimed at improving insulin sensitivity (e.g., exercise) (3). Individuals in class 3 had an early but high glucose peak—features that were associated both with lower first-phase insulin secretion and insulin sensitivity. Serum insulin and C-peptide concentrations were highest in class 3 compared with the other groups despite the lower first-phase insulin secretion measured during the IVGTT. Parallel to previous findings of a discrepancy between insulin responses from oral versus intravenous glucose testing (19), this finding is likely to be explained by the release of incretin hormones, which stimulate glucose-induced insulin response during an OGTT but not during intravenous testing. Individuals in class 4 had a slightly later glucose peak than those in class 3, potentially driven by the lower degree of insulin sensitivity. Thus, a novel finding of this study is that whole-body insulin sensitivity determines almost exclusively the time to glucose peak during the OGTT (Fig. 2*B*), whereas first-phase insulin secretion also plays a substantial role in determining the size of the glucose peak (Fig. 2*C*). A previous analysis of the RISC cohort found that individuals with normal glucose tolerance who had a high glucose peak (≥8.95 mmol/L) had a risk profile similar to that of participants with IGT (20), which underscores that a higher glucose peak reflects an abnormal metabolic state.

In line with this observation, we found that individuals belonging to class 3, which was characterized by the highest and earliest glucose peak, were likely to be men and smokers. This observation is in accordance with previous findings of individuals belonging to a group with similar OGTT curve characteristics (13). The finding is also in line with previous findings from the RISC study, showing higher total glucose and C-peptide AUCs during OGTTs among men who smoke (21). There is also evidence for higher intermediate glucose levels in those who smoke, despite similar FPG and lower 2hPG (22). Possible explanations include a delayed insulin response among smokers (23) and a faster gastric emptying rate (24). In a study of another cohort, we showed that individuals with a glucose curve pattern characterized by a high glucose peak had an increased risk for future diabetes and all-cause mortality independent of age, sex, smoking, and other cardiometabolic risk factors (8). Together, these findings and the pathophysiological differences (e.g., between classes 2 and 3 despite very similar FPG and 2hPG concentrations; <6%) suggest that the shape of the glucose curve carries information that cannot be captured by FPG and/or 2hPG. Because of the relatively short follow-up, we could not study incidence of diabetes and cardiovascular disease, and therefore we cannot conclude which of the glucose curve features are most detrimental to health.

Among cardiometabolic risk factors, obesity, triglycerides, and markers of subclinical inflammation all were associated with the glucose pattern classes in both cross-sectional and prospective analyses. Fat mass was more strongly associated with the glucose curve classes than BMI, and, of interest, low levels of adiponectin, which is downregulated in obese and insulin-resistant individuals (25,26), were related to a higher probability of being in classes 3 and 4. Although the underlying mechanism is not fully understood, adiponectin is consistently found to be the only anti-inflammatory cytokine with a decreased level before the diagnosis of type 2 diabetes (27). Also, markers of low-grade

inflammation, such as CRP and IL-6, were associated with classes 3 and 4, which included individuals with the lowest insulin sensitivity and poorest insulin secretion. Markers of subclinical inflammation have previously been prospectively associated with the development of increased glycemia, insulin resistance, and β-cell dysfunction (27,28). Now we demonstrate that these markers are also related to different glucose curve characteristics during an OGTT in individuals who do not have diabetes. Differences in CRP levels, but not in IL-6 levels, between the identified classes were robust for confounder adjustment. In line with previous studies, this suggests that CRP might indicate an earlier stage of glycemic defects than IL-6 (28).

Previous investigations used predefined and rather arbitrary approaches (e.g., mono-/biphasic curves, time to the return to FPG level) to assess the shape of glucose curves (29). Our study takes advantage of methodologies routinely used in longitudinal epidemiology by considering change over time while accounting for measurement error and by not relying on predefined characterizations. This method captures change over time in a more intuitive way than the mono-/biphasic classification, and it provides class membership probabilities indicating the certainty of the estimates. Recent studies have focused on the role of 1hPG in the risk of diabetes and diabetes complications (7). Considering our results, however, a cutoff value at 1hPG would not be able to distinguish between classes 3 and 4, just as 2hPG cannot distinguish between classes 2 and 3. Our comparisons between classes are based on individuals who mostly have high class membership probabilities but not perfect classification with 100% probability. In such a theoretical comparison based on perfectly matching members, we would expect even larger differences between the classes.

The RISC study offers a unique data set achieved with "gold standard" measures of insulin sensitivity and secretion, and a repeated five–time point OGTT, but such detailed data are seldom available. An online application was developed to facilitate the dissemination of our methodology and to provide a tool for classification of individuals outside of our study, even with fewer than five measurements. Further research is needed to investigate whether accurate classification can be achieved by measuring glucose at fewer time points but by adding to the model other determinants as concomitant variables (30).

The OGTTs at follow-up allowed us to investigate the robustness of the latent class method. These analyses showed reassuring results in the sense that the same latent classes found at baseline could be identified at follow-up. Thus, the appearance of very similar patterns at follow-up and similarities to our previous study (13) confirm the utility of latent class trajectory analysis in finding heterogeneous subgroups of individuals without diabetes based on a detailed characterization of their glucose response. Our sensitivity analysis of transitions showed that movements between classes are due to random variation only to a modest degree.

In conclusion, we identified four glucose curve patterns that differed from each other with regard to insulin sensitivity, insulin secretion, obesity, plasma lipids, and low-grade inflammatory markers. In general, whole-body insulin sensitivity was a more important determinant than insulin secretion of time to glucose peak during the OGTT, whereas both seemed to contribute substantially to the size of the glucose peak. The identified glucose curve patterns were robust over time, and transitions between classes were associated with changes in cardiometabolic risk factors, suggesting that the latent class trajectory method may be useful to stratify risk. Future research is warranted to explore in more detail genetic and lifestyle determinants, as well as long-term consequences of different glucose curve patterns, such as incidence of diabetes, cardiovascular disease, and response to interventions.

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Author Contributions. A.H. performed the analysis. A.H., D.R.W., K.F., and M.M. conceived the study. A.H., K.F., and M.M. wrote the first draft of the manuscript. B.B., J.M.D., M.H., and T.K. collected data. All authors interpreted the data and revised the manuscript. A.H. 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.

Appendix

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More information on the RISC study and participating centers can be found at www.egir.org.

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