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Heterogeneity in Glucose Response Curves During an Oral Glucose Tolerance Test and Associated Cardiometabolic Risk

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Abstract

Purpose: To examine heterogeneity in glucose response curves during an OGTT with multiple measurements and to compare cardiometabolic risk profiles between identified glucose response curve groups.

Methods: We analyzed data from 1,267 individuals without diabetes from five studies in Denmark, the Netherlands and the USA. Each study included between five and eleven measurements at different time points during a 2-hour OGTT, resulting in 9,602 plasma glucose measurements. Latent class trajectories with a cubic specification for time were fitted to identify different patterns of plasma glucose change during the OGTT. Cardiometabolic risk factor profiles were compared between the identified groups.

Results: Using latent class trajectory analysis, five glucose response curves were identified. Despite similar fasting and 2-hour values, glucose peaks and peak times varied greatly between groups, ranging from 7 to 12 mmol/L, and 35 to 70 minutes. The group with the lowest and earliest plasma glucose peak had the lowest estimated cardiovascular risk, while the group with the most delayed plasma glucose peak and the highest 2-hour value had the highest estimated risk. One group, with normal fasting and 2-hour values, exhibited an unusual profile, with the highest glucose peak and the highest proportion of smokers and men.

Conclusions: The heterogeneity in glucose response curves and the distinct cardiometabolic risk profiles may reflect different underlying physiologies. Our results warrant more detailed studies to identify the source of the heterogeneity across the different phenotypes and whether these differences play a role in the development of type 2 diabetes and cardiovascular disease.

Keywords: oral glucose tolerance test; glucose response curve; cardiometabolic risk; latent class trajectory analysis

Introduction

The development of type 2 diabetes is a heterogeneous process [1]. Some types of diabetes are predominantly associated with obesity and insulin resistance, while others exhibit relatively modest insulin resistance, but with a loss of beta cell function as the primary abnormality [2]. The relative contribution of these pathophysiological mechanisms is usually discernable by estimation of beta cell function and insulin resistance. However, the exact determination of these mechanisms using several insulin measurements is expensive and complicated. Attention has recently focused on other methods of delineating pathophysiological mechanisms. Examination of heterogeneity in glucose response curves during an oral glucose tolerance test (OGTT) is an alternative approach to identifying a range of features that may reflect underlying pathophysiological mechanisms. For example, intermediate glucose measures, such as 30-minute or 1-hour concentrations, and glucose curve features, including time to peak concentration or the number of peaks, are associated both with pathophysiological mechanisms and risk of incident diabetes [3-8]. Furthermore, examining whether different glucose response curves represent distinct cardiometabolic risk profiles may contribute to elucidating the heterogeneity in the underlying pathophysiological pathways to incident diabetes. Previous research examining heterogeneity in OGTT response curves has been hampered by the use of pre-defined characteristics to categorize glucose curves and a limited number of time points during an OGTT. Indeed, there are few large-scale studies where multiple measures of glucose are captured during an OGTT. These are necessary to examine glucose response curves in detail. In order to identify subgroups of glucose response curves, we used the data-driven method of latent class mixed-effects modelling. With this approach we set out to examine heterogeneity in glucose response curves during frequently sampled OGTTs in more than 1,200 individuals without diabetes from five different studies, and to describe and compare the cardiometabolic risk profiles associated with each identified glucose response curve.

We hypothesize that there will be significant heterogeneity in glucose response curves in this large composite population, and that the group with the latest and highest glucose peak will have the highest cardiometabolic risk.

Materials and Methods

Study populations

We included data from five cross-sectional studies where glucose levels were measured at least ≥ 5 time points during a 2-hour OGTT among individuals without type 2 diabetes: the Inter99 sub-study [9], Hoorn Meal Study [10], CPH/CLE study [11], ADIGEN [12], and the Danish Family Study [13]. The Inter99 study is an age- and sex-stratified random population-based sample from Copenhagen County, Denmark where study participants were recruited for a non-pharmacological intervention [14]. The Hoorn Meal study is a population-based cohort among men and women aged 40-65 years in the Netherlands with participants sampled randomly from the city of Hoorn's municipal register. The CPH/CLE study is a clinical convenience sample of participants recruited by newspaper/radio advertisement from local municipal areas in Copenhagen, Denmark, and Cleveland, Ohio, USA. ADIGEN participants are a subset of a longitudinal case-cohort selected from Caucasian men attending military draft boards in Copenhagen and its surrounding areas. The subset includes a range of extremely overweight to moderately obese young men. The Danish Family study is a cohort of individuals with one parent suffering from verified type 2 diabetes and one parent without known diabetes. Study participants were identified through the outpatient clinic at Steno Diabetes Center (Gentofte, Denmark) or through an ongoing family study at the University of Copenhagen (Copenhagen, Denmark). All studies were performed in accordance with the Helsinki Declaration and approved by local ethics committees. Written informed consent was obtained from all participants.

All studies used a standard 75g frequently sampled OGTT following an overnight fast for each participant. Plasma glucose concentrations were measured using the hexokinase/G6P-DH technique in the Inter99 (Boehringer Mannheim, Mannheim, Germany) [9], Hoorn Meal (Gluco-quant, Roche Diagnostics) [10] and ADIGEN studies (Boehringer and Mannheim GmbH Diagnostica, Germany) [12]. In the CPH/CLE study, plasma glucose concentration was measured using a bedside analyser (YSI Stat, Yellow Springs; ABL, Radiometer Medical, Brønshøj, Denmark) [11], and in the Danish Family Study by a glucose oxidase method (Granutest; Merck, Darmstadt, Germany) [13]. Plasma insulin was measured with a fluoro-immunoassay technique in the Inter99 study [9]

(AutoDELFIA;Perkin Elmer-Wallac, Turku, Finland), by immunometric assays in the Hoorn Meal study [10] (ACS Centaur, Bayer Diagnostics, Mijdrecht, Netherlands), using a Dako insulin kit K6219 in the ADIGEN study (Dako Diagnostics, Ely, UK) [12], by electrochemiluminescence immunoassay in the CPH/CLE study [11] (E-modular; Roche, Basel,Switzerland), and by ELISA with a narrow specificity in the Danish Family Study [15,13]. In all studies, height, body weight, blood pressure and plasma lipids were measured according to standard procedures. Information on smoking status was obtained by questionnaire. We excluded individuals with doctor-diagnosed, self-reported or screen-detected diabetes from our analyses.

Statistical analysis

We performed an individual-level pooled analysis of data from a total of 1,267 participants from the five described studies (**Table 1**). We used latent class trajectory analysis to identify heterogeneous glucose response curve groups during the frequently sampled OGTTs, using all available glucose measurements [16]. The latent class mixed-effects model was specified with linear, quadratic and cubic time terms to model non-linear change over time. Coefficients were allowed to vary between latent groups. We controlled for between study differences by including a categorical study variable in the model. A random intercept was included in the model to account for the correlation between measurements from the same individual. This method allowed us to pool data from all five studies at the individual level, as it does not require all individuals to have the same number of measurements at the same time points. In order to delineate the optimal number of latent groups, we started with one group (equivalent to fitting a linear mixed-effects model) and then increased the number of groups one-by-one. We repeated this until group sizes remained sufficiently large and the model's Bayesian Information Criteria (BIC) stabilized. After determining the best fitting model, each individual was assigned to the glucose response curve group with the highest membership probability.

Glucose response curves were then refitted with standard linear mixed-effects models including group membership and its interaction with all terms within each of the glucose curve groups. We also added a random intercept for each of the five studies to account for potential between-study differences. In a

sensitivity analysis, we examined these models stratified by sex and BMI (above/below 25kg/m^2). We also conducted a sensitivity analysis to see if the results reamain stable after excluding the CPH/CLE cohort that included only non-smokers, which might influence our CVD risk estimates. Next, we plotted plasma insulin levels over the course of the frequently sampled OGTT for each glucose response curve group. We then described the cardiometabolic characteristics (age, sex, smoking status, BMI, lipid profile and blood pressure) of study participants stratified by glucose response curve group. We calculated the 10-year risk of fatal CVD using the formula for low-risk countries from the SCORE project that was established to develop a tool for risk stratification in the primary prevention of CVD [17]. We chose this measure, because it was developed using European data and our pooled cohort is predominantly from low-risk European countries (Denmark and the Netherlands). We calculated the Matsuda index [18], the insulinogenic index [19], and the disposition index (as the product of the Matsuda and the insulinogenic index) to estimate insulin sensitivity and insulin secretion. Medians were compared with the Kruskal-Wallis test for continuous variables, and proportions were compared with the chi-square test. Statistical analyses were performed using the lcmm, lme4 and Epi packages in R version 3.2.0 [20].

Results

Our analysis included 9,602 plasma glucose measurements taken from 1,267 individuals in five studies (**Table 1**). Data were collected between 1993 and 2012 and there was a median of 7 (IQR 6-9) glucose measurements per person. Study population sizes ranged from 117 (Inter99 sub-study) to 422 (ADIGEN) individuals. The proportion of men ranged from 42% to 100%, the median age from 40 to 56 years, and the proportion of smokers from 0% to 39% across the five studies.

In individual-level pooled analyses, the median (IQR) age of the study population was 49 (42 to 57) years; 68% were men and 24% reported smoking **(Table 2)**. The median BMI was 27.0 kg/m^2, with median systolic blood pressure of 129 mmHg and median values in the normal range for levels of total cholesterol, triglycerides, HDL-cholesterol and diastolic blood pressure.

We identified five latent groups, each including between 7% and 29% of the study population (**Figure 1**). The corresponding plasma glucose curve equations are included in the electronic supplement (**eTable 1**). Average posterior group membership probabilities were high, ranging between 0.85 and 0.93, indicating a good discrimination between the groups. Peak plasma glucose levels varied greatly between groups ranging from 7 to 12 mmol/L, and peak times ranged from 35 to 70 minutes, indicating considerable heterogeneity. Similar latent class groups and patterns were observed when we stratified the models by sex and BMI, and when we excluded the CPH/CLE cohort in the sensitivity analysis (data available on request).

Between these latent groups of glucose responses, there was marked heterogeneity in serum insulin levels during the OGTT. Peak levels varied from 306 to 442 pmol/L, and peak times between 47 and 78 minutes. Group 1 demonstrated the earliest and lowest insulin peak, while Group 5 had the latest and highest peaks. While insulin levels were clustered more tightly than the glucose peaks around 45 minutes to one hour, there was marked variation in the final 2-hour values. Insulin levels at 2-hours were statistically significantly higher than fasting insulin values for Groups 3, 4 and 5. In general, the insulin response during the OGTT corresponded to the same configuration as the glucose response curves (**Figure 1**). The ranking of peak times between the glucose and insulin curves was consistent. Differences in timing between glucose response curve peaks and insulin peaks ranged from 3 to 12 minutes. Similarly, peak values were very consistent between glucose and insulin, with only Groups 4 and 5 changing order.

Characteristics of study participants stratified by glucose response curve groups are shown in **Table 2**. Group 1, characterized by the lowest and earliest plasma glucose peak, included the highest proportion of women (41% compared to other groups with 20-34%, $p<0.001$) and the lowest median age (46 years). Overall, this group had the lowest estimated CVD risk, with the lowest values for BMI, total cholesterol and triglycerides, and the highest HDL-cholesterol and Matsuda index. When moving from Group 1 to Group 5, there was a statistically significant increase in estimated CVD risk (from a median SCORE of 0.5% in Group 1 to 1.5% in Group 5). In Group 2 there was a high

proportion of men, with normal values for blood pressure and cholesterol, but slightly reduced insulin sensitivity and insulin secretion. Group 3 had a median BMI of 28.0 kg/m^2. They had elevated levels of triglycerides and low HDL-cholesterol, as well as lower insulin sensitivity and secretion compared to Group 1. Group 4, characterized by the highest plasma glucose peak but one of the lowest 2-hour values, demonstrated a different trend. This group had the highest proportion of smokers (50% compared to 25-37% in other groups, $p<0.001$), a very high proportion of men (80%) and the highest estimated CVD risk (median SCORE 1.6%), but with low BMI and blood pressure values, and high HDL-cholesterol. Group 5, characterized by the latest plasma glucose peak and highest 2-hour value (fulfilling IGT criteria), had the highest values for age, estimated CVD risk, BMI, total cholesterol, triglycerides and blood pressure, and the lowest HDL-cholesterol and Matsuda index.

Discussion

We found statistically and clinically significant heterogeneity in glucose response curves during a frequently sampled OGTT in a large pooled population of middle-aged individuals using a latent class trajectory analysis approach. Each group had a distinct cardiometabolic risk profile.

Heterogeneity in glucose response curves

We identified five different glucose response curves, which showed marked variation in peak plasma glucose levels and times, even between groups with similar fasting and 2-hour values. While it is not possible to directly compare our results with other studies due to our novel latent class approach, researchers have found similar patterns of glucose and insulin during an OGTT [3,8]. For example, Abdul-Ghani *et al* reported four distinct glucose response curves during a 4-point OGTT among 1,110 individuals with normal glucose tolerance [3]. They linked their findings to incident diabetes and showed that the more quickly plasma glucose concentrations returned to, or below, the fasting glucose levels, the lower the future risk of diabetes. Their group stratification also identified physiologically distinct groups with abnormalities in insulin sensitivity and insulin secretion.

Groups 2 to 5 had significantly reduced insulin sensitivity and insulin secretion compared to Group 1. However, the differences in the estimates of insulin sensitivity and insulin secretion across the groups were small despite large variations in the shape of the glucose and insulin curves. In addition to insulin sensitivity and insulin secretion, factors contributing to differences in postprandial glucose and insulin responses include genetics [21], lifestyle [22,23] and body height [24,25], as well as the rate of gastric emptying [26], glucose absorption [27] and the release of incretin hormones [28]. Despite only small observed differences in the estimates of insulin secretion between the groups, it is likely that differences in the secretion of the gut incretin hormones were present between the groups as previously illustrated in individuals with diverse glucose responses during an OGTT [9]. Group 2 was characterized by a rapid increase in circulating insulin levels peaking around 50 minutes after oral glucose intake. By contrast Group 3 had more gradual fluctuations in both insulin and glucose concentrations. These differences could not be explained by differences in insulin sensitivity and secretion as estimated by the OGTT data. This underscores the fact that simple surrogate markers are not always sufficient to understand the complexity of postprandial glucose regulation. Group 4 had a rapid rise in glucose levels after glucose ingestion during the first 30 to 45 minutes. However, the corresponding early insulin secretion was slightly delayed, giving rise to a significantly lower insulinogenic index in Group 4 compared to Group 1. This low early phase insulin secretion may also explain the high glucose peak observed after approximately 45 to 60 minutes in Group 4. By contrast, Group 5 had a high degree of insulin resistance, which likely contributed to their elevated 2-hour glucose levels as previously shown in individuals with IGT [9].

The consistency in patterns of glucose curves identified in most studies (including our analysis) suggests that we are observing variability that exists across populations. While we do not know exactly which abnormality is driving individual membership of each glucose response curve, we have identified true heterogeneity in human glucose metabolism rather than a pattern spuriously introduced by assumptions based on the shape of the curves. It would be useful to validate our results and examine whether the same or similar groups of glucose response curves are observed in other large, heterogeneous populations.

Cardiometabolic risk profiles

We observed the lowest estimated cardiometabolic risk in Group 1, which was characterized by the lowest and earliest plasma glucose peak, and the highest risk in Group 5, with the latest plasma glucose peak and the highest 2-hour value. This finding supports previous literature. A Finnish study of 504 individuals showed that 2-hour plasma glucose was a better marker of CVD risk than fasting plasma glucose and 1-hour plasma glucose among people with prediabetes [29]. Similarly, two-hour glucose values are stronger risk factors for incident diabetes [3], cardiovascular disease [30] and mortality compared to fasting levels. Group 4, characterized by the highest plasma glucose peak but one of the lowest 2-hour glucose values, had an unusual cardiometabolic risk profile. This group had the highest proportion of smokers and a very high proportion of men but a low BMI and high HDL-cholesterol levels. The high smoking prevalence may reflect increased gastric motor activity [31], but it could also be a marker for other unhealthy lifestyle behaviors.

By examining glucose response curves during a frequently sampled OGTT and comparing risk profiles between identified curves, we detected sub-groups with differences in cardiometabolic risk not revealed using standard fasting and two-hour cut-offs. Only Group 5 (who, on average, have IGT) would currently be identified as being at high risk based on standard fasting and 2-hour glucose cut-off points. Individuals in Group 4 would be considered low risk according to fasting and 2-hour values but they had the highest peak glucose concentrations. A similar result was observed in cross-sectional data from 1,205 healthy volunteers in the RISC study [32]. Increased 1-hour plasma glucose (>8.95mmol/l) on an OGTT identified a sub-group of NGT individuals with increased insulin resistance and beta-cell dysfunction compared to NGT participants with lower 1-hour glucose (≤8.95mmol/l). The authors concluded that these individuals might represent an intermediate condition between NGT and IGT, and constitute a group who might benefit from lifestyle interventions analogous to the state of IGT [33]. Overall, these data and our results suggest that some high-risk individuals will not be captured using classical OGTT measurements. To confirm our hypothesis, further research might examine the association between latent classes and incidence of

type 2 diabetes or cardiovascular outcomes, as well as the stability of groups and membership probabilities across populations and throughout the life course.

Strengths and limitations

This is a very large pooled analysis of population studies comprising multiple OGTT measurements. Data were drawn from studies in three different countries, allowing us to assemble a sample that was large enough to carry out multi-level models. We used a data-driven approach to examine heterogeneity in glucose response curves rather than specify pre-defined characteristics. This approach allowed us to derive and discover the characteristics (peak level and time to peak) of maximally different curves rather than simply examine the profiles of pre-defined curves. The latent class trajectory approach also allowed us to investigate glucose change over time while taking measurement error into account. Our study population was largely comprised of White Caucasians, which may limit the generalizability of our findings. Although the majority of the studies used population-based recruitment, the frequently sampled OGTTs were generally performed in a subset of the population in people at elevated diabetes risk. As such, the proportion of individuals assigned to the five identified glucose response curve groups may be different in an unselected general population. However, the positive selection for slightly higher diabetes risk individuals may have strengthened our capacity to discover patterns that may not have been discernable in an unselected population. While the same standard 75g OGTT was carried out in all studies, the technique and assay used to analyze plasma glucose concentrations differed. Any differences introduced by these differing methods were likely to have primarily affected the intercepts, an effect that we controlled for in the modeling process. Similarly, while height, body weight, blood pressure and plasma lipids were measured according to standard procedures, there were likely to be small differences in measurement protocol between studies. In the CPH/CLE cohort, there was a subset of individuals recruited in the US, a country with high risk of CVD. In spite of this, we used the European SCORE charts to estimate their CVD risk, because the proportion of these individuals in the pooled sample was minor.

In conclusion, these data represent a unique opportunity to observe the heterogeneity in glucose response to an OGTT, even between groups with normal fasting and 2-hour plasma glucose values. The different glucose response curves were linked to distinct cardiometabolic risk profiles, which suggests a complex underlying pathophysiology. Deeper phenotyping to accurately diagnose metabolic disease may be required for some individuals.

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Compliance with Ethical Standards

The authors have no conflict of interest related to this manuscript.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

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Fig. 1 Latent class trajectories identifying different plasma glucose response curves during an OGTT and corresponding insulin responses among 1,267 participants from five study populations

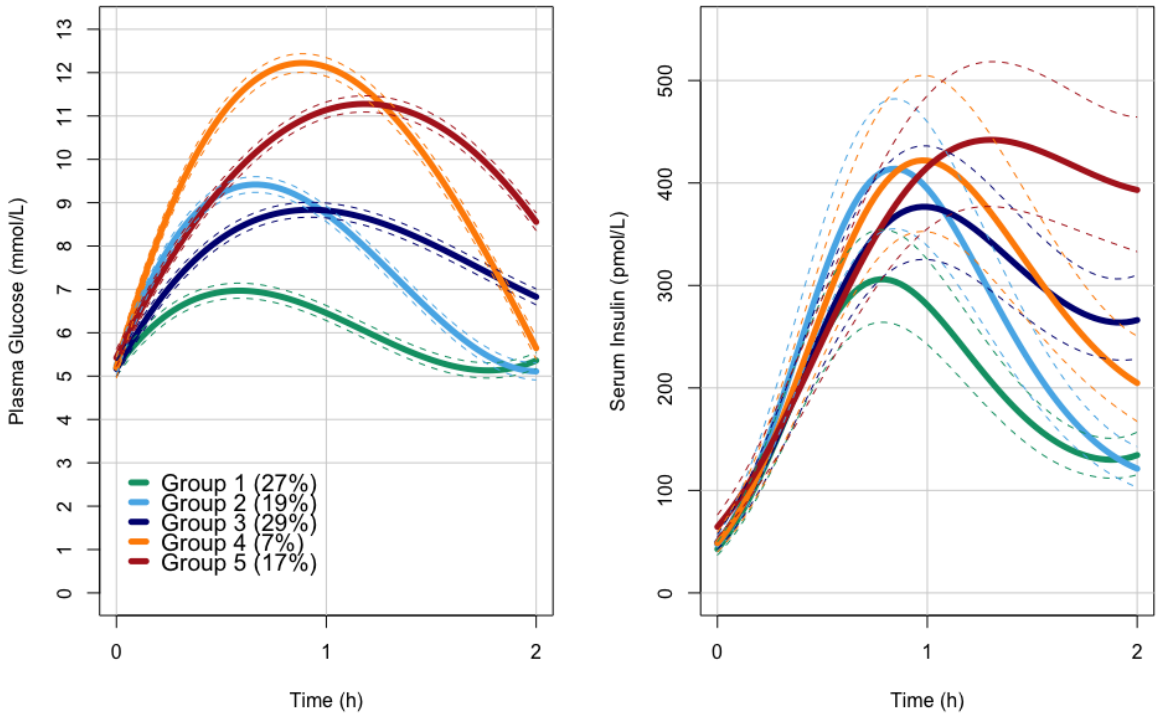

Table 1 Study population characteristics

	Inter99 sub-study	**Hoorn study**	**CPH/CLE study**	**ADIGEN study**	**Family study**	**Pooled**
Year of data collection	2005-2006	2005-2006	2005-2012	1998-2000	1993-1998	1993-2012
Time points during OGTT (mins)	0, 10, 20, 30, 45, 60, 75, 90, 120	0, 15, 30, 60, 90, 120	0, 30, 60, 90, 120	0, 15, 30, 45, 60, 90, 120	0, 10, 20, 30, 40, 50, 60, 75, 90, 105, 120	
N (participants)	117	185	234	422	309	1267
Sex (male %)	62	48	64	100	42	68
Age (years), median (IQR)	56 (46-61)	54 (48-59)	56 (38-66)	48 (44-52)	40 (34-50)	49 (42-57)
Smoking (yes %)	20	25	0 (by design)	39	52	31
BMI (kg/m^2), median (IQR)	26.5 (24.8-28.9)	26.5 (24.2-28.7)	28.4 (23.9-32.7)	28.4 (24.8-33.2)	25.6 (22.8-28.6)	27.0 (24.0-31.0)
FPG (mmol/L)	5.4 (5.1-5.7)	5.4 (5.1-5.7)	5.2 (4.9-5.6)	5.7 (5.4-6.0)	5.2 (4.8-5.6)	5.4 (5.1-5.8)
2hPG (mmol/L)	6.1 (5.1-6.8)	4.9 (4.0-5.8)	6.9 (5.7-8.4)	6.5 (5.5-7.9)	6.1 (5.2-7.1)	6.2 (5.1-7.4)
FSI (pmol/L)	34 (25-54)	40 (27-60)	59 (33-97)	38 (24-61)	33 (21-49)	39 (25-63)
2hSI (pmol/L)	245 (147-413)	134 (80-229)	306 (166-530)	175 (90-299)	182 (124-304)	188 (109-349)

BMI: body mass index; FPG: fasting plasma glucose; 2hPG: 2-hour plasma glucose; FSI: fasting serum insulin; 2hSI: 2-hour serum insulin

Table 2 Characteristics of study participants stratified by glucose response curve groups

	Overall	**Group 1**	**Group 2**	**Group 3**	**Group 4**	**Group 5**
N (%)	1,267 (100)	347 (27)	240 (19)	369 (29)	92 (7)	219 (17)
Sex (male %)	68	59	80	66	80	69
Age (years)	49 (42-57)	46 (39-54)	47 (42-54)	50 (43-59)	53 (46-59)	52 (45-62)
Smoking (yes %)	31	28	37	25	50	29
Body Mass Index (kg/m^2)	27.0 (24.0-31.0)	25.1 (22.8-28.5)	26.2 (23.8-28.9)	28.0 (25.0-32.3)	26.4 (24.0-30.0)	30.3 (27.0-35.0)
Total cholesterol (mmol/L)	5.3 (4.6-6.0)	5.1 (4.5-5.8)	5.2 (4.5-5.9)	5.3 (4.6-6.0)	5.4 (4.7-5.9)	5.5 (5.0-6.3)
Triglycerides (mmol/L)	1.20 (0.88-1.69)	1.00 (0.79-1.35)	1.20 (0.90-1.56)	1.27 (0.90-1.80)	1.27 (0.93-1.60)	1.51 (1.14-2.10)
HDL-cholesterol (mmol/L)	1.22 (1.02-1.46)	1.31 (1.11-1.60)	1.21 (1.04-1.41)	1.18 (1.00-1.41)	1.29 (1.08-1.55)	1.10 (0.95-1.35)
Systolic blood pressure (mmHg)	129 (118-140)	128 (118-139)	125 (116-136)	130 (119-140)	127 (116-145)	133 (123-145)
Diastolic blood pressure (mmHg)	80 (72-87)	79 (72-87)	77 (71-84)	81 (73-87)	80 (71-89)	81 (75-90)
10-year estimated risk of fatal CVD (%)[†]	0.8 (0.3-1.6)	0.5 (0.1-1.2)	0.7 (0.3-1.7)	0.9 (0.3-1.9)	1.6 (0.8-2.6)	1.4 (0.6-2.9)
Fasting plasma glucose (mmol/L)	5.4 (5.1-5.8)	5.1 (4.8-5.4)	5.5 (5.2-5.8)	5.4 (5.1-5.7)	5.6 (5.3-6.0)	5.7 (5.3-6.1)
2-h plasma glucose (mmol/L)	6.2 (5.1-7.4)	5.3 (4.4-6.0)	5.2 (4.3-5.9)	6.9 (6.2-7.6)	5.9 (5.0-6.7)	8.7 (8.0-9.6)
Matsuda index[‡]	6.1 (3.8-9.4)	8.9 (6.3-12.1)	6.4 (4.5-9.1)	5.5 (3.6-8.5)	4.6 (3.3-8.3)	3.8 (2.1-6.1)
Insulinogenic $index_{0-30}$	0.7 (0.4-1.0)	0.9 (0.6-1.5)	0.5 (0.4-0.8)	0.7 (0.4-1.1)	0.3 (0.2-0.5)	0.5 (0.3-0.8)
Disposition index	3.8 (2.4-6.2)	7.7 (6.0-10.4)	3.7 (2.8-4.7)	3.8 (2.9-5.3)	1.8 (1.2-2.2)	2.0 (1.5-2.6)

Values are median (IQR) unless otherwise indicated.

Cardiometabolic risk factors were compared between latent groups using Kruskal-Wallis tests for continuous variables and chi-square tests for categorical variables, and for all differences P-values were <0.001, which remained robust after correction for multiple testing (Benjamini & Hochberg, J R Statist Soc B 1995, 57:289-300); [†]Estimated using the formula for low-risk countries from the SCORE project. Conroy et al., Eur Heart J (2003) 24: 987-1003; [‡]See formula in Matsuda & DeFronzo, Diab Care (1999) 22: 1462-1470.

eTable 1 Coefficients for plasma glucose curve equations (the unit of time is in hours)

	Group 1	**Group 2**	**Group 3**	**Group 4**	**Group 5**
Intercept	5.16	5.24	5.17	5.20	5.42
Time	6.86	14.18	9.03	17.28	9.96
$Time^2$	-7.79	-14.23	-6.65	-12.17	-4.29
$Time^3$	2.20	3.55	1.28	1.82	0.05

Example: Group 1 equation is $5.16 + 6.86\ Time - 7.79\ Time^2 + 2.20\ Time^3$