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## RESEARCH: EPIDEMIOLOGY

# Clinical profiles of post-load glucose subgroups and their association with glycaemic traits over time: An IMI-DIRECT study

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## Abstract

**Aim:** To examine the hypothesis that, based on their glucose curves during a seven-point oral glucose tolerance test, people at elevated type 2 diabetes risk can be divided

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into subgroups with different clinical profiles at baseline and different degrees of subsequent glycaemic deterioration.

**Methods:** We included 2126 participants at elevated type 2 diabetes risk from the Diabetes Research on Patient Stratification (IMI-DIRECT) study. Latent class trajectory analysis was used to identify subgroups from a seven-point oral glucose tolerance test at baseline and follow-up. Linear models quantified the associations between the subgroups with glycaemic traits at baseline and 18 months.

**Results:** At baseline, we identified four glucose curve subgroups, labelled in order of increasing peak levels as 1–4. Participants in Subgroups 2–4, were more likely to have higher insulin resistance (homeostatic model assessment) and a lower Matsuda index, than those in Subgroup 1. Overall, participants in Subgroups 3 and 4, had higher glycaemic trait values, with the exception of the Matsuda and insulinogenic indices. At 18 months, change in homeostatic model assessment of insulin resistance was higher in Subgroup 4 ( $\beta = 0.36$ , 95% CI 0.13–0.58), Subgroup 3 ( $\beta = 0.30$ ; 95% CI 0.10–0.50) and Subgroup 2 ( $\beta = 0.18$ ; 95% CI 0.04–0.32), compared to Subgroup 1. The same was observed for C-peptide and insulin. Five subgroups were identified at follow-up, and the majority of participants remained in the same subgroup or progressed to higher peak subgroups after 18 months.

**Conclusions:** Using data from a frequently sampled oral glucose tolerance test, glucose curve patterns associated with different clinical characteristics and different rates of subsequent glycaemic deterioration can be identified.

## 1 | INTRODUCTION

Impaired glucose regulation is typically defined as having higher than normal blood glucose levels that do not meet the thresholds for diabetes<sup>1</sup> and therefore at increased risk for developing diabetes. However, only approximately 50% of those with impaired glucose regulation will progress to diabetes.<sup>2</sup> This suggests that it is a heterogeneous condition explained by different pathophysiological mechanisms,<sup>3</sup> which may have a long lead time before the diagnosis of diabetes.<sup>4</sup> Hence, information on these pathophysiologies is essential for personalized diabetes prevention and underlines the need for stratification in individuals with impaired glucose regulation.

Recent studies reported variation in the glucose response curves following an oral glucose tolerance test (OGTT).<sup>5–7</sup> One cross-sectional study identified five heterogeneous glucose response curves, using latent class trajectory analysis.<sup>6</sup> However, to date, only two prospective studies associated such curves with incident type 2 diabetes,<sup>5,7</sup> and they only used three-point OGTTs, which could lead to insufficient information on the curves' heterogeneity. We therefore aimed to test the hypothesis that, based on their glucose curves during a seven-point OGTT, people at elevated type 2 diabetes risk

### What's new?

- There is marked variation in the glucose curves following an oral glucose tolerance test (OGTT).
- It is possible to stratify individuals at elevated risk of type 2 diabetes into subgroups with different glycaemic traits and rates of subsequent glycaemic deterioration based on their glucose curves during a seven-point OGTT.
- The number of OGTT time points influences the number of identified subgroups.
- The glucose subgroups had good discriminative ability for incident type 2 diabetes (area under the curve 0.70).
- These subgroups with potentially different pathophysiology could be crucial for personalized diabetes prevention strategies.

can be divided into subgroups with different clinical profiles at baseline and different rates of subsequent glycaemic deterioration.

## 2 | MATERIALS AND METHODS

### 2.1 | Study population

We used data from the Innovative Medicines Initiative - Diabetes Research on Patient Stratification (IMI-DIRECT) study, which has been described in detail elsewhere.<sup>8</sup> In brief, the IMI-DIRECT study is a European study initiated in 2012 that consisted of individuals at elevated risk of glycaemic deterioration from several European prospective cohort studies. The study included 2247 participants, but we excluded 121 individuals, leaving us with 2126 participants at baseline and with 1988 at 18 months after excluding those lost to follow-up ( $n = 138$ ; Figure S1).

### 2.2 | Oral glucose tolerance test assessment

A 75-g OGTT was performed at baseline and 18 months. Blood was sampled at 0, 15, 30, 45, 60, 90 and 120 min after a 10-h overnight fast. Plasma glucose (mmol/l), C-peptide (nmol/l), insulin (pmol/l) and HbA<sub>1c</sub> (mmol/mol) were also determined.<sup>8</sup> More detail is provided in the Supporting information.

The Matsuda index was calculated as  $10\,000/[\text{fasting glucose} * \text{fasting insulin}]/[\text{mean glucose} * \text{mean insulin during OGTT}]^{0.5}$  and insulin resistance was estimated using homeostatic model assessment of insulin resistance (HOMA-IR), calculated as  $\text{fasting insulin (mU/ml)} \times \text{fasting glucose (mmol/l)}/22.5$ . From the OGTT, a mathematical model that describes the association between glucose concentration and insulin secretion was used to estimate insulin secretion rates and total insulin secretion rate (nmol/m<sup>2</sup>).<sup>9</sup> The insulinogenic index was calculated as  $(\text{Insulin}_{30} - \text{Insulin}_0)/(\text{Glucose}_{30} - \text{Glucose}_0)$ .<sup>10</sup>

### 2.3 | Assessment of covariates

Questionnaires were used to collect data on parental history of diabetes, smoking and alcohol status. Waist circumference (cm), weight (kg) and height (m) were also measured. Body mass index (BMI) was calculated as  $\text{weight (kg)}/\text{height (m)}^2$ . Blood pressure (mmHg) was also measured. ActiGraph triaxial accelerometers were used to assess physical activity.

### 2.4 | Data analysis

Latent class trajectory analysis with cubic polynomials for the specification of time was used to identify glucose

curve subgroups from OGTTs at baseline and follow-up. The best-fitting classification model was determined based on the Bayesian Information Criterion (BIC) and the Akaike Information Criterion (AIC). The lowest BIC and AIC suggesting the best fit, and a difference of at least 10 points regarded as sufficient improvement. Additionally, groups were selected if mean membership probabilities, i.e. the probability of an individual belonging to a particular group, were  $>0.8$  (Table S1). More details of the latent class trajectory analysis are described elsewhere.<sup>11-13</sup> The `lcmm` function in the `lcmm` package in R (version 3.2.1) was used to conduct the latent class trajectory analysis.

Baseline clinical characteristics were compared between subgroups using univariate analysis. We used linear models to calculate coefficients ( $\beta$ ) and 95% CIs to estimate if any of the identified subgroups were associated with glycaemic traits at baseline and with change in glycaemic traits at 18 months, i.e. using actual trait at follow-up as the outcome while conditioning on the baseline value. The lowest glucose peak subgroup (Subgroup 1) was used as the reference. We adjusted for multiple testing using the Bonferroni method and thereafter a pairwise comparison of subgroup means using Tukey's honestly significant difference test<sup>14</sup> for continuous traits that remained significant after Bonferroni correction. For prospective analysis, two multivariable linear models were formulated. Model 1 adjusted for age, sex, follow-up time, study centre and respective baseline glycaemic traits. Model 2 additionally adjusted for smoking and physical activity. Moreover, we produced plots of serum insulin levels corresponding to each glucose response curve at baseline and follow-up. Finally, by using areas under the curve (AUCs), we compared the subgroups' discriminative ability to predict incident diabetes with the clinical model proposed by Wilson *et al.* from the Framingham offspring study.<sup>15</sup> Missing values were below 5% for all variables except for physical activity (19%). Therefore, missing values for physical activity were imputed using a predictive mean matching method in the MICE (multivariate imputation by chained equations) package in R.<sup>16</sup>

For sensitivity analyses, we identified the baseline glucose subgroups stratified by centre and sex. We also identified the curves using less but commonly used five time points (0, 30, 60, 90 and 120 min) of the OGTT and we fitted cubic polynomials to specify time for these analyses.

All curves were smoothed using locally estimated scatterplot smoothing estimates and all statistical analyses were conducted using R software (version 3.0.1). All statistical tests were two-sided, with significance ascertained at 5% (see Supporting information for more details on Methods).

## 2.5 | Ethics

All participants signed informed consents and each centre's ethics review boards separately approved this study. The study also conformed to the Declaration of Helsinki standards.

## 3 | RESULTS

### 3.1 | Population characteristics

At baseline, we included 2126 participants (76% men) with a mean  $\pm$ sd age of  $61.6 \pm 6.3$  years. The mean  $\pm$  SD BMI was  $27.9 \pm 3.9$  kg/m<sup>2</sup>, HbA<sub>1c</sub> was  $37 \pm 2.9$  mmol/mol (5.5%), fasting glucose was  $5.6 \pm 0.5$  mmol/l and 2-h glucose was  $5.9 \pm 1.6$  mmol/l (Table 1).

### 3.2 | Baseline clinical profiles

We identified four subgroups with different glucose patterns, labelled in order of increasing 1-h glucose peak levels as Subgroup 1 to Subgroup 4 (Table 1 and Figure 1). We had 577 participants (27%) in Subgroup 1, 1012 (48%) in Subgroup 2, 327 (15%) in Subgroup 3 and 210 (10%) in Subgroup 4. Membership probabilities were all  $>0.80$ , ranging from 0.86 to 0.89. Subgroup 1 had the earliest and lowest glucose peak (7.3 mmol/l), while Subgroup 3 took the longest (1-h) to reach peak and had the highest 2-h level (8.3 mmol/l). Subgroup 4 had the highest peak (14.5 mmol/l) and 1-h level (12.4 mmol/l). Similar glucose subgroups, i.e. four subgroups, were observed when we stratified the models by sex and centre. Three subgroups were identified as optimum when using five OGTT time points, with all membership probabilities  $>80\%$  (Figure S2).

Although the insulin curves between 0 to 45 min were more tightly clustered compared to the glucose curves, there was marked variation between 1 h and 2 h. Subgroups 1 and 2 had the earliest and lowest insulin peaks, while Subgroups 3 and 4 had the latest and highest insulin peaks, respectively (Figure S3).

Participants in higher glucose peak subgroups, i.e. Subgroups 2–4, were more likely to be men and to have a significantly higher BMI, insulin resistance, systolic blood pressure, total insulin secretion rate and glucose values, compared to individuals in Subgroup 1 (Table 1). In general, with the exception of the Matsuda and insulinogenic indices, individuals in higher glucose peak subgroups had higher glycaemic trait values and hence a less favourable glycaemic trait profile, compared to participants in Subgroup 1. Subgroups 2 and 4 had similar fasting and 2-h glucose levels and took the same time to peak (45 min). However, participants in Subgroup 4 had a worse glycaemic trait profile, with a significantly higher glucose peak value, total insulin secretion rate, BMI, HbA<sub>1c</sub> and a lower Matsuda and insulinogenic

indices, compared to participants in Subgroup 2 (Table 1). Furthermore, Subgroup 3 with the highest 2-h value, had a significantly higher 1-h glucose level, BMI, total insulin secretion rate and HOMA-IR than Subgroup 2, with similar fasting, but lower 2-h glucose level (Table 1).

### 3.3 | Prospective analyses

After a median follow-up of 18 months, 57 participants (2.8%) developed type 2 diabetes. No differences in characteristics were observed between those lost to follow-up and those in the study, with the exception of sex and centre (Table S2).

Change in HOMA-IR was significantly higher in Subgroups 2–4, compared to Subgroup 1 (Table 2). The same was observed for change in C-peptide, plasma glucose and insulin levels. Change in total insulin secretion rate, Matsuda and insulinogenic indices was lower in Subgroups 2–4, indicating reduced insulin sensitivity and insulin secretion, compared to Subgroup 1.

When comparing Subgroups 2 and 4, with similar fasting and 2-h glucose levels, relative to the reference (Subgroup 1), individuals in Subgroup 4 had significantly higher values in the change in HOMA-IR, plasma glucose and plasma insulin levels and lower values in the change in Matsuda and insulinogenic indices. The same was generally observed when comparing subgroups with the same fasting but different 2-h glucose levels, i.e. Subgroups 3 and 2, with Subgroup 3 participants progressing faster than participants in Subgroup 2 (Table 2).

The subgroups had a moderate discriminative ability for incident type 2 diabetes [AUC 0.70 (95% CI 0.64–0.76)], which was comparable to the clinical model from the Framingham offspring study [AUC 0.75 (95% CI 0.69–0.80)].

Five subgroups were identified at 18 months and labelled in order of increasing 1-h glucose peak levels as Subgroups 1–5 (Figure 2).

With respect to change in trajectory, the majority of participants remained in the same subgroup, or progressed to higher peak subgroups at follow-up (Figure 3 and Table S4). The insulin response curves had similar patterns, with corresponding glucose response curves at follow-up (Figure S4).

Using binary logistic regression, we investigated the association between baseline subgroups with incident type 2 diabetes. Higher peak subgroups, i.e. Subgroups 3 and 4, had higher odds ratios compared to Subgroup 1 (Table S3).

## 4 | DISCUSSION

Using seven-point OGTT data from 2126 participants at elevated risk of type 2 diabetes, combined with the latent class trajectory analysis approach, four glucose curve subgroups with different clinical profiles were identified.

**TABLE 1** Baseline characteristics of 2126 participants at elevated glycaemic deterioration risk stratified by glucose curve subgroups. [Correction added on 2 December 2020, after first online publication: The position of values in column 'Subgroup 4' and row with text 'Fasting plasma glucose, mmol/l' to row with text 'Insulinogenic index' are corrected.]

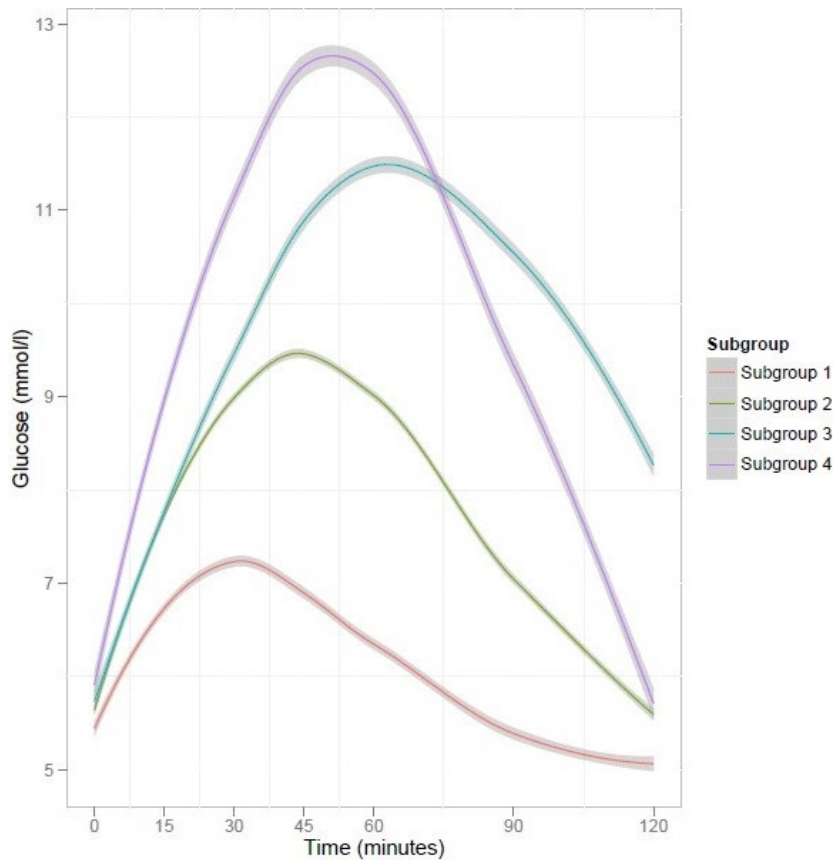
Characteristic	Total	Subgroups			
		Subgroup 1	Subgroup 2	Subgroup 3	Subgroup 4
Number of participants, <i>n</i> (%)	2126 (100)	577 (27)	1012 (48)	327 (15)	210 (10)
Age, years	61.6 (6.3)	62.1 (6.4) <sup>bd</sup>	61.2 (6.3) <sup>ac</sup>	62.4 (6.3) <sup>bd</sup>	60.5 (5.9) <sup>ac</sup>
Men, <i>n</i> (%)	1608 (76)	349 (60)	804 (79)	263 (80)	192 (91)
BMI, kg/m <sup>2</sup>	27.9 (3.9)	27.0 (3.9) <sup>bcd</sup>	27.9 (3.8) <sup>ac</sup>	29.1 (4.1) <sup>ab</sup>	28.5 (3.7) <sup>a</sup>
Waist circumference, cm	100.0 (11.1)	95.9 (11.2) <sup>bcd</sup>	100.2 (10.6) <sup>acd</sup>	104.0 (10.4) <sup>ab</sup>	103.5 (10.5) <sup>ab</sup>
Smoking status: current, <i>n</i> (%)	312 (15)	80 (14)	159 (16)	36 (11)	37 (18)
Alcohol status: never, <i>n</i> (%)	263 (12)	72 (12)	124 (12)	45 (14)	22 (10)
Average physical activity intensity, mgs	37.1 (9.6)	37.4 (9.4)	37.6 (9.9) <sup>c</sup>	34.3 (8.7) <sup>b</sup>	38.4 (9.3)
Systolic blood pressure, mmHg	131 (16)	127 (16) <sup>bcd</sup>	131 (15) <sup>acd</sup>	135 (16) <sup>abd</sup>	132 (14) <sup>abc</sup>
Family history: parents, yes, <i>n</i> (%)	606 (29)	146 (25)	285 (28)	99 (30)	76 (36)
Glucose/insulin measures					
Glucose peak values <sup>†</sup> , mmol/l	9.4	7.3 <sup>bcd</sup>	9.4 <sup>acd</sup>	11.5 <sup>abd</sup>	12.6 <sup>abc</sup>
Time to peak, min	45	30 <sup>bcd</sup>	45 <sup>acd</sup>	60 <sup>abd</sup>	45 <sup>ac</sup>
HbA <sub>1c</sub> , mmol/mol	37.0 (2.9)	36.2 (2.7) <sup>cd</sup>	37.0 (2.8) <sup>cd</sup>	37.9 (3.2) <sup>ab</sup>	38.2 (3.0) <sup>abc</sup>
HbA <sub>1c</sub> , %	5.5	5.5 <sup>cd</sup>	5.5 <sup>cd</sup>	5.6 <sup>ab</sup>	5.6 <sup>ab</sup>
Blood glucose, mmol/l	5.5 (0.6)	5.4 (0.6) <sup>cd</sup>	5.5 (0.6) <sup>cd</sup>	5.7 (0.7) <sup>abd</sup>	5.8 (0.6) <sup>ab</sup>
C-peptide, nmol/l	0.8 (0.3)	0.7 (0.3) <sup>bcd</sup>	0.8 (0.3) <sup>acd</sup>	1.0 (0.4) <sup>abd</sup>	0.9 (0.4) <sup>ab</sup>
Fasting insulin, mmol/l*	7.8 (4.7–11.5)	6.1 (4.2–9.6) <sup>c</sup>	7.8 (4.8–11.2) <sup>c</sup>	10.4 (6.3–15.0) <sup>ab</sup>	9.1 (5.5–12.5) <sup>ab</sup>
2-h postprandial insulin, pmol/l*	33.5 (19.0–59.4)	22.5 (14.1–36.2) <sup>cd</sup>	32.4 (18.9–53.2) <sup>cd</sup>	76.1 (49.3–120.0) <sup>abd</sup>	36.7 (22.0–61.6) <sup>abc</sup>
Fasting plasma glucose, mmol/l	5.6 (0.5)	5.3 (0.4) <sup>bcd</sup>	5.7 (0.4) <sup>ad</sup>	5.8 (0.5) <sup>ad</sup>	6.0 (0.4) <sup>abc</sup>
1-h postprandial glucose, mmol/l	8.9 (2.3)	6.2 (1.0) <sup>bcd</sup>	8.8 (1.1) <sup>acd</sup>	11.5 (1.2) <sup>abd</sup>	12.4 (1.3) <sup>abc</sup>
2-h postprandial glucose, mmol/l	5.9 (1.6)	5.1 (1.1) <sup>bcd</sup>	5.6 (1.3) <sup>ac</sup>	8.3 (1.3) <sup>abd</sup>	5.8 (1.4) <sup>ac</sup>
Impaired fasting glucose, <i>n</i> (%)	553 (25)	49 (8)	250 (25)	128 (39)	108 (51)
Impaired glucose tolerance, <i>n</i> (%)	285 (13)	5 (1)	48 (4)	206 (63)	20 (10)
Matsuda index	5.0 (2.9)	6.6 (3.3) <sup>bcd</sup>	4.8 (2.6) <sup>acd</sup>	3.6 (2.3) <sup>abd</sup>	3.7 (2.0) <sup>abc</sup>
HOMA-IR	2.3 (1.6)	1.8 (1.3) <sup>bcd</sup>	2.2 (1.3) <sup>cd</sup>	3.1 (2.1) <sup>abd</sup>	2.8 (2.1) <sup>abc</sup>
Total insulin secretion rate, nmol/m <sup>2</sup>	51.9 (17.4)	42.5 (12.8) <sup>bcd</sup>	51.8 (15.5) <sup>acd</sup>	62.7 (19.1) <sup>ab</sup>	61.3 (19.3) <sup>ab</sup>
Insulinogenic index	119.7 (90.4)	192.5 (67.1) <sup>bcd</sup>	101.4 (67.8) <sup>acd</sup>	86.1 (57.8) <sup>abd</sup>	67.6 (51.2) <sup>abc</sup>
Centre, <i>n</i> (row %)					
Copenhagen	322 (14)	45 (16)	136 (49)	64 (23)	30 (11)
Kuopio	1274 (57)	236 (19)	633 (51)	205 (17)	165 (13)
Lund	147 (7)	71 (51)	42 (30)	24 (17)	2 (1)
Hoorn	490 (22)	225 (48)	201 (42)	34 (7)	13 (3)

Abbreviation: HOMA-IR, homeostatic model assessment of insulin resistance.

Data are mean ± SD for continuous data and all such values, unless stated otherwise. \*Values are median (interquartile range). †We assessed the highest glucose value in each subgroup as the peak and the corresponding time point as time to peak. All *P* values <0.001 except for smoking (0.26). For all other traits, *P* values remained robust after Bonferroni correction for multiple testing. Superscript letters a to d denote the subgroups that are statistically significantly different using Tukey *post hoc* test. Superscript letters a, b, c and d represent Subgroups 1, 2, 3 and 4, respectively.

These differences were evident even in subgroups with similar fasting and 2-h glucose levels. In short, participants in subgroups with the highest glucose peaks and the highest 1-h glucose levels (Subgroups 2–4) had the worst glycaemic traits profile compared to participants in the subgroup with the lowest and earliest glucose peak. Similarly,

participants in the subgroup with the highest 2-h glucose level had a worse glycaemic traits profile compared to participants in the subgroup with similar fasting glucose but lower 2-h glucose level. This is consistent with previous literature showing that, among people with impaired glucose regulation, the 2-h concentration was a stronger predictor of



**FIGURE 1** Four glucose curve subgroups following an oral glucose tolerance test depicting estimated mean trajectories with 95% CIs identified by the latent class trajectory analysis

type 2 diabetes risk than fasting glucose.<sup>17,18</sup> Nevertheless, some data suggest that fasting glucose and 2-h glucose are similarly associated with risk of diabetes.<sup>19</sup> The subgroups with the worst glycaemic profiles also had the highest 1-h glucose levels, which is consistent with recent studies suggesting that 1-h plasma glucose is a stronger predictor of incident type 2 diabetes risk compared to other standard measures, i.e. fasting and 2-h glucose.<sup>20–24</sup> Based on conventional measures, such as the current WHO criteria for the diagnosis of impaired glucose regulation, only 39% and 63% of the participants in subgroup 3, and 51% and 10% in subgroup 4, would be considered as having impaired fasting glucose (IFG) or impaired glucose tolerance (IGT), respectively. Consequently, it is plausible that there are individuals at high risk for type 2 diabetes and its associated complications, who would be missed if only one or two time points of the OGTT were used. This is supported by our sensitivity analyses, with a loss of one subgroup when using fewer time points to identify subgroups. Therefore, our results underscore the necessity of including characteristics of intermediate time points in the identification of individuals at high risk of type 2 diabetes.

Our findings are in line with previous studies that used a similar latent class trajectory analysis approach and identified four similar subgroups.<sup>5,7,25</sup> This highlights the robustness of the latent class trajectory analysis approach. Two of the studies, one conducted in an Indian<sup>5</sup> and one in a European

population,<sup>7</sup> reported that the subgroup with the highest plasma glucose peak had the least favourable cardiometabolic risk profile at baseline and was associated with a higher risk of incident type 2 diabetes, compared to the subgroup with the lowest glucose response. In contrast to the present study, the identified subgroups in these studies were based on three-point OGTT measurements and were missing 1-h glucose. This may lead to an underestimation of type 2 diabetes risk in these studies. One study<sup>25</sup> additionally looked at the stability of glucose curves after 3 years of follow-up and identified similar glucose curve patterns at follow-up and baseline. This is somewhat similar to our study because we found similar patterns at follow-up, with the exception that we found an additional subgroup, making it five subgroups in total. This study also showed that the latent class trajectory analysis approach can be used to classify new individuals not part of the development data.

Another study using the latent class trajectory analysis approach identified five glucose curve subgroups with distinct cardiometabolic profiles.<sup>6</sup> That study included participants from the general population, while we selected those at elevated type 2 diabetes risk. Consequently, our population was more homogenous at baseline, which may explain the lower number of subgroups in the present study. Higher heterogeneity might have increased the number of subgroups at follow-up because participants progressed in different degrees. Moreover, the fifth group probably includes those who

TABLE 2 Association between glucose curve subgroups and glycaemic traits measured at 18 months in 1988 participants.

Characteristic	Subgroups				P
	Subgroup 1	Subgroup 2	Subgroup 3	Subgroup 4	
Number of subjects, <i>n</i> (%)	532 (26.5)	954 (47.5)	303 (15.1)	199 (9.9)	
Mean (SD) follow-up, months	17.7 (1.5)	18.2 (1.3)	18.3 (1.4)	18.4 (1.3)	
HOMA-IR					
Model 1	1.7 <sup>bc</sup>	0.2 (0.0–0.3)	0.3 (0.1–0.5) <sup>a</sup>	0.3 (0.1–0.5) <sup>a</sup>	<0.001
Model 2	3.6 <sup>abc</sup>	0.2 (0.0–0.3) <sup>a</sup>	0.3 (0.1–0.5) <sup>a</sup>	0.4 (0.1–0.6) <sup>a</sup>	<0.001
Plasma C-peptide (nmol/l)					
Model 1	0.2 <sup>ab</sup>	0.0 (0.0–0.1) <sup>a</sup>	0.1 (0.0–0.1) <sup>a</sup>	0.0 (0.0–0.1)	<0.001
Model 2	0.6 <sup>abc</sup>	0.0 (0.0–0.1) <sup>a</sup>	0.1 (0.0–0.1) <sup>a</sup>	0.0 (0.0–0.1) <sup>a</sup>	<0.001
Plasma insulin (pmol/l)					
Model 1	5.7 <sup>abc</sup>	0.7 (0.2–1.2) <sup>ad</sup>	1.2 (0.5–1.9) <sup>a</sup>	1.3 (0.5–2.1) <sup>ab</sup>	<0.001
Model 2	13.2 <sup>abc</sup>	0.7 (0.2–1.2) <sup>ad</sup>	1.2 (0.5–1.9) <sup>a</sup>	1.4 (0.7–2.1) <sup>ab</sup>	<0.001
Plasma glucose (mmol/l)					
Model 1	3.3 <sup>abc</sup>	0.1 (0.1–0.2) <sup>ad</sup>	0.2 (0.1–0.3) <sup>a</sup>	0.2 (0.2–0.3) <sup>ab</sup>	<0.001
Model 2	3.3 <sup>abc</sup>	0.1 (0.09–0.2) <sup>ad</sup>	0.2 (0.1–0.3) <sup>a</sup>	0.2 (0.2–0.3) <sup>ab</sup>	<0.001
Matsuda index					
Model 1	−4.2 <sup>abc</sup>	−1.4 (−1.7 to −1.0) <sup>acd</sup>	−2.4 (−2.8 to −2.0) <sup>ab</sup>	−2.4 (−2.9, −1.9) <sup>ab</sup>	<0.001
Model 2	−2.8 <sup>abc</sup>	−1.2 (−1.6 to −0.9) <sup>acd</sup>	−2.2 (−2.4 to −1.5) <sup>ab</sup>	−2.2 (−2.7, −1.7) <sup>ab</sup>	<0.001
Total insulin secretion (nmol/m <sup>2</sup> )					
Model 1	17.3 <sup>abc</sup>	−1.2 (−3.4 to −0.6) <sup>ac</sup>	−3.0 (−4.5 to −1.0) <sup>ab</sup>	−2.0 (−3.9, −0.1) <sup>a</sup>	<0.001
Model 2	19.4 <sup>abc</sup>	−1.8 (−3.3 to −0.4) <sup>ac</sup>	−2.9 (−4.8 to −0.9) <sup>ab</sup>	−2.3 (−4.4, −0.5) <sup>a</sup>	<0.001
Insulinogenic index					
Model 1	196.2 <sup>abc</sup>	−41.5 (−61.2 to −21.9) <sup>a</sup>	−46.6 (−72.4 to −20.7) <sup>a</sup>	−62.1 (−91.8, −32.4) <sup>ab</sup>	<0.001
Model 2	228.7 <sup>abc</sup>	−41.3 (−61.0 to −21.7) <sup>a</sup>	−49.5 (−75.5 to −23.5) <sup>a</sup>	−61.3 (−91.1, −31.5) <sup>ab</sup>	<0.001

Abbreviation: HOMA-IR, homeostatic model of insulin resistance.

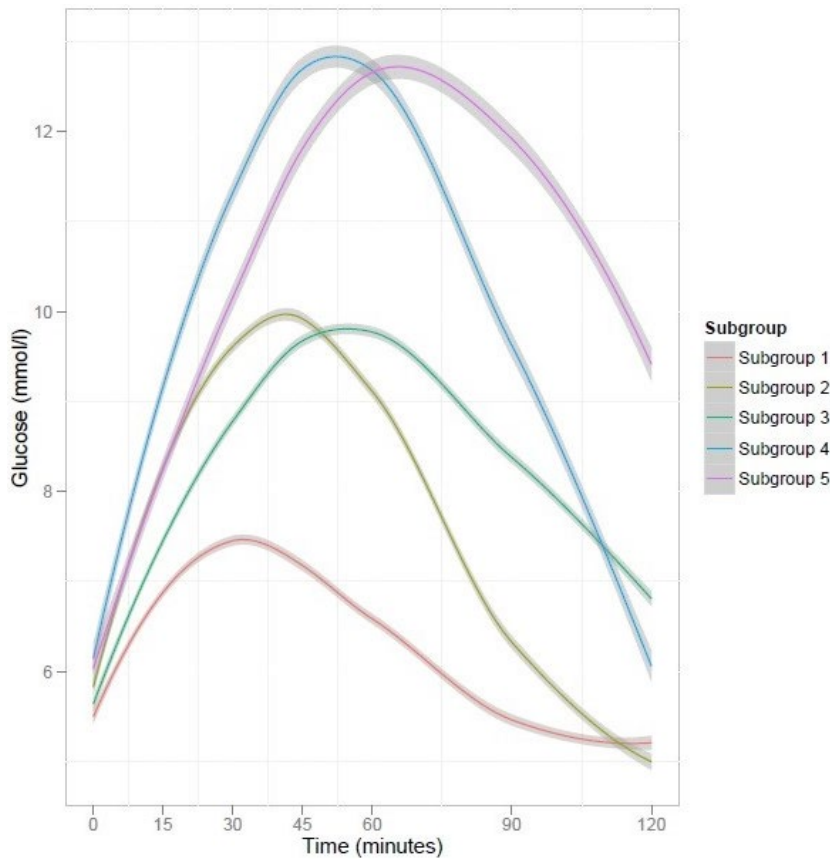
Values are  $\beta$  coefficients (95% CIs), unless otherwise indicated, with intercept values in Subgroup 1 (reference category). For all traits, *P* values remained robust after Bonferroni correction for multiple testing. Superscript letters a to d denote the subgroups' adjusted means that are statistically significantly different using Tukey *post hoc* test. Superscript letters a, b, c and d represent Subgroups 1, 2, 3 and 4, respectively. Model 1 was adjusted for age in years, sex (men or women), follow-up time in months, study centre (categories) and respective baseline glycaemic traits. Model 2 was additionally adjusted for smoking status (categories) and physical activity (in mgs).

progressed to diabetes and we excluded those with diabetes at baseline, possibly explaining why we had four subgroups at baseline instead of five. Another study,<sup>26</sup> like the present study, showed that heterogeneous glucose response curve patterns can be estimated with fewer time points during the OGTT.

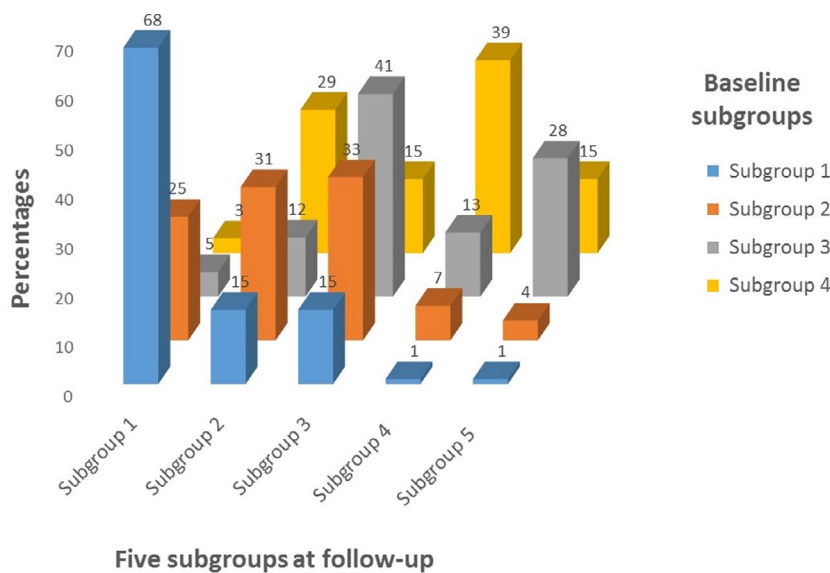
Several plausible mechanisms that could explain the heterogeneity in subgroups, include insulin secretion and insulin resistance. In the present study, participants in Subgroups 3 and 4 were the most insulin-resistant and had the highest 1-h glucose level. This suggests they had the lowest early-phase insulin secretion, compared to other subgroups. Early-phase glucose increase during an OGTT may be indicative of impaired  $\beta$ -cell function, and in particular, defects in early-phase insulin secretion and reduced insulin sensitivity could also help explain the variation in the subgroups.<sup>8</sup> Those with IGT have elevated 2-h levels and previous literature has shown that, although both IGT and IFG are associated with reduced early-phase insulin secretion, individuals with IGT

additionally have an impaired late-phase insulin secretion and a lower insulin sensitivity compared to those with normal glucose tolerance or with IFG.<sup>27–29</sup> These differences may help to explain why the subgroup with high 2-h glucose had a worse glycaemic traits profile compared to subgroups with similar fasting glucose but lower 2-h levels. These findings suggest that more emphasis should be put on identifying individuals at risk using the frequently sampled OGTT curve patterns and IGT, compared to using IFG only. Moreover, the glucose curve subgroups had a moderately good discriminatory ability for type 2 diabetes. In addition, the Diabetes Prevention Program study, indicates that those with IGT benefit from lifestyle interventions.<sup>30</sup> So, those high-risk individuals with elevated 1-h and 2-h glucose levels would benefit from lifestyle interventions similar to those offered to people with IGT, thus preventing and delaying the development of diabetes and associated complications. Nevertheless, no clear differences in physical activity between subgroups





**FIGURE 2** Five glucose curve subgroups following an oral glucose tolerance test depicting estimated mean trajectories with 95% CIs identified by the latent class trajectory analysis at 18-month follow-up



**FIGURE 3** Percentages of 2126 participants from the four glucose curve subgroups at baseline contained in the five glucose curve subgroups identified at follow-up: the IMI-DIRECT study

was observed in the present study. However, other modifiable factors such as BMI, waist circumference and blood pressure, which are correlates of physical activity, increased with increasing subgroups, hence lifestyle modification could still help to prevent and/or improve glycaemic control of those at diabetes risk.

The present study has some limitations. First, the inclusion of only white European adults may limit the generalizability of the results. Second, we only had a short follow-up time of

18 months; hence, it was not possible to investigate associations with incident type 2 diabetes (due to low numbers, i.e. 57) and cardiovascular outcomes. Longer follow-up time is needed to assess these associations to draw strong conclusions. Nevertheless, we attempted the analysis with incident type 2 diabetes (Table S3), and higher peak subgroups had higher odds ratios compared to Subgroup 1. Lastly, more men were lost to follow-up than women and more participants from two study centres were lost to follow-up, which could have led to selection bias.

The present study also has several strengths. First, the use of a large sample of participants at an elevated risk of type 2 diabetes enabled us to perform a mixed-model analysis and achieve a detailed characterization of the identified subgroups. Second, instead of using predefined groups to identify the different glucose curve subgroups, we used the data-driven latent class trajectory analysis approach. Using predefined characteristics to categorize glucose curves might spuriously introduce heterogeneous patterns. Although the latent class trajectory analysis method can provide insight into the heterogeneity among those at risk of diabetes, which might be significant in creating more personalized prevention strategies, it should be noted that one cannot predict the subgroup of a specific individual based on their single glucose profile, hence limiting the direct clinical utility of the latent class trajectory analysis method. Finally, we included participants at an elevated type 2 diabetes risk, which made it possible to identify glucose subgroups that may otherwise be indiscernible in an unselected population.

In conclusion, using data from a frequently sampled OGTT, glucose curve subgroups with different clinical characteristics and different rates of subsequent glycaemic deterioration can be identified using latent class trajectory analysis. The heterogeneity in glucose response curves and glycaemic trait profiles may reflect different underlying pathophysiologies, which cannot be determined using only fasting and 2-h levels. Furthermore, the number of time points in the OGTT influenced the number of subgroups identified, with a seven-point OGTT having more subgroups than a five-point OGTT.

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## COMPETING INTERESTS

None declared.

## DATA AVAILABILITY STATEMENT

This study analysed already existing data obtained upon request and subject to licence restrictions from a number of different sources and, due to ethical and legal concerns, data cannot be made openly available. To access data, a data access form should be completed and returned to the Data Access Committee Executive Group (DIRECTdataaccess@Dundee.ac.uk).

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

Supplementary Material  
Supplementary Material

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