Title: Determinants of diagnostic discordance for non-diabetic hyperglycaemia and type 2 diabetes using paired glycated haemoglobin measurements in a large English primary care population: cross sectional study

Running title: Diagnostic discordance for non-diabetic hyperglycaemia and type 2 diabetes

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Novelty statement

- What is already known? Diagnosis of non-diabetic hyperglycaemia is a key component of diabetes prevention programmes and clinical practice. Non-diabetic hyperglycaemia diagnosis with a single test often changes to normality when re-tested.
- What has this study found? Classification based on both HbA1c and fasting plasma glucose independently predicted discordant diagnosis of non-diabetic hyperglycaemia and type 2 diabetes.
- What are the clinical implications of the study? Diagnosis of non-diabetic hyperglycaemia and type 2 diabetes should be based on two HbA1c measurements.

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Abstract

Aim: To investigate factors influencing diagnostic discordance for non-diabetic hyperglycaemia and type 2 diabetes.

Methods: 10,000 adults at increased risk of diabetes were screened with HbA1c and fasting plasma glucose (FPG). The 2,208 with initial HbA1c ≥42 mmol/mol (≥6.0%) or FPG ≥ 6.1 mmol/L were retested after a median 40 days. We compared the first and second HbA1c results, and consequent diagnoses of non-diabetic hyperglycaemia and type 2 diabetes, and investigated predictors of discordant diagnoses.

Results: Of 1,463 participants with non-diabetic hyperglycaemia and 394 with type 2 diabetes on first testing, on repeated testing 28.4% and 21.1%, respectively, had discordant diagnoses. Initial diagnosis of non-diabetic hyperglycaemia and/or impaired fasting glucose according to both HbA1c and FPG criteria, or to FPG only, made reclassification as type 2 diabetes more likely than initial classification according to HbA1c alone. Initial diagnosis of type 2 diabetes according to both HbA1c and FPG criteria made reclassification much less likely than initial classification according to HbA1c alone. Age, and anthropometric and biological measurements independently but inconsistently predicted discordant diagnoses and changes in HbA1c.

Conclusions: Diagnosis of non-diabetic hyperglycaemia or type 2 diabetes with a single measurement of HbA1c in a screening programme for entry to diabetes prevention trials is unreliable. Diagnosis of non-diabetic hyperglycaemia and type 2 diabetes should be confirmed by repeat testing. FPG results could help prioritise retesting. These findings do not apply to people classified as normal on a single test, who were not retested.

Keywords: diagnosis, non-diabetic hyperglycaemia, reproducibility, type 2 diabetes

Introduction

The prevalence of type 2 diabetes mellitus is increasing rapidly worldwide [1,2]. This has prompted population-wide national diabetes prevention programmes, usually based on identifying people at highest risk of Type 2 diabetes using plasma glucose or haemoglobin A1c (HbA1c; glycated haemoglobin) data, who are then offered a lifestyle intervention to reduce the risk of progression to type 2 diabetes [3]. Randomised trials have shown that such interventions can be effective in preventing diabetes, but identification of the highest risk people can be problematic because of imperfect validity and reliability of diagnostic tests and because of recognised analytical and biological variation [4]. Changes in the diagnostic criteria for diabetes, from glucose based criteria (fasting plasma glucose (FPG) or oral glucose tolerance test) to measurement of haemoglobin A1c (HbA1c; glycated haemoglobin), has generated a large population with non-diabetic hyperglycaemia who are deemed to be at increased risk of type 2 diabetes [5-7]. In England the National Health Service (NHS) launched a national diabetes prevention programme in 2015, in which people diagnosed with non-diabetic hyperglycaemia are offered dietary and lifestyle counselling [5-6]. There are equivalent models in the United States [7].

An important but neglected problem with diagnosis of non-diabetic hyperglycaemia is that people diagnosed with non-diabetic hyperglycaemia on the basis of a single test may have normal values if retested soon after. NHS policy is that asymptomatic adults must have paired HbA1c testing before diagnosis of type 2 diabetes [8], as recommended by the World Health Organisation and the American Diabetes Association [9,10]. However for non-diabetic hyperglycaemia only one test is required to be eligible for the diabetes prevention programme [11]. If incorrectly diagnosed as having non-diabetic hyperglycaemia they may be unnecessarily labelled as being at high risk of diabetes, and exposed to costly and inconvenient preventive interventions. Population based diabetes programmes need evidence about the repeatability of non-diabetic hyperglycaemia screening, to help decide whether and in whom screening tests should be repeated before starting lifestyle interventions and treatment.

The present study is based on targeted screening data from the Norfolk Diabetes Prevention Study (NDPS, ISRCTN34805606) [12]. The study entailed testing over 12,000 adults with known risk factors for previously undiagnosed non-diabetic hyperglycaemia, impaired fasting glucose (IFG) and type 2 diabetes. Those whose HbA1c or FPG measurements indicated that they had non-diabetic hyperglycaemia, impaired fasting glucose (IFG) or type 2 diabetes were tested again for HbA1c and FPG a median of 40 days later. If their second test confirmed non-diabetic hyperglycaemia, IFG or type 2 diabetes they were invited to participate in various trials. We report elsewhere on the results of screening, including the prevalence of non-diabetic hyperglycaemia, IFG and type 2 diabetes, participant characteristics associated with these diagnostic classifications, and differences between initial and repeated diagnostic classifications, in the first 10,000 participants screened [13]. In the present analysis we focus on the anthropometric and biochemical factors associated with discordant non-diabetic hyperglycaemia or type 2 diabetes classification, and with discrepancies in HbA1c, on retesting. The purpose of this analysis is to investigate whether one can identify individuals who most need repeated testing, because they are most likely to have a change in diagnosis if retested.

The objectives of the present study were i) to compare initial and second HbA1c values recorded in each individual, ii) to estimate the probabilities of concordant or discordant diagnoses of non-diabetic hyperglycaemia and type 2 diabetes, iii) to investigate how initial HbA1c and FPG values, alone and in combination, predicted change from non-diabetic hyperglycaemia to normal glycaemic classification or to type 2 diabetes, and iv) to investigate whether other participant characteristics, anthropometric measurements and biochemical

measurements independently predicted change in HbA1c and discordant classification of non-diabetic hyperglycaemia and type 2 diabetes.

PARTICIPANTS AND METHODS

Design and population

This was a cross sectional study based on data gathered from the NDPS [12]. NDPS evaluates the efficacy of dietary and lifestyle counselling interventions which aim to prevent progression of non-diabetic hyperglycaemia or IFG to type 2 diabetes, and to improve management of newly diagnosed type 2 diabetes. NDPS aimed to screen over 10,000 people at highest risk of non-diabetic hyperglycaemia, IFG or type 2 diabetes and to randomize approximately 1,600 to several clinical trials. The size of the sample to be screened was calculated to enable differences in the primary outcomes to be estimated with 5% significance and 80% power [12].

The NDPS population comprised adults with known risk factors for previously undiagnosed non-diabetic hyperglycaemia, IFG or type 2 diabetes in the East Anglia region of England. Participants were initially identified through general practice electronic medical records as being at high risk of non-diabetic hyperglycaemia, IFG or type 2 diabetes, as defined below, and tested by HbA1c and fasting plasma glucose (FPG). If they initially tested positive for non-diabetic hyperglycaemia, IFG or type 2 diabetes, they were tested again to confirm their diagnosis. NDPS contacted 194 general practices in Norfolk, Suffolk, and North East Essex. By March 2016, 135 general practices participated, with a combined practice population of 1.8 million. All individuals were contacted if their general practice electronic health records indicated no known diabetes and a) age ≥ 50 and body mass index (BMI) ≥ 30kg/m^2; or b) age ≥ 50 years and BMI ≥ 25kg/m^2 and recorded first degree family history of type 2 diabetes, coronary artery disease, or gestational diabetes; or c) any previous record of IFG, impaired glucose tolerance (IGT) or FPG 6.1-7.0 mmol/L; or d) any record of HbA1c 42-48 mmol/mol (6.0-6.5%) and FPG 5.6-6.9 mmol/L. 141,973 people satisfying these criteria were contacted, and 12,778 (9%) registered for participation. The present study included all individuals who had non-diabetic hyperglycaemia, IFG or type 2 diabetes on initial HbA1c or FPG test, among the first 10,000 tested.

Data collection

Following an overnight fast, participants underwent venesection for FPG and HbA1c, and demographic, anthropometric and biochemical data were recorded. Follow-up tests for both HbA1c and FPG were conducted for all individuals whose initial HbA1c or FPG results indicated non-diabetic hyperglycaemia, IFG or type 2 diabetes. Repeated venesection for measurement of HbA1c and FPG was carried out a median of 40 (interquartile range 27-69) days after the first venesection. For this study non-diabetic hyperglycaemia was defined as HbA1c 42 to 47 mmol/mol (6.0% to 6.4%), IFG was defined as FPG $\geq$6.1 or $\geq$5.6 to <7.0 mmol/L (depending on classification criteria at the time of testing), and type 2 diabetes was defined as HbA1c $\geq$48 mmol/mol ($\geq$6.5%) or FPG $\geq$7.0 mmol/L [14-16]. We used the latter definition of non-diabetic hyperglycaemia, instead of the American Diabetes Association's definition of prediabetes (39-47 mmol/mol (5·7-6·4%) [17]), so as to conform to current practice in the English National Health Service (NHS) where the range 42-47 mmol/mol (6.0% to 6.4%) is used in national diabetes prevention policy guidance [14], in the national vascular screening programme [18], and in the NHS diabetes prevention programme [19], which determined the choice of this range in the original programme protocol [12]. We were unable to use the American Diabetes Association's definition of prediabetes [17] for the statistical analyses reported in this paper because participants with initial HbA1c 39-41 mmol/mol (5.7-5.9%) were not retested unless they also had initial FPG$\geq$5.6 or $\geq$6.1 mmol/L.

Anthropometric measurements (weight, body mass index, body fat mass, visceral fat, and body fat percentage) were measured with a Tanita body fat composition analyser (TANITA – Hoogoorddreef, 1011 BE, Amsterdam, the Netherlands. Model BC-420 MA). HbA1c was measured using Affinity high performance liquid chromatography (Hb9210: Menarini Diagnostics Ltd., Wokingham, UK). FPG was measured by a hexokinase/G-6-PDH method on an automated platform (Architect c8000: Abbott Diagnostics, Maidenhead, UK).

Statistical analysis

The statistical analysis aimed to estimate the prevalences, and to identify predictors, of discordant or confirmed diagnosis of non-diabetic hyperglycaemia and type 2 diabetes and changes in HbA1c. Statistical analysis was performed with STATA version 15 (StataCorp, Texas) software. A 5% significance level was used.

Discordant non-diabetic hyperglycaemia was defined as diagnosis of non-diabetic hyperglycaemia on initial HbA1c test combined with diagnosis of normality or type 2 diabetes on the second HbA1c test. Discordant type 2 diabetes was defined as diagnosis of type 2 diabetes on initial HbA1c test combined with diagnosis of normality or type 2 diabetes on the second HbA1c test.

Summary statistics were computed as means and standard deviations, or counts and proportions. We tested whether participant characteristics, anthropomorphic measurements or biochemical measurements were associated with discordant diagnoses of non-diabetic hyperglycaemia or type 2 diabetes, first using chi square and t tests.

We assessed the added value of FPG in predicting discordant diagnosis of non-diabetic hyperglycaemia and of type 2 diabetes, as follows. We cross-tabulated the initial classification of non-diabetic hyperglycaemia and/or IFG based on initial HbA1c and/or FPG (5.6-7.0 mmol/L) results with classification of normality, non-diabetic hyperglycaemia or type 2 diabetes based on second HbA1c results. We then tested the independent associations between these initial classifications and the three possible classifications based on second HbA1c results, using multinomial logistic regression. Non-diabetic hyperglycaemia was defined as the base outcome category. In this model we included baseline covariates that were associated with discordant non-diabetic hyperglycaemia at 10% significance level (Table 1), and weeks from first to second HbA1c test. However, because body mass index and body fat mass were highly correlated with each other (Pearson R^2=0.88) and because both are measures of adipositity, we excluded body fat mass from the models.

We cross-tabulated the initial classification of type 2 diabetes, based on initial HbA1c and/or FPG results, with subsequent classification of normality, non-diabetic hyperglycaemia or type 2 diabetes, based on second HbA1c results. Because very few participants changed from type 2 diabetes to normality we pooled them with those who changed to non-diabetic hyperglycaemia to create a binary outcome indicating discordance. We constructed a logistic regression model with discordant classification of type 2 diabetes as outcome. Model covariates were initial HbA1c and/or FPG classification of type 2 diabetes, baseline variables associated with discordant type 2 diabetes at 10% significance level (Table 1), except for body fat mass, and weeks from first to second HbA1c test.

We calculated the difference between the second and first HbA1c results, and tested whether this difference was independently associated with initial HbA1c, initial FPG or with other participant characteristics, biological or anthropomorphic measurements, using multiple linear regression models. Linear regression analyses were conducted separately for participants with initial diagnoses of non-diabetic hyperglycaemia or type 2 diabetes. All variables listed in Table 1 were initially included as potential explanatory variables, and then removed if they were not independently associated with change in HbA1c in either subgroup

at 10% significance level. We retained the same covariates in the final models for both subgroups to enable comparison between the subgroups.

Although various regression-based methods could be used to evaluate the incremental value of additional assays for diagnosis [20,21], they were unsuitable for our purpose of examining factors associated with discordant results of a single assay.

All participants gave written informed consent to participate. Ethical review and approval was provided by the National Research Ethics Service (NRES), Essex 1 Research Ethics Committee (10/H0301/55; 13.1.2011). The study was carried out according to NRES permissions and with research governance approval from the sponsor organisation (Norfolk and Norwich University Hospital NHS Foundation Trust).

RESULTS

A total of 2208 participants whose initial HbA1c or FPG results indicated non-diabetic hyperglycaemia, IFG or type 2 diabetes were retested and comprised the sample described in the present study. These participants were mostly white British nationals, with mean age 65 years, mean BMI 31 Kg/m^2, and 42% had a family history of type 2 diabetes (Table 1).

Discordant classification of non-diabetic hyperglycaemia was more likely in participants with higher BMI, body fat mass, diastolic blood pressure, triglycerides and weeks between tests, and with lower age, initial HbA1c and initial FPG (Table 1). Discordant classification of type 2 diabetes was more likely in participants with lower BMI, waist circumference, body fat percentage, body fat mass, initial HbA1c, initial FPG, and weeks between tests (Table 1).

Of 1463 with initial HbA1c values indicating non-diabetic hyperglycaemia, on repeated testing 71.6% had non-diabetic hyperglycaemia confirmed, 21.3% had lower values indicating normality and 7.1% had values indicating type 2 diabetes. When classification of IFG or non-diabetic hyperglycaemia based on initial FPG and HbA1c results were considered together (Table 2), those with IFG and non-diabetic hyperglycaemia according to both assays were slightly more likely to be classified as having non-diabetic hyperglycaemia on repeated testing, compared to those with non-diabetic hyperglycaemia according to HbA1c only (74.4% vs. 68.3%), but were much more likely than those initially with IFG according to FPG only (24.0%).

Of 394 with initial HbA1c values indicating type 2 diabetes, 21.1% had lower values indicating NDH or normality later. When classification of type 2 diabetes based on initial FPG and HbA1c results were considered together (Table 2), those with type 2 diabetes according to both assays were more likely to be classified as having type 2 diabetes on repeated testing, compared to those with type 2 diabetes according to HbA1c only (90.7% vs. 71.7%), and much more likely than those initially with type 2 diabetes according to FPG only (11.5%).

Multinomial logistic regression (Table 3) showed that, after adjustment for baseline covariates, those initially classified as having non-diabetic hyperglycaemia were not significantly moreor less likely to be reclassified as normal if they also initially had impaired fasting glucose than if they only had non-diabetic hyperglycaemia (relative risk ratio (RRR) 0.91 (95%CI 0.63-1.31)). They were more likely to be reclassified as having type 2 diabetes (RRR 1.62 (95%CI 0.94-2.80)), but this association was not statistically significant (P=0.081). Without adjustment for covariates the respective RRRs were 0.58 (95%CI 0.45-0.76), P<0.001) and 5.0 (95%CI 3.8-6.5), P<0.001), indicating that participants initially classified with both tests were less likely to be reclassified as normal and were more likely to be reclassified as type 2 diabetes on second HbA1c testing. Those with impaired fasting glucose only were much more likely to be reclassified as normal (adjusted RRR 8.41) or type 2 diabetes (adjusted RRR 17.7). Age and weeks between tests were inversely associated with reclassification as normal.

Multiple logistic regression (Table 4) showed that, after adjustment for baseline covariates, those initially classified as having type 2 diabetes according to both FPG and HbA1c were much less likely to be reclassified as normal or non-diabetic hyperglycaemia than those classified according to HbA1c alone (odds ratio 0.28). Smaller waist circumference and more weeks between tests were independently associated with reclassification.

Multiple linear regression (Table 5) showed that, in participants with an initial diagnosis of non-diabetic hyperglycaemia, initial FPG, BMI and weeks between tests were independently associated with increased HbA1c between initial and second tests, and initial HbA1c and body fat mass were associated with decreased HbA1c. In participants with an initial diagnosis of type 2 diabetes, initial FPG and total cholesterol were independently associated with increased HbA1c, and initial HbA1c and low density lipoprotein were independently associated with decreased HbA1c (Table 5).

Discussion

This study shows that, in a population-based screening study to diagnose non-diabetic hyperglycaemia for entry into a diabetes prevention trial, high proportions of those initially classified by HbA1c as having non-diabetic hyperglycaemia (28%) and type 2 diabetes (21%) had different classifications when retested a few weeks later. Because HbA1c and fasting plasma glucose are known to vary randomly within individuals over time, it was predictable that individuals found to have high glucose or HbA1c levels on initial testing would tend to have lower levels on retesting, because of regression to the mean. Regression to the mean occurs when measurements are repeated which include some random variation, due either to true variation in the parameter being measured, or to measurement error, or both [22,23]. Individuals with initial measurements that are higher or lower than the average would tend to have repeated measurements that are closer to the average, due to chance alone. As participants in the present study were selected because they had HbA1c measurements that were higher than the average, it was to be expected that their repeated measurements would be lower, on average, than before, and more so for those with the highest initial values. The negative associations between initial HbA1c and change in HbA1c (Table 5) confirm that such regression to the mean did occur. We also found that decreases in HbA1c, and the probability of discordant classifications, were greater with more time between tests (Tables 3-5), which could be due to secular trends in true glycaemic levels [23], for example if participants' diet and activity changed after initial testing. .

Because repeated testing was carried out only in participants with elevated HbA1c or FPG, and not in those with normal test results, this study does not therefore provide complete evidence about the test-retest reliability of glycaemic classification based on HbA1c. What it provides is evidence about how reliable this classification is among participants initially classified at abnormal in a screening study. Screening programmes typically follow an abnormal screening test with a second, confirmatory, test before delivering an intervention. They do not typically repeat tests in those initially classified as normal, which would add to the cost of screening and further complicate decisions about appropriate management of participants with discordant classifications. The results of this study show that, to increase certainty that participants in screening truly have type 2 diabetes or non-diabetic hyperglycaemia that is not transient, it is desirable to repeat the test.

This study adds to our previous report [13] by investigating the value of participant characteristics other than initial HbA1c results in predicting whether individuals had discordant non-diabetic hyperglycaemia and type 2 diabetes diagnoses on retesting. The study showed that initial diagnosis of prediabetes according to both HbA1c and FPG criteria made reclassification as normal less likely, and reclassification as type 2 diabetes more likely, than initial classification according to HbA1c alone. Initial diagnosis of type 2 diabetes according to both HbA1c and FPG criteria also made reclassification much less likely than initial

classification according to HbA1c alone. Although age and various anthropometric and biological measurements independently predicted discordant diagnoses and changes in HbA1c, these associations were inconsistent and so do not help to identify individuals who most need retesting.

This approach is important in scoping capacity for national prevention programmes [5], and to normal clinical practice. It is estimated from the Health Survey for England that 10.7% of adults in England have non-diabetic hyperglycaemia [24] and national policy is that all such people should have diabetes prevention advice [5]. In the UK, this workload would fall largely on primary care and current workload pressures are such that some form of risk stratification and targeted intervention seems clinically essential. These data support modelling to develop a more focussed risk stratified approach.

When interpreting HbA1c data for diagnosis and monitoring, it is vital to understand Uncertainty of Measurement (UoM), which includes Biological Variation and the Total Analytical Error. The Total Analytical Error comprises the analytical imprecision and bias of the method and can be assessed using Sigma-metrics. Sigma-metrics targets for HbA1c have been published [25]. The HbA1c method used in the NDPS conforms to this quality standard and is standardized to the international Reference Measurement Procedure [26] as recommended in the worldwide consensus statement [27]. The analytical imprecision for the HbA1c method used is <3% coefficient of variation [87]; within-individual biological variation is relatively small compared to the between-person variation in people without diabetes [29]. The analytical imprecision of the HbA1c assay in routine clinical use at the laboratory where the present study was carried out is as follows. Internal Quality Control (IQC) material is analysed at regular intervals throughout the day. The running mean and standard deviation (SD) are continuously updated and the between-day imprecision for one month (236 data points at each level) calculated. The low IQC target value is 37 mmol/mol and the running mean was 36.8 mmol/mol; SD 0.7 mmol/mol; coefficient of variation (CV) 1.9%. The high IQC target value is 100 mmol/mol and the running mean was 100.0 mmol/mol; SD 2.0 mmol/mol; CV 2.0%. Based on UoM, a change of >5 mmol/mol in HbA1c measurement reflects a true change in glycemic category and a difference of 42 to 48 mmol/mol (6.0% to 6.5%) in a repeat measurement may simply be accounted for by UoM. This UoM has to be recognised when categorizing participants, and reinforces the value of paired confirmatory data for glycemic categorization, particularly for participants with results close to a diagnostic threshold. Lifestyle and genetic variance to glycation and HbA1c variability (independent of glycemic profiles) are also reported to have an effect on the measured HbA1c [30]

The study had several limitations. Only people at risk of diabetes were invited to be tested, only 9% of them consented to be tested, and only those with elevated HbA1c or FPG were retested, so the results are not generalizable to the whole East of England population. However the participants in this study represent people who would be most likely to participate in a diabetes prevention programme and to be identified as having non-diabetic hyperglycaemia or type 2 diabetes. As 96% of participants were white British from one region of England, generalisability would be affected if cultural, behavioural or genetic factors influence HbA1c variability over time. To assess the repeatability of these diagnostic tests more generally it would have been better to have had retest data on all 10,000 participants in screening, but these data were not available. Alternative analyses using the American Diabetic Association definition of prediabetes [17], may have produced different results but would not be directly relevant to the NHS and its Diabetes Prevention Programme [19].

Population-based diabetes prevention and screening programmes need to address this problem of reproducibility of diagnostic testing. Confirmation of diagnosis by repeated testing is necessary and clear policies are needed for management of individuals with discordant test results.

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Table 1. Characteristics of participants, and of those with discordant or concordant classification of non-diabetic hyperglycaemia or type 2 diabetes

	All participants	Discordant classification of non-diabetic hyperglycaemia	Concordant classification of non-diabetic hyperglycaemia	P*	Discordant classification of type 2 diabetes	Concordant classification of type 2 diabetes	P*	Participants with impaired fasting glucose and without non-diabetic hyperglycaemia or type 2 diabetes on initial test
	No. (%)	No. (%)	No. (%)		No. (%)	No. (%)		No. (%)
Total participants	2208 (100)	416 (100)	1047 (100)		83 (100)	311 (100)		351 (100)
Demographic characteristics and medical history								
Female	928 (42.0)	187 (45.0)	458 (43.7)	0.675	31 (37.4)	129 (41.5)	0.496	118 (33.6)
Ethnicity				0.180			0.477	
• White British	2070 (93.4)	392 (95.2)	993 (96.0)		74 (89.2)	284 (91.3)		326 (92.9
• Any other white background	39 (1.8)	8 (1.9)	24 (2.3)		6 (7.2)	11 (3.5)		11 (3.1)
• Other ethnic group	69 (3.1)	12 (2.9)	17 (1.6)		2 (2.4)	11 (3.5)		7 (2.0)
• Not recorded	30 (1.4)	0 (0.0)	0 (0.0)		1 (1.2)	5 (1.6)		7 (2.0)
History of gestational diabetes	61 (5.5%)	10 (2.4)	29 (2.8)	0.695	3 (3.6)	9 (2.9)	0.734	9 (2.6)
Family history type 2 diabetes	934 (42.3%)	168 (40.4)	444 (42.4)	0.479	32 (38.6)	140 (45.0)	0.292	145 (41.3)
Family history cardiovascular disease	357 (16.2%)	59 (14.2)	188 (18.0)	0.082	11 (13.3)	53 (17.0)	0.406	46 (13.1)
	Mean (SD)	Mean (SD)	Mean (SD)		Mean (SD)	Mean (SD)		
Age (years)	65.0 (9.5)	64.2 (9.9)	66.5 (9.1)	<0.001	66.2 (9.2)	64.3 (10.1)	0.129	64.2 (9.5)
Weeks between first and second test	11.9 (15.1)	16.3 (17.4)	13.9 (16.5)	0.012	8.6 (10.9)	5.8 (5.6)	0.002	5.2 (5.1)
Anthropometric measurements								
Body mass index	31 (5.7)	31.8 (5.9))	31.1 (5.4)	0.032	31.0 (6.3)	33.3 (6.8)	0.003	30.8 (5.6)
Waist circumference (cm)	107 (14)	106 (14)	106 (14)	0.152	106.0 (13.3)	111.7 (13.8)	0.001	105 (14.1)
Body fat percentage	36 (12)	36.8 (9.1)	36.4 (14.0)	0.534	35.5 (8.7)	38.5 (8.7)	0.008	34.4 (9.3)
Visceral fat percentage	15 (5)	15.3 (5.2)	15.2 (4.7)	0.757	15.6 (5.1)	16.7 (5.3)	0.099	15.1 (4.4)
Body fat mass (Kg)	33 (12)	34 (12)	32 (12)	0.023	32.2 (11.9)	37.1 (12.9)	0.002	31.6 (12.5)
Systolic blood pressure (mm Hg)	142 (17)	142 (17)	141 (17)	0.485	140 (18)	143 (18)	0.416	142 (17)
Diastolic blood pressure (mm Hg)	82 (10)	82 (10)	81 (10)	0.050	82 (10)	83 (11)	0.305	82 (10)
Biochemical measurements								
Initial HbA1c (mmol/mol)	44.7 (6.0)	43.5 (1.6)	44.0 (1.5)	<0.001	48.9 (1.5)	54.7 (8.9)	<0.001	39.1 (2.3)
Initial HbA1c (%)	6.2 (0.5)	6.1 (0.1)	6.2 (0.1)	<0.001	6.6 (0.8)	7.2 (0.9)	<0.001	5.7 (4.7)
Initial fasting plasma glucose (mmol/L)	6.1 (1)	5.7 (0.7)	5.9 (0.6)	0.002	6.2 (1.3)	7.3 (1.8)	<0.001	6.3 (0.3)
Total cholesterol (mmol/L)	5.1 (1.2)	5.2 (1.2)	5.1 (5.1)	0.422	5.2 (0.3)	5.2 (1.3)	0.777	5.1 (1.1)
High density lipoprotein (mmol/L)	1.3 (0.3)	1.3 (0.3)	1.3 (0.3)	0.983	1.3 (0.9)	1.2 (0.3)	0.277	1.3 (0.3
Low density lipoprotein (mmol/L)	3.1 (1.0)	3.2 (1.0)	3.1 (1.0)	0.661	3.1 (0.7)	3.1 (1.1)	0.966	3.1 (0.9)
Triglycerides (mmol/L)	1.6 (0.9)	1.7 (0.8)	1.6 (0.7)	0.045	1.7 (0.7)	1.9 (1.1)	0.127	1.6 (1.1)

Legend: * Participants with discordant and concordant classifications were compared with chi square or t test

Table 2. Comparison between initial classification of non-diabetic hyperglycaemia and/or impaired fasting glucose *, or type 2 diabetes, based on initial HbA1c and/or fasting plasma glucose, and second classification, based on second HbA1c

Initial classification	Second classification based on HbA1c							
	Normal (<42 mmol/mol, <6.0%)		Non-diabetic hyperglycaemia (42-47 mmol/mol, 6.0-6.4%)		Type 2 diabetes (>47 mmol/mol, >6.4%)		Total	
Non-diabetic hyperglycaemia and/or impaired fasting glucose * based on:	No.	%	No.	%	No.	%	No.	%
• HbA1c only	177	26.6	455	68.3	34	5.1	666	100.0
• HbA1c and fasting plasma glucose	135	17.0	592	74.3	70	8.8	797	100.0
• Fasting plasma glucose only	250	46.6	129	24.0	158	29.4	534	100.0
Total	562	28.1	1176	58.8	262	13.1	2000	100.0
	Chi^2=407.2 df=4, P<0.001							
Type 2 diabetes, based on:								
• HbA1c only	1	0.4	68	27.8	175	71.7	244	100.0
• HbA1c and fasting plasma glucose	0	0.0	14	9.3	136	90.7	150	100.0
• Fasting plasma glucose only	8	13.1	46	75.4	7	11.5	61	100.0
Total	9	2.0	128	28.1	318	70.0	455	100.0
	Chi^2=150.7 df=4, P<0.001							

* Impaired fasting glucose if fasting plasma glucose $\geq$5.6 and <7.0 mmol/L.

Table 3. Prediction of discordant classification (normality or type2 diabetes versus non-diabetic hyperglycaemia), based on second HbA1c test, in participants with classification of non-diabetic hyperglycaemia and/or impaired fasting glucose * based on initial HbA1c and/or fasting plasma glucose: multinomial logistic regression model

Outcome: Normal vs. non-diabetic hyperglycaemia			
Baseline explanatory variables	Relative risk ratio	(95% confidence interval)	P
Non-diabetic hyperglycaemia and/or impaired fasting glucose * based on:			
• HbA1c only (reference)	1.00		
• HbA1c and fasting plasma glucose	0.91	(0.63 - 1.31)	0.622
• Fasting plasma glucose only	8.41	(5.8 - 12.2)	<0.001
Age (years)	0.98	(0.96 - 0.99)	0.002
Body mass index	1.00	(0.98 - 1.02)	0.966
Triglycerides (mmol/L)	0.99	(0.84 - 1.17)	0.911
Diastolic blood pressure (mm Hg)	1.01	(0.94 - 0.98)	0.234
Weeks between first and second test	0.96	(0.11 - 4.36)	<0.001
Outcome: Type 2 diabetes vs. non-diabetic hyperglycaemia			
Baseline explanatory variables	Relative risk ratio	(95% confidence interval)	P
Non-diabetic hyperglycaemia and/or impaired fasting glucose based on:			
• HbA1c only (reference)	1.00		
• HbA1c and fasting plasma glucose	1.62	(0.94 - 2.80)	0.081
• Fasting plasma glucose only	17.7	(10.3 - 30.5)	<0.001
Age (years)	0.99	(0.97 - 1.01)	0.248
Body mass index	1.05	(1.02 - 1.08)	0.001
Triglycerides (mmol/L)	1.15	(0.97 - 1.37)	0.116
Diastolic blood pressure (mm Hg)	1.01	(0.99 - 1.03)	0.281
Weeks between first and second test	1.00	(0.99 - 1.02)	0.687

* Non-diabetic hyperglycaemia if HbA1c 42-47 mmol/mol (6.0-6.4%), and/or impaired fasting glucose if fasting plasma glucose 5.6-7.0 mmol/L.

Table 4. Prediction of discordant classification (normality or non-diabetic hyperglycaemia versus type 2 diabetes) based on second HbA1c test, in participants with classification of type 2 diabetes based on initial HbA1c and/or fasting plasma glucose: logistic regression model

Baseline explanatory variables	Odds ratio	(95% confidence interval)	P
Type 2 diabetes, based on:			
• HbA1c only (reference)	1.00		
• HbA1c and fasting plasma glucose	0.28	(0.15 - 0.54)	<0.001
• Fasting plasma glucose only	20.5	(8.8 - 48.1)	<0.001
Body mass index	1.00	(0.94 - 1.08)	0.910
Waist circumference (cm)	0.96	(0.92 - 1.00)	0.029
Visceral fat percentage	1.07	(0.99 - 1.15)	0.084
Weeks between first and second test	1.03	(1.00 - 1.07)	0.035

Table 5. Association between baseline measurements and change in HbA1c value (mmol/mol) in participants with initial diagnosis of non-diabetic hyperglycaemia or type 2 diabetes: linear regression models

	Participants with initial diagnosis of non-diabetic hyperglycaemia			Participants with initial diagnosis of type 2 diabetes		
Explanatory variable	**Coefficient**	**(95% CI)**	**P**	**Coefficient**	**(95% CI)**	**P**
Initial HbA1c (mmol/mol)	-0.16	(-0.25, -0.08)	<0.001	-0.17	(-0.25, -0.09)	<0.001
Initial fasting plasma glucose (mmol/L)	0.49	(0.27, 0.72)	<0.001	0.67	(0.25, 1.09)	0.002
Body mass index	0.05	(0.00, 0.11)	0.038	0.05	(-0.08, 0.17)	0.493
Body fat mass (Kg)	-0.03	(-0.05, 0.00)	0.023	-0.02	(-0.08, 0.04)	0.588
Total cholesterol (mmol/L)	0.32	(-0.12, 0.76)	0.158	1.10	(0.20, 2.00)	0.016
High density lipoprotein (mmol/L)	-0.46	(-0.97, 0.05)	0.077	-0.53	(-1.86, 0.80)	0.434
Low density lipoprotein (mmol/L)	-0.19	(-0.68, 0.30)	0.451	-1.15	(-2.18, -0.12)	0.028
Weeks between first and second test	0.04	(0.03, 0.05)	<0.001	-0.05	(-0.11, 0.02)	0.139