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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

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Abstract

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

Methods Some 10 000 adults at increased risk of diabetes were screened with HbA_{1c} and fasting plasma glucose (FPG). The 2208 participants with initial $HbA_{1c} \geq 42$ mmol/mol (≥ 6.0%) or FPG ≥ 6.1 mmol/l were retested after a median 40 days. We compared the first and second HbA_{1c} results, and consequent diagnoses of non-diabetic hyperglycaemia and Type 2 diabetes, and investigated predictors of discordant diagnoses.

Results Of 1463 participants with non-diabetic hyperglycaemia and 394 with Type 2 diabetes on first testing, 28.4% and 21.1% respectively had discordant diagnoses on repeated testing. Initial diagnosis of non-diabetic hyperglycaemia and/or impaired fasting glucose according to both HbA_{1c} and FPG criteria, or to FPG only, made reclassification as Type 2 diabetes more likely than initial classification according to HbA_{1c} alone. Initial diagnosis of Type 2 diabetes according to both HbA_{1c} and FPG criteria made reclassification much less likely than initial classification according to HbA_{1c} alone. Age, and anthropometric and biological measurements independently but inconsistently predicted discordant diagnoses and changes in HbA_{1c}.

Conclusions Diagnosis of non-diabetic hyperglycaemia or Type 2 diabetes with a single measurement of HbA_{1c} 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.

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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 HbA_{1c} data, who are then offered a lifestyle intervention to reduce the risk of progression to Type 2 diabetes [3]. Randomized trials have shown that such interventions can be effective in preventing diabetes, but identification of people at the highest risk can be problematic because of the imperfect validity and reliability of diagnostic tests, and recognized analytical and biological variation [4]. Changes in the diagnostic criteria for diabetes, from glucose based—fasting plasma glucose (FPG) or oral glucose tolerance test—to measurement of HbA_{1c}, 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 USA [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

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What's new?

- 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.
- Classification based on both HbA_{1c} and fasting plasma glucose independently predicted discordant diagnosis of non-diabetic hyperglycaemia and Type 2 diabetes.
- Diagnosis of non-diabetic hyperglycaemia and Type 2 diabetes should be based on two HbA_{1c} measurements.

have normal values if retested soon after. NHS policy is that asymptomatic adults must have paired HbA_{1c} testing before diagnosis of Type 2 diabetes [8], as recommended by the World Health Organization and the American Diabetes Association (ADA) [9,10]. However, for non-diabetic hyperglycaemia only one test is required to be eligible for the diabetes prevention programme [11]. People diagnosed incorrectly as having non-diabetic hyperglycaemia 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.

This 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 HbA_{1c} or FPG measurements indicated that they had non-diabetic hyperglycaemia, IFG or Type 2 diabetes were tested again for HbA_{1c} 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 people screened [13]. In this analysis, we focus on the anthropometric and biochemical factors associated with discordant non-diabetic hyperglycaemia or Type 2 diabetes classification, and with discrepancies in HbA_{1c} 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 study were to: (1) compare initial and second HbA_{1c} values recorded in each individual; (2) estimate the probabilities of concordant or discordant diagnoses of non-diabetic hyperglycaemia and Type 2 diabetes; (3) investigate how initial HbA_{1c} and FPG values, alone and in combination, predicted change from non-diabetic hyperglycaemia to normal glycaemic classification or to Type 2 diabetes; and (4) to investigate whether other participant characteristics, anthropometric measurements and biochemical measurements independently predicted change in HbA_{1c} 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 that 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 ~ 1600 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 HbA_{1c} and FPG. If participants 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 had 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 they fulfilled the following criteria: (1) age $\geq$ 50 years and BMI $\geq$ 30 kg/m^2; (2) age $\geq$ 50 years, BMI $\geq$ 25 kg/m^2 and recorded first-degree family history of Type 2 diabetes, coronary artery disease or gestational diabetes; (3) any previous record of IFG, impaired glucose tolerance or FPG 6.1–7.0 mmol/l; or (4) any record of HbA_{1c} 42–48 mmol/mol (6.0–6.5%) and FPG 5.6–6.9 mmol/l. Some 141 973 people satisfying these criteria were contacted, and 12 778 (9%) registered for participation. The study included all individuals who had non-diabetic hyperglycaemia, IFG or Type 2 diabetes on initial HbA_{1c} or FPG test, among the first 10 000 tested.

Data collection

Following an overnight fast, participants underwent venesection for FPG and HbA_{1c}, and demographic,

anthropometric and biochemical data were recorded. Follow-up tests for both HbA_{1c} and FPG were conducted for all individuals whose initial HbA_{1c} or FPG results indicated non-diabetic hyperglycaemia, IFG or Type 2 diabetes. Repeated venesection for measurement of HbA_{1c} and FPG was carried out a median of 40 [interquartile range (IQR) 27–69] days after the first venesection. For this study non-diabetic hyperglycaemia was defined as HbA_{1c} 42–47 mmol/mol (6.0– 6.4%), IFG was defined as FPG ≥ 6.1 or ≥ 5.6 to < 7.0 mmol/l (depending on classification criteria at the time of testing), and Type 2 diabetes was defined as HbA_{1c} ≥ 48 mmol/mol (≥ 6.5%) or FPG ≥ 7.0 mmol/l [14–16]. We used the latter definition of non-diabetic hyperglycaemia, instead of the ADA 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 (6.0–6.4%) 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 ADA definition of prediabetes [17] for the statistical analyses reported here because participants with initial HbA_{1c} 39–41 mmol/mol (5.7–5.9%) were not retested unless they also had initial FPG ≥ 5.6 or ≥ 6.1 mmol/l.

Anthropometric measurements (weight, BMI, body fat mass, visceral fat and body fat percentage) were measured with a Tanita body fat composition analyser (TANITA – Hoogoorddreef, Amsterdam, the Netherlands; model BC-420 MA). HbA_{1c} 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 identify predictors of discordant or confirmed diagnosis of non-diabetic hyperglycaemia and Type 2 diabetes and changes in HbA_{1c}. Statistical analysis was performed with STATA version 15 (StataCorp, College Station, TX, USA) software. A 5% significance level was used.

Discordant non-diabetic hyperglycaemia was defined as diagnosis of non-diabetic hyperglycaemia on initial HbA_{1c} test combined with diagnosis of normality or Type 2 diabetes on the second HbA_{1c} test. Discordant Type 2 diabetes was defined as diagnosis of Type 2 diabetes on initial HbA_{1c} test combined with diagnosis of normality or Type 2 diabetes on the second HbA_{1c} test.

Summary statistics were computed as means and standard deviations (SD), 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 HbA_{1c} and/or FPG (5.6–7.0 mmol/l) results with classification of normality, non-diabetic hyperglycaemia or Type 2 diabetes based on second HbA_{1c} results. We then tested the independent associations between these initial classifications and the three possible classifications based on second HbA_{1c} 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 HbA_{1c} test. However, because BMI and body fat mass were highly correlated (Pearson $R^2 = 0.88$) and because both are measures of adiposity, we excluded body fat mass from the models.

We cross-tabulated the initial classification of Type 2 diabetes, based on initial HbA_{1c} and/or FPG results, with subsequent classification of normality, non-diabetic hyperglycaemia or Type 2 diabetes, based on second HbA_{1c} 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 HbA_{1c} 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 HbA_{1c} test.

We calculated the difference between the second and first HbA_{1c} results, and tested whether this difference was independently associated with initial HbA_{1c}, 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 HbA_{1c} in either subgroup at the 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.

Results

A total of 2208 participants whose initial HbA_{1c} or FPG results indicated non-diabetic hyperglycaemia, IFG or Type 2

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*-value*	Discordant classification of Type 2 diabetes	Concordant classification of Type 2 diabetes	*P*-value*	Participants with impaired fasting glucose and without non-diabetic hyperglycaemia or Type 2 diabetes on initial test
Total number of 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 (2.9)
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)
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†								
BMI	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 BP (mmHg)	142 (17)	142 (17)	141 (17)	0.485	140 (18)	143 (18)	0.416	142 (17)
Diastolic BP (mmHg)	82 (10)	82 (10)	81 (10)	0.050	82 (10)	83 (11)	0.305	82 (10)
Biochemical measurements†								
Initial HbA_{1c} (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 HbA_{1c} (%)	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)
HDL cholesterol (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
LDL cholesterol (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)

Values are given n (%), except †mean (SD).
*Participants with discordant and concordant classifications were compared with chi square or *t* test.

diabetes were retested and comprised the sample. These participants were mostly white British nationals, with a mean age of 65 years and mean BMI of 31 kg/m^2; 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 BP, triglycerides and weeks between tests, and with lower age, initial HbA_{1c} 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 HbA_{1c}, initial FPG and weeks between tests (Table 1).

Of 1463 participants with initial HbA_{1c} 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 HbA_{1c} results were considered together (Table 2); participants 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 with those with non-diabetic hyperglycaemia according to HbA_{1c} only (74.4% vs. 68.3%), but were much more likely than those initially with IFG according to FPG only (24.0%).

Of 394 participants with initial HbA_{1c} values indicating Type 2 diabetes, 21.1% had lower values indicating non-diabetic hyperglycaemia or normality later. When classifications of Type 2 diabetes based on initial FPG and HbA_{1c} results were considered together (Table 2), participants with Type 2 diabetes according to both assays were more likely to be classified as having Type 2 diabetes on repeated testing, compared with those with Type 2 diabetes according to HbA_{1c} 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, participants initially classified as having non-diabetic hyperglycaemia were not significantly more or less likely to be reclassified as normal if they also initially had IFG than if they only had non-diabetic hyperglycaemia [relative risk ratio (RRR) 0.91, 95% confidence interval (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 RRR values 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 HbA_{1c} testing. Those with IFG 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 HbA_{1c} were much less likely to be reclassified as normal or non-diabetic hyperglycaemia than those classified according to HbA_{1c} 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

Table 2 Comparison between initial classification of non-diabetic hyperglycaemia and/or impaired fasting glucose or Type 2 diabetes, based on initial HbA_{1c} and/or fasting plasma glucose, and second classification, based on second HbA_{1c}

	Second classification based on HbA_{1c}							
Initial classification	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								
HbA_{1c} only	177	26.6	455	68.3	34	5.1	666	100.0
HbA_{1c} 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								
HbA_{1c} only	1	0.4	68	27.8	175	71.7	244	100.0
HbA_{1c} 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 ≥ 5.6 and < 7.0 mmol/l.
Values are given as *n* (%).

Table 3 Prediction of discordant classification (normality or Type 2 diabetes vs. non-diabetic hyperglycaemia), based on second HbA_{1c} test, in participants with classification of non-diabetic hyperglycaemia and/or impaired fasting glucose based on initial HbA_{1c} and/or fasting plasma glucose: multinomial logistic regression model

Baseline explanatory variables	Relative risk ratio	95% confidence interval	*P*-value
Outcome: Normal vs. non-diabetic hyperglycaemia			
Non-diabetic hyperglycaemia and/or impaired fasting glucose based on			
HbA_{1c} only (reference)	1.00		
HbA_{1c} 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
BMI	1.00	0.98–1.02	0.966
Triglycerides (mmol/l)	0.99	0.84–1.17	0.911
Diastolic BP (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			
Non-diabetic hyperglycaemia and/or impaired fasting glucose based on			
HbA_{1c} only (reference)	1.00		
HbA_{1c} 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
BMI	1.05	1.02–1.08	0.001
Triglycerides (mmol/l)	1.15	0.97–1.37	0.116
Diastolic BP (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 HbA_{1c} 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 vs. Type 2 diabetes) based on second HbA_{1c} test, in participants with classification of Type 2 diabetes based on initial HbA_{1c} and/or fasting plasma glucose: logistic regression model

Baseline explanatory variables	Odds ratio	95% confidence interval	*P*-value
Type 2 diabetes, based on			
HbA_{1c} only (reference)	1.00		
HbA_{1c} and fasting plasma glucose	0.28	0.15–0.54	< 0.001
Fasting plasma glucose only	20.5	8.8–48.1	< 0.001
BMI	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

independently associated with increased HbA_{1c} between initial and second tests, and initial HbA_{1c} and body fat mass were associated with decreased HbA_{1c}. In participants with an initial diagnosis of Type 2 diabetes, initial FPG and total cholesterol were independently associated with increased HbA_{1c}, and initial HbA_{1c} and LDL cholesterol were independently associated with decreased HbA_{1c} (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 HbA_{1c} as having non-diabetic hyperglycaemia (28%) and Type 2 diabetes (21%) had different classifications when retested a few weeks later. Because HbA_{1c} and FPG are known to vary randomly within individuals over time, it was predictable that individuals found to have high glucose or HbA_{1c} 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 that 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 HbA_{1c} 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 HbA_{1c} and change in HbA_{1c} (Table 5) confirm that such regression to the mean did occur. We also found that decreases in HbA_{1c}, 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 HbA_{1c} or FPG, and not in those with normal test results, this study does not provide complete evidence about the test–retest reliability of glycaemic classification based on HbA_{1c}. What it does provide is evidence about how reliable this classification is among participants initially classified as 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 the 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.

Table 5 Association between baseline measurements and change in HbA_{1c} 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% confidence intervals	*P*-value	Coefficient	95% confidence intervals	*P*-value
Initial HbA_{1c} (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
BMI	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

This study adds to our previous report [13] by investigating the value of participant characteristics other than initial HbA_{1c} 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 HbA_{1c} and FPG criteria made reclassification as normal less likely, and reclassification as Type 2 diabetes more likely, than initial classification according to HbA_{1c} alone. Initial diagnosis of Type 2 diabetes according to both HbA_{1c} and FPG criteria also made reclassification much less likely than initial classification according to HbA_{1c} alone. Although age and various anthropometric and biological measurements independently predicted discordant diagnoses and changes in HbA_{1c}, 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 focused risk stratified approach.

When interpreting HbA_{1c} 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 HbA_{1c} have been published [25]. The HbA_{1c} 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 HbA_{1c} method used is < 3% coefficient of variation [28]; within-individual biological variation is relatively small compared with the between-person variation in people without diabetes [29]. The analytical imprecision of the HbA_{1c} 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 SD are updated continuously and the between-day imprecision for 1 month (236 data points at each level) calculated. The low IQC target value is 37 mmol/mol and the running mean was 36.8 (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 (SD 2.0) mmol/mol; CV 2.0%. Based on UoM, a change of > 5 mmol/mol in HbA_{1c} measurement reflects a true change in glycaemic category and a difference of 42–48 mmol/mol (6.0–6.5%) in a repeat measurement may simply be accounted for by UoM. This UoM has to be recognized when categorizing participants, and reinforces the value of paired confirmatory data for glycaemic categorization, particularly for participants with results close to a diagnostic threshold. Lifestyle and genetic variance in glycation and HbA_{1c} variability (independent of glycaemic profiles) are also reported to have an effect on the measured HbA_{1c} [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 HbA_{1c} 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, generalizability would be affected if cultural, behavioural or genetic factors influence HbA_{1c} 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 ADA 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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Competing interests

None declared.

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Ethical approval

Ethical review and approval was provided by the National Research Ethics Service (NRES), Essex 1 Research Ethics Committee (10/H0301/55; 13.1.2011) and informed consent was obtained from all participants. 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). This research study was conducted in accordance with the guidelines of the Declaration of Helsinki.

References

1 NCD Risk Factor Collaboration [NCD-RisC]. Worldwide trends in diabetes since 1980: a pooled analysis of 751 population-based studies with 4.4 million participants. *Lancet* 2016; **387**: 1513–1530.
2 World Health Organization. Global Action Plan for the Prevention and Control of Non-Communicable Diseases 2013–2020. Available at http://www.who.int/nmh/events/ncd_action_plan/en/ Last accessed 27 August 2019.
3 Editorial. Beat diabetes: an urgent call for global action. *Lancet* 2016; **387**: 1483.
4 Barry E, Roberts S, Oke J, Vijayaraghavan S, Normansell R, Greenhalgh T. Efficacy and effectiveness of screen and treat policies in prevention of type 2 diabetes: systematic review and meta-analysis of screening tests and interventions. *Br Med J* 2017; **356**: i6538.
5 Maruthappu M, Sood H, Keogh B. Radically upgrading diabetes prevention in England. *Lancet Diabet Endocrinol* 2015; **3**: 312–313.
6 Torjesen I. NHS England rolls out world's first national diabetes prevention programme. *Br Med J* 2016; **352**: i1669.
7 Ely EK, Gruss SM, Luman ET, Gregg EW, Ali MK, Nhim K *et al*. A national effort to prevent Type 2 diabetes: participant-level evaluation of CDC's national diabetes prevention programme. *Diabetes Care* 2017; **40**: 1331–1341.
8 National Institute of Health and Care Excellence. Type 2 Diabetes in Adults: Management. Clinical guideline 28. Available at https://cks.nice.org.uk/diabetes-type-2#!diagnosissub Last accessed 2 August 2018.
9 World Health Organization. Use of Glycated Haemoglobin (HbA_{1c}) in the Diagnosis of Diabetes Mellitus. Abbreviated report of a WHO consultation. Available at http://www.who.int/diabetes/publications/report-hba1c_2011.pdf Last accessed 5 September 2018.
10 American Diabetes Association. Standards of medical care in diabetes-2010. *Diabetes Care* 2010; **33**(Suppl 1): S11–S61.
11 NHS England. Eligibility Changes relating to the NHS Diabetes Prevention Programme: Revised Guidance, 2017. Available at https://www.england.nhs.uk/wp-content/uploads/2017/03/nhs-diabetes-prevention-prog-mar17.pdf Last accessed 2 August 2018.
12 Pascale M, Murray N, Bachmann M, Barton G, Clark A, Howe A *et al*. The Norfolk Diabetes Prevention Study [NDPS]: a 46 month multi-centre, randomised, controlled parallel group trial of a lifestyle intervention [with or without additional support from lay lifestyle mentors with Type 2 diabetes] to prevent transition to Type 2 diabetes in high risk groups with non-diabetic hyperglycemia, or impaired fasting glucose. *BMC Public Health* 2017; **17**: 31.
13 Sampson M, Elwell-Sutton T, Bachmann MO, Clark A, Dhatariya KK, Ferns C *et al*. Discordance in glycemic categories and regression to normality at baseline in 10,000 people in a Type 2 diabetes prevention trial. *Sci Rep* 2018; **8**: 6240.
14 National Institute for Health and Clinical Excellence. Public Health Draft Guidance. Preventing Type 2 Diabetes: Risk Identification and Interventions for Individuals at High Risk, 2017. Available at https://www.nice.org.uk/guidance/ph38 Last accessed 2 August 2018.
15 Forouhi NG, Balkau B, Borch-Johnsen K, Dekker J, Glumer C, Qiao Q *et al*. The threshold for diagnosing impaired fasting glucose: a position statement by the European Diabetes Epidemiology Group. *Diabetologia* 2006; **49**: 822–827.
16 Morris DH, Khunti K, Achana F, Srinivasan B, Gray LJ, Davies MJ *et al*. Progression rates from HbA_{1c} 6.0–6.4% and other prediabetes definitions to type 2 diabetes: a meta-analysis. *Diabetologia* 2003; **56**: 1489–1493.
17 American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2010; **33**(Suppl 1): S62–S69.
18 Robson J, Dostal I, Sheikh A, Eldridge S, Madurasinghe V, Griffiths C *et al*. The NHS Health Check in England: an evaluation of the first 4 years. *BMJ Open* 2016; **6**: e00884.
19 Barron E, Clark R, Hewings R, Smith J, Valabhji J. Progress of the Healthier You: NHS Diabetes Prevention Programme: referrals, uptake and participant characteristics. *Diabet Med* 2018; **35**: 513–518.
20 Pencina MJ, D' Agostino RB, D' Agostino RB, Ramachandran S, Vasan RS. Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond. *Stat Med* 2008; **27**: 157–172.

21 Steyerberg EW, Pencina MJ, Lingsma HF, Kattan MW, Vickers AJ, Van Calster B. Assessing the incremental value of diagnostic and prognostic markers: a review and illustration. *Eur J Clin Invest* 2012; **42**: 216–228.
22 Yudkin PL, Stratton IM. How to deal with regression to the mean in intervention studies. *Lancet* 1996; **347**: 241–243.
23 McDonald TJ, Warren R. Diagnostic confusion? Repeat HbA_{1c} for the diagnosis of diabetes. *Diabetes Care* 2014; **37**: e135–e136.
24 National Cardiovascular Intelligence Network NHS Diabetes Prevention Programme (NHS DPP). *Non-Diabetic Hyperglycaemia*. London: Public Health England, 2015.
25 Weykamp C, John G, Gillery P, English E, Ji L, Lenters-Westra E *et al*. Investigation of 2 models to set and evaluate quality targets for HbA_{1c}: biological variation and sigma-metrics. *Clin Chem* 2015; **61**: 752–759.
26 Hoelzel W, Weykamp C, Jeppsson J-O, Miedema K, Barr J, Goodall I *et al*. IFCC reference system for measurement of HbA_{1c} in human blood and the national standardization schemes in the United States, Japan and Sweden: a method-comparison study. *Clin Chem* 2004; **50**: 166–174.
27 Consensus Committee. Consensus statement on the worldwide standardisation of the HbA_{1c} measurement. *Diabetologia* 2007; **50**: 2042–2043.
28 John WG, Little R, Sacks DB, Weykamp C, Lenters-Westra E, Hornsby T *et al*. Multicentre evaluation of the Premier Hb9210 HbA_{1c} analyser. *Clin Chem Lab Med* 2015; **53**: 319–327.
29 Lenters-Westra E, Røraas T, Schindhelm RK, Slingerland RJ, Sandberg S. Biological variation of hemoglobin A_{1c}: consequences for the diagnosis of diabetes mellitus. *Clin Chem* 2014; **60**: 1570–1572.
30 Jansen H, Stolk RP, Nolte IM, Kema IP, Wolffenbuttel BH, Snieder H. Determinants of HbA_{1c} in nondiabetic Dutch adults: genetic loci and clinical and lifestyle parameters, and their interactions in the Lifelines Cohort Study. *J Intern Med* 2013; **273**: 283–293.