Follow-up duration influences the relative importance of OGTT and optimal timing of glucose measurements for predicting future type 2 diabetes

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Abstract

Objective: To examine the impact of follow-up duration on the incremental prognostic yield of a baseline oral glucose tolerance test (OGTT) for predicting type 2 diabetes and to assess the discrimination ability of blood glucose (BG) obtained at different time points during OGTT.

Design: A prospective, population-based cohort study (Malmö Preventive Project) with inclusion of subjects from 1974 to 1992.

Methods: A total of 5256 men without diabetes, who had BG measured at 0, 20, 40, 60, 90, and 120 min during OGTT (30 g/m² glucose), were followed for 30 years. Incident type 2 diabetes was recorded using registries. The performance of OGTT added to a clinical prediction model (age, body mass index (BMI), diastolic blood pressure, fasting BG, triglycerides, and family history of diabetes) was assessed using Harrell's concordance index (C-index) and integrated discrimination improvement (IDI).

Results: Median age was 48 years, mean BMI 24.9 kg/m², and mean fasting BG 4.7 mmol/L. Models with added post-load BG performed better than the clinical model (C-index: $P = 0.08$ for BG at 120 min at 5 years, otherwise $P \leq 0.045$; IDI: $P \geq 0.06$ for BG at 60 and 90 min at 5 years, otherwise $P \leq 0.01$). With a longer follow-up duration, C-index decreased, and the C-index increase associated with OGTT was attenuated. Models including BG at 60 or 90 min performed significantly better than the model with BG at 120 min, evident beyond follow-up of 10 and 5 years, respectively.

Conclusions: OGTT provided incremental prognostic yield for type 2 diabetes prediction. BG measured at 60 or 90 min provided better discrimination than BG at 120 min.

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Introduction

The prevalence of type 2 diabetes is rapidly increasing and is likely to approach 600 million worldwide by 2035 (<http://www.idf.org/diabetesatlas> (cited 2015 July 20)). The rise is closely linked to the obesity epidemic (1) and constitutes a heavy economic burden on every health care system.

The importance of predicting subjects at high risk for developing type 2 diabetes is further emphasized by the fact that lifestyle modification and pharmacological intervention can prevent progression into overt diabetes, at least when high risk is defined as overweight subjects with impaired glucose tolerance (IGT) detected by oral glucose

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tolerance test (OGTT) (2, 3, 4). Nonetheless, the cost and time consumption of OGTT has prompted the development of prediction models based on easily measureable risk factors, e.g. age, gender, fasting plasma glucose (FPG), serum lipids, BP, family history of diabetes, and anthropometric measurements (5). Clinical prediction models consisting of risk factors seem to be equivalent to OGTT in the prediction of future type 2 diabetes in younger subjects at short follow-up, but not in older subjects or during longer follow-up (6). To our knowledge, however, only very few studies have explored these relationships at follow-up beyond 10 years.

Furthermore, previous studies including the Malmö Preventive Project (MPP) and the Botnia Study have demonstrated that glucose obtained at 60 min during OGTT may be a superior predictor of the incident type 2 diabetes when compared with both the traditionally used 120 min glucose measurement as well as a clinical prediction model without FPG (7). In addition, it was indicated that adding early OGTT-derived blood glucose (BG) measurements to clinical risk factors may provide incremental predictive benefit in specific populations and during certain circumstances. Finally, the predictive values of all models were lower in MPP, except for FPG, suggesting that the differences in predictive properties might not be only due to the differences in follow-up duration (23.5 years in MPP vs 4.94 years in the Botnia Study). This study was undertaken with the following purposes: (i) To evaluate whether the addition of OGTT provides incremental prognostic value on top of clinical risk factors, including FBG, for the prediction of type 2 diabetes; (ii) to assess whether BG measured at an earlier time point during OGTT provides better prognostic yield than BG measured at 120 min; (iii) to explore whether the prognostic properties of these prediction models vary according to the follow-up time.

Subjects and methods

Study population

The Malmö Preventive Project (1974–1992, $n=33\,346$) was a population-based cohort study conducted among the inhabitants in Malmö, Sweden, belonging to pre-specified birth cohorts between 1921 and 1949 (8). Progressively older subjects were recruited later during the inclusion process. A total of 22 444 males and 10 902 females attended the screening program, with an overall attendance rate of 71% (75% for men). All subjects answered a self-administered questionnaire on lifestyle,

family history of diabetes (first-degree relatives) and cardiovascular disease, medical history, and current medication. Current smoking was defined as smoking at least one cigarette a day, and sedentary lifestyle was defined as leisure time mostly spent on sedentary activities. Prevalent diabetes was defined as self-reported diabetes or according to the 1985 WHO criteria (9, 10). Height and weight in light indoor clothing were measured, from which body mass index (BMI) was calculated. Blood pressure was measured twice in the right arm after 10 min of supine rest, with the mean value recorded for analysis. Moreover, blood samples were obtained after ≥ 10 h overnight fasting for measurement of BG, plasma insulin, serum lipids, and serum creatinine. BG was analyzed by the glucose-oxidase method (1974–1977) or the hexokinase method (1977–1992) (11). The serum samples were analyzed using the laboratory's standard methods. Plasma insulin was analyzed by a standard radioimmunoassay method (12). MPP was conducted in accordance with the Declaration of Helsinki.

Oral glucose tolerance test

In 18 960 participants without prevalent diabetes, a 120 min OGTT was performed by ingestion of 30 g glucose per m^2 body surface area (BSA; DuBois equation) in a 10% aqueous solution within 5 min. BG levels were determined at 0, 20, 40, 60, 90, and 120 min.

Recording of events

The follow-up time for each subject was defined as time elapsed from baseline screening to the date of type 2 diabetes diagnosis (type 2 diabetes event), death, emigration, or last follow-up date, although no more than 30 years. Type 2 diabetes events were recorded by 14 national and local registries and comprised International Classification of Diseases (ICD-10) codes E11.0–E11.9. The method to ascertain type 2 diabetes diagnosis in the Swedish Inpatient Register has been previously validated (13). Mortality follow-up was based on the national registry on causes of mortality at the Central Bureau of Statistics, Sweden.

Final study population

Subjects with a missing BG value ($n=27\,728$) were excluded from this study. By far, most subjects did not undergo BG measurements at 20, 40, 60, and/or 90 min during OGTT, which was due to financial issues. This was especially

evident for the female study subjects as they were mostly screened during the latter half of the inclusion period, i.e. 1981–1992. The remaining 132 females with BG measurements at these intermediate time points during OGTT were therefore excluded as well. The remaining subjects with missing information regarding family history of diabetes ($n=26$), other baseline variables ($n=23$), or incomplete follow-up due to emigration ($n=70$) were likewise excluded. Of the 5367 subjects left, 111 had prevalent diabetes, leaving a final study population comprising 5256 males (Fig. 1). The subjects receiving cardiovascular medication were kept in the study, as they comprised less than 5% of the study population, and their exclusion did not significantly alter the study results.

Statistical analysis

Continuous variables were summarized by means and standard deviations (normally distributed variables) and medians and interquartile ranges (IQR; non-normally distributed variables), whereas categorical variables were presented by frequencies and corresponding percentages. Groupwise comparisons were performed using independent samples *t*-test, Mann–Whitney *U*-test, and Pearson's χ^2 -test. A simple multivariable Cox proportional hazards regression model (clinical prediction model) for predicting incident type 2 diabetes was derived using a panel of clinically important and statistically significant baseline characteristics as the independent variables, and type 2 diabetes events recorded until 30 years of follow-up or censoring. Furthermore, model

Figure 1

Flowchart depicting the selection of the study population. SBP, systolic blood pressure; TG, triglycerides.

discrimination was assessed using Harrell's concordance index (C-index) (14) with all calculations repeated using cumulated events (5, 10, 20, and 30 years of follow-up). The number of subjects still at risk at each of these time points was 5103, 4806, 3997, and 2850 respectively. Since the last subject was included on November 2nd 1983, complete 30-year follow-up data were available for all subjects. In addition, the incremental predictive value of each BG measurement during OGTT was evaluated using integrated discrimination improvement (IDI) (15). The analyses were focused on BG measured at 60 min, 90 min, and 120 min because of the better overall predictive capacity compared with BG measured at 20 and 40 min (analyses available upon request). Since the distributions of serum triglycerides (TG) and creatinine were moderately positively skewed, they were both natural log-transformed for the regression analyses. All analyses were carried out using IBM SPSS Statistics 23 (IBM) and Stata/IC 14 (StataCorp LP, College Station, TX, USA).

Results

Characteristics of the study population

At baseline, subjects had a median age of 48 (48–49) years, mean BP 131 ± 16 mmHg systolic and 88 ± 11 mmHg diastolic, and mean BMI 24.9 ± 3.3 kg/m². Total cholesterol was 5.8 ± 1.0 mmol/L. During follow-up (median 30 (20–30) years), 676 incident cases of type 2 diabetes were detected, corresponding to 5.3 cases per 1000 person-years. The subjects who developed type 2 diabetes had significantly greater baseline BMI, systolic BP (SBP), diastolic BP (DBP), total cholesterol, TG, FBG, and BG values at 20, 40, 60, 90, and 120 min (Table 1). Peak BG levels were achieved at 40 min. There were no significant between-group differences regarding smoking status and sedentary lifestyle.

Fasting blood glucose and the clinical prediction model

Using Cox regression modeling at 30-year follow-up, the following variables were statistically significant on univariable analysis for prediction of incident type 2 diabetes: BMI, SBP, DBP, FBG, total cholesterol, TG, sedentary lifestyle, and family history of diabetes. The initial clinical model included all of these except SBP, since SBP and DBP displayed significant colinearity; however, with DBP performing better than SBP. Total cholesterol

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Table 1 Baseline characteristics in subjects with and without incident type 2 diabetes detected at follow-up. Categorical variables are given as *n* (%), whereas continuous variables are given as mean $\pm$ s.d. (approximately normally distributed variables) or median (IQR) (non-normally distributed variables).

Variable	All subjects (<i>n</i> = 5256)	No incident type 2 diabetes (<i>n</i> = 4580)	Incident type 2 diabetes (<i>n</i> = 676)	P-values
Age (years)	48 (48–49)	48 (48–49)	48 (48–49)	0.002*
Family history of diabetes	703 (13%)	553 (12%)	150 (22%)	<0.0001†
Sedentary lifestyle	3005 (57%)	2597 (57%)	408 (60%)	0.07†
Active smoking	2754 (52%)	2408 (53%)	346 (51%)	0.50†
BMI (kg/m ²)	24.9 $\pm$ 3.3	24.6 $\pm$ 3.2	26.6 $\pm$ 3.4	<0.0001‡
Systolic BP (mmHg)	131 $\pm$ 16	130 $\pm$ 16	134 $\pm$ 17	<0.0001‡
Diastolic BP (mmHg)	88 $\pm$ 11	88 $\pm$ 10	91 $\pm$ 11	<0.0001‡
Fasting blood glucose (mmol/L)	4.7 $\pm$ 0.6	4.6 $\pm$ 0.5	4.9 $\pm$ 0.6	<0.0001‡
Blood glucose at 20 min (mmol/L)	7.6 $\pm$ 1.4	7.6 $\pm$ 1.4	8.0 $\pm$ 1.4	<0.0001‡
Blood glucose at 40 min (mmol/L)	9.1 $\pm$ 1.8	8.9 $\pm$ 1.8	10.0 $\pm$ 1.7	<0.0001‡
Blood glucose at 60 min (mmol/L)	8.1 $\pm$ 2.1	7.9 $\pm$ 2.0	9.4 $\pm$ 2.0	<0.0001‡
Blood glucose at 90 min (mmol/L)	6.6 $\pm$ 1.7	6.4 $\pm$ 1.6	7.6 $\pm$ 1.9	<0.0001‡
Blood glucose at 120 min (mmol/L)	5.4 $\pm$ 1.5	5.3 $\pm$ 1.4	6.1 $\pm$ 1.6	<0.0001‡
Total cholesterol (mmol/L)	5.8 $\pm$ 1.0	5.8 $\pm$ 1.0	5.9 $\pm$ 1.0	0.049‡
Triglycerides (mmol/L)	1.4 (1.1–1.9)	1.4 (1.0–1.8)	1.7 (1.3–2.3)	<0.0001*
Creatinine (μ mol/L)	94 (86–102)	94 (86–102)	94 (86–102)	0.59*
Follow-up time (years)	30 (20–30)	30 (22–30)	21 (15–26)	<0.0001*

*Mann–Whitney *U* test; †Pearson's χ^2 -test; ‡independent samples *t*-test.

and sedentary lifestyle did not result in significant model improvement and were excluded. Age was forced into the model, as older subjects on average had shorter follow-up due to the inclusion process. Therefore, the final optimized clinical Cox regression model included age, BMI, DBP, FBG, TG, and family history of diabetes (clinical prediction model, likelihood-ratio χ^2 =449.44). Supplementary analyses using type 2 diabetes events at shorter time points revealed that the statistically optimized clinical prediction model would include fewer variables at the two shortest pre-specified follow-up periods, i.e. age, FBG, and TG at 5 years (Cox regression likelihood-ratio χ^2 =57.00; C-index: 0.821), and age, BMI, FBG, TG, and family history at 10 years (Cox regression likelihood-ratio χ^2 =116.10; C-index: 0.764). However, all the following analyses were based on the model derived using 30-year follow-up (Table 2).

Table 2 Cox proportional hazards regression model (clinical prediction model) for the prediction of type 2 diabetes.

Variable	Hazard ratio (95% CI)	P-value
Age (years)	1.02 (0.98–1.06)	0.25
BMI (kg/m ²)	1.14 (1.11–1.16)	<0.0001
DBP (mmHg)	1.01 (1.002–1.02)	0.01
FBG (mmol/L)	1.98 (1.72–2.28)	<0.0001
TG (mmol/L)*	1.66 (1.40–1.96)	<0.0001
Family history of diabetes	1.84 (1.53–2.21)	<0.0001

Likelihood-ratio χ^2 =449.44; *After logarithmic transformation.

Oral glucose tolerance test

Both 60 (OGTT₆₀) and 90 min BG (OGTT₉₀) consistently performed better than 120 min BG (OGTT₁₂₀) ($P \geq 0.10$ at 5 years, $P \leq 0.04$ at 10, 20, and 30 years). There were no significant differences in C-index between OGTT₆₀ and OGTT₉₀, regardless of follow-up duration. The predictive capacity (i.e. C-index) declined with increasing follow-up time. In addition, when comparing OGTT alone with the clinical prediction model, the predictive capacities were similar at 5 years ($P \geq 0.40$ for all comparisons); however, at 10 years, OGTT₆₀ and OGTT₉₀, but not OGTT₁₂₀ performed significantly better than the clinical model ($P=0.02$ for OGTT₆₀, $P=0.04$ for OGTT₉₀, and $P=0.58$ for OGTT₁₂₀, respectively). Beyond 10 years, the clinical model performed increasingly better than OGTT, although nonsignificantly for most comparisons (Table 3).

Multivariable prediction models with oral glucose tolerance test

The multivariable prediction models were constructed by adding a single BG obtained during OGTT, i.e. BG at 60, 90, or 120 min, to the clinical prediction model. All these multivariable models, regardless of follow-up duration, performed significantly better than the clinical model ($P \leq 0.045$ for all comparisons), except the multivariable model incorporating OGTT₁₂₀ for 5-year risk prediction ($P=0.08$). With increasing

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follow-up time, C-index decreased in all models, although mostly in the multivariable models (Table 3). Supporting the influence of follow-up duration on the additive predictive value of OGTT, only OGTT₁₂₀ was

associated with a significant IDI at 5 years ($P=0.01$), whereas OGTT₆₀, OGTT₉₀, and OGTT₁₂₀ displayed a significant IDI at 10 years and beyond ($P\leq 0.0001$ for all comparisons) (Table 4).

Table 3 Harrell's concordance index (C-index) for each prediction model, and P -values for between-model comparisons.

	C-index	OGTT ₆₀	OGTT ₉₀	OGTT ₁₂₀	Multi ₆₀	Multi ₉₀	Multi ₁₂₀
Incident type 2 diabetes at 5 years							
FBG	0.805	0.26	0.16	0.42			
Clinical	0.838	0.59	0.40	0.87	0.006	0.045	0.08
OGTT ₆₀	0.866		0.48	0.60			
OGTT ₉₀	0.883			0.10			
OGTT ₁₂₀	0.846						
Multi ₆₀	0.884					0.30	0.96
Multi ₉₀	0.909						0.15
Multi ₁₂₀	0.885						
Multi ₆₀₊₉₀	0.913				0.23	0.35	
Multi ₆₀₊₁₂₀	0.904				0.32		0.10
Multi ₉₀₊₁₂₀	0.903					0.25	0.15
Incident type 2 diabetes at 10 years							
FBG	0.717	<0.0001	0.001	0.06			
Clinical	0.765	0.02	0.04	0.58	<0.0001	<0.0001	0.004
OGTT ₆₀	0.832		0.95	0.04			
OGTT ₉₀	0.831			0.005			
OGTT ₁₂₀	0.783					0.10	0.83
Multi ₆₀	0.824						0.02
Multi ₉₀	0.850						
Multi ₁₂₀	0.821						
Multi ₆₀₊₉₀	0.856				0.04	0.02	
Multi ₆₀₊₁₂₀	0.848				0.09		<0.0001
Multi ₉₀₊₁₂₀	0.849					0.50	0.01
Incident type 2 diabetes at 20 years							
FBG	0.690	<0.0001	<0.0001	0.4			
Clinical	0.774	0.82	0.29	<0.0001	<0.0001	0.001	0.02
OGTT ₆₀	0.778		0.17	<0.0001			
OGTT ₉₀	0.764			<0.0001			
OGTT ₁₂₀	0.709						
Multi ₆₀	0.808					0.99	0.03
Multi ₉₀	0.809						0.01
Multi ₁₂₀	0.792						
Multi ₆₀₊₉₀	0.818				0.12	<0.0001	
Multi ₆₀₊₁₂₀	0.814				0.28		<0.0001
Multi ₉₀₊₁₂₀	0.809					0.53	0.01
Incident type 2 diabetes at 30 years							
FBG	0.648	<0.0001	<0.0001	0.231			
Clinical	0.732	0.39	0.06	<0.0001	<0.0001	<0.0001	0.008
OGTT ₆₀	0.731		0.10	<0.0001			
OGTT ₉₀	0.713			<0.0001			
OGTT ₁₂₀	0.662						
Multi ₆₀	0.764					0.45	0.002
Multi ₉₀	0.760						0.009
Multi ₁₂₀	0.743						
Multi ₆₀₊₉₀	0.770				0.15	<0.0001	
Multi ₆₀₊₁₂₀	0.768				0.17		<0.0001
Multi ₉₀₊₁₂₀	0.760					0.84	0.006

FBG, fasting blood glucose; Clinical, clinical prediction model (age, BMI, diastolic BP, fasting blood glucose, triglycerides, family history of diabetes); OGTT₆₀, blood glucose measured at 60 min during oral glucose tolerance test; Multi₆₀, multivariable prediction model (clinical prediction model+blood glucose measured at 60 min during oral glucose tolerance test).

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Table 4 Absolute integrated discrimination improvement for the various multivariable prediction models (including *P*-values) at 5, 10, 20, and 30 years.

	5 years	10 years	20 years	30 years
Multi ₆₀ vs clinical	0.006 (0.11)	0.015 (0.0001)	0.0334 (<0.0001)	0.0306 (<0.0001)
Multi ₉₀ vs clinical	0.017 (0.057)	0.0447 (<0.0001)	0.0391 (<0.0001)	0.0301 (<0.0001)
Multi ₁₂₀ vs clinical	0.018 (0.01)	0.0328 (<0.0001)	0.0212 (<0.0001)	0.0159 (<0.0001)
Multi ₆₀₊₉₀ vs Multi ₆₀	0.1175 (0.81)	0.02854 (<0.0001)	0.01027 (<0.0001)	0.00496 (0.0001)
Multi ₆₀₊₁₂₀ vs Multi ₆₀	0.01508 (0.03)	0.02513 (<0.0001)	0.00900 (0.0005)	0.00539 (0.0001)
Multi ₉₀₊₆₀ vs Multi ₉₀	0.0003 (0.77)	-0.00102 (0.44)	0.00454 (0.007)	0.00548 (<0.0001)
Multi ₉₀₊₁₂₀ vs Multi ₉₀	0.00418 (0.22)	0.00258 (0.11)	0.00023 (0.42)	0.00012 (0.57)
Multi ₁₂₀₊₆₀ vs Multi ₁₂₀	0.00278 (0.52)	0.00745 (0.03)	0.02120 (<0.0001)	0.02008 (<0.0001)
Multi ₁₂₀₊₉₀ vs Multi ₁₂₀	0.00336 (0.55)	0.01446 (0.004)	0.01816 (<0.0001)	0.01428 (<0.0001)

Clinical, clinical prediction model (age, BMI, diastolic BP, fasting blood glucose, triglycerides, family history of diabetes); Multi₆₀, multivariable prediction model (clinical prediction model + blood glucose measured at 60 min during oral glucose tolerance test).

Addition of another OGTT-derived BG to the above did not improve the predictive value any further, except at 10 years, for which a subtle, but statistically significant benefit was observed for the combination of the clinical prediction model and both OGTT₆₀ and OGTT₉₀ (Table 3). Consistent with the high sensitivity of the IDI method, significant additional effects were noted for most combinations of two OGTT-derived BG beyond 5 years, although the numerical increases were minimal (Table 4).

Multivariable models including either OGTT₆₀ or OGTT₉₀ outperformed the multivariable model with OGTT₁₂₀ at more than 10 and 5 years of follow-up respectively, whereas the differences between multivariable models with OGTT₆₀ and OGTT₉₀ were subtle (Table 3).

Discussion

Concordant with our hypotheses, the main findings of our study were: (i) Addition of OGTT to a clinical prediction model including FBG provided incremental prognostic value for the prediction of type 2 diabetes; (ii) BG measured at 60 and 90 min during OGTT provided better prognostic yield than BG measured at 120 min, both isolated and in addition to the clinical prediction model, at follow-up longer than 10 and 5 years respectively; and (iii) The performance of all prediction models decreased over time, especially evident for models with OGTT₁₂₀ and less so for models without OGTT.

Several prediction models for incident type 2 diabetes based on clinical risk factors have been suggested (6, 16, 17) because of the ease of administration, convenience, acceptability to patients, and lower cost in comparison with OGTT. Data from The San Antonio Heart Study demonstrated that a simple clinical prediction model resulted in better identification of subjects at risk than OGTT

during a 7- to 8-year follow-up period (16). In contrast, although in accordance with our results, McNeely *et al.* (6) found no significant difference between a clinical model and OGTT for the 5- to 6-year prediction of type 2 diabetes in subjects aged ≤55 years, whereas Alyass *et al.* (7) found a superior discrimination ability of OGTT; however, their clinical prediction model did not include FPG, which by itself had an area under the receiver-operating characteristics curve comparable with OGTT₁₂₀, but lower than OGTT₆₀. One possible explanation for the heterogeneous results between studies could be the use of different diagnostic criteria for type 2 diabetes. For instance, one would assume a relatively better prognostic yield of OGTT if more incident type 2 diabetes cases were diagnosed according to OGTT criteria. As the diagnostic threshold for FPG was lowered in 1997, relatively more subjects received a diagnosis of diabetes based on the OGTT criteria before 1997, during which most cases in this study were identified. This may explain why OGTT performed well in our study, especially at a short-term follow-up (18).

As a substantial number of subjects who eventually develop type 2 diabetes have normal glucose tolerance (NGT) and/or normal fasting glucose at baseline, and IGT and impaired fasting glucose (IFG) display very limited overlap (19, 20, 21), the combination of a clinical prediction model with OGTT may potentially identify a larger percentage of high risk subjects. However, Stern *et al.* (16) found only a minor improvement, and Wilson *et al.* (22) did not find additional capability to discriminate persons who developed type 2 diabetes from those who did not, when adding OGTT₁₂₀ to a clinical model. Possible explanations may be the use of a 75 g glucose loading dose, shorter follow-up duration (7.5 years and 7 years, respectively), subjects with greater mean BMI (27.1 kg/m² in both studies), the inclusion of both male and female subjects, and the use of clinical prediction models with

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a larger number of co-variables, many of which were not significantly associated with the outcome in multivariable analysis. In this study, addition of any one OGTT-derived BG to the clinical prediction model resulted in significant model improvement for most comparisons, but the benefit of further adding a second OGTT-derived BG was very limited, i.e. a single measurement may be a reliable indicator of glucose tolerance, at least for prediction purposes.

Timing of glucose measurements during the oral glucose tolerance test

The cut-offs for glucose intolerance and diabetes after glucose loading in non-pregnant subjects have traditionally been based on OGTT₁₂₀ (23, 24). However, this topic has been debated for at least 40 years (23), and a growing body of evidence supports the use of OGTT₆₀. In addition to the study by Alyass *et al.* (7) which demonstrated better capability of OGTT₆₀ compared with OGTT₁₂₀, both isolated and combined with other clinical risk factors, for predicting incident type 2 diabetes at 4.94 and 23.5 years, authors Abdul-Ghani *et al.* (21, 25, 26, 27, 28) showed that OGTT₆₀ performed superiorly to OGTT₁₂₀ for identifying subjects at high risk of developing type 2 diabetes, and that addition of OGTT₆₀ to multivariable prediction models resulted in significant model improvement similar to adding OGTT₁₂₀, during a 7 to 8-year follow-up period. Likewise, Kuang *et al.* (29) demonstrated a better predictive ability of OGTT₆₀ compared with OGTT₁₂₀ at 10-year follow-up. In this study, both OGTT₆₀ and OGTT₉₀ performed significantly better than OGTT₁₂₀ for most comparisons, including the multivariable models. Lastly, Janghorbani *et al.* (30) found almost equal discriminating abilities of OGTT₆₀ and OGTT₁₂₀ during a follow-up time of 3–5 years. However, the study population was highly selected and included only first-degree relatives of patients with type 2 diabetes. The generally superior predictive ability of BG obtained at earlier time points during OGTT may reflect the crucial role of the first-phase insulin response in postprandial glucose homeostasis (31, 32).

Importance of follow-up duration

Most previous similar studies have not assessed the predictive value of clinical models with additional OGTT beyond 10 years of follow-up. Furthermore, the impact of follow-up duration on the discriminating ability of prediction models has not been well studied. Nonetheless,

McNeely *et al.* (6) found no significant difference between a clinical model and OGTT₁₂₀ for neither 5 to 6-year nor 10-year prediction of type 2 diabetes in subjects ≤ 55 years. In this study, the predictive capacity of all models decreased, when follow-up increased. This is not surprising since at least some of the risk factors are modifiable and show considerable changes over time (33). The fact that the clinical prediction model performed better than OGTT alone beyond 10 years may therefore simply be the inclusion of non-modifiable risk factors, which also explains why the relative incremental predictive ability of OGTT decreased with increasing follow-up time. Also, a substantial proportion of individuals with IGT may revert to NGT on a subsequent OGTT (34). Finally, the clinical model included several components of the metabolic syndrome and may identify subjects who are more prone to develop diabetes in the long-term, even before demonstrating elevated BG (35), which was supported by the fact that the optimal clinical prediction model at short-term follow-up mainly included FBG. Beyond the 5-year-mark, OGTT₆₀ and OGTT₉₀ were consistently significantly better predictors than OGTT₁₂₀. The multivariable models with OGTT₆₀ or OGTT₉₀ displayed better predictive properties than the clinical models at all time points. However, IDI was greatest for the multivariable model with OGTT₁₂₀ at 5-year follow-up, but was surpassed by the multivariable model with OGTT₉₀ at 10-year follow-up and by the multivariable model with OGTT₆₀ at 20-year follow-up, whereby one might speculate that BG obtained at earlier time points during OGTT particularly predicts late incident type 2 diabetes, whereas OGTT₁₂₀ predicts early incident type 2 diabetes. This may additionally explain the controversies concerning the optimal timing of BG measurement during OGTT.

Limitations

In the last few decades, there has been a number of changes in the definition of type 2 diabetes, e.g. the lowering of the diagnostic threshold for FPG-defined diabetes in 1997 and the introduction of HbA_{1c}-defined diabetes in 2011 (18, http://www.who.int/diabetes/publications/diagnosis_diabetes2011/en/ (cited 2015 July 20)). The subjects defined according to these different measures only partially overlap, both with respect to pre-diabetes and diabetes (36, 37). However, the predictive capability of risk factors associated with incident type 2 diabetes defined by either FPG or HbA_{1c} seems comparable (38). The addition of HbA_{1c} to our study would have been desirable, but these

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measurements were not done. Furthermore, at the time of inclusion, a standard OGTT comprised a 30 g/m² glucose load which, however, seems to yield results similar to the 75 g load (39). Our exclusion of a significant proportion of the original study population, although based on financial reasons, constitutes a major limitation and prevents us from extending our results beyond middle-aged Caucasian men, and due to the large decline in subjects at risk in the latter half of the study, the proportional hazards assumption assessed using Schoenfeld residuals was violated at 20- and 30-year follow-up. Still, the choice of comparing C-indices derived from Cox models was superior to comparing areas under the curves derived from logistic models, and additional analyses revealed the same patterns for OGTT when using a composite endpoint comprising diabetes and death. Furthermore, we found no significant interaction between OGTT and death. It should also be remembered that prediction models generally tend to overestimate the actual event risk (5). Lastly, differences between-study results might further be due to variations in sex ratio, ethnicity, sample sizes, inclusion criteria, co-variables included, glucose load used for OGTT, and follow-up duration.

Clinical perspectives

Identifying subjects at an increased risk for future diabetes has become more relevant because of the proven preventive effects of lifestyle modification and medication in subjects with IGT (2, 3, 4). Our findings may carry future clinical implications, as shorter examination time is associated with lower cost and greater convenience for both patients and health care professionals. Although the introduction of HbA_{1c} as a diagnostic tool for diabetes has led to fewer OGTTs in general, it is well known that these methods do not always identify diabetes in the same subjects (36, 37). Therefore, a role for OGTT likely exists in subjects with a high normal or an only slightly elevated HbA_{1c}, in order to reach a definitive diagnostic decision. Supplementary analyses evaluating the role of OGTT in subjects with and without IFG, defined according to the current World Health Organization and American Diabetes Association criteria, revealed no significant interaction for the predictive value of OGTT between subjects with NFG and IFG, respectively. This indicates that our findings in the entire study population are also applicable to the subgroup of patients with IFG who to some degree may reflect the subjects with borderline HbA_{1c}, i.e. subjects in whom an OGTT could

be considered in daily clinical practice. However, our findings need further replication and external validation. In addition, interventional studies are necessary in order to prove the benefit of secondary prevention in the subjects found through multivariable screening, including the use of OGTT₆₀ or OGTT₉₀, and not only those with IGT defined by OGTT₁₂₀.

Such trials are also prudent in order to address the real magnitude of effect of adding screening with the OGTT to the other risk factors in the relevant clinical setting.

In conclusion, in this prospective population-based cohort study, we found that the addition of an OGTT-derived BG to a clinical prediction model provided incremental prognostic value, including IDI, in the prediction of type 2 diabetes, but relatively less so at longer follow-up. BG measured at 60 or 90 min during OGTT provided better prognostic yield than BG measured at 120 min at follow-up longer than 10 and 5 years respectively.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

Mette Lundgren Nielsen, Manan Pareek and Michael Hecht Olsen contributed substantially to the conception of the hypothesis, the design of the work, analysis of the work, interpretation of data for the work, drafting the work. Margrét Leósdóttir, Karl-Fredrik Eriksson and Peter M Nilsson contributed substantially to the acquisition of data for the work whilst Kurt Højlund contributed substantially to the interpretation of data for the work. All authors contributed equally to critical revision for important intellectual content, final approval of the version to be published, and agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

All authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

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