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Article

The 2-Hour Plasma Glucose Levels during OGTT, Conducted in the Postprandial Period between 4 and 7.9 Hours, Are Associated with the Diagnosis of Diabetes, Diabetes Mortality, and Cardiovascular Mortality

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Abstract: Postprandial plasma glucose between 4 and 7.9 hours is associated with the diagnosis of diabetes, diabetes mortality, and cardiovascular mortality. However, it is unknown whether 2-hour plasma glucose during the oral glucose tolerance test conducted in this postprandial period (4–7.9 hours), termed as 2-h $PG_{OGTT@4–7.9h}$, can accurately classify diabetes diagnosis and predict mortality risks. This study aimed to address these questions using 2,347 adult participants. Diabetes was defined as HbA_{1c} ≥6.5%, and the ability of 2-h $PG_{OGTT@4–7.9h}$ to classify diabetes was analyzed using receiver operating characteristic curves. Cox proportional hazards models were employed to estimate mortality hazard ratios (HRs) and 95% confidence intervals (CIs). The results showed that 2-h $PG_{OGTT@4–7.9h}$ could classify diabetes with 92% accuracy. Participants were followed up for a mean of 21.4 years. A 1-natural-log higher 2-h $PG_{OGTT@4–7.9h}$ was associated with an increased risk of mortality from diabetes (adjusted HR, 21.1; 95% CI, 9.2–48.0) and cardiovascular disease (adjusted HR, 1.47; 95% CI, 1.13–1.91). Simulation analysis indicated that future studies may require at least 100 participants to investigate 2-h $PG_{OGTT@4–7.9h}$ for diabetes diagnosis. In conclusion, 2-h $PG_{OGTT@4–7.9h}$ may be useful for diabetes classification and prediction of mortality risk.

Keywords: postprandial; oral glucose tolerance test; cardiovascular disease; diabetes; survival

1. Introduction

Diabetes is a metabolic disease characterized by elevated blood glucose levels [1]. As of 2021, approximately 529 million people worldwide were living with diabetes, a number projected to rise to 1.31 billion by 2050 [2]. This condition contributes to about 1.5 million deaths annually [3]. It imposes a substantial economic burden, costing $1.3 trillion globally in 2015, which figure is estimated to climb to around $2.2 trillion by 2030 [4].

In 2021, about half of diabetic cases in adults remained undiagnosed [5]. Those with undiagnosed diabetes are developing diabetes-related complications, leading to increased healthcare expenditure [6]. Individuals with undiagnosed diabetes face a 60% higher risk of mortality compared to those without diabetes [7]. Timely diagnosis is crucial for initiating appropriate medical interventions to prevent or delay diabetes-related complications [8]. Therefore, enhanced efforts are needed to improve diabetes detection.

Currently, diabetes diagnosis relies on fasting plasma glucose levels, 2-h plasma glucose during an oral glucose tolerance test (OGTT), and hemoglobin A_{1c} (HbA_{1c}) [9]. However, fasting

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requirements for tests such as fasting plasma glucose and OGTT can be inconvenient and may induce hypoglycemia in vulnerable individuals [10]. Exploring the diagnostic potential of non-fasting plasma glucose and non-fasting OGTT could therefore offer valuable insights.

Recent research highlights postprandial glucose levels measured between 4 and 7.9 h after a meal ($PPG_{4–7.9h}$) as a promising biomarker for diagnosis. Computed $PPG_{4–7.9h}$ demonstrates an 87% accuracy in diagnosing diabetes [11], falling within the optimal accuracy range of 80% to 90% [12]. Moreover, $PPG_{4–7.9h}$ has been linked to predicting mortality from both diabetes and cardiovascular disease (CVD) [13]. Importantly, it remains stable throughout this postprandial period, as evidenced by consistent hourly measurements [13,14].

Supporting this finding, Eichenlau et al's study showed that plasma glucose returned to baseline levels within 4 h after a meal, regardless of meal type (standard meal or high carbohydrate meal) and meal time (breakfast, lunch or dinner) in healthy individuals [15]. These clinical results underscore the potential of the postprandial period between 4 and 7.9 h to reflect an individual's glucose homeostasis state, offering a promising window for diabetes diagnosis.

Yet, the diagnostic and prognostic value of 2-h plasma glucose during OGTT conducted within this postprandial period between 4 and 7.9 h (2-h $PG_{OGTT@4–7.9h}$) remains unknown. This study aimed to explore whether 2-h $PG_{OGTT@4–7.9h}$ was associated with diabetes diagnosis and predicted mortality risks. It utilized data from 2,347 adult participants who attended the third National Health and Nutrition Examination Survey (NHANES III) during 1988–1994. Additionally, 3,865 participants from the same survey with 2-h plasma glucose during OGTT conducted in the fasting period (fasting time ≥8 h [9,16,17]), termed as 2-h $PG_{OGTT@fasting}$, were included in the analysis.

2. Materials and Methods

2.1. Participants

This study included adult participants (aged ≥20 years) from NHANES III (1988–1994) [18]. Two cohorts of participants were selected from the participants: the postprandial cohort (fasting time, 4–7.9 h) and the fasting cohort (fasting time, ≥ 8 h [9,16,17]).

The postprandial cohort included all participants who had 2-h plasma glucose during OGTT conducted in the postprandial period between 4 and 7.9 h (n = 2410). This 2-h plasma glucose was termed as 2-h $PG_{OGTT@4–7.9h}$. Participants missing follow-up time or with a follow-up of 0 months (n = 2) were excluded. Individuals who lacked the following data were also excluded: HbA_{1c} (n = 13), body mass index (n = 4), systolic blood pressure (n = 4), total cholesterol (n = 21), and high-density lipoprotein (HDL) cholesterol (n = 19). Therefore, the remaining 2347 participants were included in the final analysis for the postprandial cohort (Figure 1).

The fasting cohort included all participants who had 2-h plasma glucose during OGTT conducted in the fasting period (fasting time, ≥ 8 h; n = 3961). The 2-h plasma glucose in this cohort was termed as 2-h $PG_{OGTT@fasting}$. Participants missing follow-up time or with a follow-up of 0 months (n = 2) were excluded. Individuals who lacked the following data were also excluded: HbA_{1c} (n = 16), body mass index (n = 5), systolic blood pressure (n = 4), total cholesterol (n = 41), and HDL cholesterol (n = 28). Therefore, the remaining 3865 participants were included in the final analysis for the fasting cohort (Figure 1).

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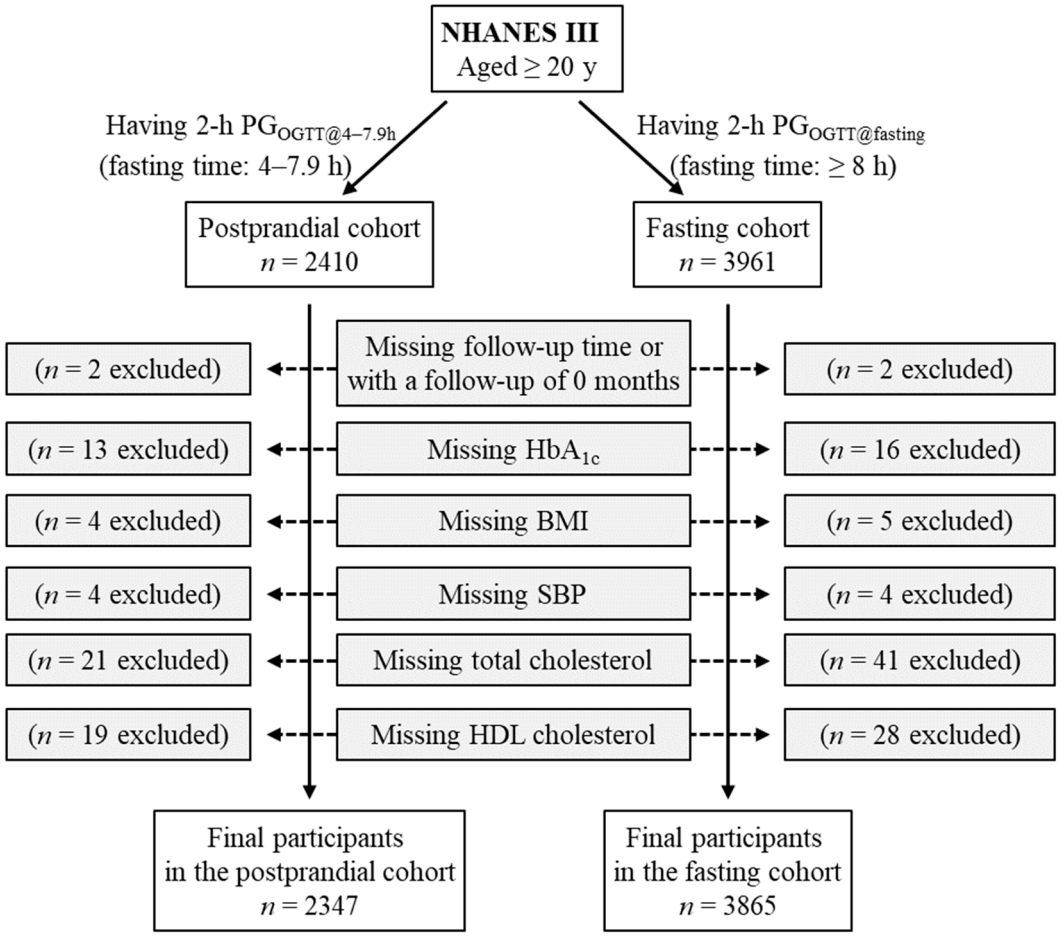

Figure 1. Flow diagram of the study participants. 2-h PG$_{OGTT@4–7.9h}$, 2-h plasma glucose during OGTT conducted in the postprandial period between 4 and 7.9 h; 2-h PG$_{OGTT@fasting}$, 2-h plasma glucose during OGTT conducted in the fasting period (fasting time, ≥ 8 h); BMI, body mass index; HbA$_{1c}$, hemoglobin A$_{1c}$; HDL, high-density lipoprotein; NHANES III, the third National Health and Nutrition Examination Survey; OGTT, oral glucose tolerance test; PG, plasma glucose; SBP, systolic blood pressure.

2.2. Exposure Variable

The exposure variable of this study was 2-h plasma glucose during OGTT which was conducted in the postprandial period between 4 and 7.9 h or in the fasting period (fasting time, ≥ 8 h [9,16,17]). During the OGTT test, participants were administered a glucose challenge containing the equivalent of 75 grams of glucose [19]. Two hours later, a blood specimen was drawn to measure 2-h plasma glucose levels using the hexokinase method [20,21].

2.3. Outcome Variables

The outcome variables of this study were HbA$_{1c}$, diabetes diagnosis, and various types of mortality.

HbA$_{1c}$ was measured using the Bio-Rad DIAMAT glycosylated hemoglobin analyzer system [20]. Currently, diabetes in the clinic is diagnosed using HbA$_{1c}$, fasting plasma glucose and 2-h plasma glucose during OGTT which was conducted in the fasting period. However, participants in the postprandial cohort lacked fasting plasma glucose and OGTT that was conducted in the fasting period. Therefore, diabetes in the current study was defined as HbA$_{1c}$ ≥ 6.5 only in the main analyses. Diabetes was also defined as a self-reported diagnosis in additional analyses.

Data on mortality from diabetes, CVD, cancer, and all causes were directly retrieved from NHANES-linked mortality files [18]. To evaluate mortality status and the cause of death, the National

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Center for Health Statistics linked the NHANES data with death certificate records from the National Death Index records [22]. Follow-up time was the duration from the time when the individual was examined at the Mobile Examination Center until death or until the conclusion of follow-up (31 December 2019), whichever occurred first [23].

2.4. Covariables

Covariables were described previously [11,14] and included age, sex (female or male), ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, or other), body mass index, poverty–income ratio (<130%, 130%–349%, ≥350%, or unknown), education (<high school, high school, >high school, or unknown), smoking status (current smoker, past smoker, or non-smoker), alcohol consumption (never, <1 drink per week, 1–6 drinks per week, ≥7 drinks per week, or unknown), physical activity (inactive, insufficiently active, or active), survey periods (1988–1991 or 1991–1994), systolic blood pressure, total cholesterol, high-density lipoprotein (HDL) cholesterol, and family history of diabetes (yes, no, or unknown).

2.5. Statistical Analyses

The baseline characteristics of these two cohorts of participants were presented as median and interquartile range for not normally distributed continuous variables, mean and standard deviation for normally distributed continuous variables, or number and percentage for categorical variables [24]. Differences in continuous variables were analyzed via the Mann-Whitney U test (not normally distributed) [25] and Student's T-test (normally distributed) [26], and differences among categorical variables were analyzed via Pearson's chi-square test [27].

The associations of 2-h plasma glucose with HbA_{1c} and diabetes diagnosis were analyzed by multiple linear regression and binary logistic regression [28], respectively. Receiver operating characteristic curves were constructed and the area under the curve (AUC) was calculated to assess the association of 2-h plasma glucose with diabetes diagnosis [29], and the Youden Index was used to determine the optimal cutoff [30].

Cox proportional hazards models were used to calculate hazard ratios (HRs) and 95% confidence intervals (CIs) of 2-h plasma glucose for mortality from diabetes, CVD, cancer, and all causes [31]. 2-h plasma glucose was treated as a continuous variable (natural log-transformed) or categorical variable (≥ versus < 200 mg/dL). Kaplan–Meier curves were constructed to estimate the survival rates of participants between the two 2-h plasma glucose categories (≥ versus < 200 mg/dL), which were compared using the log-rank test [32]. To improve data distribution, body mass index, total cholesterol, HDL cholesterol, and systolic blood pressure were natural log-transformed in all the regression analyses [33].

Power estimation was conducted by simulations employing 10,000 randomly generated samples with various sample sizes (ranging from 50 to 200) derived from the postprandial cohort of 2347 participants [34,35]. Diabetes prediction was defined as a 2-h $PG_{OGTT@4–7.9h}$ ≥ 200 mg/dL, and actual diabetes status was defined as HbA_{1c} ≥ 6.5% [36]. Within each sample, the diagnostic accuracy, sensitivity, and specificity of 2-h $PG_{OGTT@4–7.9h}$ for diabetes diagnosis were then calculated [37-39].

A diagnostic accuracy of 80%, which is deemed a minimum threshold for an excellent diagnostic marker [12], was used for power estimation. The percentage of samples exhibiting ≥ 80% accuracy out of 10,000 random samples was assigned as the diagnostic power of 2-h $PG_{OGTT@4–7.9h}$ in classifying diabetes. Mean sensitivity and specificity values were calculated from the 10,000 samples, and their 95% confidence intervals were generated from the 2.5th and 97.5th percentiles of the 10,000 sensitivity and specificity values [40]. In addition, a diagnostic accuracy of 81% was also used to estimate power and sample size.

The null hypothesis was rejected for two-sided values of $p < 0.05$. The estimation of power and sample size were conducted using the R program, and the remaining analyses were conducted using SPSS version 27.0 (IBM SPSS Statistics for Windows, Armonk, NY, USA, IBM Corporation) [41].

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3. Results

3.1. Baseline Characteristics

This study included two cohorts of participants: the postprandial cohort (fasting time, 4–7.9 h; *n* = 2347) and the fasting cohort (fasting time, ≥ 8 h; *n* = 3865). Both cohorts had a mean age of 56 years. Participants with higher 2-h plasma glucose during OGTT were older, and had higher levels of HbA_{1c}, body mass index, systolic blood pressure, and total cholesterol, and had lower levels of HDL-cholesterol, education, and income (Tables 1 and 2).

Table 1. Baseline characteristics of the postprandial cohort (fasting time, 4–7.9 h).

	2-h $PG_{OGTT@4–7.9h}$		All	*p*
	< 200 mg/dL	≥ 200 mg/dL		
Sample size	1797	550	2347	NA
Age, y, mean (SD)	55 (11)	61 (9)	56 (11)	<0.001
Sex (male), *n* (%)	869 (48.4)	246 (44.7)	1115 (47.5)	0.14
2-h $PG_{OGTT@4–7.9h}$, mg/dL, median (IQR)	131 (105–158)	247 (218–303)	147 (114–196)	<0.001
HbA_{1c}, %, median (IQR)	5.4 (5.1–5.7)	6.0 (5.4–7.1))	5.5 (5.1–5.8)	<0.001
BMI, kg/m², median (IQR)	27 (24–30)	28 (25–32)	27 (24–31)	<0.001
TC, mg/dL, median (IQR)	212 (185–242)	226 (199–253)	215 (189–245)	<0.001
HDL-C, mg/dL, median (IQR)	49 (40–60)	47 (38–57)	49 (40–59)	0.003
SBP, mm Hg, median (IQR)	127 (117–140)	136 (126–151)	129 (118–142)	<0.001
Ethnicity, *n* (%)				
Non-Hispanic white	928 (51.6)	245 (44.5)	1173 (50)	<0.001
Non-Hispanic black	408 (22.7)	100 (18.2)	508 (21.6)	
Hispanic	436 (24.3)	198 (36.0)	634 (27.0)	
Other	25 (1.4)	7 (1.3)	32 (1.4)	
Education, *n* (%)				
<High school	674 (37.5)	276 (50.2)	950 (40.5)	<0.001
High school	551 (30.7)	151 (27.5)	702 (29.9)	
>High school	561 (31.2)	120 (21.8)	681 (29.0)	
Unknown	11 (0.6)	3 (0.5)	14 (0.6)	
Poverty–income ratio, *n* (%)				
<130%	372 (20.7)	139 (25.3)	511 (21.8)	0.002
130%–349%	746 (41.5)	236 (42.9)	982 (41.8)	
≥350%	553 (30.8)	127 (23.1)	680 (29.0)	
Unknown	126 (7.0)	48 (8.7)	174 (7.4)	
Physical activity, *n* (%)				
Active	667 (37.1)	192 (34.9)	859 (36.6)	0.56
Insufficiently active	796 (44.3)	247 (44.9)	1043 (44.4)	
Inactive	334 (18.6)	111 (20.2)	445 (19.0)	
Alcohol consumption, *n* (%)				
0 drinks/week	277 (15.4)	121 (22.0)	398 (17.0)	<0.001
<1 drink/week	228 (12.7)	53 (9.6)	281 (12.0)	
1–6 drinks/week	345 (19.2)	71 (12.9)	416 (17.7)	
≥7 drinks/week	222 (12.4)	62 (11.3)	284 (12.1)	
Unknown	725 (40.3)	243 (44.2)	968 (41.2)	
Smoking status, *n* (%)				
Current smoker	459 (25.5)	78 (14.2)	537 (22.9)	<0.001
Past smoker	547 (30.4)	209 (38.0)	756 (32.2)	
Non-smoker	791 (44.0)	263 (47.8)	1054 (44.9)	
Survey period, *n* (%)				

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1988–1991	873 (48.6)	253 (46.0)	1126 (48.0)	0.29
1991–1994	924 (51.4)	297 (54.0)	1221 (52.0)	
Family history of diabetes, *n* (%)				
Yes	787 (43.8)	290 (52.7)	1077 (45.9)	<0.001
No	990 (55.1)	249 (45.3)	1239 (52.8)	
Unknown	20 (1.1)	11 (2.0)	31 (1.3)	
Selt reported diagnosis, *n* (%)				
Yes	29 (1.6)	152 (27.6)	181 (7.7)	<0.001
No	1767 (98.3)	398 (72.4)	2165 (92.2)	
Unknown	1 (0.1)	0 (0.0)	1 (0.0)	

Abbreviations: 2-h $PG_{OGTT@4–7.9h}$, 2-hour plasma glucose during OGTT which was conducted in the postprandial period between 4 and 7.9 h; BMI, body mass index; HbA_{1c}, hemoglobin A_{1c}; HDL-C, high-density lipoprotein cholesterol; IQR, interquartile range; NA, not applicable; OGTT, oral glucose tolerance test; SBP, systolic blood pressure; SD, standard deviation; TC, total cholesterol.

Table 2. Baseline characteristics of the fasting cohort (fasting time, ≥8 h).

	2-h $PG_{OGTT@fasting}$		All	*p*
	< 200 mg/dL	≥ 200 mg/dL		
Sample size	3287	578	3865	NA
Age, y, mean (SD)	55 (10)	60 (9)	56 (10)	<0.001
Sex (male), *n* (%)	1614 (49.1)	292 (50.5)	1906 (49.3)	0.53
2-h $PG_{OGTT@fasting}$, mg/dL, median (IQR)	114 (93–140)	275 (224–353)	121 (97–162)	<0.001
HbA_{1c}, %, median (IQR)	5.4 (5.1–5.7)	6.7 (5.9–8.4)	5.5 (5.2–5.9)	<0.001
BMI, kg/m², median (IQR)	27 (24–31)	30 (26–33)	27 (24–31)	<0.001
TC, mg/dL, median (IQR)	213 (188–240)	222 (194–251)	214 (189–242)	<0.001
HDL-C, mg/dL, median (IQR)	49 (40–60)	44 (36–54)	48 (39–59)	<0.001
SBP, mm Hg, median (IQR)	126 (115–139)	136 (126–150)	128 (117–141)	<0.001
Ethnicity, *n* (%)				
Non-Hispanic white	1527 (46.5)	216 (37.4)	1743 (45.1)	<0.001
Non-Hispanic black	864 (26.3)	126 (21.8)	990 (25.6)	
Hispanic	847 (25.8)	231 (40.0)	1078 (27.9)	
Other	49 (1.5)	5 (0.9)	54 (1.4)	
Education, *n* (%)				
<High school	1350 (41.1)	332 (57.4)	1682 (43.5)	<0.001
High school	977 (29.7)	150 (26.0)	1127 (29.2)	
>High school	939 (28.6)	96 (16.6)	1035 (26.8)	
Unknown	21 (0.6)	0 (0)	21 (0.5)	
Poverty–income ratio, *n* (%)				
<130%	766 (23.3)	197 (34.1)	963 (24.9)	<0.001
130%–349%	1308 (39.8)	218 (37.7)	1526 (39.5)	
≥350%	902 (27.4)	101 (17.5)	1003 (26.0)	
Unknown	311 (9.5)	62 (10.7)	373 (9.7)	
Physical activity, *n* (%)				
Active	1198 (36.4)	187 (32.4)	1385 (35.8)	0.08
Insufficiently active	1397 (42.5)	249 (43.1)	1646 (42.6)	
Inactive	692 (21.1)	142 (24.6)	834 (21.6)	
Alcohol consumption, *n* (%)				
0 drinks/week	467 (14.2)	118 (20.4)	585 (15.1)	<0.001
<1 drink/week	391 (11.9)	50 (8.7)	441 (11.4)	
1–6 drinks/week	612 (18.6)	76 (13.1)	688 (17.8)	
≥7 drinks/week	409 (12.4)	66 (11.4)	475 (12.3)	

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Unknown	1408 (42.8)	268 (46.4)	1676 (43.4)	
Smoking status, *n* (%)				
Current smoker	910 (27.7)	112 (19.4)	1022 (26.4)	<0.001
Past smoker	1015 (30.9)	231 (40.0)	1246 (32.2)	
Non-smoker	1362 (41.4)	235 (40.7)	1597 (41.3)	
Survey period, *n* (%)				
1988–1991	1584 (48.2)	261 (45.2)	1845 (47.7)	0.18
1991–1994	1703 (51.8)	317 (54.8)	2020 (52.3)	
Family history of diabetes, *n* (%)				
Yes	1435 (43.7)	309 (53.5)	1744 (45.1)	<0.001
No	1814 (55.2)	262 (45.3)	2076 (53.7)	
Unknown	38 (1.2)	7 (1.2)	45 (1.2)	
Selt reported diagnosis, *n* (%)				
Yes	76 (2.3)	212 (36.7)	288 (7.5)	<0.001
No	3207 (97.6)	365 (63.1)	3572 (92.4)	
Unknown	4 (0.1)	1 (0.2)	5 (0.1)	

Abbreviations: 2-h $PG_{OGTT@fasting}$, 2-hour plasma glucose during OGTT which was conducted in the fasting period (fasting time, ≥ 8 h); BMI, body mass index; HbA_{1c}, hemoglobin A_{1c}; HDL-C, high-density lipoprotein cholesterol; IQR, interquartile range; NA, not applicable; OGTT, oral glucose tolerance test; SBP, systolic blood pressure; SD, standard deviation; TC, total cholesterol.

3.2. Association of 2-h Plasma Glucose during OGTT with HbA_{1c}

2-h $PG_{OGTT@4–7.9h}$ was positively associated with HbA_{1c} without adjustment (Model 1, β = 0.544, p <0.001, Table 3). This association remained significant after adjustment for all the tested confounders (Model 6, β = 0.530, p <0.001, Table 3). Similarly, 2-h $PG_{OGTT@fasting}$ was positively associated with HbA_{1c} in the absence (β = 0.603) and presence of adjustment (β = 0.574, Table 3).

Table 3. Association of 2-h plasma glucose during OGTT[1] (independent variable) with HbA_{1c}[1] (dependent variable)

Models	2-h $PG_{OGTT@4–7.9h}$		2-h $PG_{OGTT@fasting}$	
	β	*p*	β	*p*
Model 1	0.544	<0.001	0.603	<0.001
Model 2	0.545	<0.001	0.590	<0.001
Model 3	0.530	<0.001	0.578	<0.001
Model 4	0.537	<0.001	0.583	<0.001
Model 5	0.533	<0.001	0.578	<0.001
Model 6	0.530	<0.001	0.574	<0.001

2-h $PG_{OGTT@4–7.9h}$, 2-hour plasma glucose during OGTT which was conducted in the postprandial period between 4 and 7.9 h; 2-h $PG_{OGTT@fasting}$, 2-hour plasma glucose during OGTT which was conducted in the fasting period (fasting time, ≥ 8 h); HbA_{1c}, hemoglobin A_{1c}; OGTT, oral glucose tolerance test. [1] Natural log-transformed. Model 1 was not adjusted; Model 2 was adjusted for age, sex, and ethnicity; Model 3 was adjusted for all the factors in Model 2 plus body mass index, poverty–income ratio, and education; Model 4 was adjusted for all the factors in Model 3 plus physical activity, alcohol consumption, smoking status, and survey period; Model 5 was adjusted for all the factors in Model 4 plus total cholesterol, HDL cholesterol, and systolic blood pressure; and Model 6 was adjusted for all the factors in Model 5 plus family history of diabetes.

3.3. Association of 2-h Plasma Glucose during OGTT with Diabetes Diagnosis

A 1-natural-log increase in 2-h $PG_{OGTT@4–7.9h}$ was associated with a higher risk of HbA_{1c}-diagnosed diabetes after adjustment for all the tested confounders (Model 6; OR = 687; 95% CI, 310–1523; p <0.001; Table 4). 2-h $PG_{OGTT@fasting}$ was associated with HbA_{1c}-diagnosed diabetes to a similar extent (Model 6; OR = 655; 95% CI, 356–1204; p <0.001; Table 4).

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ROC curve analysis showed that 2-h $PG_{OGTT@4–7.9h}$ predicted HbA_{1c}-diagnosed diabetes with an accuracy of 92% as indicated by the AUC value, and the accuracy for 2-h $PG_{OGTT@fasting}$ was 95% (Figure 2). The optimal cutoff for 2-h $PG_{OGTT@4–7.9h}$ to predict HbA_{1c}-diagnosed diabetes was 206.8 mg/dL, and the corresponding cutoff for 2-h $PG_{OGTT@fasting}$ was 203.6 mg/dL (Figure 2).

In further analyses, we defined diabetes as a self-reported diagnosis. The results showed that both 2-h $PG_{OGTT@4–7.9h}$ and 2-h $PG_{OGTT@fasting}$ remained significantly associated with diabetes diagnosis (Table 5 and Figure 3).

Table 4. Association of 2-h plasma glucose during OGTT (natural log-transformed) with diabetes diagnosis (defined as $HbA_{1c} \geq 6.5\%$).

Models	2-h $PG_{OGTT@4–7.9h}$			2-h $PG_{OGTT@fasting}$		
	OR	95% CI	*p*	OR	95% CI	*p*
Model 1	451	229–885	<0.001	402	242–669	<0.001
Model 2	589	284–1221	<0.001	632	357–1119	<0.001
Model 3	563	270–1176	<0.001	609	341–1090	<0.001
Model 4	695	320–1511	<0.001	714	390–1305	<0.001
Model 5	688	313–1513	<0.001	658	359–1207	<0.001
Model 6	687	310–1523	<0.001	655	356–1204	<0.001

2-h $PG_{OGTT@4–7.9h}$, 2-hour plasma glucose during OGTT which was conducted in the postprandial period between 4 and 7.9 h; 2-h $PG_{OGTT@fasting}$, 2-hour plasma glucose during OGTT which was conducted in the fasting period (fasting time, ≥ 8 h); CI, confidence interval; HbA_{1c}, hemoglobin A_{1c}; OGTT, oral glucose tolerance test; OR, odds ratio. Model 1 was not adjusted; Model 2 was adjusted for age, sex, and ethnicity; Model 3 was adjusted for all the factors in Model 2 plus body mass index, poverty–income ratio, and education; Model 4 was adjusted for all the factors in Model 3 plus physical activity, alcohol consumption, smoking status, and survey period; Model 5 was adjusted for all the factors in Model 4 plus total cholesterol, HDL cholesterol, and systolic blood pressure; and Model 6 was adjusted for all the factors in Model 5 plus family history of diabetes.

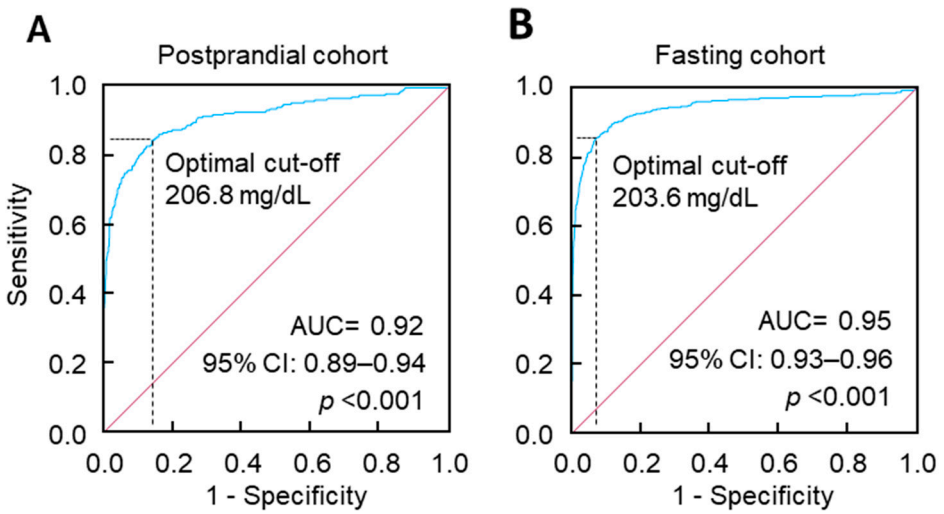

Figure 2. ROC curves of 2-h plasma glucose to classify diabetes, defined as $HbA_{1c} \geq 6.5\%$. **A**, OGTT was conducted in the postprandial period between 4 and 7.9 h. The optimal cutoff was 206.8 mg/dL, with a sensitivity of 84.8%, specificity of 86.1%, and an area under the curve (AUC) of 0.92. **B**, OGTT was conducted in the fasting period (fasting time, ≥ 8 h). The optimal cutoff was 203.6 mg/dL, with a sensitivity of 85.8%, specificity of 93.1%, and an AUC of 0.95. HbA_{1c}, hemoglobin A_{1c}; OGTT, oral glucose tolerance test; ROC, receiver operating characteristic.

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Table 5. Association of 2-h plasma glucose (natural log-transformed) with diabetes (defined as a self-reported diagnosis).

Models	2-h $PG_{OGTT@4–7.9h}$ (n = 2346[1])			2-h $PG_{OGTT@fasting}$ (n = 3860[2])		
	OR	95% CI	p	OR	95% CI	p
Model 1	31.2	23.0–42.3	<0.001	20.7	16.6–25.7	<0.001
Model 2	33.2	23.4–46.9	<0.001	17.9	14.2–22.5	<0.001
Model 3	33.9	23.3–49.3	<0.001	17.1	13.5–21.7	<0.001
Model 4	38.6	25.9–57.4	<0.001	17.6	13.8–22.4	<0.001
Model 5	40.2	26.8–60.3	<0.001	16.6	13.0–21.2	<0.001
Model 6	39.7	26.3–59.9	<0.001	15.7	12.2–20.1	<0.001

2-h $PG_{OGTT@4–7.9h}$, 2-hour plasma glucose during OGTT which was conducted in the postprandial period between 4 and 7.9 h; 2-h $PG_{OGTT@fasting}$, 2-hour plasma glucose during OGTT which was conducted in the fasting period (fasting time, ≥ 8 h); CI, confidence interval; OGTT, oral glucose tolerance test; OR, odds ratio. [1] Out of 2347 participants, the self-reported diabetes status was missing in one participant. Therefore, the remaining 2346 participants were included in the analysis. [2] Out of 3865 participants, the self-reported diabetes status was missing in 5 participants. Therefore, the remaining 3860 participants were included in the analysis. Model 1 was not adjusted; Model 2 was adjusted for age, sex, and ethnicity; Model 3 was adjusted for all the factors in Model 2 plus body mass index, poverty–income ratio, and education; Model 4 was adjusted for all the factors in Model 3 plus physical activity, alcohol consumption, smoking status, and survey period; Model 5 was adjusted for all the factors in Model 4 plus total cholesterol, HDL cholesterol, and systolic blood pressure; and Model 6 was adjusted for all the factors in Model 5 plus family history of diabetes.

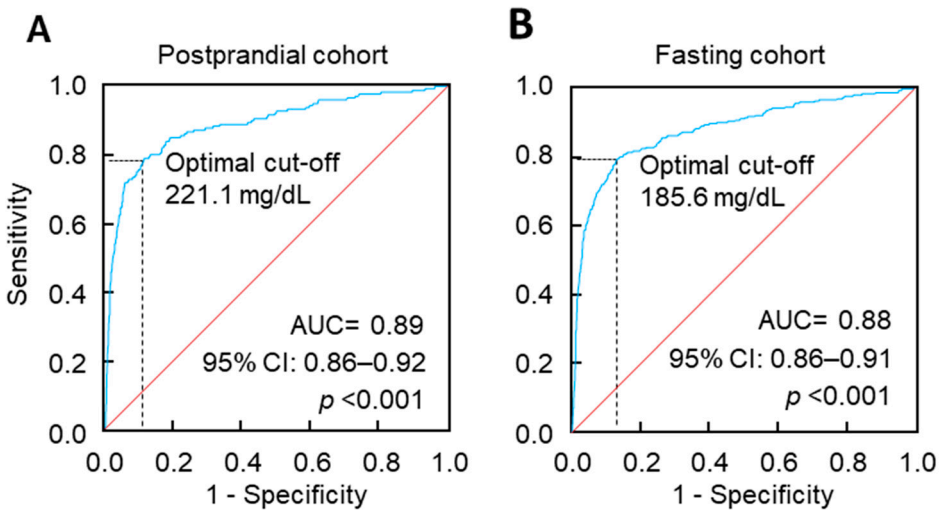

Figure 3. ROC curves of 2-h plasma glucose to classify diabetes, defined as a self-reported diagnosis. **A**, OGTT was conducted in the postprandial period between 4 and 7.9 h. The optimal cutoff was 221.1 mg/dL, with a sensitivity of 79.0%, specificity of 88.7%, and an area under the curve (AUC) of 0.89. **B**, OGTT was conducted in the fasting period (fasting time, ≥ 8 h). The optimal cutoff was 185.6 mg/dL, with a sensitivity of 79.5%, specificity of 87.0%, and an AUC of 0.88. OGTT, oral glucose tolerance test; ROC, receiver operating characteristic.

3.4. Association of 2-h Plasma Glucose during OGTT with Diabetes Mortality

The postprandial cohort was followed up for 50,185 person-years with a mean follow-up of 21.4 years. The fasting cohort was followed up for 82,039 person-years with a mean follow-up of 21.2 years. During the follow-up, diabetes led to 40 and 62 deaths in the postprandial and the fasting cohorts, respectively (Table 6).

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Table 6. Numbers of mortality during the follow-up.

Mortality	Postprandial cohort	Fasting cohort	All
All causes	1299	2144	3443
Diabetes	40	62	102
CVD	432	734	1166
Cancer	319	562	881

CVD, cardiovascular disease.

A 1-natural-log increase in 2-h $PG_{OGTT@4–7.9h}$ was associated with a 21.1-fold increase in diabetes mortality risk after adjustment for all the tested confounders (Model 6; HR, 21.1; 95% CI, 9.2–48.0; $p < 0.001$; Table 7). A 1-natural-log increase in 2-h $PG_{OGTT@fasting}$ was associated with a 7.1-fold increase in diabetes mortality risk after adjustment for all the tested confounders (Model 6; HR, 7.1; 95% CI, 4.2–11.9; $p < 0.001$; Table 7).

Table 7. Association of 2-h plasma glucose (natural log transformed) with diabetes mortality.

Models	2-h $PG_{OGTT@4–7.9h}$			2-h $PG_{OGTT@fasting}$		
	HR	95% CI	*p*	HR	95% CI	*p*
Model 1	18.1	9.5–34.6	<0.001	10.4	6.6–16.4	<0.001
Model 2	17.2	8.4–35.1	<0.001	8.7	5.4–13.9	<0.001
Model 3	16.0	7.6–33.6	<0.001	8.2	5.0–13.3	<0.001
Model 4	22.0	10.2–47.5	<0.001	8.1	4.9–13.4	<0.001
Model 5	21.7	9.6–49.4	<0.001	7.3	4.4–12.2	<0.001
Model 6	21.1	9.2–48.0	<0.001	7.1	4.2–11.9	<0.001

2-h $PG_{OGTT@4–7.9h}$, 2-hour plasma glucose during OGTT which was conducted in the postprandial period between 4 and 7.9 h; 2-h $PG_{OGTT@fasting}$, 2-hour plasma glucose during OGTT which was conducted in the fasting period (fasting time, ≥ 8 h); CI, confidence interval; HR, hazard ratio; OGTT, oral glucose tolerance test. Model 1 was not adjusted; Model 2 was adjusted for age, sex, and ethnicity; Model 3 was adjusted for all the factors in Model 2 plus body mass index, poverty–income ratio, and education; Model 4 was adjusted for all the factors in Model 3 plus physical activity, alcohol consumption, smoking status, and survey period; Model 5 was adjusted for all the factors in Model 4 plus total cholesterol, HDL cholesterol, and systolic blood pressure; and Model 6 was adjusted for all the factors in Model 5 plus family history of diabetes.

Further analysis was conducted by treating 2-h plasma glucose as a categorical variable using the clinical cutoff of 200 mg/dL. The Kaplan–Meier survival curves showed that those with 2-h plasma glucose of ≥ 200 mg/dL (versus < 200 mg/dL) had an increased risk of diabetes mortality in both cohorts (p <0.001, Figure 4). The positive association remained after further adjustment for all the tested confounders (Table 8)

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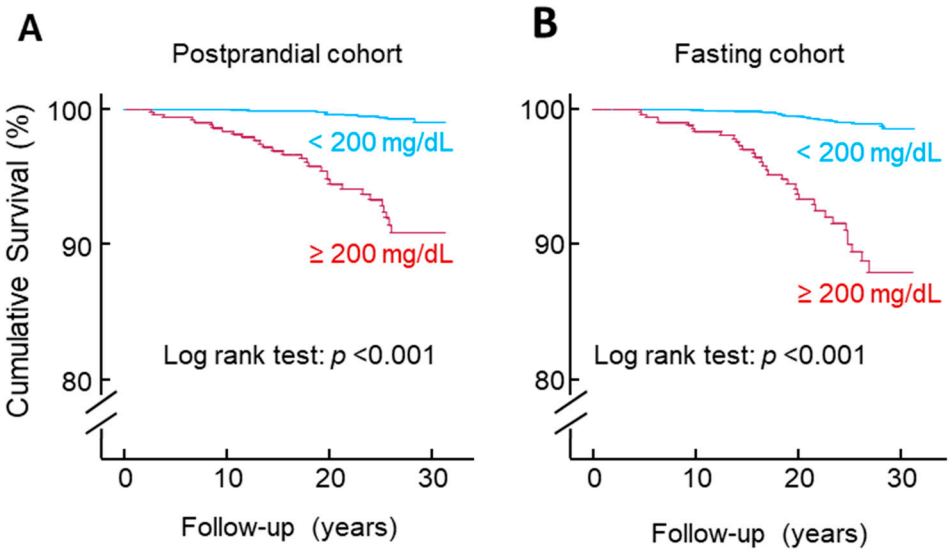

Figure 4. Kaplan–Meier survival curves of 2-h plasma glucose for diabetes mortality. **A**, The postprandial cohort; **B**, The fasting cohort. The 2-h plasma glucose during OGTT was stratified as ≥ versus < 200 mg/dL. OGTT, oral glucose tolerance test.

Table 8. Association of 2-h plasma glucose during OGTT (≥ versus < 200 mg/dL) with diabetes mortality.

Models	2-h $PG_{OGTT@4–7.9h}$			2-h $PG_{OGTT@fasting}$		
	HR	95% CI	*p*	HR	95% CI	*p*
Model 1	12.4	6.0–25.4	<0.001	10.4	6.3–17.2	<0.001
Model 2	10.0	4.7–20.9	<0.001	8.1	4.8–13.7	<0.001
Model 3	9.0	4.3–19.1	<0.001	7.3	4.3–12.4	<0.001
Model 4	13.7	6.2–30.6	<0.001	7.3	4.3–12.5	<0.001
Model 5	12.0	5.3–27.1	<0.001	6.1	3.6–10.4	<0.001
Model 6	12.3	5.4–27.9	<0.001	5.9	3.4–10.1	<0.001

2-h $PG_{OGTT@4–7.9h}$, 2-hour plasma glucose during OGTT which was conducted in the postprandial period between 4 and 7.9 h; 2-h $PG_{OGTT@fasting}$, 2-hour plasma glucose during OGTT which was conducted in the fasting period (fasting time, ≥ 8 h); CI, confidence interval; HR, hazard ratio; OGTT, oral glucose tolerance test. Model 1 was not adjusted; Model 2 was adjusted for age, sex, and ethnicity; Model 3 was adjusted for all the factors in Model 2 plus body mass index, poverty–income ratio, and education; Model 4 was adjusted for all the factors in Model 3 plus physical activity, alcohol consumption, smoking status, and survey period; Model 5 was adjusted for all the factors in Model 4 plus total cholesterol, HDL cholesterol, and systolic blood pressure; and Model 6 was adjusted for all the factors in Model 5 plus family history of diabetes.

3.5. Association of 2-h Plasma Glucose during OGTT with All-Cause Mortality, CVD Mortality, and Cancer Mortality

We further analyzed the association of 2-h $PG_{OGTT@4–7.9h}$ with mortality from all causes and CVD. The results showed that a 1-natural-log increase in 2-h $PG_{OGTT@4–7.9h}$ was associated with a 41% increase in multivariable-adjusted risk of all-cause mortality (Model 6; HR, 1.41; 95% CI, 1.22–1.64; p <0.001; Table 9) and a 47% increase in multivariable-adjusted risk of CVD mortality (Model 6; HR, 1.47; 95% CI, 1.13–1.91; p <0.001; Table 10). 2-h $PG_{OGTT@fasting}$ predicted mortality from all causes and CVD to a similar extent (Tables 9–10). In addition, neither 2-h $PG_{OGTT@4–7.9h}$ nor 2-h $PG_{OGTT@fasting}$ was independently associated with cancer mortality (Table 11).

Table 9. Association of 2-h plasma glucose during OGTT (natural log transformed) with all-cause mortality.

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Models	2-h $PG_{OGTT@4–7.9h}$			2-h $PG_{OGTT@fasting}$		
	HR	95% CI	*p*	HR	95% CI	*p*
Model 1	1.97	1.72–2.24	<0.001	1.96	1.79–2.15	<0.001
Model 2	1.38	1.20–1.60	<0.001	1.45	1.31–1.60	<0.001
Model 3	1.37	1.19–1.58	<0.001	1.44	1.30–1.59	<0.001
Model 4	1.50	1.30–1.74	<0.001	1.52	1.37–1.68	<0.001
Model 5	1.41	1.22–1.64	<0.001	1.42	1.28–1.57	<0.001
Model 6	1.41	1.22–1.64	<0.001	1.40	1.26–1.55	<0.001

2-h $PG_{OGTT@4–7.9h}$, 2-hour plasma glucose during OGTT which was conducted in the postprandial period between 4 and 7.9 h; 2-h $PG_{OGTT@fasting}$, 2-hour plasma glucose during OGTT which was conducted in the fasting period (fasting time, ≥ 8 h); CI, confidence interval; HR, hazard ratio; OGTT, oral glucose tolerance test. Model 1 was not adjusted; Model 2 was adjusted for age, sex, and ethnicity; Model 3 was adjusted for all the factors in Model 2 plus body mass index, poverty–income ratio, and education; Model 4 was adjusted for all the factors in Model 3 plus physical activity, alcohol consumption, smoking status, and survey period; Model 5 was adjusted for all the factors in Model 4 plus total cholesterol, HDL cholesterol, and systolic blood pressure; and Model 6 was adjusted for all the factors in Model 5 plus family history of diabetes.

Table 10. Association of 2-h plasma glucose during OGTT (natural log transformed) with CVD mortality.

Models	2-h $PG_{OGTT@4–7.9h}$			2-h $PG_{OGTT@fasting}$		
	HR	95% CI	*p*	HR	95% CI	*p*
Model 1	2.37	1.89–2.96	<0.001	2.24	1.92–2.61	<0.001
Model 2	1.63	1.28–2.09	<0.001	1.59	1.35–1.87	<0.001
Model 3	1.54	1.21–1.98	<0.001	1.54	1.31–1.82	<0.001
Model 4	1.64	1.27–2.11	<0.001	1.60	1.35–1.89	<0.001
Model 5	1.49	1.15–1.93	0.003	1.43	1.20–1.70	<0.001
Model 6	1.47	1.13–1.91	0.004	1.41	1.19–1.68	<0.001

2-h $PG_{OGTT@4–7.9h}$, 2-hour plasma glucose during OGTT which was conducted in the postprandial period between 4 and 7.9 h; 2-h $PG_{OGTT@fasting}$, 2-hour plasma glucose during OGTT which was conducted in the fasting period (fasting time, ≥ 8 h); CI, confidence interval; CVD, cardiovascular disease; HR, hazard ratio; OGTT, oral glucose tolerance test. Model 1 was not adjusted; Model 2 was adjusted for age, sex, and ethnicity; Model 3 was adjusted for all the factors in Model 2 plus body mass index, poverty–income ratio, and education; Model 4 was adjusted for all the factors in Model 3 plus physical activity, alcohol consumption, smoking status, and survey period; Model 5 was adjusted for all the factors in Model 4 plus total cholesterol, HDL cholesterol, and systolic blood pressure; and Model 6 was adjusted for all the factors in Model 5 plus family history of diabetes.

Table 11. Association of 2-h plasma glucose during OGTT (natural log transformed) with cancer mortality.

Models	2-h $PG_{OGTT@4–7.9h}$			2-h $PG_{OGTT@fasting}$		
	HR	95% CI	*p*	HR	95% CI	*p*
Model 1	1.13	0.86–1.49	0.37	1.23	1.02–1.50	0.03
Model 2	0.89	0.67–1.19	0.43	0.97	0.80–1.19	0.79
Model 3	0.94	0.70–1.25	0.65	0.99	0.81–1.22	0.93
Model 4	1.05	0.78–1.41	0.76	1.08	0.88–1.32	0.48
Model 5	1.00	0.74–1.36	0.99	1.03	0.84–1.27	0.78
Model 6	1.03	0.76–1.39	0.87	1.01	0.82–1.24	0.95

2-h $PG_{OGTT@4–7.9h}$, 2-hour plasma glucose during OGTT which was conducted in the postprandial period between 4 and 7.9 h; 2-h $PG_{OGTT@fasting}$, 2-hour plasma glucose during OGTT which was conducted in the fasting period (fasting time, ≥ 8 h); CI, confidence interval; HR, hazard ratio; OGTT, oral glucose tolerance test. Model 1 was not adjusted; Model 2 was adjusted for age, sex, and ethnicity; Model 3 was adjusted for all the factors in Model 2 plus body mass index, poverty–income ratio, and education; Model 4 was adjusted for all the factors in Model 3 plus physical activity, alcohol consumption, smoking status, and survey period; Model 5 was adjusted for all

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the factors in Model 4 plus total cholesterol, HDL cholesterol, and systolic blood pressure; and Model 6 was adjusted for all the factors in Model 5 plus family history of diabetes.

3.6 Power and Sample Size Estimation for 2-h PG$_{OGTT@4–7.9h}$ to Diagnose Diabetes

Power analysis for using 2-h PG$_{OGTT@4–7.9h}$ to diagnose diabetes was conducted through the simulation of 10,000 random samples, and each simulation had a certain sample size ranging from 50 to 200 participants. The accuracy of predicted diagnoses for each of the 10,000 random samples was assessed by comparing them with the actual diabetes status.

A diagnostic accuracy within the range of 80% to 90% is considered excellent [12]. This study employed an accuracy threshold of 80% to conduct power and sample size estimations. Additionally, a slightly improved accuracy of 81% was also explored for these estimations (Table 12).

Analysis revealed that when the sample size increased, the analysis power increased and the confidence interval for sensitivity and specificity narrowed (Table 12). The findings suggested that a sample size of 100 participants may be necessary to achieve over 80% power in detecting a diagnostic accuracy of 81% (Table 12).

Table 12. Power estimation for 2-h PG$_{OGTT@4–7.9h}$ to diagnose diabetes[1].

Sample size	*n* = 50	*n* = 90	*n* = 100	*n* = 150	*n* = 175	*n* = 200
Power for 80% accuracy	82.8%	86.4%	87.8%	91.8%	92.8%	94.1%
Power for 81% accuracy	71.2%	79.3%	81.7%	83.1%	85.8%	88.0%
Sensitivity (95% CI)	86.5% (50.0%–100%)	86.5% (60.0%–100%)	86.6% (61.5%–100%)	86.5% (66.7%–100%)	86.5% (68.8%–100%)	86.6% (70.0%–100%)
Specificity (95% CI)	83.5% (72.1%–93.5%)	83.4% (75.3%–91.0%)	83.4% (75.8%–90.4%)	83.4% (77.3%–89.4%)	83.4% (77.6%–88.7%)	83.4% (78.0%–88.5%)

2-h PG$_{OGTT@4–7.9h}$, 2-hour plasma glucose during OGTT which was conducted in the postprandial period between 4 and 7.9 h; CI, confidence interval; OGTT, oral glucose tolerance test. [1] Diabetes was defined as HbA_{1c} ≥6.5%. Power was estimated using simulations on 10,000 random samples for each sample size.

4. Discussion

Using a cohort of US adults (*n* = 2347), this study demonstrated, for the first time, that OGTT conducted during the postprandial period between 4 and 7.9 h may serve as a valuable tool for diabetes diagnosis and predicting mortality risk.

2-h PG$_{OGTT@4–7.9h}$ classified HbA_{1c}-diagnosed diabetes with 92% accuracy (95% CI 89%–94%), falling within the outstanding accuracy range (>90%) [12]. This accuracy was comparable to its fasting counterpart, 2-h PG$_{OGTT@fasting}$, which achieved 95% accuracy (95% CI, 93%–96%). Further analysis using self-reported diagnosis confirmed similar diagnostic accuracies between 2-h PG$_{OGTT@4–7.9h}$ and 2-h PG$_{OGTT@fasting}$ (89% versus 88%). These findings suggest that 2-h PG$_{OGTT@4–7.9h}$ holds promise as a diagnostic marker for diabetes.

In epidemiological studies, self-reported diagnosis of diabetes is widely accepted due to its relatively higher accuracy compared to many other chronic conditions such as stroke, heart disease, and hypertension [42,43]. Studies across diverse populations have consistently shown that self-

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reported diagnosis exhibits a sensitivity of approximately 70%–75% in identifying true diabetes, with specificity exceeding 95% [44-47].

The slightly lower accuracy of 2-h plasma glucose in classifying self-reported diabetes compared to HbA_{1c}-diagnosed diabetes (e.g., 88% versus 95% for 2-h $PG_{OGTT@fasting}$) may be attributed to inherent limitations in the accuracy of self-reported diagnosis.

As fasting plasma glucose and OGTT conducted during fasting were not available in participants from the postprandial cohort, these parameters were not used as diagnostic criteria in this study. Therefore, the full diagnostic potential of 2-h $PG_{OGTT@4–7.9h}$ requires further investigation in well-designed studies where all three diabetes diagnostic criteria are concurrently assessed in each participant.

The optimal cutoff for predicting HbA_{1c}-diagnosed diabetes with 2-h $PG_{OGTT@4–7.9h}$ was 206.8 mg/dL, aligning closely with the cutoff for 2-h $PG_{OGTT@fasting}$ at 203.6 mg/dL. This suggests that the clinical cutoff of 200 mg/dL used for 2-h $PG_{OGTT@fasting}$ [9,16,17] may be applicable to 2-h $PG_{OGTT@4–7.9h}$ as well. Participants with 2-h $PG_{OGTT@4–7.9h}$ ≥ 200 mg/dL demonstrated a significantly higher risk of diabetes mortality (HR, 12.3; 95% CI, 5.4–27.9) compared to those with lower values (< 200 mg/dL).

Regarding mortality predictions, both 2-h $PG_{OGTT@4–7.9h}$ and 2-h $PG_{OGTT@fasting}$ effectively forecasted mortality from CVD and all causes. This is consistent with literature suggesting that 2-h $PG_{OGTT@fasting}$ serves as an independent predictor for CVD [48-51] and all-cause mortality [52-55]. Furthermore, 2-h $PG_{OGTT@4–7.9h}$ also demonstrated comparable predictive ability for mortality from CVD and all causes.

Interestingly, neither 2-h $PG_{OGTT@4–7.9h}$ nor 2-h $PG_{OGTT@fasting}$ predicted cancer mortality in this study, consistent with some reports in the literature regarding 2-h $PG_{OGTT@fasting}$ [56-58]. Notably, other studies have reported associations between 2-h $PG_{OGTT@fasting}$ and cancer mortality [59,60].

Moreover, both 2-h $PG_{OGTT@4–7.9h}$ and 2-h $PG_{OGTT@fasting}$ predicted mortality specifically from diabetes, consistent with a previous report that 2-h $PG_{OGTT@fasting}$ predicted diabetes mortality [61]. In fact, 2-h $PG_{OGTT@4–7.9h}$ exhibited potentially greater sensitivity for predicting diabetes mortality compared to its fasting counterpart, evidenced by an adjusted HR of 21.1 (95% CI, 9.2–48.0) versus 7.1 (95% CI, 4.2–11.9) per 1-natural-log increase. A similar trend was observed when analyzing 2-h plasma glucose as a categorical variable (≥ versus < 200 mg/dL), with adjusted HRs of 12.3 (95% CI, 5.4–27.9) and 5.9 (95% CI, 3.4–10.1) for higher $PG_{OGTT@4–7.9h}$ and $PG_{OGTT@fasting}$, respectively. Notably, $PG_{OGTT@4–7.9h}$ and $PG_{OGTT@fasting}$ data were mutually exclusive in this study, necessitating future research to directly compare their predictive capacities within the same participant cohort.

A limitation of this study was its reliance on HbA_{1c} alone for defining diabetes, excluding fasting plasma glucose and OGTT from fasting periods due to their absence in participants with 2-h $PG_{OGTT@4–7.9h}$. Nevertheless, analyses involving self-reported diagnosis yielded similar findings, indicating comparable diagnostic utility between 2-h $PG_{OGTT@4–7.9h}$ and 2-h $PG_{OGTT@fasting}$ for diabetes. Future investigations should incorporate all three diagnostic criteria to comprehensively assess the diagnostic value of 2-h $PG_{OGTT@4–7.9h}$. This study provides valuable insights into sample size estimation for future research aimed at elucidating the full diagnostic potential of 2-h $PG_{OGTT@4–7.9h}$.

5. Conclusions

This study found that 2-h $PG_{OGTT@4–7.9h}$ classified HbA_{1c}-diagnosed diabetes with an outstanding accuracy of 92%, similar to that of 2-h $PG_{OGTT@fasting}$ (i.e., 95%). 2-h $PG_{OGTT@4–7.9h}$ predicted mortality risk from diabetes, CVD and all causes. Therefore, 2-h $PG_{OGTT@4–7.9h}$, a non-fasting test, might be useful for diabetes diagnosis and risk prediction.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the NHANES Institutional Review Board. Approval codes: NHANES III 1988–1994.

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Informed Consent Statement: All participants provided written informed consent. The participants' records were anonymized before being accessed by the author.

Data Availability Statement: All data in the current analysis are publicly available on the NHANES website (https://www.cdc.gov/nchs/nhanes/index.htm, accessed on 1 March 2023).

Conflicts of Interest: The author declares no conflicts of interest.

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