Hemoglobin A1c Levels and the Risk of Cardiovascular Disease in People Without Known Diabetes

A Population-Based Cohort Study in Japan

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Abstract: High hemoglobin A1c (HbA1c) levels are strongly associated with an increased risk of cardiovascular disease (CVD) in people with and without diabetes. However, information regarding the relationship between low HbA1c levels and the risk of CVD among people without known diabetes is limited. The aim of this large-scale, prospective, population-based cohort study was to clarify the association between HbA1c levels and CVD risk among people without known diabetes.

We followed up 10,980 men and 18,079 women (46–80 years old and free of CVD and cancer at baseline) in the Japan Public Health Center-based Prospective Study. Using Cox models, we estimated the hazard ratios for CVD risk with adjustments for age, sex, geographic areas, body mass index, smoking status, sports and physical exercise, alcohol intake, systolic blood pressure, non-high-density lipoprotein cholesterol, and high-density lipoprotein cholesterol.

During the median follow-up of 9.4 years, 935 CVD events (770 strokes and 165 coronary heart diseases) occurred. We observed a nonlinear association between HbA1c levels and CVD risk in participants without known diabetes. Compared with HbA1c levels of 5.0 to 5.4% (31–36 mmol/mol), the hazard ratios for CVD in participants without known diabetes were 1.50 (95% confidence interval: 1.15–1.95), 1.01 (0.85–1.20), 1.04 (0.82–1.32), and 1.77 (1.32–2.38) for HbA1c levels of <5.0% (<31 mmol/mol), 5.5 to 5.9% (37–41 mmol/ mol), 6.0 to 6.4% (42–47 mmol/mol), and ≥6.5% (≥48 mmol/mol), respectively (P value for nonlinear trend: <0.001). In addition, the hazard ratio for CVD was 1.81 (1.43–2.29) in patients with known diabetes compared with participants with HbA1c levels of 5.0 to 5.4% and without known diabetes. This nonlinear relation persisted after excluding people with kidney dysfunction, liver dysfunction, anemia, body mass index <18.5 kg/m², or early events within 3 years of follow-up (P value for nonlinear trend: <0.01 for all tests).

In conclusion, both low and high levels of HbA1c were associated with a higher risk of CVD in a Japanese general population without known diabetes.
and smoking), CVD remains to be the leading cause of death globally.1–3 Biomarkers, such as hemoglobin A1c (HbA1c), may be useful for identifying people with increased risk of CVD and eventually help reduce the global burden of CVD.4,5 It has been well established that high HbA1c levels are strongly associated with a high risk of CVD in people with4 and without5,6 diabetes. Accordingly, several researchers have suggested that HbA1c measurement may be useful for identifying people with an increased risk of CVD.5,7 However, the association between low HbA1c levels and CVD risk is not well understood. In some studies,8–10 but not all,6 it has been suggested that patients with type 2 diabetes and low HbA1c levels may have a higher CVD risk, which is consistent with the observation that severe hypoglycemia is associated with an increased CVD risk among patients with type 2 diabetes.11 However, the association between low HbA1c levels in people without known diabetes and CVD risk remains unknown. Although a possible association between low HbA1c levels and increased mortality in populations without known diabetes has been previously reported,4,12,13 the biological mechanisms underlying this association are currently unknown.13–15 Investigating the association between low HbA1c levels and CVD risk may improve our understanding of health risks associated with low HbA1c levels. The aim of this large-scale, prospective, population-based cohort study was to address the question of whether low HbA1c levels are associated with a higher CVD risk among people without known diabetes using strictly standardized HbA1c levels and detailed measurements of covariates in a general Japanese population free of CVD and cancer at baseline.

METHODS

Study Design and Population

The Japan Public Health Centre-based Prospective Study (JPHC Study) was initiated in 1990 for cohort I and in 1993 to 1994 for cohort II. All subjects were Japanese inhabitants from 11 public health center areas, and aged 40 to 59 years in 1990 (cohort I) and 40 to 69 years in 1993 (cohort II). Details of the study design have been described elsewhere.14 The JPHC Diabetes Study, involving HbA1c measurements and an additional questionnaire concerning diabetes and lifestyle, was conducted among JPHC participants at the time of their health check-ups (the first survey in 1998–2000 and the second survey in 2003–2005).17 Two public health center areas from Tokyo and Osaka were excluded because information regarding the incidence of coronary heart disease and stroke was not available. Therefore, this present study involved subjects from 9 areas (cohort I: 4 areas; cohort II: 5 areas). Individuals who participated in either of the JPHC Diabetes Study surveys were included in the present study. Among the 35,197 participants from the JPHC Diabetes Study, we excluded 1004 and 984 participants with a history of CVD and cancer, respectively, as well as 4150 participants with missing anthropometric or laboratory data. In total, we analyzed data for 29,059 participants. All participants provided written informed consent before participating in this study. The JPHC Diabetes Study was approved by the institutional review board of the National Center for Global Health and Medicine, Japan.

Measurements

Detailed procedures for the HbA1c measurements have been described previously,17 and these were performed using high-performance liquid chromatography or immunochemical assays. The overall intra-assay coefficient of variation for HbA1c ranged from 0.0 to 3.4%, and the maximal inter-assay coefficient of variation among the various laboratories ranged from 2.2 to 2.8%. The HbA1c measurement methods differed according to the public health center areas, and therefore, HbA1c values were strictly standardized to minimize inter-laboratory variation. For the calibration procedure, standard samples (approved by the Japan Diabetes Society) were provided to each public health center area before the surveys. HbA1c values were converted to National Glycohemoglobin Standardization Program values.18 Censoring events (which defined the individual’s final data point) were defined as the first CVD event, death, change of residence, loss to follow-up, December 31, 2009 (cohort I), or December 31, 2008 (cohort II). For individuals who participated in both surveys of the JPHC Diabetes Study before the censoring events (35.1% of the study population), the average HbA1c levels were used for analyses to capture their long-term exposure.19 The sensitivity analyses using the time-dependent Cox proportional hazard models to update the HbA1c levels and diabetes status did not materially change the estimates.

Each participant completed a self-administered questionnaire at the 5-year and/or 10-year follow-up of the JPHC Study, which comprised questions regarding previously diagnosed medical conditions, medication, and lifestyle factors, including physical activity, alcohol intake, dietary intake, and smoking.10 In the present study, we used data from the JPHC Study questionnaire at the time of entry into the JPHC Diabetes Study, except for participants from cohort I who only participated in the second JPHC Diabetes Study survey (10% of the study population). These participants completed the JPHC Study questionnaire 5 years before their entry into the JPHC Diabetes Study, and these data were used in the current analysis. Sensitivity analyses for excluding these participants did not materially change study findings. At the time of the 2 JPHC Diabetes Study surveys, blood pressure, weight, height, hemoglobin, serum creatinine, alanine aminotransferase, and lipid levels were measured. Body mass index was calculated as weight (kg) divided by height squared (m²).

We defined CVD as either stroke or coronary heart disease, including myocardial infarction or sudden cardiac death. CVD events were documented based on active patient notifications from the local hospitals, hospital record reviews for participants who reported CVD in the follow-up questionnaires, and a review of death certificates.20 A total of 78 major hospitals capable of treating patients with acute CVD were included in the registry of CVD events within the 9 public health center areas. Overall, 97% of stroke and 92% of myocardial infarction cases in the 9 areas were treated at these registry hospitals. CVD events were included in this study if they occurred between the time of entry into the JPHC Diabetes Study and December 31, 2009 (cohort I) or December 31, 2008 (cohort II). Changes in residential status, including survival status, were identified using the residential registry in each area. During the follow-up period, 1273 (4.4%) participants died, 420 (1.4%) moved out of the study areas, and 28 (0.1%) were lost to follow-up.

Stroke diagnoses were confirmed according to the National Survey of Stroke criteria21 by the presence of sudden or rapid onset focal neurological deficits that last >24h or until death. Strokes were classified according to subtype: hemorrhagic or ischemic (lacunar or nonlacunar).22 A diagnosis of definite myocardial infarction was confirmed according to the Monitoring Trends and Determinants of Cardiovascular Disease Project.
criteria on the basis of typical chest pain and evidence from electrocardiograms and/or cardiac enzyme levels. For cases of typical prolonged chest pain (>20 min) that were not confirmed by electrocardiograms or cardiac enzymes (8.5% of the total myocardial infarctions), a diagnosis of possible myocardial infarction was made, and these cases were included in the myocardial infarction cases. Sensitivity analyses for excluding cases with possible myocardial infarction did not materially change the findings. In the absence of myocardial infarction diagnoses, deaths that occurred within 1 h from symptom onset were considered as sudden cardiac deaths. Only the first CVD event during the follow-up was included in the analysis; recurrent events were excluded.

Statistical Methods

We followed-up 29,059 participants (46–80 years old) and calculated their person-years from the time of entry into the JPHC Diabetes Study until their censoring event. If individuals participated in both JPHC Diabetes Study surveys, the time of entry at the first survey was considered the starting point. We also calculated the baseline characteristics for patients with diabetes and 5 groups of people without known diabetes categorized by their HbA1c levels: <5.0% (<31 mmol/mol), 5.0 to 5.4% (31–36 mmol/mol), 5.5 to 5.9% (37–41 mmol/mol), 6.0 to 6.4% (42–47 mmol/mol), and ≥6.5% (≥48 mmol/mol) and had self-reported diabetes or were receiving treatment. We defined participants as having known diabetes if they had self-reported diabetes or were receiving treatment for diabetes. Following conventional practice, the HbA1c category of 5.0 to 5.4% (31–36 mmol/mol) was used as the reference category.

To examine the CVD risk in the 6 groups of people, we used Cox proportional hazards models and estimated the hazard ratios and 95% confidence intervals (categorical models). These models were adjusted for age, sex, health center areas, body mass index, smoking status (never smoked, past smoker, or current smoker), alcohol intake (current nondrinker, occasional drinker, or current drinker), sports and physical exercise (≥1 day/week or other), systolic blood pressure (mmHg), high-density lipoprotein cholesterol levels (mmol/L), and non-high-density lipoprotein cholesterol levels (mmol/L). Slightly different physical activity questionnaires were used during the 5-year and 10-year follow-ups of the JPHC Study. Therefore, we first calculated separate estimates for participants who completed the 5-year follow-up questionnaire and for those who completed the 10-year follow-up questionnaire. Because there was no apparent difference in the estimates between these 2 groups, we computed the pooled results using the fixed-effects model with inverse variance weighting.

Among the participants without known diabetes, we computed 2-sided P values for linear trends by assigning a mean HbA1c value for each category and including the variables as continuous variables in the models. We also computed 2-sided P values for quadratic trends (P value for quadratic trend) by including a quadratic term in each linear trend model. The proportional hazards assumption was assessed using the scaled Schoenfeld residuals and found to be appropriate. For sensitivity analysis, we further examined the association between HbA1c levels and CVD after excluding people with kidney dysfunction (estimated glomerular filtration <60.0 mL/min 1.73 m 2), liver dysfunction (alanine aminotransferase ≥100 IU/L), anemia (hemoglobin <100 g/L), or low body mass index (<18.5 kg/m 2). Further analyses were also conducted after excluding CVD cases with an early diagnosis (within 3 years of follow-up) from both the numerator and denominator (278 participants). To examine the shape of the association between continuous HbA1c levels and CVD risk among people without known diabetes, we fitted restricted cubic spline models by including transformed variables of HbA1c levels in the Cox models, with adjustment for the same covariates that were used in the categorical models. We fitted the models using 3, 4, and 5 knots at percentiles, and chose the number of knots that produced the smallest Akaike Information Criterion. The level of significance was set at P value <0.05. Analyses were performed using Stata version 12.1 (StataCorp, College Station, TX).

RESULTS

The baseline characteristics of the study population according to the 6 groups are shown in Table 1. Compared with participants with lower HbA1c levels, participants with higher HbA1c levels or known diabetes tended to be older; current or past smokers; have a higher body mass index, blood pressure, and non-high-density lipoprotein cholesterol levels; have lower high-density lipoprotein cholesterol levels; use lipid-lowering medication(s); be engaged in physical activity; and consume more calories.

During the median follow-up of 9.4 years (238,456 person-years), 770 strokes (226 lacunar infarctions, 232 nonlacunar infarctions, 311 hemorrhagic strokes, and 1 stroke of undetermined type) and 165 coronary heart diseases (129 definite myocardial infarctions, 12 possible myocardial infarctions, and 24 sudden cardiac deaths) were documented. Table 2 shows the associations for CVD, coronary heart disease, and stroke risk in the 6 groups. After multivariable adjustment for potential confounding factors, we observed a nonlinear relation between HbA1c levels and CVD risk (model 2; P value for quadratic trend: <0.001). The nonlinear trend was observed even after excluding people with kidney dysfunction, liver dysfunction, anemia, and low body mass index (P value for quadratic trend: <0.05 for all tests). Further adjustment for the use of lipid-lowering medication(s) or total energy intake resulted in similar results (P value for quadratic trend: <0.001 for all tests, data not shown). Similar findings were observed when CVD cases with an early diagnosis (within 3 years of follow-up) were excluded (P value for quadratic trend: 0.002). Spline curves indicated a U-shaped relation, with increased CVD risk observed in participants with low and high levels of HbA1c (Figure 1A). A similar pattern was observed for stroke risk (Table 2, Figure 1B). Because nonlinear associations were observed, particularly for stroke, we further examined the association between HbA1c levels and stroke subtypes. We observed nonlinear trends for the risks of hemorrhagic and nonlacunar ischemic stroke (model 2; P values for quadratic trend: 0.018 and 0.006, respectively), and a similar pattern was suggested for lacunar stroke (model 2; P value for quadratic trend: 0.066). Participants with high HbA1c levels (≥6.5%, ≥48 mmol/mol) or known diabetes had an increased risk of ischemic stroke (both lacunar and nonlacunar; model 2). A linear relation was suggested for coronary heart disease risk (Table 2, Figure 1C), although the number of coronary heart disease cases was likely too small to examine its risk in participants with low HbA1c levels. Participants with known diabetes had increased risks of CVD, stroke, and coronary heart disease (model 2).

Stratified analysis according to sex suggested an increased CVD risk existed in men with low and high levels of HbA1c (Table 3). Among women, increased CVD risk was apparent in...
TABLE 1. Baseline Characteristics According to Hemoglobin A1c Levels and Known Diabetes

| Characteristic                        | <5.0% (≤31 mmol/mol) | 5.0–5.4% (31–36 mmol/mol) | 5.5–5.9% (37–41 mmol/mol) | 6.0–6.4% (42–47 mmol/mol) | 6.5% (48 mmol/mol) | ≥6.5% (≥48 mmol/mol) | Known Diabetes |
|--------------------------------------|----------------------|----------------------------|----------------------------|----------------------------|-------------------|---------------------|-----------------|
|                                     | N = 2008             | N = 8177                   | N = 12,450                 | N = 3635                   | N = 1009          | N = 1780            |
| HbA1c, %                            | 4.8 ± 0.2            | 5.2 ± 0.1                  | 5.7 ± 0.1                  | 6.1 ± 0.1                  | 7.1 ± 0.9         | 7.0 ± 1.3           |
| Age, years                          | 61.5 ± 8.1           | 62.1 ± 7.3                 | 62.8 ± 6.6                 | 63.3 ± 6.4                 | 63.5 ± 6.5        | 64.2 ± 6.4          |
| Men*, %                             | 43.2                 | 36.3                       | 34.5                       | 39.5                       | 47.6              | 51.3                |
| Body mass index*, kg/m²              | 23.4 ± 3.1           | 23.4 ± 3.1                 | 23.7 ± 3.1                 | 24.3 ± 3.3                 | 25.0 ± 3.6        | 24.3 ± 3.4          |
| Diabetes treatment, %                |                      |                            |                            |                            | 64.1              |                     |
| No medication, %                    |                      |                            |                            |                            |                   | 46.1                |
| Oral hypoglycemic agent only, %     |                      |                            |                            |                            |                   | 47.4                |
| Insulin, %                          |                      |                            |                            |                            |                   | 6.5                 |
| Current smoking*, %                 | 13.9                 | 12.8                       | 14.6                       | 18.8                       | 25.1              | 19.2                |
| Past smoking*, %                    | 10.1                 | 9.7                        | 10.4                       | 11.8                       | 12.6              | 15.1                |
| Sports and physical exercise*, ≥1 day/week, % | 34.8          | 40.7                       | 46.5                       | 48.1                       | 50.3              | 48.9                |
| Current alcohol drinking*, %        | 35.6                 | 31.7                       | 30.2                       | 34.6                       | 39.3              | 37.2                |
| Ethanol intake*, g/week             | 5 (0–190)            | 6 (0–161)                  | 16 (0–162)                 | 42 (1–252)                 | 78 (1–262)        | 40 (1–252)          |
| Systolic blood pressure*, mmHg      | 130 ± 17             | 130 ± 17                   | 130 ± 17                   | 132 ± 17                   | 135 ± 18          | 133 ± 17            |
| Diastolic blood pressure*, mmHg     | 77 ± 11              | 77 ± 11                    | 78 ± 10                    | 79 ± 11                    | 79 ± 11           | 77 ± 10             |
| Non-high-density lipoprotein        | 3.52 ± 0.85          | 3.75 ± 0.84                | 3.92 ± 0.85                | 4.01 ± 0.88                | 4.13 ± 0.95       | 3.89 ± 0.89         |
| cholesterol*, mmol/L                |                      |                            |                            |                            |                   |                     |
| High-density lipoprotein            | 1.53 ± 0.39          | 1.55 ± 0.39                | 1.52 ± 0.38                | 1.49 ± 0.38                | 1.41 ± 0.35       | 1.45 ± 0.39         |
| cholesterol*, mmol/L                |                      |                            |                            |                            |                   |                     |
| Lipid-lowering medication use*, %   | 3.3                  | 5.9                        | 8.8                        | 11.6                       | 12.3              | 13.1                |
| Total energy*, kcal/day             | 1865 (1471–2401)     | 1926 (1530–2420)           | 1970 (1564–2468)           | 1987 (1597–2497)          | 2027 (1615–2532)  | 1891 (1505–2382)    |

Data are presented as mean ± standard deviation, percentage, or median (interquartile range). HbA1c = hemoglobin A1c.

* All variables (with the exception of age and HbA1c) were adjusted for age.
| HbA1c Levels in Participants Without Known Diabetes | Known Diabetes |
|-------------------------------------------------|----------------|
| <5.0% (<31 mmol/mol) | N = 2008 | N = 8177 |
| 5.0–5.4% (31–36 mmol/mol) | N = 12,450 | N = 3635 |
| 5.5–5.9% (37–41 mmol/mol) | N = 10,097 | |
| 6.0–6.4% (42–47 mmol/mol) | N = 1,070 | |
| ≥6.5% (≥48 mmol/mol) | N = 1780 |

| Cardiovascular disease | Person-years | No. of events | Crude incidence rate | P for Linear Trend | P for Quadratic Trend |
|------------------------|--------------|---------------|----------------------|--------------------|-----------------------|
| Stroke                 | 17,043       | 80            | 7.7                  | <0.001             | 1.92 (1.52–2.42) |
| | 70,311        | 228           | 3.2                  | 0.001               | 1.78 (1.41–2.26) |
| | 101,292       | 352           | 3.5                  | <0.001             | 1.81 (1.43–2.29) |
| | 28,226        | 108           | 3.8                  | <0.001             | 1.49 (0.92–2.03) |
| | 7700          | 60            | 7.8                  | <0.001             | 1.53 (1.37–2.44) |
| Hemorrhagic stroke     | 17,043       | 71            | 4.2                  | <0.001             | 1.55 (1.38–2.31) |
| | 70,311        | 193           | 2.7                  | <0.001             | 1.55 (1.43–2.38) |
| | 101,292       | 288           | 2.8                  | <0.001             | 1.55 (1.43–2.38) |
| | 28,226        | 83            | 2.9                  | <0.001             | 1.55 (1.43–2.38) |
| | 7700          | 49            | 6.4                  | <0.001             | 1.55 (1.43–2.38) |
| Ischemic stroke        | 17,043       | 33            | 1.9                  | 0.010              | 1.74 (1.07–2.81) |
| | 70,311        | 85            | 1.2                  | <0.001             | 1.74 (1.07–2.81) |
| | 101,292       | 125           | 1.2                  | 0.010              | 1.74 (1.07–2.81) |
| | 28,226        | 32            | 1.1                  | <0.001             | 1.74 (1.07–2.81) |
| | 7700          | 13            | 1.7                  | <0.001             | 1.74 (1.07–2.81) |
| Lacunar stroke         | 17,043       | 38            | 2.2                  | 0.018              | 1.72 (1.07–2.81) |
| | 70,311        | 108           | 1.5                  | <0.001             | 1.72 (1.07–2.81) |
| | 101,292       | 162           | 1.6                  | <0.001             | 1.72 (1.07–2.81) |
| | 28,226        | 51            | 1.8                  | <0.001             | 1.72 (1.07–2.81) |
| | 7700          | 36            | 4.7                  | <0.001             | 1.72 (1.07–2.81) |
| Nonlacunar ischemic stroke | 17,043 | 48            | 1.1                  | 0.024              | 1.40 (0.81–2.44) |
| | 70,311        | 53            | 0.8                  | <0.001             | 1.40 (0.81–2.44) |
| | 101,292       | 77            | 0.8                  | <0.001             | 1.40 (0.81–2.44) |
| | 28,226        | 27            | 1.0                  | <0.001             | 1.40 (0.81–2.44) |
| | 7700          | 17            | 2.2                  | <0.001             | 1.40 (0.81–2.44) |

*Crude incidence rate calculated as number of events per person-year.

**Model 1:** Adjusted for age and sex.

**Model 2:** Adjusted for age, sex, smoking, and alcohol consumption.

**Model 2 + excluding participants:** Adjusted for age, sex, smoking, and alcohol consumption, and excluding participants with a history of stroke or heart disease at baseline.
HbA1c Levels in Participants Without Known Diabetes

| HbA1c (%) | Crude Incidence Rate | Model 1 | N | Model 2 | N | N | P for Linear Trend | P for Quadratic Trend |
|----------|----------------------|---------|---|---------|---|---|-------------------|----------------------|
| <5.0%    | 1.2 (0.88–2.56)      | 1.50 (0.88–2.56) | 2008 | 1.00 | 1.50 (0.89–2.58) | 8177 | 0.018 | 0.005 |
| 5.0–5.4% | 0.8 (0.8–1.56)       | 1.10 (0.78–1.56) | 12,450 | 1.07 (0.75–1.52) | 12,450 | 2.81 (1.65–4.79) | 1.65–4.79 |
| 5.5–5.9% | 0.8 (0.66–1.78)      | 1.08 (0.66–1.78) | 3635  | 1.02 (0.62–1.69) | 3635  | 0.066 | 0.006 |
| 6.0–6.4% | 0.9 (1.43–4.25)      | 2.47 (1.43–4.25) | 1009  | 2.56 (1.33–5.07) | 1009  | 0.004 | 0.82 |
| 6.5%     | 2.5                  | 2.47 (1.43–4.25) | 1009  | 1.92 (0.96–3.85) | 1009  | 0.046 | 0.61 |

Model 1 was adjusted for age, sex, and public health center areas. Model 2 was further adjusted for body mass index, smoking status, alcohol intake, systolic blood pressure, non-high-density lipoprotein cholesterol, and high-density lipoprotein cholesterol. Data are presented as hazard ratios (95% confidence interval) unless otherwise indicated. HbA1c = hemoglobin A1c.

Coronary heart disease

| HbA1c (%) | Crude Incidence Rate | Model 1 | N | Model 2 | N | N | P for Linear Trend | P for Quadratic Trend |
|----------|----------------------|---------|---|---------|---|---|-------------------|----------------------|
| <5.0%    | 0.5                  | 0.5     | 21 | 1.23 (0.57–2.56) | 1.23 (0.57–2.56) | 3635 | 1.94 (1.11–3.41) | 1.11–3.41 |
| 5.0–5.4% | 0.6                  | 0.6     | 11 | 1.23 (0.57–2.56) | 1.23 (0.57–2.56) | 1009 | 2.33 (1.33–4.06) | 1.33–4.06 |
| 5.5–5.9% | 0.9                  | 0.9     | 21 | 1.23 (0.57–2.56) | 1.23 (0.57–2.56) | 1009 | 2.33 (1.33–4.06) | 1.33–4.06 |
| 6.0–6.4% | 1.4                  | 1.4     | 21 | 1.23 (0.57–2.56) | 1.23 (0.57–2.56) | 1009 | 2.33 (1.33–4.06) | 1.33–4.06 |

Model 1 was adjusted for age, sex, and public health center areas. Model 2 was further adjusted for body mass index, smoking status, alcohol intake, systolic blood pressure, non-high-density lipoprotein cholesterol, and high-density lipoprotein cholesterol. Data are presented as hazard ratios (95% confidence interval) unless otherwise indicated. HbA1c = hemoglobin A1c.

FIGURE 1. Hazard ratios for cardiovascular events according to continuous hemoglobin A1c (HbA1c) levels among participants without known diabetes. Restricted cubic spline models with the inclusion of transformed variables in the Cox model were used to estimate hazard ratios (solid curve) with point-wise 95% confidence intervals (grey shaded area) for (A) cardiovascular disease, (B) stroke, and (C) coronary heart disease. An HbA1c level of 5.3% (ie, the mean HbA1c level in people with HbA1c levels of 5.0–5.5%) was used to estimate all hazard ratios. We chose the number of knots that produced the smallest Akaike Information Criterion. Hazard ratios were adjusted for age, sex, public health center areas, body mass index, smoking status, alcohol intake, systolic blood pressure, non-high-density lipoprotein cholesterol, and high-density lipoprotein cholesterol.
### TABLE 3. Sex-Stratified Incidence of Cardiovascular Disease According to Hemoglobin A1c Levels and Known Diabetes

| HbA1c Levels in Participants Without Known Diabetes | Known Diabetes |
|---------------------------------------------------|----------------|
| <5.0% (<31 mmol/mol)                              | N = 855        |
| 5.0–5.4% (31–36 mmol/mol)                         | N = 2946       |
| 5.5–5.9% (37–41 mmol/mol)                         | N = 4312       |
| 6.0–6.4% (42–47 mmol/mol)                         | N = 1452       |
| ≥6.5% (≥48 mmol/mol)                              | N = 485        |
| **P for Linear Trend**                            | **P for Quadratic Trend** |
| Men                                               | N = 930        |
| Person-years                                      | 6948           |
| No. of events                                     | 58             |
| Crude incidence rate*                             | 8.3            |
| Model 1                                           | 1.89 (1.36–2.63) |
| Model 2                                           | 1.95 (1.40–2.71) |
| **Crude incidence rate per 1000 person-years**    | **2.2 (1.36–2.63)** |
| **Model 1 was adjusted for age and public health center areas.** |
| **Model 2 was further adjusted for body mass index, smoking status, sports and physical exercise, alcohol intake, systolic blood pressure, non-high-density lipoprotein cholesterol, and high-density lipoprotein cholesterol.** |
| **Data are presented as hazard ratios (95% confidence interval) unless otherwise indicated. HbA1c = hemoglobin A1c.** |

**Women**

| Person-years                                      | 10,095         |
| No. of events                                     | 22             |
| Crude incidence rate*                             | 2.2            |
| Model 1                                           | 0.93 (0.58–1.47) |
| Model 2                                           | 0.94 (0.59–1.50) |
| **Crude incidence rate per 1000 person-years**    | **0.93 (0.58–1.47)** |

**Model 1 was adjusted for age and public health center areas. Model 2 was further adjusted for body mass index, smoking status, sports and physical exercise, alcohol intake, systolic blood pressure, non-high-density lipoprotein cholesterol, and high-density lipoprotein cholesterol. Data are presented as hazard ratios (95% confidence interval) unless otherwise indicated. HbA1c = hemoglobin A1c.**
those with HbA1c levels of ≥6.5% (≥48 mmol/mol) or known diabetes.

**DISCUSSION**

In this large-scale, prospective, cohort study in a general Japanese population, both low and high levels of HbA1c were associated with an increased risk of CVD among participants without known diabetes. The nonlinear association between HbA1c levels and CVD risk persisted even after excluding participants with kidney dysfunction, liver dysfunction, anemia, and low body mass index. Furthermore, the patterns of the association between low HbA1c levels and each CVD outcome differed from that for diabetes or high HbA1c levels. Low HbA1c levels were associated with an increased risk of stroke, especially hemorrhagic stroke, while diabetes and high HbA1c levels were associated with increased risks of coronary heart disease and stroke, especially ischemic stroke. The observed CVD risk in individuals with diabetes in this study was also consistent with accumulating evidence of diabetes as a risk factor for CVD.4,26,27 These findings emphasize a possible increased CVD risk among people without known diabetes and with low HbA1c levels.

The observed CVD risk among people with low HbA1c levels and no known diabetes is particularly important, because this increased risk could not be related to hypoglycemia induced by diabetes treatment. Although a possible increased CVD risk or low HbA1c levels among people without known diabetes have been suggested,4,13,28 most studies did not find a statistically significant association.4,12 However, we found a significant association between low HbA1c levels and CVD, particularly stroke, possibly because of a sufficient number of stroke events in this study. Earlier studies were limited by small sample sizes and HbA1c measurements that were obtained from frozen whole blood samples that were stored for >10 years.4,12,13 A significant nonlinear association between HbA1c levels and CVD risk among people without known diabetes has been reported in a recent pooled analysis; however, the lack of assay standardization and the significant heterogeneity between assay characteristics for HbA1c measurements may have limited the interpretation of the results.28 As previously shown, low HbA1c levels are associated with increased all-cause mortality in people without diabetes.4,12,13 According to our findings, the elevated incidence of CVD may partially explain the increased mortality among people with low HbA1c levels. HbA1c level is increasingly being used to screen for diabetes and therefore, our findings may facilitate the interpretation of low HbA1c levels in the nondiabetic population.

We were also able to confirm that HbA1c levels of ≥6.5% (≥48 mmol/mol) in people without known diabetes were associated with an increased CVD risk, which was consistent with other studies that were conducted in Japan.4,7,29,30 and other countries.4,8 Although a significantly increased CVD risk was not observed for the groups with elevated HbA1c levels in the nondiabetic range, the spline analyses (Figure 1A) appeared to suggest a positive linear association between continuous HbA1c levels and CVD risk in those with HbA1c levels of ≥5.5% (≥37 mmol/mol). Earlier investigators have also documented an increased CVD risk with increasing HbA1c levels within the nondiabetic range.4,7,29,31 Furthermore, individuals with prediabetes (defined by glucose levels during oral glucose tolerance tests) may have a 20% increased risk of CVD, compared to those with normal glycemia according to recent meta-analyses.32,33 Therefore, hyperglycemia within the nondiabetic range may be associated with an increased CVD risk in a continuous manner.

The mechanisms responsible for the observed association between low HbA1c levels and increased CVD risk among people without known diabetes remain largely unknown. In addition, it is unknown whether low blood glucose levels not induced by diabetes treatment could have a direct effect on blood vessels. We observed similar results (data not shown) when we adjusted for casual blood glucose levels, which suggested that the association between low HbA1c levels and increased CVD risk may not be explained by blood glucose levels. Abnormal red-cell turnover, which can lead to low HbA1c levels,13 might explain the association. However, the association persisted after we excluded participants with factors that affect red-cell turnover, including kidney dysfunction, liver dysfunction, and anemia. Alternatively, chronic kidney disease or inadequate nutrition may explain the possible increased risk among people with low HbA1c levels. However, the total energy intake did not indicate inadequate nutrition in participants with HbA1c levels of <5.0% (<31 mmol/mol), and adjustment for total energy intake did not change the results. Further, excluding people with a low body mass index at baseline provided similar results. Therefore, confounding by these factors alone may not explain the observed association. Although the biological mechanisms underlying this association remain unresolved, our data support the notion that low HbA1c levels may be a marker for identifying people who are at increased risk of CVD.13 In addition, based on our findings, diabetic patients with low HbA1c levels (eg, <5.0%, <31 mmol/mol) may have an increased risk of CVD, possibly due to glycemic and nonglycemic factors.

This study has several strengths. First, we strictly standardized the HbA1c values using approved standard samples to reduce the possibility of measurement error, leading to less biased estimates. Second, the use of a population-based prospective cohort design with low loss to follow-up and a large sample size should minimize the possibility of selection bias. Third, the systematic surveys of CVD events likely reduced outcome misclassification in our study. Finally, we were able to examine the relation between HbA1c levels and stroke subtypes because of the large number of stroke events.

Despite these strengths, certain limitations of the present study merit consideration. First, HbA1c levels and diabetes status may have changed during the follow-up, but only single measurements of HbA1c were available for most participants (65%). If HbA1c levels during the follow-up had been available for all participants, the association between HbA1c and CVD risk would likely have been stronger. Second, the increased CVD risk observed in individuals with low HbA1c levels may not necessarily indicate a causal association. Third, information regarding socioeconomic status was not available, which might explain the increased CVD risk among people with low HbA1c levels. However, there is no clear evidence that people with low socioeconomic status have low HbA1c levels. Fourth, we could not consider the genetic background of our participants because genetic variants linked to CVD or risk factors (eg, hypertension) were not measured in our study. However, it is unlikely that the missing information on such variants would bias the association between HbA1c levels and CVD risk because such variants do not tend to affect HbA1c levels. Finally, our results may not be applicable to other populations, especially Western populations, because East Asians tend to have a higher incidence of stroke and lower incidence of coronary heart disease compared with those in Western populations.35 Therefore, the nonlinear relation between HbA1c and stroke might be especially relevant to Asians.
In conclusion, both low and high levels of HbA1c were associated with a higher risk of CVD in a general Japanese population without known diabetes. These data support the notion that very low and high HbA1c levels may be markers for identifying people with an increased health risk.

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OTHER INFORMATION: CONTRIBUTORS: AG analyzed data, drafted the manuscript, reviewed and edited the manuscript, and contributed to discussion. MN and ST conducted, designed, and supervised the study, and contributed to discussion. YM analyzed data and contributed to discussion. MG analyzed data, reviewed and edited the manuscript, and contributed to discussion. MK, AI, TM, MI, and TK conducted the research. YT, KY, IS, YK, and NS conducted the research and contributed to discussion. KK, SO, and AN contributed to discussion. HY reviewed and edited the manuscript, and contributed to discussion. All authors have read and approved the final manuscript.

REFERENCES

1. GBD 2013 Mortality and Causes of Death Collaborators. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet. 2014;385:117–171.

2. Santulli G. Epidemiology of cardiovascular disease in the 21st century: updated numbers and updated facts. J Cardiovasc Dis. 2013;1:1–2.

3. Santulli G. Coronary heart disease risk factors and mortality. JAMA. 2012;307:1137–1138.

4. Selvin E, Steffes MW, Zhu H, et al. Glycated hemoglobin, diabetes, and cardiovascular risk in nondiabetic adults. N Engl J Med. 2010;362:800–811.

5. Ridker PM, Buring JE, Rifai N, et al. Development and validation of improved algorithms for the assessment of global cardiovascular risk in women: the Reynolds Risk Score. JAMA. 2007;297:611–619.

6. Stratton IM, Adler AI, Neil HA, et al. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. BMJ. 2000;321:405–412.

7. Ikeda F, Doi Y, Ninomiya T, et al. Haemoglobin A1c even within non-diabetic level is a predictor of cardiovascular disease in a general Japanese population: the Hisayama Study. Cardi ovasc Diabetol. 2013;12:164.

8. Nichols GA, Joshua-Gotlib S, Parasaruman S. Glycemic control and risk of cardiovascular disease hospitalization and all-cause mortality. J Am Coll Cardiol. 2013;62:121–127.

9. Zoungas S, Chalmers J, Ninomiya T, et al. Association of HbA1c levels with vascular complications and death in patients with type 2 diabetes: evidence of glycaemic thresholds. Diabetologia. 2012;55:636–643.
10. Currie CJ, Peters JR, Tynan A, et al. Survival as a function of HbA(1c) in people with type 2 diabetes: a retrospective cohort study. Lancet. 2010;375:481–489.

11. Goto A, Arah OA, Goto M, et al. Severe hypoglycaemia and cardiovascular disease: systematic review and meta-analysis with bias analysis. BMJ. 2013;347:f4533.

12. Carson AP, Fox CS, McGuire DK, et al. Low hemoglobin A(1c) and risk of all-cause mortality among US adults without diabetes. Circ Cardiovasc Qual Outcomes. 2010;3:661–667.

13. Aggarwal V, Schneider AL, Selvin E. Low hemoglobin A 1c in nondiabetic adults: an elevated risk state? Diabetes Care. 2012;35:2055–2060.

14. Christman AL, Lazo M, Clark JM, et al. Low glycated hemoglobin and liver disease in the U.S. population. Diabetes Care. 2010;34:2548–2550.

15. Rutter MK. Low HbA 1c and mortality: causation and confounding. Diabetologia. 2012;55:2307–2311.

16. Tsugane S, Sawada N. The JPHC Study: design and some findings on the typical Japanese diet. Jpn J Clin Oncol. 2014;44:777–782.

17. Noda M, Kato M, Takahashi Y, et al. Fasting plasma glucose and 5-year incidence of diabetes in the JPHC Diabetes Study – suggestion for the threshold for impaired fasting glucose among Japanese. Endocr J. 2010;57:629–637.

18. Kashihara A, Kasuga M, Araki E, et al. International clinical harmonization of glycated hemoglobin in Japan: From Japan Diabetes Society to National Glycohemoglobin Standardization Program values. J Diabetes Invest. 2012;3:39–40.

19. Watanabe S, Tsugane S, Sobue T, et al. Study design and organization of the JPHC Study: Japan Public Health Center-based Prospective Study on Cancer and Cardiovascular Diseases. J Epidemiol. 2013;3:57:629–637.

20. Yamagishi K, Iso H, Kokubo Y, et al. Dietary intake of saturated fatty acids and incident stroke and coronary heart disease in the general Japanese population: the Japan Public Health Center-based prospective (JPHC) Study. Atherosclerosis. 2011;216:187–191.

21. Khaw KT, Wareham N, Bingham S, et al. Association of hemoglobin A1c with cardiovascular disease and mortality in adults: the European prospective investigation into cancer in Norfolk. Ann Intern Med. 2004;141:413–420.

22. Ford ES, Zhao G, Li C. Pre-diabetes and the risk for cardiovascular disease: a systematic review of the evidence. J Am Coll Cardiol. 2010;55:1310–1317.

23. Lee M, Saver JL, Hong KS, et al. Effect of pre-diabetes on future risk of stroke: meta-analysis. BMJ. 2012;344:e3564.