Elevated gamma-glutamyl transferase to high-density lipoprotein cholesterol ratio has a non-linear association with incident diabetes mellitus: A second analysis of a cohort study

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ABSTRACT
Objective: Evidence regarding the association between the GGT/HDL-c ratio and incident diabetes is still limited. On that account, our research aims to survey the link of the GGT/HDL-c ratio with the risk of diabetes.

Methods: In this retrospective cohort study, data of 15,171 participants who participated in the medical examination program were collected in Murakami Memorial Hospital in Japan from 2004 to 2015. The independent and dependent variables were the baseline GGT/HDL-c ratio and diabetes during the follow-up, respectively. The Cox proportional-hazards regression model was used to explore the association between the GGT/HDL-c ratio and diabetes risk. A Cox proportional hazards regression with the cubic spline smoothing was used to recognize non-linear relationships between the GGT/HDL-c ratio and incident diabetes.

Results: After adjusting covariates, the results showed that the GGT/HDL-c ratio was positively associated with incident diabetes (HR = 1.013, 95% CI: 1.002, 1.024). There was also a non-linear relationship between the GGT/HDL-c ratio and the risk of diabetes, and the inflection point of the GGT/HDL-c ratio was 6.477. The HR on the left and right sides of the inflection point was 2.568 (1.157, 5.699) and 1.012 (1.001, 1.023), respectively. The sensitivity analysis demonstrated the robustness of the results. Besides, the performance of the FPG + GGT/HDL-c ratio was better than FPG + GGT, FPG + HDL-c, and FPG in predicting diabetes.

Conclusion: This study demonstrates a positive and non-linear relationship between the GGT/HDL-c ratio and incident diabetes in the Japanese population. The GGT/HDL-c ratio is strongly related to diabetes risk when it is <6.477.

BACKGROUND
Diabetes mellitus (DM) is one of the fastest-growing diseases in the 21st century. According to the International Diabetes Federation, 463 million people were diagnosed with diabetes worldwide in 2019, and it is expected to reach 700 million by 2045. In 2014, the annual medical cost of diabetes treatment in Japan was about 73 billion dollars, ranking seventh in the world.

Diabetes mellitus with diabetic complications is becoming a crucial public health issue. Therefore, only by fully understanding the risk factors of diabetes can we effectively and in a timely manner prevent and screen for diabetes.

Type 2 diabetes mellitus is characterized by adult-onset relative insulin insufficiency and insulin resistance (IR). Some recent studies have found that high-density lipoprotein cholesterol (HDL-c) and γ-glutamyl transferase (GGT) were related to non-alcoholic fatty liver disease (NAFLD) and type 2 diabetes mellitus risk. Two recent cross-sectional studies...
have demonstrated that increased levels of the GGT/HDL-c ratio were strongly associated with NAFLD and metabolic-associated fatty liver disease (MAFLD).

However, when reviewing the literature, we noticed no evidence of the connection between GGT/HDL-c and the risk of type 2 diabetes. Studies have reported that NAFLD is closely related to an increased risk of type 2 diabetes mellitus. Meanwhile, GGT, HDL, and NAFLD are all associated with insulin resistance. Studies have also confirmed that type 2 diabetes mellitus and NAFLD share several pathophysiological mechanisms, such as inflammation, insulin resistance, and oxidative stress.

Therefore, we propose the hypothesis that elevated levels of the GGT/HDL-c ratio are related to an increased risk of diabetes through insulin resistance and other possible mechanisms. We conducted a cohort study designed to test this hypothesis and explore the specific association of the GGT/HDL-c ratio with the risk of diabetes in a large Japanese cohort.

**METHODS**

**Study design**

This study was a retrospective cohort study using the data obtained from the NAGALA (NAFLd in the Gifu Area, Longitudinal Analysis) database established by Murakami Memorial Hospital in Japan. We set the baseline GGT/HDL-c ratio as the target-independent variable and diabetes during the follow-up as the dependent variable.

**Data source**

The raw data were downloaded freely from the DATADRYAD database (www.datadryad.org) provided by Okamura et al.

Data were from: Ectopic fat obesity presents the greatest risk for incident type 2 diabetes: A population-based longitudinal study, Dryad, Dataset, https://doi.org/10.5061/dryad.8q0p192.

**Study population**

The participants were collected consecutively from Murakami Memorial Hospital in Japan to minimize selection bias. Their identity information was encoded into an untraceable code to ensure the participants’ privacy. This study was performed according to the Declaration of Helsinki, and the clinical research ethics committee approved all procedures involving humans at Murakami Memorial Hospital. All participants involved in this study have signed informed consent after the study being explained to them.

The study initially included 20,944 participants; afterward, 5,773 participants were excluded, and 15,171 persons remained for data analysis (Figure 1). Inclusion criteria were: participants who participated in physical examinations between 2004 and 2015, and had completed at least two physical examinations. Exclusion criteria were: (1) participants diagnosed with type 2 diabetes (n = 323) or with a fasting plasma glucose (FPG) over 6.1 mmol/L at baseline (n = 808); (2) participants with known liver disease, such as hepatitis B or C virus (n = 416); (3) anyone who took medication at baseline (n = 2,321); (4) participants with heavy drinking habits (more than 40 g per day for women and more than 60 g per day for men) (n = 739); (5) participants with a missed value of the covariates, including abdominal ultrasonography, exercise, alcohol intake, or laboratory variables (n = 863); (6) participants with incomplete HDL-c (n = 12); (7) those with GGT/HDL-c ratio outliers (out of the range of means plus or minus three standard deviations).

**Variables**

**GGT/HDL-c ratio**

After a night of fasting, venous blood was collected for GGT and HDL-c testing, and they were measured using an automated analyzer. We recorded the GGT/HDL ratio as a continuous variable and obtained its information at baseline. GGT/HDL ratio = GGT divided by HDL-c.

**Incident diabetes**

Diabetes was defined as a fasting plasma glucose of ≥7 mmol/L, hemoglobin A1c (HbA1c) ≥6.5%, or self-reported diabetes during follow-up. The patients were reviewed on the day of the diagnosis of diabetes or on the day of the last visit, whichever came first.

**Covariates**

The following variables were used as covariates based on the above principles: (1) continuous variables: waist circumference (WC), total cholesterol (TC), age, triglyceride (TG), systolic blood pressure (SBP), HbA1c, alanine aminotransferase (ALT), fasting plasma glucose (FPG), body mass index (BMI), aspartate aminotransferase (AST), ethanol consumption; (2) categorical variables: exercise, smoking status, sex, fatty liver.

This study’s clinical baseline information was collected through a standardized self-administered questionnaire, including medical history, smoking and alcohol habits, and physical exercise activity. The participants were divided into four groups based on their alcohol consumption: none to minimal, <40 g/week; light, 40–140 g/week; moderate, 140–280 g/week; or heavy drinking, >280 g/week. Regular exercisers were defined as those who reported any exercise more than once a week. BMI (kg/m^2) = weight (kg) divided by height^2 (m^2). After a night of fasting, venous blood was collected for hematological indicators testing, including TC, TG, ALT, AST, HbA1c, and FPG, and they were measured using an automated analyzer. Fatty liver was diagnosed through the abdominal ultrasonography findings conducted by trained technicians.

**Statistical analysis**

Quartiles of the GGT/HDL-c ratio stratified the participants. Continuous variables were expressed as mean (standard deviation) (normal distribution) or median (range) (non-normal distribution), and categorical variables as number (%). We used the one-way ANOVA test (normal distribution), the χ^2 (categorical variables), or the Kruskal-Wallis H test (non-normal
distribution) to test for differences among the different GGT/HDL-c ratio groups. The Kaplan–Meier method was used to compute the survival estimates and time-to-event variables. We used the log-rank test to compare the probability of diabetes-free survival among the GGT/HDL-c ratio groups.

To investigate the association between the GGT/HDL-c ratio and diabetes risk, three different models were performed using univariate and multivariate Cox proportional hazards regression models, including the non-adjusted model (no covariates were adjusted), minimally adjusted model (only sociodemographic variables were adjusted, including BMI, SBP, age, WC, ethanol consumption, sex, smoking status and habit of exercise) and fully adjusted model (including WC, SBP, age, ALT, BMI, sex, AST, TG, FPG, TC, HbA1c, ethanol consumption, exercise,

Figure 1 | Flowchart of study participants. The figure shows the inclusion of participants. The eligibility of 15,464 participants was assessed in the original study. We further excluded individuals with missing values of HDL-c (n = 12) and outliers of the GGT/HDL-c ratio (n = 282). The final analysis included 15,171 subjects in the present study.
smoking status, and fatty liver). Hazard ratios (HR) with 95% confidence intervals (CI) were recorded. We adjusted them when the covariates were added to the model, and the HR changed by 10% or greater. According to the results of the collinearity screening, DBP was collinear with other variables, so we did not finally include DBP in the multivariate Cox regression equation. We performed a series of sensitivity analyses to test the robustness of our results. We converted the GGT/HDL-c ratio from a continuous variable to a categorical variable based on quartiles and calculated the \( P \) for trend, in order to verify whether the GGT/HDL-c ratio as a continuous variable was consistent with the categorical variable, and to test whether there was the possibility of a non-linear relationship. Besides, we also used a generalized additive model (GAM) to insert the continuity covariate into the equation (model III) as a curve to ensure the robustness of the results. Additionally, we explored the potential for unmeasured confounding between the GGT/HDL-c ratio and diabetes risk by calculating \( E \)-values.

We used a stratified Cox proportional hazard model to explore the results’ robustness in various subgroups (sex, SBP, ethanol consumption, FPG, fatty liver, DBP, exercise, age, smoking status). Firstly, we converted the continuous variable SBP (\( <140, \geq 140 \) mmHg), DBP (\( <90, \geq 90 \) mmHg), age (\( <30, 30–40, 40–50, 50–60, \geq 60 \))\(^{34} \), FPG (\( <5.6, \geq 5.6 \) mmol/L)\(^{35} \), ethanol consumption (\( <40, 40–140, 140–280, >280 \) g/week) to a categorical variable based on the clinical cut point. Secondly, in addition to the stratification factor itself, we adjusted each stratification for all factors. Lastly, tests for interaction were addressed with the likelihood ratio test of models with and without interaction terms.\(^{36} \)

Non-linearity between the GGT/HDL-c ratio and diabetes risk was addressed using a Cox proportional hazards regression model with cubic spline functions and the smooth curve fitting. It is better to use the explanatory variable, which showed normal distribution for using the cubic spline models. Since the GGT/HDL-c ratio showed a skewed distribution, we first log-transformed it. If non-linearity was detected, we first calculated the inflection point of the GGT/HDL-c ratio using a recursive algorithm. We then performed a two-piece Cox proportional hazards regression model on either side of the inflection point. The log-likelihood ratio test was used to seek the most appropriate model describing the association between the GGT/HDL-c ratio and diabetes risk. The risk of diabetes was obviously increased in patients with impaired fasting glucose (IFG)\(^{37} \) and fatty liver.\(^{38} \) Therefore, when exploring the non-linear relationship between the GGT/HDL-c ratio and diabetes risk, we excluded participants with a FPG of \( \geq 5.6 \) mmol/L or fatty liver for the sensitivity analysis.

Finally, we constructed a receiver operating characteristic (ROC) curve to estimate the ability of the FPG + GGT/HDL-c ratio, FPG + HDL-c, FPG + GGT, and FPG to predict the occurrence of diabetes. Harrell’s C statistic concordance index (C-index)\(^{39} \) was applied to evaluate the discrimination of different variables. The C-index value ranges from 0.5 to 1.0, with 0.5 indicating random chance and 1 indicating perfect concordance. Generally, a C-index >0.7 is considered to show relatively good discrimination.\(^{40} \) We also calculated net reclassification improvement (NRI) and integrated discrimination improvement (IDI) to compare the predictive values among different models.\(^{41} \) All results were written following the STROBE statement.\(^{42} \)

Modeling was performed using the statistical packages R (http://www.R-project.org; The R Foundation) and EmpowerStats (http://www.empowerstats.com; X&Y Solutions, Inc, Boston, MA, USA). \( P \) values <0.05 (two-sided) were considered statistically significant.

**RESULTS**

**Participants’ baseline characteristics**

Baseline characteristics are depicted in Table 1. Of the 15,171 included participants, the mean age was 43.69 ± 8.91 years, and 53.75% were male. The mean baseline GGT/HDL-c ratio was 14.09 ± 10.30. We assigned the adults into subgroups based on the quartile of GGT/HDL-c ratio (\(<7.148, \geq 7.148 \) to \(<10.610, \geq 10.610 \) to \(<17.412, \geq 17.412 \)). In the comparison based on the Q1 (\(<7.148 \) group, the higher value or proportion of age, AST, BMI, ALT, WC, GGT, HbA1c, SBP, TC, ethanol consumption, TG, male, DBP, fatty liver, ex-smoker, and current smoker were detected in the Q4 (\( \geq 17.412 \)) group. In comparison, the lower value and proportion of HDL-c, regular exercisers, females, and non-smokers were observed.

Figure S1 shows the distribution of the GGT/HDL-c ratio. It presents a skewed distribution ranging from 1.572 to 62.708, with a median level of 10.611. Participants were divided into two groups based on whether they had experienced diabetes. The GGT/HDL-c ratio distribution in the two groups is shown in Figure S2. The results showed that the GGT/HDL-c ratio distribution level in the diabetes group was higher. Across the 10 age stratifications, male subjects had higher rates of diabetes than female subjects, regardless of age group (Figure S3). It also found that the incidence of diabetes increased with age in both male and female subjects.

**The results of univariate analyses**

The univariate analysis was conducted on the available data, showing that the factor in terms of the habit of exercise was not associated with diabetes risk, but age, ethanol consumption, BMI, WC, AST, ALT, GGT, GGT/HDL-c ratio, TC, TG, HbA1c, FPG, SBP, DBP, male, fatty liver, ex-smokers, and current smokers were positively connected to incident diabetes, and HDL-c was negatively linked with incident diabetes (Table S1).

There was a significant difference in the probability of diabetes-free survival between the GGT/HDL-c ratio groups (log-rank test, \( P < 0.0001 \)). As the GGT/HDL-c ratio increased, the probability of diabetes-free survival gradually decreased, indicating that the group with the highest GGT/HDL-c ratio had the highest future risk of diabetes (Figure 2).
Table 1 | The baseline characteristics of participants

| GGT/HDL-c ratio quartile | Q1 (<7.148) | Q2 (7.148–10.610) | Q3 (10.610–17.412) | Q4 (≥17.412) | P-value |
|--------------------------|-------------|-------------------|-------------------|--------------|---------|
| Participants             | 3,793       | 3,792             | 3,793             | 3,793        |         |
| Age, years               | 42.31 ± 8.37| 43.09 ± 9.04      | 44.30 ± 9.27      | 45.07 ± 8.69 | <0.001  |
| Ethanol consumption, g/week | 1.00 (0.00–12.00) | 1.00 (0.00–36.00) | 7.43 (0.00–84.00) | 36.00 (1.00–126.00) | <0.001   |
| BMI, kg/m²               | 20.36 ± 2.31| 21.24 ± 2.64      | 22.48 ± 2.94      | 24.19 ± 3.05 | <0.001  |
| WC, cm                   | 69.81 ± 6.83| 73.83 ± 7.32      | 78.17 ± 7.90      | 83.40 ± 7.93 | <0.001  |
| AST, IU/L                | 15.57 ± 4.63| 16.80 ± 5.12      | 18.23 ± 6.52      | 21.93 ± 8.39 | <0.001  |
| ALT, IU/L                | 13.00 (10.00–15.00) | 15.00 (12.00–18.00) | 18.00 (14.00–22.00) | 25.00 (20.00–34.00) | <0.001   |
| GGT, IU/L                | 10.04 ± 2.28| 13.30 ± 2.76      | 18.01 ± 4.79      | 33.43 ± 14.46 | <0.001  |
| HDL-c, mmol/L            | 1.82 ± 0.37 | 1.53 ± 0.30       | 1.34 ± 0.32       | 1.18 ± 0.30  | <0.001  |
| TC, mmol/L               | 5.08 ± 0.82 | 4.98 ± 0.83       | 5.10 ± 0.88       | 5.31 ± 0.87  | <0.001  |
| TG, mmol/L               | 0.51 (0.37–0.69) | 0.61 (0.44–0.85) | 0.82 (0.60–1.19) | 1.19 (0.84–1.72) | <0.001 |
| FPG, mmol/L              | 5.14 ± 0.31 | 5.16 ± 0.31       | 5.18 ± 0.32       | 5.21 ± 0.34  | <0.001  |
| SBP mmHg                 | 107.47 ± 13.16 | 111.76 ± 13.89 | 116.42 ± 14.28 | 121.42 ± 14.30 | <0.001  |
| DBP mmHg                 | 66.41 ± 9.17 | 69.44 ± 9.48      | 72.98 ± 10.02     | 76.85 ± 10.00 | <0.001  |
| Male, %                  | 419 (11.05%) | 507 (13.97%)      | 7295 (73.69%)     | 3433 (90.51%) | <0.001  |
| Fatty liver, n (%)       | 82 (2.16%)  | 245 (6.46%)       | 692 (18.24%)      | 1576 (41.55%) | <0.001  |
| Regular exercisers, n (%)| 645 (17.01%) | 706 (18.62%)     | 730 (19.25%)      | 593 (15.63%)  | <0.001  |
| Smoking status, n (%)    |            |                   |                   |              |         |
| Non-smoker               | 3,151 (83.07%) | 2,624 (69.20%) | 1,808 (47.67%)   | 1,345 (35.46%) | <0.001  |
| Ex-smoker                | 368 (9.70%)  | 579 (15.27%)      | 891 (23.49%)      | 1,020 (26.89%) | <0.001  |
| Current smoker           | 274 (7.22%)  | 589 (15.53%)      | 1,094 (28.84%)    | 1,428 (37.65%) |         |

Values are n (%) or mean ± SD or medians (quartiles). ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; DBP, diastolic blood pressure; FPG, fasting plasma glucose; GGT, gamma-glutamyl transferase; GGT/HDL-c ratio, γ-glutamyl transpeptidase to high-density lipoprotein cholesterol ratio; HbA1c, hemoglobin A1c; HDL-c, high-density lipoprotein cholesterol; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride; WC, waist circumference.

The results of multivariate analyses

Table 2 revealed that during a median follow-up time of 64.9 months, 350 participants developed diabetes. The total incidence rate of all persons was 3.77 per 1,000 person-years. In particular, the incidence rate of the four GGT/HDL-c ratio groups were 0.85, 1.66, 3.26, and 9.14 per 1,000 person-years, respectively. Participants with a high GGT/HDL-c ratio had higher incidence rates of diabetes compared with the group with the lowest GGT/HDL-c ratio (P < 0.0001 for trend) (Figure S4).

The non-linear relationship

Through the Cox proportional hazards regression model with the penalized spline method, we observed that the association trend was consistent with the result when GGT/HDL-c ratio was a continuous variable. In addition, we used a GAM to insert the continuity covariate into the equation as a curve. The result of Model III in Table 2 showed this generally remained consistent with the fully adjusted model (HR = 1.013, 95% CI: 1.002–1.025, P = 0.0203). Besides, we developed an E-value to determine sensitivity to unmeasured confounding. The E-value was 1.13. The E-value was greater than the relative risk of unmeasured confounders on the risk of diabetes, suggesting that unknown or unmeasured confounders had little impact on the relationship between the GGT/HDL-c ratio and diabetes risk.

The results of subgroup analyses

There was no significant interaction in all the prespecified or exploratory subgroups (Table S2) in sex, SBP, ethanol consumption, FPG, age, DBP, fatty liver, exercise, and smoking status (all P for interaction above 0.05). These results indicated that the relationship between the GGT/HDL-c ratio and incident diabetes was robust, both in general and specific populations.
between the log-transformed GGT/HDL-c ratio and diabetes risk was non-linear (Figure S5). The data were fit using a standard Cox proportional hazards model and the best fit model determined using a log-likelihood ratio test. In our study, the $P$ for the log-likelihood ratio test was $<0.05$. Through a recursive algorithm, we first obtained the inflection point of the GGT/HDL-c ratio of 6.477. We then calculated the HR and 95% CI around the inflection point through a two-piece Cox proportional hazards regression model. On the left and right sides of the inflection point, the HR and 95% CI were 2.568 (1.157, 5.699) and 1.012 (1.001, 1.023), respectively (Table S3).
We also excluded participants with fatty liver or FPG ≥5.6 mmol/L for sensitivity analysis when exploring the non-linear relationship between the log-transformed GGT/HDL-c ratio and incident diabetes. The results showed that the non-linear relationship between log-transformed GGT/HDL-c ratio and diabetes still existed in participants without FPG ≥5.6 mmol/L or fatty liver. Specifically, the inflection point of the GGT/HDL-c ratio was 6.568 in the participants without a FPG of ≥5.6 mmol/L. On the left and right sides of the inflection point, the HR and 95% CI were 2.305 (1.103, 4.820) and 1.011 (0.994, 1.030), respectively. Besides, when we excluded subjects with fatty liver, the inflection point of the curve became 6.445. On the left and right sides of the inflection point, the HR and 95% CI were 3.766 (1.081, 13.117) and 1.002 (0.984, 1.022), respectively (Table S4 and Figure S6a,b).

Comparison of the predictive value of FPG + GGT, FPG + HDL-c, FPG + GGT/HDL-c ratio, and FPG for DM

In addition, we drew a ROC curve and calculated the C-index to measure the ability of the FPG + GGT/HDL-c ratio, FPG + HDL-c, FPG + GGT, and FPG to predict the risk of diabetes (Figures S7 and S8). The C-index of each variable were as follows: GGT: 0.702 < HDL-c: 0.717 < GGT/HDL-c ratio: 0.745 < FPG: 0.807 (Figure S8). The results also suggested that the C-index of each variable were as follows: FPG: 0.807 < FPG + GGT: 0.814 < FPG + HDL-c: 0.830 < FPG + GGT/HDL-c ratio: 0.831 (Figure S7). We also calculated the net reclassification improvement (NRI) and integrated discrimination improvement (IDI) to compare the predictive values among the different models. Compared with FPG, FPG + HDL-c, and FPG + GGT, the c-index of the FPG + GGT/HDL-c ratio was the highest, and its IDI and NRI were all increased. Combining the c-index, IDI, and NRI, we concluded that the predictive value of the FPG + GGT/HDL-c ratio for type 2 diabetes was superior to that of FPG, FPG + HDL-c, and FPG + GGT (Table 3).

DISCUSSION

Our findings suggest that an increased GGT/HDL-c ratio was associated with a significantly increased risk of diabetes. Besides, a threshold effect curve was found as well, and a different relationship of the GGT/HDL-c ratio on the diabetes risk was detected on both sides of the inflection point. In addition, the predictive ability of the FPG + GGT/HDL-c ratio to incident diabetes was better than FPG + HDL-c and FPG + GGT.

Several recent studies have found a positive association between GGT and the risk of diabetes43,44,47. A retrospective study found that after adjusting for confounders, the HR of diabetes in individuals with a high GGT was 3.05 (95% CI, 2.73–3.41) in women and 2.60 (95% CI, 2.47–2.73) in men compared with those who had a normal level of GGT. Another study suggested that a higher HDL-c was strongly and independently associated with a lower DM risk (OR = 0.55, 95% CI: 0.47–0.64) after adjusting BMI, family history of diabetes, hypertension, age, alcohol, sex, and smoking status43. Although there is no report on the relationship between the GGT/HDL-c ratio and diabetes risk, the GGT/HDL-c ratio increase reflects an increase of GGT or a decrease of HDL-c. Therefore, our findings were consistent with the results mentioned above. It should be pointed out that, compared with the study of Abbasi et al.43, when we analyzed the association between GGT/HDL-c ratio and diabetes, we additionally adjusted for confounding variables such as WC, ALT, FPG, TG, fatty liver, etc. However, recent studies have identified these variables as factors associated with diabetes40,44–47.

Furthermore, the present study observed a non-linear relationship between the log-transformed GGT/HDL-c ratio and the risk of diabetes for the first time. When the GGT/HDL-c ratio was below 6.477, a 1 unit increase in the GGT/HDL-c ratio level was associated with a 1.6 times greater adjusted HR of the risk of diabetes. However, when the GGT/HDL-c ratio >6.477, a 1 unit increase in the GGT/HDL-c ratio level was only associated with a 1.2% greater adjusted HR. The reason is that other variables in the participants’ baseline may also have influenced the diabetes risk. It could be found that compared with a GGT/HDL-c ratio of <6.477 group, people with a GGT/HDL-c ratio of >6.477 have generally higher levels or proportion of WC, age, ALT, BMI, AST, FPG, SBP, TG, DBP, HbA1c, fatty liver, ex-smokers and current smokers (Table S5). However, the above indicators were closely related to DM38,44–50.

Table 3 | Model performance compared with the FPG

| Variables        | FPG          | FPG + GGT     | FPG + HDL-c    | FPG + GGT/HDL-c ratio |
|------------------|--------------|---------------|----------------|-----------------------|
| C-index          | 0.807 (0.784 to 0.831) | 0.814 (0.791 to 0.837) | 0.830 (0.808 to 0.852) | 0.831 (0.809 to 0.852) |
| P-value          | –             | 0.0003        | <0.0001        | <0.0001               |
| NRI              | –             | 0.0103 (-0.0164 to 0.0371) | 0.0543 (0.0203 to 0.0833) | 0.0601 (0.0263 to 0.0940) |
| P-value          | –             | 0.4484        | 0.0018         | 0.0005                |
| IDI              | –             | 0.0103 (-0.0164 to 0.0371) | 0.0543 (0.0202 to 0.0833) | 0.0601 (0.0262 to 0.0941) |
| P-value          | –             | 0.4491        | 0.0018         | 0.0005                |

C-index, Harrell’s C statistic concordance index; FPG, fasting plasma glucose; GGT, gamma-glutamyl transpeptidase; GGT/HDL-c ratio, gamma-glutamyl transpeptidase to high-density lipoprotein cholesterol ratio; HDL-c, high-density lipoprotein cholesterol; IDI, integrated discrimination improvement; NRI, net reclassification improvement.
When the GGT/HDL-c ratio was >6.477, due to the presence of these diabetes risk factors, the GGT/HDL-c ratio had a relatively weak effect on diabetes risk. On the contrary, when the GGT/HDL-c ratio was <6.477, the level of the risk factors for diabetes, such as BMI, SBP, ALT, TG, FPG, and WC was lower, and the impact on diabetes was weakened, at this time the effect of the GGT/HDL-c ratio was relatively enhanced. Simultaneously, after excluding participants with a FPG of ≥5.6 mmol/L or fatty liver, we found that the non-linear relationship between GGT/HDL-c ratio and diabetes still existed. The inflection point is similar, which further confirms the stability of the curve relationship between the GGT/HDL-c ratio and diabetes risk.

Although the exact mechanism of the association between GGT/HDL-c ratio and diabetes risk remains unclear, insulin resistance may be involved. Studies have confirmed that GGT, HDL, and NAFLD are all associated with insulin resistance. Also, recent studies have found that increased levels of the GGT/HDL-c ratio were strongly related to the risk of NAFLD and MAFLD. Therefore, we propose that the GGT/HDL-c ratio may affect the development of diabetes by mediating insulin resistance. In the future, we can further verify the specific mechanism of action between the GGT/HDL-c ratio and diabetes by detecting the level of insulin resistance.

Our study has some strengths, and are listed as follows. (1) One strength of our study is the large sample size that allows such analysis. (2) This is the first study to explore the relationship between the GGT/HDL-c ratio and diabetes risk. (3) Research on the exploration of non-linear relationships is a significant advance. (4) In this study, we verified the robustness of the results by performing a series of sensitivity analyses (target independent variable transformation, subgroup analysis, using a GAM to insert the continuity covariate into the equation as a curve, calculating E-values to explore the potential for unmeasured confounding, and reanalyzing the non-linear relationship between the GGT/HDL-c ratio and diabetes after excluding participants with a FPG of ≥5.6 mmol/L or fatty liver). (5) We drew a ROC curve and calculated the c-index, IDI, and NRI, to compare the ability of FPG, FPG + HDL-c, FPG + GGT, and FPG + GGT/HDL-c ratio to predict the risk of diabetes.

Our research has the following shortcomings, which need to be noted. First, since all participants are of Japanese descent, further research is required to explore the association between the GGT/HDL-c ratio and diabetes in populations with different genetic backgrounds. Second, since this study is a secondary analysis, it is impossible to adjust variables not included in the original dataset, such as insulin resistance and renal function. However, we computed the E-value to quantify the potential impact of unmeasured confounders on our results and found that unmeasured confounders were unlikely to affect the GGT/HDL-c ratio relationship with diabetes risk. Third, the incidence rate of diabetes may be underestimated due to the lack of an oral glucose tolerance test. However, it is not feasible to perform oral glucose tolerance testing on all participants for various practical reasons. Besides, since the incidence of type 1 diabetes in Japan is very low (~2 cases/year/100,000 people), we did not differentiate diabetes as type 1 or type 2. In addition, this is a secondary study, and the original study did not provide detailed measurement information on GGT, HDL-C, and other samples.

CONCLUSION
This study demonstrates a positive and non-linear relationship between the GGT/HDL-c ratio and the risk of diabetes in the Japanese population. There is a threshold effect between the GGT/HDL-c ratio level and diabetes risk. When the GGT/HDL-c ratio is lower than 6.477, there is a significant positive association with the risk of diabetes. The result is expected to provide a reference for the clinical control of the GGT/HDL-c ratio. When the GGT/HDL-c ratio level is below the inflection point, lowering the GGT/HDL-c ratio level can significantly reduce the risk of developing diabetes in the future. Fasting plasma glucose combined with GGT/HDL-c ratio has a better predictive ability for the occurrence of diabetes.

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DISCLOSURE
The authors declare that they have no competing interests. Approval of the research protocol: The study was conducted following the Declaration of Helsinki and was approved by the ethics committee of Murakami Memorial Hospital. Informed consent: Obtained from all participants. Registry and the registration no. of the study/trial: N/A. Animal studies: N/A.

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Figure S1** | Distribution of GGT/HDL-c ratio.

**Figure S2** | Data visualization of all participants’ GGT/HDL-c ratio from diabetes and non-diabetes groups.

**Figure S3** | Diabetes incidence by age at 10 intervals.

**Figure S4** | Incidence of diabetes according to the quartiles of the GGT/HDL-c ratio.

**Figure S5** | The non-linear relationship between the log-transformed GGT/HDL-c ratio and the risk of diabetes.

**Figure S6** | The non-linear relationship between the log-transformed GGT/HDL-c ratio and diabetes risk in participants without fasting plasma glucose ≥5.6 mmol/L or fatty liver.

**Figure S7** | The results of ROC curve analysis for comparing the ability of FPG, FPG + GGT, FPG + HDL-c, and FPG + GGT/HDL-c ratio to predict the risk of diabetes.
Figure S8 | The results of ROC curve analysis for comparing the ability of HDL-c, GGT, FPG, and the GGT/HDL-c ratio to predict the risk of diabetes.

Table S1 | The results of the univariate analysis

Table S2 | The effect size of GGT/HDL-c ratio on diabetes in prespecified and exploratory subgroups

Table S3 | The result of the two-piecewise linear regression model in the complete cohort population

Table S4 | The result of the two-piecewise Cox regression model in different sensitivity analyses

Table S5 | The baseline characteristics of participants on both sides of the inflection point