Serum Ethylamine Levels as an Indicator of L-Theanine Consumption and the Risk of Type 2 Diabetes in a General Japanese Population: The Hisayama Study

Toshiharu Ninomiya,[1] Noriyuki Kanzaki,[2] Yoichiro Hirakawa,[3] Masahito Yoshinari,[1] Mayu Higashioka,[1] Takanori Honda,[1] Mao Shibata,[4] Satoko Sakata,[4] Daigo Yoshida,[1] Takanori Teramoto,[2] Susumu Takemoto,[2] Shozo Nishimoto,[2] Jun Hata,[1] and Takanari Kitazono[3]

Diabetes Care 2019;42:1234–1240 | https://doi.org/10.2337/dc18-2655

EPIDEMIOLOGY/HEALTH SERVICES RESEARCH

OBJECTIVE

This study investigated the association between serum ethylamine levels as an indicator of L-theanine consumption and the development of type 2 diabetes in a Japanese community.

RESEARCH DESIGN AND METHODS

A total of 2,253 community-dwelling Japanese individuals aged 40–79 years without diabetes were monitored for 7 years. Serum ethylamine levels were divided into quartiles: ≤0.86, 0.87–2.10, 2.11–5.28, and ≥5.29 ng/mL. Kinetic analysis of serum ethylamine concentrations was performed after ingestion of L-theanine–rich green tea products containing 8 mg of L-theanine by 12 healthy volunteers.

RESULTS

During follow-up, 282 subjects developed type 2 diabetes. The age- and sex-adjusted cumulative incidence of type 2 diabetes decreased significantly with elevating levels of serum ethylamine (*P* for trend = 0.04). This association remained unchanged after adjusting for potential confounding factors. The multivariable-adjusted hazard ratio (HR) for type 2 diabetes was significantly lower in the fourth quartile of serum ethylamine than in the first quartile (HR 0.69, 95% CI 0.49–0.98). This trend of decrease in diabetic risk across serum ethylamine levels was more prominent in middle-aged subjects and in subjects with prediabetes, obesity, or insulin resistance. Kinetic analysis estimated that the minimum concentration at the steady state was >5.90 ng/mL in the case of twice-daily ingestion with an interval of 12 h.

CONCLUSIONS

Higher serum ethylamine was significantly associated with lower risk of the development of type 2 diabetes in a general Japanese population. The measurement of serum ethylamine concentration would be a useful biomarker for the objective estimation of L-theanine consumption.

[1]Department of Epidemiology and Public Health, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan
[2]Development & Design Department, Japan Business Division, Suntory Beverage & Food Limited, Kanagawa, Japan
[3]Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan
[4]Center for Cohort Studies, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

Corresponding author: Toshiharu Ninomiya, nino@eph.med.kyushu-u.ac.jp

Received 29 December 2018 and accepted 15 April 2019

This article contains Supplementary Data online at http://care.diabetesjournals.org/lookup/suppl/doi:10.2337/dc18-2655/-/DC1.

T.N. and N.K. contributed equally to this work.

The number of patients with type 2 diabetes (T2DM) is increasing worldwide (1). T2DM deteriorates quality of life through macrovascular and microvascular complications and causes >2 million deaths globally each year (2,3). Moreover, it has been reported that diabetes is associated with greater risk of cognitive impairment and dementia (4). Therefore, the prevention of diabetes should be a public health priority (5). Dietary factors have been well acknowledged to play an important role for the prevention of T2DM. Several epidemiological studies have reported that higher tea consumption is associated with a lower incidence of T2DM (6,7). However, the tea consumptions in these studies were evaluated using a self-reported food questionnaire rather than by objective measures such as the serum concentrations of tea ingredients.

L-Theanine is a non–protein-forming amino acid that is principally synthesized in the roots and accumulated in the leaves of teas, especially green teas (8,9). L-Theanine is also an important ingredient in the taste and flavor of green teas (8). In addition, L-theanine has been considered to have several physiologically favorable effects on the prevention of lifestyle-related diseases (e.g., cardiovascular disorders, obesity, dyslipidemia, and hypertension) in addition to promoting stress relief, tumor suppression, and antioxidation effects, although these conclusions were mainly based on the findings from animal experiments (9). In contrast, few population-based epidemiological studies have addressed the influence of L-theanine on lifestyle-related diseases in humans, although favorable effects of green tea consumption, as estimated by a self-reported food questionnaire, have been reported. In the human body, L-theanine is rapidly degraded within 24 h after ingestion and is hydrolyzed to ethylamine and glutamic acid, the serum concentrations of which can be detected after 24 h (8). Therefore, it can be assumed that the serum ethylamine concentration is an indicator of L-theanine consumption.

The aim of the current study was to investigate the association between serum ethylamine levels and the development of T2DM in a general Japanese population. We additionally sought to clarify the kinetics of the serum ethylamine concentration after the ingestion of L-theanine–rich green tea.

RESEARCH DESIGN AND METHODS

Prospective Cohort Study for the Development of T2DM

Study Population

The Hisayama Study is a population-based prospective study that has been ongoing since 1961 in the town of Hisayama, a suburb of Fukuoka, in Japan (10,11). Health examinations for residents have been repeated every year since 1961, and participants have been encouraged to undergo a 75-g oral glucose tolerance test (OGTT) since 1988 (10). In 2007, 2,957 residents aged 40–79 years underwent a health examination (participation rate, 77.1%). After excluding 8 subjects who did not provide consent to participate, 490 with diabetes at baseline, 36 with no fasting blood samples, 160 who were not followed up, and 10 who lacked measurement of serum ethylamine concentration, the remaining 2,253 subjects (930 men and 1,323 women) were enrolled in the current study. The current study was performed with the approval of the Kyushu University Institutional Review Board for Clinical Research, and written informed consent was obtained from all participants.

Quantitation of Serum Ethylamine Concentrations

At the examination in 2007, portions of the serum specimens were stored at −80°C. In 2017, the serum ethylamine concentrations were measured by liquid chromatograph–tandem mass spectrometry, the detailed methods of which are described in the Supplementary Appendix. The coefficient of variation for the serum ethylamine concentration from five repeated measurements by this method was 2.3%. The study participants were divided into four groups according to the quartiles of serum ethylamine levels: Q1, ≤0.86 ng/mL; Q2, 0.87–2.10 ng/mL; Q3, 2.11–5.28 ng/mL; and Q4, ≥5.29 ng/mL.

Determination of Glucose Tolerance Status at Baseline

In total, 95.7% (2,155 of 2,253) of participants underwent an OGTT (blood sampling at 0 min, 30 min, and 2 h) after an overnight fast of at least 12 h at baseline. According to the 1998 World Health Organization criteria (12), glucose tolerance status at baseline was determined by the OGTT (or only fasting plasma glucose [FPG] levels among 98 subjects without OGTT) as follows: normal glucose tolerance, FPG <6.1 mmol/L and 2-h postload glucose (2hPG) <7.8 mmol/L; prediabetes, impaired fasting glycemia (FPG 6.1–6.9 mmol/L and 2hPG <7.8 mmol/L) or impaired glucose tolerance (FPG <7.0 mmol/L and 2hPG 7.8–11.0 mmol/L); and diabetes, FPG ≥7.0 mmol/L, 2hPG ≥11.1 mmol/L and/or the use of antidiabetic medications. Serum insulin concentrations were measured by a chemiluminescent enzyme immunoassay. The insulinogenic index and the HOMA-insulin resistance (HOMA-IR) were calculated with the following formulas, respectively: (serum insulin at 30 min − serum insulin at 0 min)/(plasma glucose at 30 min − plasma glucose at 0 min) and (plasma glucose at 0 min × serum insulin at 0 min/22.5), where the units of variables were serum insulin in μU/mL and plasma glucose in mmol/L (13,14).

Measurements of Other Risk Factors at Baseline in the Prospective Cohort Study for the Development of T2DM

Each participant completed a self-administered questionnaire, including medical history; family history; use of antihypertensive, antidiabetic, and lipid-modifying medications; smoking habits; alcohol intake; and regular exercise. A family history of diabetes was defined as the presence of diabetes in the first- or second-degree relatives of the subjects. Smoking habits and alcohol intake were classified as current use or not. Regular exercise was defined as sports at least three times per week during their leisure time. Total energy intake per day and green tea consumption per day were estimated using a brief-type self-administered diet history questionnaire (15). Obesity was defined as a BMI ≥25.0 kg/m^2. Blood pressure was measured three times using an automated sphygmomanometer (BP-203RV III; Colin, Tokyo, Japan) with the subject in the sitting position after >5 min of rest, and the mean value of the three measurements was calculated. Hypertension was defined as a systolic and diastolic blood pressure ≥140/90 mmHg or use of antihypertensive medications. Lipids were measured enzymatically.

Determination of New-Onset T2DM During Follow-up

The study participants were monitored at yearly health examinations until

30 November 2014 (median follow-up period: 6.9 years; range: 0.2–7.4 years). During the follow-up period, new onset of T2DM was determined based on the OGTT data or the measurements of fasting or casual plasma glucose in the annual health examinations, plus the clinical information—namely, medical records and the use of antidiabetic medications. T2DM was defined as FPG ≥7.0 mmol/L, 2hPG or casual plasma glucose ≥11.1 mmol/L, and/or the use of antidiabetic medications (oral hypoglycemic agents, injectable glucagon-like peptide analogs, or insulin) (12). The participants underwent an average of 5.3 ± 2.2 follow-up examinations.

Statistical Analysis

Serum triglycerides, insulinogenic index, and HOMA-IR were log-transformed owing to the skewed distributions. Trends in the risk factors at baseline across quartiles of serum ethylamine levels were tested using a linear regression for mean values and logistic regression for frequencies. The incidence rates of T2DM were estimated using the person-years method. Participants were censored at the time of death, the latest occasion of the follow-up survey, or the date of the diagnosis of type 1 diabetes (one subject was diagnosed with type 1 diabetes during follow-up). The age- and sex-adjusted incidence rates of T2DM were calculated using a Poisson regression model including age and sex, where the variables of log-transformed person-years were used as the offset term (16). The hazard ratios (HRs) with their 95% CIs of the quartiles of serum ethylamine levels for the development of T2DM were computed by using a Cox proportional hazards model. The age- and sex-adjusted cumulative incidence of T2DM across serum ethylamine levels was calculated by using regression estimates from a relevant Cox model including age and sex (17). The trends in the age- and sex-adjusted cumulative incidence and HRs were tested using a Cox model including serum ethylamine levels with ordinal numbers (1, 2, 3, and 4) and the relevant covariates. The heterogeneity in the association between subgroups of risk factors was assessed by adding multiplicative interaction terms of the serum ethylamine level with each subgroup in the relevant Cox model. All statistical analyses were conducted using SAS 9.4 software (SAS Institute, Cary, NC). A value of two-tailed $P < 0.05$ was considered statistically significant in all analyses.

Kinetic Study of Serum Ethylamine After the Consumption of L-Theanine–Rich Green Tea Products by Healthy Volunteers

Study Design

Twelve healthy middle-aged to elderly Japanese men and women, aged 40–69 years, participated in the kinetic study. In the open-label single-arm study, which was performed in October 2018, subjects agreed to be hospitalized for 4 days at a clinical center. On the morning of the day after the 1st day of hospitalization, subjects ingested 105 mL of L-theanine–rich green tea products (manufactured by Suntory Beverage & Food, Tokyo, Japan), 30 min after a light breakfast. The L-theanine–rich green tea products contained 8 mg of L-theanine per 105 mL. To avoid uptake of L-theanine from other food sources, subjects were instructed not to consume any foods, including L-theanine (e.g., green tea, oolong tea, tea-based products, L-theanine–rich functional foods, and a particular type of mushroom) from 7 days before the day of study products ingestion. Subjects were required to fast for 4 h after the ingestion of the green tea products. Subjects were prohibited from having any food or drink other than those provided by the clinical center, from smoking, and from leaving the clinical center during the study. Blood samples were collected before ingestion (baseline) and 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 30, 36, and 48 h after ingestion of the study products (8). After 30 min at room temperature, blood was centrifuged at 3,000*g* for 10 min at room temperature to obtain serum, which was stored at −80°C until analysis.

The study protocol was approved by the Clinical Research Network Fukuoka Ethics Committee (Fukuoka, Japan). Medical investigators explained the study to all subjects, and written informed consent was obtained from all subjects before enrollment.

Kinetic Analysis

Serum ethylamine concentrations were measured by liquid chromatograph–tandem mass spectrometry as described in the Supplementary Appendix. Serum kinetic parameters were calculated using noncompartmental analysis (Phoenix WinNonlin version 7.0) (18). The maximum concentration of ethylamine in serum (C_{max}) and the time at C_{max} (t_{max}) were determined directly from the observed values. The terminal elimination rate constant (λ_z) was estimated by linear regression of the log-linear decline in individual serum concentration–time data. The terminal half-life ($t_{1/2}$) was calculated for each individual as follows: $t_{1/2} = \ln(2)\lambda_z^{-1}$. The area under the serum concentration-time curve (AUC) was calculated by the linear trapezoidal rule (for AUC_{0-t}), plus the quotient of serum concentration at the predicted last point divided by λ_z (for $AUC_{0-\infty}$). The simulation curves of the serum ethylamine concentrations after putative multiple ingestions of the green tea products were calculated by using the superposition principle (18). The effect of time on serum ethylamine concentrations was investigated by ANOVA, followed by a Bonferroni adjustment for multiple comparisons of time points. These statistical analyses were performed using Microsoft Office Excel 2013 and SPSS Statistics (Subscription version; IBM, Tokyo, Japan).

RESULTS

Prospective Cohort Study for the Development of T2DM

The distribution of serum ethylamine concentrations was right skewed, and the log-transformed serum ethylamine concentrations were almost normally distributed (Supplementary Fig. 1). Median values of serum ethylamine concentrations were 2.11 ng/mL (interquartile range 0.87–5.29) in overall subjects. Serum ethylamine concentrations were distributed at higher levels in men than in women. The baseline characteristics of the study population according to the quartiles of serum ethylamine concentrations are summarized in Table 1. The mean values of age, FPG, systolic and diastolic blood pressure, and total energy intake increased significantly with higher serum ethylamine levels, while the geometric mean values of the insulinogenic index and HOMA-IR decreased significantly. Subjects with greater serum ethylamine levels were more likely to be male, to be prediabetic, to use antihypertensive agents, to consume alcohol, or to have a smoking habit. The proportion of subjects drinking ≥1 cup/day

Table 1—Baseline characteristics according to serum ethylamine levels

Variables	Serum ethylamine level, ng/mL				*P* for trend
	Q1 (≤0.86) (*n* = 563)	Q2 (0.87–2.10) (*n* = 563)	Q3 (2.11–5.28) (*n* = 563)	Q4 (≥5.29) (*n* = 564)	
Age, years	57.5 (10.5)	59.5 (10.1)	61.0 (10.6)	63.2 (9.8)	<0.001
Men	26.6	42.5	48.5	47.5	<0.001
Family history of diabetes	16.7	21.5	14.4	15.4	0.12
Prediabetes	26.1	28.6	30.6	30.5	0.08
FPG, mmol/L	5.4 (0.5)	5.5 (0.5)	5.5 (0.5)	5.5 (0.5)	0.002
2hPG, mmol/L	6.7 (1.5)	6.8 (1.6)	6.7 (1.7)	6.8 (1.6)	0.65
Insulinogenic index[a]	0.75 (0.15–3.77)	0.69 (0.13–3.62)	0.66 (0.11–4.06)	0.67 (0.11–3.98)	0.02
HOMA-IR[a]	1.30 (0.42–4.00)	1.29 (0.40–4.15)	1.25 (0.42–3.73)	1.21 (0.38–3.81)	0.03
Systolic blood pressure, mmHg	127.5 (18.9)	128.3 (17.7)	129.2 (18.4)	131.0 (18.3)	<0.001
Diastolic blood pressure, mmHg	78.2 (11.0)	78.7 (10.2)	78.9 (11.0)	79.8 (10.3)	0.01
Use of antihypertensive agents	22.4	24.0	24.9	28.9	0.01
Use of ARB	12.8	11.8	11.8	11.6	0.54
Serum total cholesterol, mmol/L	5.52 (0.90)	5.40 (0.94)	5.45 (0.94)	5.44 (0.99)	0.30
Serum HDL cholesterol, mmol/L	1.78 (0.45)	1.75 (0.46)	1.75 (0.46)	1.77 (0.48)	0.80
Serum triglycerides, mmol/L[a]	1.11 (0.39–3.11)	1.15 (0.39–3.39)	1.18 (0.42–3.33)	1.16 (0.43–3.18)	0.09
Use of statin	10.4	11.8	9.9	13.5	0.23
BMI, kg/m^2	23.1 (3.5)	22.9 (3.1)	22.8 (3.1)	22.7 (3.2)	0.06
Obesity	25.8	22.0	22.4	23.2	0.36
Smoking habits	15.1	21.5	21.8	23.4	<0.001
Alcohol intake	45.3	50.6	58.8	49.4	0.03
Regular exercise	11.4	12.1	13.0	12.9	0.37
Total energy intake, kcal/day	1,715.3 (522.9)	1,796.9 (543.1)	1,835.8 (550.9)	1,833.7 (528.8)	<0.001
Green tea consumption					
≥1 cup/day	50.7	73.4	76.8	88.8	<0.001
≥4 cup/day	18.4	27.2	34.9	55.8	<0.001
No. of health examinations received during follow-up, times	5.3 (2.1)	5.1 (2.3)	5.2 (2.2)	5.4 (2.2)	0.38

Data are mean (SD), percent, or as indicated. ARB, angiotensin II receptor blockers. [a]Insulinogenic index, HOMA-IR, and serum triglycerides are shown as geometric mean values (95% CIs).

of green tea was higher in subjects with higher serum ethylamine levels. Higher frequency of green tea consumption was significantly associated with greater mean values of serum ethylamine levels (Supplementary Fig. 2). There was no evidence of a significant association between serum ethylamine levels and other risk factors. Pearson correlation coefficients of serum ethylamine concentrations with risk factors taken as a continuous variable are reported in Supplementary Table 1.

During the median 6.9-year follow-up, 282 subjects developed T2DM. The age- and sex-adjusted incidence rate (per 100 person-years) and cumulative incidence of T2DM decreased significantly with elevating levels of serum ethylamine (both *P* for trend <0.05) (Table 2 and Fig. 1). This association remained unchanged after adjusting for potential confounding factors—namely, age, sex, family history of diabetes, systolic blood pressure, use of antihypertensive agents, use of angiotensin II receptor blockers, prediabetes, serum total cholesterol, serum HDL cholesterol, serum triglycerides, use of statins, obesity, current smoking, current drinking, and regular exercise (Table 2, model 2). The multivariable-adjusted HR for T2DM was significantly lower in the third (HR 0.71, 95% CI 0.50–0.99) and fourth (HR 0.69, 95% CI 0.49–0.98) quartile of serum ethylamine than in the first quartile. These significant associations were still observed after additional adjustment for total energy intake and green tea consumption (model 3), or the insulinogenic index (model 4) (both *P* for trend <0.05). Meanwhile, this association was modestly attenuated after additional adjustment for HOMA-IR (model 5) (*P* for trend = 0.06). In the sensitivity analysis using the variable "BMI" in place of the variable "obesity," the findings were not altered substantially: HR 0.76 (95% CI 0.54–1.07) for serum ethylamine of 0.87–2.10 ng/mL, 0.71 (0.51–1.00) for 2.11–5.28 ng/mL, and 0.70 (0.50–0.99) for ≥5.29 ng/mL (*P* for trend = 0.049) compared with ≤0.86 ng/mL.

Next, we addressed the difference in the association between serum ethylamine levels and the risk of incident T2DM between the subgroups according to age, sex, glucose tolerance status, obesity, insulinogenic index, and HOMA-IR (Supplementary Table 2). The multivariable-adjusted HRs on the development of T2DM decreased significantly or marginally with higher serum ethylamine levels in subjects aged <65 years, subjects with prediabetes, obese subjects, and subjects with higher HOMA-IR: there was significant heterogeneity in the association between subjects with and without obesity (*P* for heterogeneity = 0.048), whereas the differences in the association did not reach the statistically significant level

Table 2—Associations between the serum ethylamine level and risk of the development of T2DM

	Serum ethylamine level, ng/mL				
	Q1 (≤0.86)	Q2 (0.87–2.10)	Q3 (2.11–5.28)	Q4 (≥5.29)	*P* for trend
Median (ng/mL)	0.46	1.35	3.29	9.56	
No. of events/PYs at risk	76/3,344	67/3,323	70/3,369	69/3,355	
Incidence rate (per 100 PYs)					
Unadjusted	2.27	2.02	2.08	2.06	0.60
Age- and sex-adjusted	2.53	1.91	1.83	1.73	0.03
HR (95% CI)					
Model 1	1.00 (reference)	0.75 (0.54–1.05)	0.72 (0.52–1.00)	0.69 (0.49–0.97)*	0.04
Model 2	1.00 (reference)	0.76 (0.54–1.07)	0.71 (0.50–0.99)*	0.69 (0.49–0.98)*	0.04
Model 3	1.00 (reference)	0.74 (0.52–1.05)	0.68 (0.48–0.97)*	0.68 (0.48–0.97)*	0.04
Model 4	1.00 (reference)	0.76 (0.53–1.08)	0.67 (0.48–0.96)*	0.69 (0.48–0.98)*	0.03
Model 5	1.00 (reference)	0.74 (0.53–1.05)	0.71 (0.50–0.99)*	0.71 (0.51–1.01)	0.06

PYs, person-years. Model 1: Adjusted for age and sex. Model 2: Adjusted for age, sex, family history of diabetes, systolic blood pressure, use of antihypertensive agents, use of angiotensin II receptor blockers, prediabetes, serum total cholesterol, serum HDL cholesterol, serum triglycerides (log-transformed), use of statins, obesity, current smoking, current drinking, and regular exercise. Model 3: Adjusted for covariates included in Model 2 + total energy intake and green tea consumption. Model 4: Adjusted for covariates included in Model 2 + insulinogenic index (log-transformed). Model 5: Adjusted for covariates included in Model 2 + HOMA-IR (log-transformed). *$P < 0.05$ vs. Q1.

between subgroups of age, glucose tolerance status, and HOMA-IR (all *P* for heterogeneity >0.16). There was no evidence of heterogeneity in the association between subgroups of sex and insulinogenic index (both *P* for heterogeneity >0.60).

Kinetic Study of Serum Ethylamine After Consumption of L-Theanine–Rich Green Tea Products by Healthy Volunteers

The characteristics of the subjects are presented in Supplementary Table 3. The time course of the serum concentration of ethylamine is shown in Fig. 2. The corresponding kinetic parameters are summarized in Supplementary Table 4. The mean serum ethylamine concentration of the subjects increased with a peak concentration at 2 h ($P < 0.05$ compared with baseline), with a mean t_{max} and C_{max} of 1.67 h and 5.86 ng/mL, respectively. The concentration then returned to a level equivalent to the baseline value after 36 h with an average $t_{1/2}$ of 12.4 h.

Based on the results from the kinetic analysis, we computed the steady-state serum ethylamine concentration using a simulation after putative multiple ingestion. The serum ethylamine concentration reached a steady state by day 7. The value of the minimum concentration at the steady state was estimated to be >5.90 ng/mL in the case of twice-daily ingestion with an interval of 12 h (Supplementary Fig. 3*A*) and >10.23 ng/mL in the case of five-times daily consecutive ingestion at intervals of 1 h (Supplementary Fig. 3*B*).

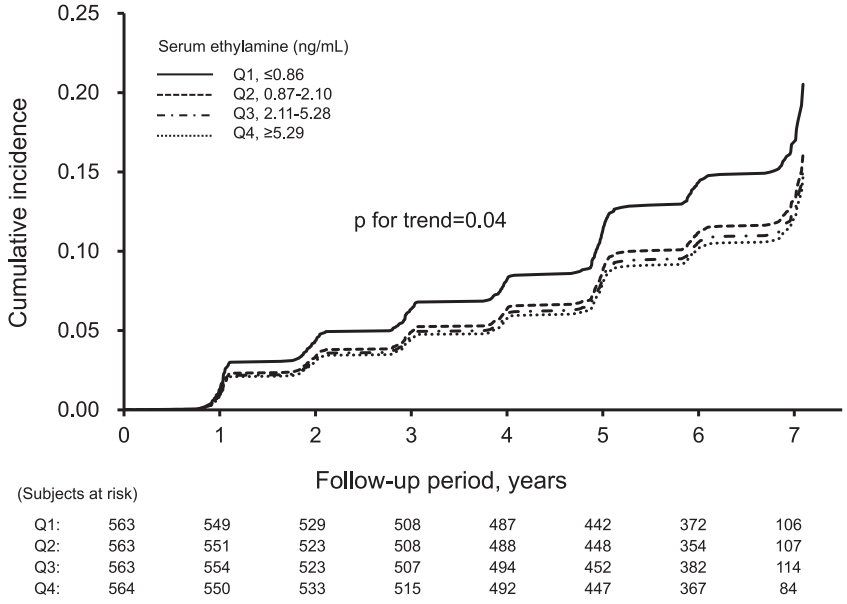

Figure 1—Age- and sex-adjusted cumulative incidence of T2DM according to serum ethylamine levels.

CONCLUSIONS

The current study demonstrated that elevated levels of serum ethylamine were significantly associated with lower risk of the development of T2DM in a general Japanese population. This decreasing tendency of the diabetic risk across serum ethylamine levels was more likely to be prominent in middle-aged subjects or in subjects with prediabetes, obesity, or insulin resistance. Our kinetic study additionally revealed that serum ethylamine levels were a possible indicator of the presence of L-theanine, which is one of the bioactive amino acids contained in green tea, because ethylamine is a stable metabolite of L-theanine in serum. Certainly, self-reported green tea consumption was significantly associated with serum ethylamine concentrations. To the best of our knowledge, this is the first study addressing the association of a serum biomarker of L-theanine consumption with diabetic risk.

A typical cup of brewed green tea has been reported to contain an average of 7.9 (SD 3.8) mg of L-theanine (19). In contrast, shade-grown green tea

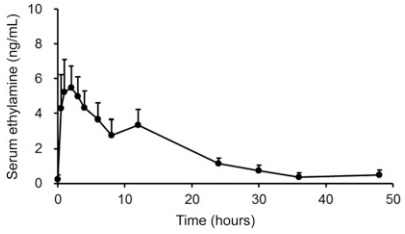

Figure 2—Serum ethylamine concentrations after ingestion of L-theanine–rich green tea products containing 8 mg of L-theanine. Data are expressed as the mean ± SD.

(gyokuro, matcha) brewed under the recommended conditions contains 40 mg of L-theanine per serving (8). Several epidemiological studies have reported that tea consumption was significantly associated with a reduced risk of T2DM. A systematic review from 12 cohort studies showed that daily tea consumption of ≥3 cups/day was associated with a 16% lower risk of T2DM, but there was a statistically significant heterogeneity across the selected studies (6). This negative association was observed mainly in Asian populations. In the current study, we found an ~30% lower risk of T2DM in subjects with serum ethylamine concentrations of ≥2.11 ng/mL, which corresponds to at least 2–3 cups/day in the regression analysis of self-reported green tea consumption with serum ethylamine concentrations. In addition, twice-daily ingestion of green tea products containing 8 mg of L-theanine was expected to result in a serum ethylamine concentration of 5.90 ng/mL at the steady state in our simulation analysis, which corresponded to approximately the fourth quartile level of serum ethylamine concentration in the current study. These findings may suggest that a daily green tea consumption of two or more cups has a protective role on the incidence of T2DM.

The mechanism by which tea consumption ameliorates the risk of developing T2DM may involve an improvement in insulin resistance (20,21). A green tea extract reduced the levels of hyperglycemia and hyperinsulinemia in an animal model of insulin resistance (20). In support of this finding, the current study revealed that the significant negative association between serum ethylamine levels and diabetic risk was attenuated after adjusting for HOMA-IR and was prominent in subjects with prediabetes, obesity, or insulin resistance. It has also been reported that tea protects pancreatic β-cells and the insulin secretory machinery (22).

The direct effect of L-theanine or serum ethylamine on diabetic risk is unclear. An animal experiment found that the administration of L-theanine inhibited glucose uptake by downregulating the expression of related genes, including *SGLT2* and *GLUT5*, in the small intestine of rats (23). In addition, L-theanine has been suggested to ameliorate lipopolysaccharide-induced inflammation by inhibiting inflammatory cytokine production in mice (3,24). Recently, lipopolysaccharides derived from the gut microbiota have been acknowledged to contribute to a low-grade system associated with an increased risk of insulin resistance and subsequent diabetes (25–27). In our study, the risks of incident T2DM were similar among the higher three quartiles of serum ethylamine levels, suggesting the existence of threshold effects of green tea or L-theanine on the diabetic risk. Since L-theanine has been reported to improve sleep quality or reduce appetite (28,29), these changes may account for the reduced risk of T2DM.

Certainly, the significant association between serum ethylamine and diabetic risk was attenuated by adjusting for HOMA-IR and was apparent in the subgroup with obesity or higher HOMA-IR level in the current study. Therefore, L-theanine may improve the insulin resistance by modifying the inflammatory response. Alternatively, we cannot rule out the possibility that our findings reflect the impact of other ingredients of green tea or the Japanese diet. Further interventional studies using L-theanine will be needed to clarify the direct effects of L-theanine on glucose tolerance status. Nevertheless, based on the present findings, we can at least state that green tea consumption has an ameliorating effect on the risk of T2DM.

The strengths of our study include the prospective population-based design, longer duration of follow-up, and precise diagnosis of T2DM based on OGTT. In addition, the consumption of L-theanine was estimated by using a validated serum biomarker for the metabolite of L-theanine rather than by using the information from a self-reported food questionnaire.

The current study had some limitations. First, our findings were based on a single measurement of serum ethylamine concentration at baseline. Moreover, information on the change of serum ethylamine concentration during the follow-up was not available. These limitations might weaken the association, biasing the results toward the null hypothesis.

Second, our study could not directly assess the association between serum L-theanine levels and diabetic risk, because serum L-theanine concentrations were hardly detected in the sera from fasting blood samples (data not shown). We therefore could not distinguish whether L-theanine or ethylamine had a favorable influence on diabetic risk.

Third, the adjustment for green tea consumption did not alter the significant association between serum ethylamine and diabetic risk, but we could not deny the possibility that other ingredients of green tea (e.g., epigallocatechin, epigallocatechin gallate, and epichatechin) were related to the lower diabetic risk. Nevertheless, it was hard to address the influence of these ingredients of green tea on the disease in this large-scale epidemiological study, because, like L-theanine, these ingredients are degraded to below the detectable level in plasma within 24 h of consumption (8,30).

Fourth, there is a possibility that the higher serum ethylamine levels merely reflected a healthier lifestyle.

Fifth, the current study was performed in a single Japanese population.

Finally, because our findings may reflect specific Japanese dietary habits, the generalizability of the findings to other ethnic populations is limited. Our results should thus be verified in other populations with different genetic and nutritional backgrounds.

In conclusion, the current study demonstrated that higher serum ethylamine was significantly associated with lower risk of the development of T2DM in a general Japanese population. These findings raise the possibility that the dietary intake of L-theanine through the consumption of green tea may reduce the risk of the development of T2DM. Specifically, consumption of two or more cups of L-theanine–rich green tea (e.g., matcha and gyokuro) per day may be a good lifestyle choice for reducing the risk of T2DM. In addition, the measurement of serum ethylamine concentrations would be a useful biomarker for objectively estimating the consumption of L-theanine. Finally, we note that the present findings showed a significant association between serum ethylamine

and diabetic risk but did not demonstrate causation. Further experimental, epidemiological, and clinical studies will be needed to elucidate the causal effect of consumption of two or more cups L-theanine on the prevention of T2DM.

Acknowledgments. The authors thank the residents of the town of Hisayama for their participation in the survey and the staff of the Division of Health and Welfare of Hisayama for their cooperation with this study. The authors also thank Nobuhiro Ohkubo and Ko Igami of Kyushu Pro Search LLP for the measurement of the serum ethylamine concentrations, and Dr. Yasuhiko Koezuka and Dr. Go Ichien of HuBit Genomix for the scientific advice.

Funding. This study was supported in part by Grants-in-Aid for Scientific Research (*A*) JP16H02644 and JP16H02692; (*B*) JP16H05850, JP16H05557, JP17H04126, and JP18H02737; (*C*) JP16K09244, JP17K09114, JP17K09113, JP17K01853, JP18K07565, and JP18K09412; and Early-Career Scientists (JP18K17925 and JP18K17382) from the Ministry of Education, Culture, Sports, Science and Technology of Japan; by Health and Labour Sciences Research Grants of the Ministry of Health, Labour and Welfare of Japan (H29-Junkankitou-Ippan-003 and H30-Shokuhin-[Sitei]-005); and by the Japan Agency for Medical Research and Development (JP18dk0207025, JP18ek0210082, JP18gm0610007, JP18ek0210083, JP18km0405202, JP18ek0210080, and JP18fk0108075). This study was additionally sponsored by Suntory Beverage & Food Limited (Tokyo, Japan).

Duality of Interest. T.N. obtained the funding support from Suntory Holdings Limited (Tokyo, Japan). N.K., T.T., and S.T. are employed by Suntory Beverage & Food Limited (Tokyo, Japan), which was the sponsor of this study and markets green tea products. S.N. is a managing executive officer of Suntory Beverage & Food Limited (Tokyo, Japan). No other potential conflicts of interest relevant to this article were reported.

Author Contributions. T.N. contributed to the data collection, data analysis, data interpretation, and drafting of the manuscript and performed study supervision for the prospective cohort study of T2DM. N.K. contributed to the data collection, the data analysis, the data interpretation, and drafting of the manuscript for the kinetic study of serum ethylamine. Y.H., M.Y., M.H., T.H., M.S., S.S., D.Y., J.H., and T.K. contributed to the data collection and the data interpretation for the prospective cohort study of T2DM and to the critical revision of the manuscript. T.T., S.T., and S.N. contributed to the critical revision of the manuscript. T.N. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

References

1. Ogurtsova K, da Rocha Fernandes JD, Huang Y, et al. IDF Diabetes Atlas: global estimates for the prevalence of diabetes for 2015 and 2040. Diabetes Res Clin Pract 2017;128:40–50
2. NCD Risk Factor Collaboration (NCD-RisC). Worldwide trends in diabetes since 1980: a pooled analysis of 751 population-based studies with 4.4 million participants [published correction appears in Lancet 2017;389:e2]. Lancet 2016;387:1513–1530
3. Global Burden of Metabolic Risk Factors for Chronic Diseases Collaboration. Cardiovascular disease, chronic kidney disease, and diabetes mortality burden of cardiometabolic risk factors from 1980 to 2010: a comparative risk assessment. Lancet Diabetes Endocrinol 2014;2:634–647
4. Ninomiya T. Diabetes mellitus and dementia. Curr Diab Rep 2014;14:487
5. Weber MB, Narayan KM. Diabetes prevention should be a public-health priority. Lancet 2008; 371:473–474
6. Yang J, Mao QX, Xu HX, Ma X, Zeng CY. Tea consumption and risk of type 2 diabetes mellitus: a systematic review and meta-analysis update. BMJ Open 2014;4:e005632
7. Yang WS, Wang WY, Fan WY, Deng Q, Wang X. Tea consumption and risk of type 2 diabetes: a dose-response meta-analysis of cohort studies. Br J Nutr 2014;111:1329–1339
8. Scheid L, Ellinger S, Alteheld B, et al. Kinetics of L-theanine uptake and metabolism in healthy participants are comparable after ingestion of L-theanine via capsules and green tea. J Nutr 2012;142:2091–2096
9. Adhikary R, Vivekananda Mandal V. L-Theanine: a potential multifaceted natural bioactive amide as health supplement. Asian Pac J Trop Biomed 2017;7:842–848
10. Mukai N, Hata J, Hirakawa Y, et al. Trends in the prevalence of type 2 diabetes and prediabetes in a Japanese community, 1988-2012: the Hisayama Study. Diabetol Int. 3 November 2018 [E-pub ahead of print]. DOI: 10.1007/s13340-018-0380-0
11. Hata J, Ninomiya T, Hirakawa Y, et al. Secular trends in cardiovascular disease and its risk factors in Japanese: half-century data from the Hisayama Study (1961-2009). Circulation 2013;128:1198–1205
12. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med 1998;15:539–553
13. Phillips DI, Clark PM, Hales CN, Osmond C. Understanding oral glucose tolerance: comparison of glucose or insulin measurements during the oral glucose tolerance test with specific measurements of insulin resistance and insulin secretion. Diabet Med 1994;11:286–292
14. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412–419
15. Kobayashi S, Honda S, Murakami K, et al. Both comprehensive and brief self-administered diet history questionnaires satisfactorily rank nutrient intakes in Japanese adults. J Epidemiol 2012;22:151–159
16. SAS/STAT 15.1 User's Guide. The GENMOD Procedure. Poisson Regression [Internet]. SAS Institute Inc. Available from http://documentation.sas.com/?docsetId=statug&docsetTarget=statug_genmod_gettingstarted01.htm&docsetVersion=15.1&locale=ja. Accessed 10 March 2019
17. SAS/STAT 15.1 User's Guide. The PHREG Procedure. Example 89.8 Survival Curves [Internet]. SAS Institute Inc. Available from http://documentation.sas.com/?docsetId=statug&docsetTarget=statug_phreg_examples08.htm&docsetVersion=15.1&locale=ja. Accessed 10 March 2019
18. Certara. Phoenix WinNonlin [Internet]. Available from https://www.certara.com/wp-content/uploads/Resources/Brochures/BR_PhoenixWinNonlin.pdf. Accessed 10 March 2019
19. Keenan EK, Finnie MDA, Jones PS, Roger PJ, Priestley CM. How much theanine in a cup of tea? Effects of tea type and method of preparation. Food Chem 2011;125:588–594
20. Hininger-Favier I, Benaraba R, Coves S, Anderson RA, Roussel AM. Green tea extract decreases oxidative stress and improves insulin sensitivity in an animal model of insulin resistance, the fructose-fed rat. J Am Coll Nutr 2009; 28:355–361
21. Lin CL, Lin JK. Epigallocatechin gallate (EGCG) attenuates high glucose-induced insulin signaling blockade in human hepG2 hepatoma cells. Mol Nutr Food Res 2008;52:930–939
22. Manikandan R, Sundaram R, Thiagarajan R, Sivakumar MR, Meiyalagan V, Arumugam M. Effect of black tea on histological and immunohistochemical changes in pancreatic tissues of normal and streptozotocin-induced diabetic mice (Mus musculus). Microsc Res Tech 2009; 72:723–726
23. Yan Q, Tong H, Tang S, Tan Z, Han X, Zhou C. L-theanine administration modulates the absorption of dietary nutrients and expression of transporters and receptors in the intestinal mucosa of rats. BioMed Res Int 2017;2017:9747256
24. Wang D, Gao Q, Zhao G, et al. Protective effect and mechanism of theanine on lipopolysaccharide-induced inflammation and acute liver injury in mice. J Agric Food Chem 2018;66:7674–7683
25. Manco M, Putignani L, Bottazzo GF. Gut microbiota, lipopolysaccharides, and innate immunity in the pathogenesis of obesity and cardiovascular risk. Endocr Rev 2010;31:817–844
26. Cani PD, Amar J, Iglesias MA, et al. Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes 2007;56:1761–1772
27. Pussinen PJ, Havulinna AS, Lehto M, Sundvall J, Salomaa V. Endotoxemia is associated with an increased risk of incident diabetes. Diabetes Care 2011;34:392–397
28. Sarris J, Byrne GJ, Cribb L, et al. L-theanine in the adjunctive treatment of generalized anxiety disorder: a double-blind, randomised, placebo-controlled trial. J Psychiatr Res 2019;110:31–37
29. Yamada T, Nishimura Y, Sakurai T, et al. Administration of theanine, a unique amino acid in tea leaves, changed feeding-relating components in serum and feeding behavior in rats. Biosci Biotechnol Biochem 2008;72:1352–1355
30. Chow HH, Cai Y, Alberts DS, et al. Phase I pharmacokinetic study of tea polyphenols following single-dose administration of epigallocatechin gallate and polyphenon E. Cancer Epidemiol Biomarkers Prev 2001;10:53–58