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Association between equol producers and type 2 diabetes mellitus among Japanese older adults

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ABSTRACT

Aims/Introduction: Equol, which is produced by enteric bacteria from soybean isoflavones, has a chemical structure similar to estrogen. Both *in vivo* and *in vitro* studies have shown the beneficial metabolic effects of equol. However, its effects on type 2 diabetes remain unclear. We investigated the association between the equol producers/non-producers and type 2 diabetes.

Materials and Methods: The participants included 147 patients with type diabetes mellitus aged 70–89 years, and 147 age- and sex-matched controls. To ascertain the equol producers or non-producers, we used the comparative logarithm between the urinary equol and daidzein concentrations (cut-off value −1.75).

Results: The urinary equol concentration was significantly lower in the diabetes group compared with the non-diabetes group ($P = 0.01$). A significant difference in the proportion of equol producers was observed among all participants (38.8% in the diabetes group and 53.1% in the non-diabetes group; $P = 0.01$). The proportion of equol producers among women was significantly lower in the diabetes group (31.4%) than in the non-diabetes group (52.8%; $P < 0.01$). Additionally, the frequency of dyslipidemia in female equol producers was significantly lower than that in female non-equol producers ($P < 0.01$). Among men, no such differences were observed. We found a significant positive correlation between the urinary equol and daidzein concentrations among equol producers ($r = 0.55$, $P < 0.01$).

Conclusions: Our study findings showed that postmenopausal women had a low proportion of equol producers with diabetes and dyslipidemia.

INTRODUCTION

In Japan, aging is progressing at an unprecedented speed[1]. Concurrently, the number of older patients with diabetes is increasing rapidly. Diabetes causes atherosclerotic angiopathies, such as coronary heart disease, stroke and obstructive atherosclerosis, as well as microangiopathic complications, such as neurological disorders, nephropathy and retinopathy. Additionally, older people with diabetes often incur complications of the so-called "geriatric syndromes" compared with individuals without diabetes, including dementia, sarcopenia, falling and depression[2]. These complications not only reduce their quality of life, but are also an important social issue due to the high medical costs incurred and the burden of long-term care.

Sex-based differences in the frequency of diabetes have been reported. The International Diabetes Federation Diabetes Atlas (8th edition) indicates that the global diabetes prevalence rate in women is estimated to be 8.4%, which is slightly lower than that rate in men (9.1%). The number of female patients with diabetes

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is estimated to be approximately 17,100,000 fewer than that of male patients[3]. One of the possible reasons for the lower prevalence rate of diabetes in women is the impact of sex hormones on glucose metabolism. Estrogen increases insulin sensitivity, whereas progesterone decreases insulin sensitivity[4]. The decrease in estrogen levels associated with menopause accelerates the development of insulin resistance and type 2 diabetes[5]. Clinical trials have reported an improvement of insulin sensitivity and reduction in the incidence of type 2 diabetes in postmenopausal women who use estrogen replacement therapy[6].

The Japan Public Health Center-based Prospective Study, a large-scale cohort study carried out by the research team at the Ministry of Health, Labor and Welfare, has verified that a higher consumption of isoflavones from food lowers the risk of breast cancer, cerebral infarction and myocardial infarction in women[7,8]. A previous study reported that in obese or postmenopausal women, the risk of developing type 2 diabetes was lower in groups that consumed a higher amount of soybean food and soybean isoflavones than in groups that consumed a lower amount[9].

Equol is a metabolite similar to estrogen, and is produced by enteric bacteria from daidzein, a type of soybean isoflavones[10]. As a result, equol not only has estrogen-like effects, but also shows anti-androgen and anti-oxidant effects, and its role in the prevention and improvement of prostate cancer, breast cancer, and menopausal symptoms has been reported previously[11]. Equol is not necessarily produced in every person, and its production is affected by differences in an individual's dietary content and the condition of the enteric bacterial thickets. This was observed in a previous study on healthy participants, which reported that just 30–50% of the participants were equol producers[12].

In animal experiments using mice, the oral administration of equol caused improvements in insulin resistance and lipid metabolism[13]. It is possible that equol is involved in the development and progression of diabetic conditions and complications in humans; however, there are few surveys on the proportion of equol producers and their influence on complications in type 2 diabetes cases. In the present study, we measured urinary equol and daidzein concentrations to determine equol producers among Japanese older adults, and investigated the proportion of equol producers among participants with and without diabetes. Furthermore, we examined the association between equol producers/non-producers and the clinical background in diabetes patients.

MATERIALS AND METHODS

The present cross-sectional study was carried out in accordance with the Declaration of Helsinki and the Ethical Guidelines on Medical and Health Research involving Human Participants, and was approved by the Hirosaki University School of Medicine Ethics Committee (approval number: 2018-132). Written informed consent was obtained from all participants.

Study design and participants

Hirosaki University, Hirosaki City and the Aomoriken Sougou Kenshin Center, Aomori City, have been carrying out the Iwaki Health Promotion Project (Large Scale Joint Resident Health Examinations) in Iwaki District, Hirosaki City, since 2005. We targeted 1,113 individuals who underwent resident health examinations in 2017 and classified them as the control group, as well as 1,395 patients with type 2 diabetes who were examined at the Hirosaki University Hospital from 1 April to 30 June 2017. The following cases were excluded from the study: clinical cases in which we were unable to determine the equol producers or non-producers owing to the lower concentrations of equol and/or daidzein than the limit of measurement (40 cases), cases identified to be diabetic from the control group (93), type 1 diabetes cases (102), pancreatic disease cases (120), diabetes caused due to endocrine disease complications (six cases) and cases receiving hormone replacement therapy (7).

As the estrogen-like effects of equol are weaker than that of endogenic estradiol, we assumed that its effect would become apparent only after menopause. Hence, we limited the target participants of the present study to those aged 70–89 years, ultimately enrolling 147 participants without diabetes and 431 patients with diabetes.

Participants' characteristics

For the control group, the participants' fasting blood samples were collected early in the morning for the measurement of hemoglobin A1c (HbA1c) levels. The mean HbA1c value of the diabetes group was obtained for this study by collecting their blood samples for HbA1c measurement at least four times during the year. In the diabetes group, 323 patients were taking oral hypoglycemic medication and 178 were taking insulin.

The blood test results and data, such as height and weight, were collected from the resident health examinations for the non-diabetes group and from the outpatient electronic medical records for the diabetes group. Dyslipidemia was defined as a low-density lipoprotein cholesterol (LDL-C) level >140 mg/dL, triglyceride level >150 mg/dL or treatment for dyslipidemia. Hypertension was defined as the diagnosis of hypertension or current treatment with hypotensive agents.

Measuring urinary equol and daidzein concentrations

To measure the participants' urinary equol and daidzein concentrations, we used partial urine samples obtained from regular medical examinations and health checkups. The urine concentrations were calculated by extracting isoflavones and its metabolite (equol) from the urine samples using ISOLUTE® SLE+ (Biotage Japan Ltd., Tokyo, Japan) after enzymatic deconjugation treatments, followed by liquid chromatography–mass spectrometry. For this, we used the LC-30AD model (Shimadzu Corporation, Kyoto, Japan) for the high-performance liquid chromatography system, SCIEX QTRAP® 5500+ system (AB Sciex Pte. Ltd., Tokyo, Japan) for mass spectrometry detection and a reversed column SunShell C18 2.1 × 100 mm (2.7 μm;

ChromaNik Technologies Inc., Osaka, Japan) for the column. The analysis was carried out with a flow velocity of 0.3 mL/min at a column temperature of 40°C. The lower limit of quantification for equol concentration was 10.32 nmol/L, and that for daidzein was 39.34 nmol/L. To ascertain the equol producers, we used the comparative logarithm between the urinary equol and daidzein concentrations, and classified participants with values ≥−1.75 as equol producers[14].

Statistical analysis

For clinical background intergroup comparisons of equol production in the diabetes group, we used the *t*-test and χ^2-test. Confounding factors were adjusted by matching to align the patient backgrounds of the diabetes and non-diabetes groups. The propensity score was calculated using logistic regression analysis with age and sex as covariates. The matching was executed by setting the goodness of fit tolerance (caliper) of the covariates as 0.2. Participants of the diabetes and non-diabetes groups with the closest scores within the caliper limit were considered as 1:1 matches.

The data are presented as mean ± standard deviation. Statistical significance was set at $P < 0.05$. Statistical analysis was carried out using JMP® 15 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Study population

There were 431 participants in the diabetes group (227 men and 204 women; age 76.5 ± 4.8 years; HbA1c level 7.1 ± 0.9%) and 147 in the non-diabetes group (41 men and 106 women; age 75.0 ± 4.1 years; HbA1c level 5.8 ± 0.3%; Table 1). A comparison between the diabetes and non-diabetes groups before propensity score matching showed that age, body mass index (BMI) and HbA1c levels of the diabetes group were significantly higher than that of the non-diabetes group, although the proportion of women was lower. The urinary daidzein and equol concentrations were significantly lower in the diabetes group than in the non-diabetes group ($P < 0.01$ for both).

To align the participants' backgrounds, we calculated the inclination scores from the data on age and sex using logistic regression analysis and carried out 1:1 propensity score matching. The result was a study based on a type 2 diabetes group of 147 participants (45 men and 102 women; age 74.6 ± 3.8 years; HbA1c level 7.1 ± 0.9%) and a non-diabetes group of 147 participants (41 men and 106 women; age 75.0 ± 4.1 years; HbA1c level 5.8 ± 0.3%). The diabetes group had a prominently higher HbA1c level and BMI even after matching, and the equol concentration was significantly lower than that of the non-diabetes group ($P = 0.01$ for the concentrations).

Proportion of equol producers in the diabetes and non-diabetes groups

Figure 1 and Table 1 show the proportion of equol producers in the diabetes and non-diabetes groups. We observed a significant difference in the proportion of equol producers in the two groups post-matching, with 38.8% and 53.1% of equol

Table 1 | Basic characteristics of the participants before and after propensity score matching

Characteristics	All participants		*P*	Propensity-matched participants		*P*
	Non-T2D (*n* = 147)	T2D (*n* = 431)		Non-T2D (*n* = 147)	T2D (*n* = 147)	
Age (years)	75.0 ± 4.1	76.5 ± 4.8	<0.01	75.0 ± 4.1	74.6 ± 3.8	0.41
Female, *n* (%)	106 (72.1)	204 (47.3)	<0.01	106 (72.1)	102 (69.4)	0.61
Height (cm)	153.2 ± 7.9	157.8 ± 9.1	<0.01	153.2 ± 7.9	155.5 ± 8.9	0.02
Bodyweight (kg)	54.5 ± 9.5	61.1 ± 12.0	<0.01	54.5 ± 9.5	60.1 ± 11.7	<0.01
Body mass index (kg/m^2)	23.1 ± 3.1	24.5 ± 4.1	<0.01	23.1 ± 3.1	24.8 ± 4.3	<0.01
HbA1c (%)	5.8 ± 0.3	7.1 ± 0.9	<0.01	5.8 ± 0.3	7.1 ± 0.9	<0.01
Urinary isoflavones						
Daidzein (nmol/L)	20,418 ± 32,036	13,694 ± 16,203	<0.01	20,418 ± 32,036	15,834 ± 17,146	0.13
Equol (nmol/L)	8,461 ± 17,201	4,447 ± 9,697	<0.01	8,461 ± 17,201	4,396 ± 9,754	0.01
Equol-producer, *n* (%)	78 (53.1)	199 (46.2)	0.15	78 (53.1)	57 (38.8)	0.01
Hb (g/dL)	13.4 ± 1.4	13.0 ± 1.7	0.01	13.4 ± 1.4	13.0 ± 1.6	0.02
TC (mg/dL)	209.4 ± 32.7	175.1 ± 34.3	<0.01	209.4 ± 32.7	181.9 ± 33.5	<0.01
TG (mg/dL)	91.8 ± 49.6	128.5 ± 62.7	<0.01	91.8 ± 49.6	134.8 ± 65.8	<0.01
HDL (mg/dL)	66.1 ± 17.7	53.8 ± 25.7	<0.01	66.1 ± 17.7	55.2 ± 16.4	<0.01
AST (U/L)	25.3 ± 9.1	24.4 ± 13.1	0.40	25.3 ± 9.1	24.4 ± 11.1	0.42
ALT (U/L)	18.7 ± 9.1	20.9 ± 11.1	0.03	18.7 ± 9.1	21.5 ± 12.5	0.03
γGTP (U/L)	24.8 ± 14.6	29.7 ± 30.8	0.06	24.8 ± 14.6	29.7 ± 35.3	0.12
BUN (mg/dL)	17.7 ± 4.8	20.8 ± 9.7	<0.01	17.7 ± 4.8	19.8 ± 7.9	<0.01
Cre (mg/dL)	0.72 ± 0.19	1.0 ± 0.68	<0.01	0.72 ± 0.19	0.87 ± 0.34	<0.01
eGFR (mL/min/1.73 m^2)	68.5 ± 13.9	58.5 ± 20.4	<0.01	68.5 ± 13.9	60.3 ± 19.9	<0.01

The urinary equol concentrations were significantly lower in the diabetes group before and after the matching.
γGTP, gamma-glutamyl transpeptidase; ALT, alanine transaminase; AST, aspartate transaminase; BUN, blood urea nitrogen; Cre, creatinine; Hb, hemoglobin; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; TC, total cholesterol; TG, triglyceride; T2D, type 2 diabetes.

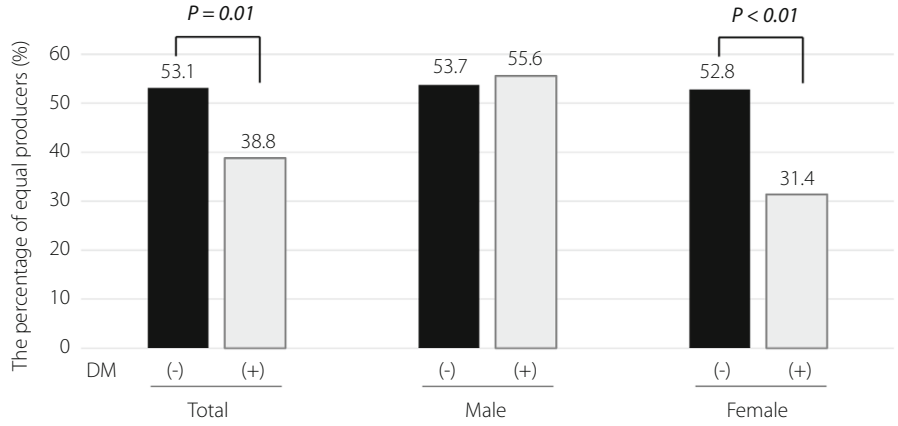

Figure 1 | The percentage of equol producers in the diabetes group compared with the non-diabetes group. Black bars represent the non-diabetes group (n = 147, 41 men and 106 women), whereas the gray bars represent the diabetes group (n = 147, 45 men and 102 women). The proportion of equol producers among women was significantly lower in the diabetes group compared with the non-diabetes group. DM, diabetes.

Table 2 | Basic characteristics of the equol producers and non-producers

Characteristics	Propensity-matched participants		*P*	Male		*P*	Female		*P*
	Epuol-producers (n = 135)	Epuol-nonproducers (n = 159)		Epuol-producers (n = 47)	Epuol-nonproducers (n = 39)		Epuol-producers (n = 88)	Epuol-nonproducers (n = 120)	
Age (years)	75.0 ± 4.1	74.7 ± 3.8	0.54	74.4 ± 3.7	74.8 ± 3.9	0.61	75.3 ± 4.3	74.7 ± 3.7	0.26
Body mass index (kg/m^2)	24.0 ± 3.7	23.9 ± 3.9	0.84	24.4 ± 3.4	23.7 ± 2.6	0.30	23.8 ± 3.9	24.0 ± 4.3	0.75
HbA1c (%)	6.4 ± 0.9	6.6 ± 0.9	0.08	6.5 ± 0.8	6.3 ± 0.7	0.20	6.3 ± 1.0	6.6 ± 1.0	0.01
Diabetes, *n* (%)	57 (42.2)	90 (56.6)	0.01	25 (53.2)	20 (51.3)	0.86	32 (36.4)	70 (58.3)	<0.01
Hypertension, *n* (%)	91 (67.4)	89 (56.0)	0.045	30 (63.8)	23 (59.0)	0.65	61 (69.3)	66 (55.0)	0.04
Dyslipidemia, *n* (%)	72 (53.3)	122 (76.7)	<0.01	24 (51.1)	26 (66.7)	0.14	48 (54.6)	96 (80.0)	<0.01
Hb (g/dL)	13.3 ± 1.3	13.1 ± 1.6	0.10	14.2 ± 1.2	14.1 ± 1.1	0.90	12.9 ± 1.2	12.7 ± 1.6	0.32
TC (mg/dL)	194.6 ± 36.8	196.6 ± 35.1	0.65	182.7 ± 38.1	186.6 ± 32.4	0.62	201.0 ± 34.6	199.8 ± 35.4	0.80
TG (mg/dL)	103.1 ± 53.6	122.1 ± 67.3	<0.01	105.2 ± 63.5	128.3 ± 69.8	0.11	101.9 ± 47.9	120.0 ± 66.6	0.03
HDL (mg/dL)	60.8 ± 18.9	60.5 ± 17.0	0.87	56.1 ± 20.2	54.2 ± 17.0	0.64	63.3 ± 17.7	62.5 ± 16.6	0.74
AST (U/L)	25.3 ± 11.1	24.5 ± 9.3	0.46	28.8 ± 15.8	26.7 ± 13.7	0.53	23.5 ± 6.9	23.7 ± 7.3	0.84
ALT (U/L)	20.6 ± 12.0	19.6 ± 10.1	0.41	24.9 ± 15.3	22.2 ± 14.0	0.40	18.4 ± 9.1	18.7 ± 8.4	0.77
γGTP (U/L)	25.4 ± 17.0	28.8 ± 33.3	0.28	31.3 ± 23.1	41.1 ± 62.3	0.32	22.3 ± 11.6	24.8 ± 13.1	0.14
BUN (mg/dL)	18.8 ± 5.7	18.7 ± 7.3	0.89	19.0 ± 5.6	17.1 ± 5.9	0.14	18.8 ± 5.7	19.3 ± 7.7	0.61
Cre (mg/dL)	0.79 ± 0.27	0.80 ± 0.30	0.75	0.93 ± 0.29	0.88 ± 0.34	0.47	0.71 ± 0.22	0.77 ± 0.28	0.10
eGFR (mL/min/1.73 m^2)	65.2 ± 15.8	63.7 ± 19.1	0.48	65.8 ± 16.6	71.2 ± 21.6	0.19	64.9 ± 15.5	61.3 ± 17.6	0.13

Among women, there was a significantly lower number of participants with diabetes and dyslipidemia, as well as a higher number of participants with hypertension in the equol producers than in the non-producers. As for men, no such differences were observed.
γGTP, gamma-glutamyl transpeptidase; ALT, alanine transaminase; AST, aspartate transaminase; BUN, blood urea nitrogen; Cre, creatinine; Hb, hemoglobin; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; TC, total cholesterol; TG, triglyceride; T2D, type 2 diabetes.

producers in the diabetes and non-diabetes groups, respectively (P = 0.01). On comparing the proportion of male equol producers between the two groups, we did not find a significant difference between the diabetes (55.6%) and non-diabetes (53.7%) groups. For women, the proportion of equol producers was 31.4% and 52.8% in the diabetes and non-diabetes groups, respectively, with the proportion of equol producers being significantly lower in the diabetes group (P < 0.01).

Characteristics of the equol producers

Table 2 shows the clinical backgrounds of the equol producers and non-producers. After propensity score matching, there were

135 equol producers (45.9%) among the total 294 participants. The frequency of diabetes and dyslipidemia were significantly lower in the equol producers than in the non-producers ($P \leq 0.01$ for both). However, we did not find significant differences in age, BMI and HbA1c levels.

We studied the differences in the clinical characteristics of men and women separately for the two groups. Among women, there was a significantly lower number of participants with diabetes and dyslipidemia ($P < 0.01$ for both), as well as a higher number of participants with hypertension ($P = 0.04$) in the equol producers than in the non-producers. As for men, we could not verify any differences in the frequency of diabetes, hypertension and dyslipidemia between equol producers and non-producers. For both men and women, there were no significant differences in age and BMI between equol producers and non-producers.

Correlation between equol and daidzein concentrations

We carried out a correlation analysis for the urinary equol and daidzein concentrations (log nmol/L), which are reflective of the soybean consumption (Figure 2), and found a significant positive correlation in these concentrations among equol producers ($r = 0.55$, $P < 0.01$). For non-producers, we could barely find any increase in the urinary equol concentration that correlated with an increase in the urinary daidzein concentration.

DISCUSSION

In the present study, we examined the association between diabetes and equol producers/non-producers in Japanese older patients with type 2 diabetes and participants without diabetes by measuring the urinary equol and daidzein concentrations. The results showed a significantly lower rate of diabetes and dyslipidemia complications in older women who were equol producers. However, this association was not observed in the male participants of this study.

Sex-based differences in the frequency of diabetes have been reported, and the prevalence rate of type 2 diabetes is significantly higher in men than in women[15–21]. Studies examining the prevalence rate of diabetes in different age groups, and the differences in the diabetes prevalence between men and women have shown an increased diabetes prevalence associated with aging in both sexes. However, the prevalence rate is higher in men before their 50s and in women after they are in their 60s[15,20]. The mechanism for this is not understood yet, although factors, such as stronger tendencies towards obesity and larger amounts of visceral fat in men than in women[22], as well as the impact of sex hormones on glycometabolism, are possible role players[4].

Equol is a compound produced when daidzein, a soybean isoflavone, is metabolized by enteric bacteria[10]. A variety of enteric bacteria produce equol, with approximately 20 types of equol-producing bacteria discovered to date, including *Lactococcus* and *Lactobacillus*[23]. Equol is not produced in every person, and its production is affected by differences in an individual's dietary content and enteric bacterial thickets.

Although equol-producing status is determined from urinary equol concentration, using an absolute equol concentration threshold is difficult, because urinary equol concentration is influenced by dietary habits. To solve this problem, Setchell and Cole[14] suggested defining equol producers using the cut-off of a urinary log10-transformed equol/daidzein ratio of −1.75. This criterion has been widely used to define equol producers.

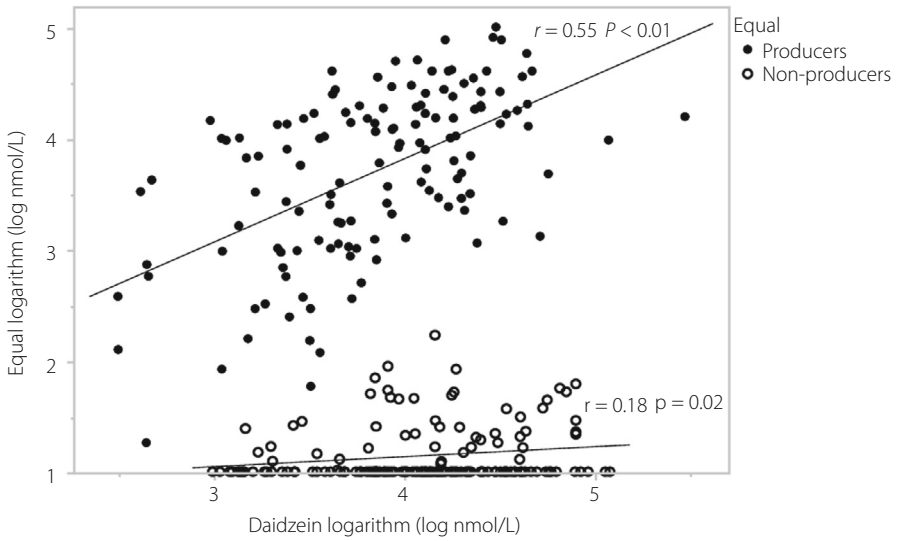

Figure 2 | The association between logarithm-transformed urinary equol and daidzein concentrations. Scatter plots and correlation analysis were separately carried out for equol producers (closed circles, $n = 135$) and non-producers (open circles, $n = 159$). A significant positive correlation was shown for equol producers, but not for non-producers.

In the present study, we also measured participants' urinary equol and daidzein concentrations using spot urine samples. Although 24-h urine samples are superior to spot urine samples, 24-h samples are difficult to obtain in large-scale studies. Franke *et al.*[24] ascertained that defining equol producers using the equol-to-daidzein ratio was useful in large-scale studies, even when using overnight urine samples instead of 24-h urine samples.

Studies investigating the equol production in healthy participants found 20–30% of equol producers in Western countries[25–30], and approximately 50% in regions with a high soybean consumption, such as Japan and China[31–36]. The proportion of equol producers in the present study was 45.9%, which is in agreement with the results of previous studies. We further investigated the proportion of equol producers in the diabetes and non-diabetes groups, and examined differences in the clinical backgrounds of equol producers and non-producers.

The main identified effects of equol are estrogen-like effects[37,38], anti-androgen effects[39] and anti-oxidant effects[40,41]. In previous epidemiological studies, equol producers showed favorable outcomes for postmenopausal symptoms and other conditions, such as osteoporosis, dyslipidemia, vascular endothelial function, breast cancer, prostatomegaly and prostate cancer, compared with non-producers[22]. In animal experiments using mice, improvements in insulin resistance and lipid metabolism due to the administration of equol have been reported[12]. However, there are limited studies examining the association between equol production capabilities and dysfunction of glucose and lipid metabolism in humans.

The results of the present study showed that the frequency of type 2 diabetes and dyslipidemia was significantly lower in women who were equol producers than in those who were non-producers. In the Guangzhou Nutrition and Health Study[42] carried out in China, 2,818 participants were tracked and studied for 3 years, with 231 participants developing diabetes. The results showed that urinary equol was significantly associated with the suppression of type 2 diabetes development. A randomized comparison study in Japan involving 49 overweight or obese participants (average age 59.4 years) found significantly lower levels of HbA1c, serum LDL-C and cardio-ankle vascular index after 12 weeks of S-equol supplementation (10 mg/day)[43]. The results of the present study did not contradict these findings, and we conclude that equol production has a positive effect on glucose tolerance and lipid metabolism. However, in the Singapore Chinese Health Study[44], a case–control study with 1,128 participants, no distinct association was found between urinary equol concentration and the risk of developing type 2 diabetes in both men and women. In that study, the average age of the participants was 59.7 years, younger than those in the present study, and included approximately 40% premenopausal participants. It was concluded that the estrogen-like effects of equol are not stronger than that of endogenic estrogen, and the effect of equol on glucose tolerance perhaps becomes clear only after menopause.

Blood pressure and blood lipid levels are reported to be significantly lower in equol producers than in non-producers. In a cross-sectional study on 648 postmenopausal women who developed untreated hypertension complications, equol producers had significantly lower systolic blood pressure (131.6 ± 16.0 vs 135.1 ± 16.6 mmHg, $P = 0.015$) and diastolic blood pressure (79.4 ± 8.7 vs 83.1 ± 9.6 mmHg, $P = 0.010$) than that of non-producers[45]. In contrast, in a study involving 743 Japanese women, no significant differences in the systolic and diastolic blood pressures were found, although the equol producers had lower triglyceride levels ($P = 0.020$) than that of non-producers[46]. Estrogen accelerates the production of vasorelaxation factors, such as nitric oxide and prostaglandin I2, by affecting vascular endothelial cells, and has a hypotensive effect by inhibiting the production of endothelins, thereby strengthening the endothelium-dependent vasorelaxation reactions[47]. Furthermore, estrogen increases the number and activates LDL receptors in the liver and peripheral tissues, lowers blood LDL-C by inhibiting the activation of hepatic triglyceride lipase, and increases blood high-density lipoprotein cholesterol by promoting the synthesis of apolipoprotein A1 in the liver and small intestine[48–50]. The same results can possibly occur with equol, which possesses estrogen-like effects; however, this cannot be distinctly established from the results of previous clinical trials due to differences in the clinical backgrounds of the target cases. In the present study, the frequency of dyslipidemia was significantly lower in women who were equol producers than in those who were non-producers, although the frequency of hypertension was rather high. It is unclear why hypertension complications were prominent among women who were equol producers, although it could be because hypertension was self-reported in the present study, based on either a pre-existing diagnosis or current treatment of hypotensive medications. In patients with diabetes who excrete microalbumin in the urine, angiotensin-converting enzyme inhibitors and angiotensin II receptor antagonists are recommended for kidney protection, and in some cases, patients who are normotensive also receive these medications[51]. Thus, a re-study involving patients who are not administered hypotensive medications is necessary for truly considering the effects of equol production on blood pressure.

The present study had several limitations that need to be considered. First, the data used in this study were obtained from a cross-sectional research in which values were measured at one point in time. The number of equol producers is known to increase with age[32]. However, it is unclear when our study participants developed their equol production capabilities. Second, a survey on dietary content was not carried out in this study. Equol is produced when daidzein in soybean isoflavones is metabolized by enteric bacteria; thus, dietary content affects the production of equol. In instances of very low soybean consumption, the urinary equol concentration becomes low, and

determining the equol producers/non-producers becomes difficult. In this study, although we used the comparison rate between the urinary equol and daidzein concentrations to determine the equol producers among the participants, we could not determine the status of four participants owing to their low urinary daidzein concentrations. Third, we might have overestimated the prevalence of dyslipidemia. In the present study, the frequency of dyslipidemia was significantly lower in women who were equol producers than in those who were non-producers. We defined dyslipidemia as the presence of high serum LDL-C, triglyceride or treatment for dyslipidemia. However, statins are commonly used for secondary prevention of cardiovascular disease in patients with diabetes mellitus. Incidentally, more female equol non-producers might undergo medical treatment for dyslipidemia compared with producers. Finally, we did not assess the current medications used by participants in this study. Hypoglycemic medications, including the biguanides, alpha-glucosidase inhibitors, glucagon-like peptide-1 receptor agonists, dipeptidyl peptidase-4 inhibitors and thiazolidinediones, can affect the gut microbiota[52]. Not only hypoglycemic medications, but also several drugs, including antibiotics, laxatives and proton-pump inhibitors, might affect human gut microbiome. It is possible that these medications could influence equol-producing status.

Despite these limitations, the present study is among the first to compare the proportions of equol producers/non-producers among patients with type 2 diabetes and among individuals without diabetes from the same region. The proportion of equol producers among older women with type 2 diabetes was lower than that among female participants without diabetes, which suggests that equol positively affects glucose tolerance. In individuals who are able to produce equol, the blood equol concentration seems to increase with the consumption of soybean products. Hence, individuals without equol production capabilities may be able to achieve a positive metabolic effect on consuming S-equol supplements.

The present study findings showed that among postmenopausal women, the proportion of equol producers was significantly low in the type 2 diabetes group in comparison with the non-diabetes group, and the rate of dyslipidemia was significantly lower in women who were equol producers. Although we need to further consider methods that would enable similar positive effects for equol non-producers, including the use of S-equol supplements and enteric bacteria supplementation as options, we expect that soybean product consumption would result in the positive effects of equol in equol producers.

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DISCLOSURE

TU and SU are employees of Otsuka Pharmaceutical Co., Ltd. The other authors declare no conflict of interest.

Approval of the research protocol: This study was approved by the Hirosaki University School of Medicine Ethics Committee.
Informed consent: Written informed consent was obtained from all participants.
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