Lower intake of saturated fatty acids is associated with persistently higher arterial stiffness in patients with type 2 diabetes

Tomoya Mita1,2,*, Yuki Someya1,3, Yusuke Osonoi4, Takeshi Osonoi1,5, Miyoko Saito6, Shiho Nakayama4, Hidenori Ishida4, Hiroaki Satō1, Masahiko Gosho5, Hirotaka Watada1,2,3,6

1Department of Metabolism & Endocrinology, Juntendo University Graduate School of Medicine, Tokyo, Japan, 2Center for Molecular Diabetology, Juntendo University Graduate School of Medicine, Tokyo, Japan, 3Sportology Center, Juntendo University Graduate School of Medicine, Tokyo, Japan, 4Naka Kinen Clinic, Ibaraki, Japan, 5Department of Biostatistics, Faculty of Medicine, University of Tsukuba, Ibaraki, Japan, and 6Center for Therapeutic Innovations in Diabetes, Juntendo University Graduate School of Medicine, Tokyo, Japan

Keywords
Arterial stiffness, Dairy products, Saturated fatty acids

*Correspondence
Tomoya Mita
Tel: +81-3-5802-1579
Fax: +81-3-3813-5976
E-mail address: tom-m@juntendo.ac.jp

J Diabetes Investig 2021; 12: 226–233
doi: 10.1111/jdi.13347
Clinical Trial Registry
University Hospital Medical Information Network Clinical Trials Registry UMIN000010932

ABSTRACT
Aims/Introduction: There are few studies to investigate the relationship between macronutrients and longitudinal changes in arterial stiffness in patients with type 2 diabetes mellitus. This exploratory study sought to determine whether macronutrients were correlated with increased arterial stiffness independently of conventional atherosclerotic risk factors.

Materials and Methods: The study participants comprised 733 type 2 diabetes outpatients who had no apparent history of cardiovascular diseases. The dietary schedule was assessed with a validated, brief, self-administered diet history questionnaire. At baseline and at years 2 and 5, brachial-ankle pulse wave velocity was measured. A multivariable linear mixed-effects model was used to determine the predictive values of macronutrients and atherosclerotic risk factors for longitudinal changes in brachial-ankle pulse wave velocity.

Results: There was a significant increase in brachial-ankle pulse wave velocity values over the 5-year follow-up period. In a multivariable linear mixed-effects model that adjusted for age and sex, lower saturated fatty acid intake was significantly correlated with persistently higher brachial-ankle pulse wave velocity, independently of other atherosclerotic risk factors. Lower intake of dairy products in particular showed this correlation.

Conclusions: Our data showed that lower saturated fatty acids intake was correlated with persistently higher brachial-ankle pulse wave velocity in type 2 diabetes patients. Among food sources of saturated fatty acids, lower dairy products specifically were correlated with elevated brachial-ankle pulse wave velocity. This might be because the consumption of dairy products in Japan is much lower than in Western countries.

INTRODUCTION
Type 2 diabetes mellitus patients are at higher risk of cardiovascular disease (CVD)1. While lifestyle modification is a fundamental aspect of diabetes care, a recent clinical trial found that CVD in obese patients with type 2 diabetes was not decreased by lifestyle interventions focused either on calorie restriction, such as fat-restricted diets, or on increased physical activity2. With respect to this, greater attention should be paid to the quantity and/or quality of diets rather than to approaches based on calorie restriction.

Nutrition therapy is the most challenging part of lifestyle management for patients with type 2 diabetes. The American Diabetes Association’s current clinical practice guideline dealing with nutrition therapy recommends a balanced variety of foods with appropriate portion sizes to achieve metabolic goals and delay or prevent diabetic complications, such as CVD3. Regarding the general population in particular, the guideline recommends limiting the intake of saturated fatty acids (SFAs) and cholesterol, and consuming higher amounts of unsaturated fatty acids.
acids, such as polyunsaturated fatty acids (PUFAs) and/or monounsaturated fatty acids (MUFAs), to delay or prevent CVD. The Japan Atherosclerosis Society Guidelines make similar recommendations. In the general population, a previous meta-analysis showed that a reduction of SFA intake was associated with a 17% reduction in the risk of CVD, and replacing SFAs with PUFAs, but not MUFAs, was correlated with a 27% risk reduction. In that study, the degree of CVD risk reduction was associated with the degree of reduced SFA intake, and the degree of increased PUF and MUF intake. In contrast, another meta-analysis showed that a higher intake of SFAs was not associated with coronary heart disease. In addition, several cohort studies, but not all, showed an inverse relationship between the intake of SFAs and the risk of CVD. Thus, the association between the types and amounts of dietary fat consumed and the risk of CVD is a matter of debate in the general population. In contrast, patients with type 2 diabetes have altered carbohydrate and lipid metabolism, and an abnormal prothrombotic profile. Thus, recommendations should be largely based on findings from individuals with this condition. In this regard, a very recent study showed that lower total mortality and CVD-related mortality in patients with type 2 diabetes was associated with higher PUFA intake, but was not correlated with carbohydrate or SFA intake. However, the association between macronutrients, including specific dietary fats and their food sources, and cardiovascular health remains largely unclarified in patients with type 2 diabetes.

Type 2 diabetes patients have increased arterial stiffness. The brachial-ankle pulse wave velocity (baPWV) is a non-invasive, convenient technique to assess arterial stiffness, and it serves as a surrogate marker for CVD in patients with type 2 diabetes. Recent cross-sectional studies showed that increased arterial stiffness was related to conventional risk factors in patients with type 2 diabetes. Conversely, a cross-sectional study showed that a diet rich in carbohydrates and MUFAs was correlated with reduced arterial stiffness in patients with type 2 diabetes. However, little is known about the association between other macronutrients, including specific dietary fats, and longitudinal changes in arterial stiffness in this population.

As it is highly beneficial to identify modifiable risk factors for atherosclerosis, we carried out an exploratory study to assess the association between dietary macronutrients and longitudinal changes in arterial stiffness in type 2 diabetes patients who were free of apparent CVD. Our goal was to gain a better understanding of the optimal types and proportions of dietary nutrients for maintaining cardiovascular health.

METHODS

Study participants

The participants were enrolled from the outpatient clinic of three medical institutions, as previously described. The study design, and inclusion and exclusion criteria were published previously. The inclusion criteria were: (i) diagnosis of type 2 diabetes; (ii) aged ≥25 and < 70 years; and (iii) provision of written informed consent for study participation. The exclusion criteria were: (i) type 1 or secondary diabetes; (ii) presence of severe infectious disease or severe trauma before or after surgery; (iii) history of myocardial infarction, angina pectoris, cerebral stroke or cerebral infarction; (iv) chronic renal failure requiring hemodialysis; (v) liver cirrhosis; (vi) moderate or severe heart failure (New York Heart Association stage III or higher); and (vii) active malignancy and so on. Among 736 outpatients enrolled between June 2013 and January 2014, two patients withdrew their consent.

The protocol of this study was approved by the ethics committee of Juntendo University Hospital. It conformed to the provision of the Declaration of Helsinki. Written informed consent was obtained from all participants. The study was registered on the University Hospital Medical Information Network Clinical Trials Registry (UMIN000010932).

Questionnaire survey

Each patient’s dietary schedule during the month preceding the initiation of the study was assessed with the validated brief-type self-administered diet history questionnaire (BDHQ). Daily caloric intake was estimated using an ad hoc computer algorithm for the 56 foods and beverages. The BDHQ has good validity and test–retest reliability.

Physical activity level was assessed with the International Physical Activity Questionnaire.

Data collection

Blood and urine samples were collected from participants once per year. Glycated hemoglobin (HbA1c), lipid levels, and renal and liver function tests were measured with standard techniques.

Measurement of baPWV

At baseline and at 2 and 5 years, baPWV was measured with use of an automatic waveform analyzer (BP-203RPE form; Colin Medical Technology, Komaki, Japan), as described previously. Briefly, measurement was carried out in the supine position after 5-min bed rest. Cuffs for occlusion and monitoring were placed snugly around both arms and both ankles. The pressure waveforms were then recorded simultaneously from the brachial arteries by the oscillometric method. All scans were carried out by well-trained observer in each institution. A previous study confirmed the high reproducibility of baPWV measurements. Participants with an ankle-brachial index ≤0.90 were considered to have peripheral artery disease, and the baPWV data of these individuals were excluded from this study.

Statistical analysis

Results are expressed as the mean ± standard deviation for continuous variables, and as the number (proportion) for categorical variables. Longitudinal baPWV was analyzed using a
linear mixed-effects model that included time, patient characteristics, dietary schedule and possible atherosclerotic risk factors at baseline as fixed effects, and patient as a random effect to account for the inherent correlation of repeated measures on the same individual over time. We added the interaction term between time and each covariate in the mixed-effects model. The correlation between SFAs and possible food sources of SFAs was evaluated using Spearman’s correlation coefficients. Statistical tests were two-sided, with a 5% significance level. All analyses were carried out using SAS software version 9.4 (SAS Institute, Cary, NC, USA).

RESULTS
Patients characteristics
The data of 734 patients at baseline were available. A total of 99 patients were lost to follow up, as previously described. One patient did not complete the BDHQ. Accordingly, the baseline characteristics of the remaining 733 type 2 diabetes patients are shown in Table 1. The mean total energy intake was 1,719 kcal/day, and the mean percentages of total energy intake from carbohydrates, proteins, fats, SFAs, MUFAs and PUFAs were 53%, 15%, 23%,7%, 9% and 6%, respectively.

Table 1 | Patient demographic and background characteristics at baseline

| Age (years) | Baseline | 57.8 ± 8.6 |
| Male (%) | | 463 (63.1) |
| Body mass index (kg/m²) | | 24.6 ± 4.0 |
| Systolic blood pressure (mmHg) | | 127 ± 14 |
| Diastolic blood pressure (mmHg) | | 77 ± 10 |
| HbA1c (%) | | 7.0 ± 1.0 |
| HbA1c (mmol/mol) | | 52.6 ± 10.9 |
| Total cholesterol (mg/dL) | | 185 ± 28 |
| HDL cholesterol (mg/dL) | | 59 ± 14 |
| Triglyceride (mg/dL) | | 125 ± 91 |
| Uric acid (mg/dL) | | 55 ± 1.2 |
| Urinary albumin excretion (mg/g creatinine) | | 69 ± 233 |
| Insulin therapy (n%) | | 80 (11) |
| Hypotensive drugs (n%) | | 348 (47.7) |
| Lipid-lowering drugs (n%) | | 444 (60.9) |
| Antplatelet agents (n%) | | 24 (3.3) |
| Physical activity (METs·h/week) | | 437 ± 73.9 |
| Current smoker (yes) | | 177 (24.1) |
| Alcohol consumption (g/day) | | 124 ± 21.6 |
| Total caloric intake (kcal/day) | | 1,719 ± 585 |
| Carbohydrate intake (g) | | 226 ± 76 |
| Carbohydrate intake (% energy) | | 53 ± 9 |
| Protein intake (g) | | 666 ± 29.3 |
| Protein intake (% energy) | | 15 ± 4 |
| Fat intake (g) | | 487 ± 204 |
| Fat intake (% energy) | | 25 ± 6 |
| SFA intake (g) | | 128 ± 6.1 |
| SFA intake (% energy) | | 7 ± 2 |
| MUFA intake (g) | | 172 ± 8.1 |
| MUFA intake (% energy) | | 9 ± 2 |
| PUFA intake (g) | | 124 ± 5.4 |
| PUFA intake (% energy) | | 6 ± 2 |
| baPWV (cm/s) | | 1545 ± 280 |

Total n = 733. Data are the mean ± standard deviation or the number of patients. P-values are derived using a mixed-effects model with repeated measures. % energy, the percentage of estimated daily total energy intake; baPWV, brachial-ankle pulse wave velocity; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; MUFA, monounsaturated fatty acid; PUFAs, polyunsaturated fatty acid; SFA, saturated fatty acid.

Relationships between longitudinal baPWV and saturated fatty acid
We used a linear mixed-effects model to examine the relationship between longitudinal baPWV, which was adjusted for age and sex, and each dietary nutrient. There was no significant interaction between time and each nutrient for longitudinal baPWV throughout the study period (Table 2 and data not shown). However, this analysis showed that a lower percentage of total calories from SFAs was significantly correlated with persistent higher baPWV (Table 2). Other nutrients were not significantly correlated with persistent higher baPWV (data not shown).

Next, we investigated whether dietary nutrients in type 2 diabetes patients were correlated with persistent increased arterial stiffness independently of conventional atherosclerotic risk factors. In a multivariable linear mixed-effects model that included both dietary nutrients and conventional atherosclerotic risk factors, a lower percentage of total calories from SFAs was still an independent predictive factor for persistently higher baPWV, in addition to older age, longer estimated duration of type 2 diabetes, lower BMI, higher systolic BP, lower high-density lipoprotein level, lower urinary albumin excretion and use of antiplatelet agents (Table 3).

We divided participants into three groups based on tertiles of the percentage of total calories from SFAs to further investigate their relationships. When replacing continuous variables with categorical variables, participants who consumed ≥7.4% of total calories from SFAs had persistent lower baPWV than those who consumed <5.8% of total calories from SFAs and those who consumed ≥5.8% and <7.4% of total calories from SFAs in the multivariable linear mixed-effects model (Table S1). These data might suggest linear relationships between saturated fatty acids intake and changes in baPWV.

Relationships between longitudinal baPWV and dairy products
Possible food sources of SFAs include dairy products, meats, processed meats, eggs, sweets, butter and nuts. However, as in
a general Japanese population, the study patients consumed almost no butter or nuts (Table 4). We evaluated the correlations between the baseline intakes of SFAs and their possible food sources, and found correlations between SFAs and each of the aforementioned food sources, excluding butter and nuts (Table 4). Next, we investigated whether different food sources of SFAs were correlated with persistent increased arterial stiffness independent of conventional atherosclerotic risk factors. Lower intake of dairy products was an independent predictive factor for persistently higher baPWV, along with older age, longer estimated duration of type 2 diabetes, higher HbA1c level, lower BMI, higher systolic BP, higher uric acid level, lower urinary albumin excretion and use of antiplatelet agents (Table 5).

Table 2 | Relationships between longitudinal brachial-ankle pulse wave velocity adjusted for age and sex, and saturated fatty acid (%) using a linear mixed-effects model

| Effect                  | Level               | Estimate (Standard error) | P value |
|-------------------------|---------------------|---------------------------|---------|
| Intercept               |                     | 778.4 (74.2)              | <0.001  |
| Time                    | 1 year              | 203 (5.8)                 | 0.0005  |
| SFA intake (% energy)   | 1 unit              | −1,614.1 (520.8)          | 0.002   |
| Time × SFA intake (%)   |                     | 406.8 (85.3)              | 0.63    |
| Age                     | 1 year              | 148.1 (1.1)               | <0.001  |
| Sex                     | Male vs female      | 243.1 (18.9)              | 0.20    |

The longitudinal brachial-ankle pulse wave velocity was analyzed with a linear mixed-effects model including time, saturated fatty acid (SFA), their interaction, and age and sex at baseline as fixed effects, and patient as a random effect. % energy, the percentage of estimated daily total energy intake.

Table 3 | Predictors of longitudinal brachial-ankle pulse wave velocity during 5-year follow up

| Comparison                                             | Regression coefficient | P-value |
|--------------------------------------------------------|------------------------|---------|
| Intercept                                              | 227.7                  | 0.12    |
| Time 1 year                                            | 20.000                 | 0.0001  |
| SFA intake (% energy) 1 unit                           | −1163.1                | 0.02    |
| Time × SFA intake (%) 1 unit                           | 49.0                   | 0.57    |
| Age 1 year                                            | 12.5                   | <0.0001 |
| Sex Male vs female                                     | −4.49                  | 0.82    |
| Physical activity                                      | 1 METs/h/week          | 0.42    |
| Current smoker                                         | Former smoker vs Current smoker | −6.7 | 0.78    |
| Estimated duration of diabetes 1 year                  | 3.0                    | 0.02    |
| Body mass index 1 kg/m²                                 | −12.5                  | <0.0001 |
| Systolic BP 5 mmHg                                      | 0.6                    | <0.0001 |
| Hba1c 1 mmol/mol                                       | 1.9                    | 0.03    |
| Total cholesterol 1 mg/dL                              | 0.5                    | 0.12    |
| HDL cholesterol 1 mg/dL                                | −1.4                   | 0.05    |
| Triglyceride: log-transformed value 1 mg/dL             | 0.2                    | 0.12    |
| Insulin therapy                                        | 12.3                   | 0.68    |
| Antihypertension drugs                                 | −3.9                   | 0.83    |
| Antihyperlipidemia drugs                               | −22.9                  | 0.19    |
| Antithrombotic drugs                                   | 108.1                  | 0.02    |
| Diabetic retinopathy                                   | 166                    | 0.37    |
| Diabetic neuropathy                                    | −23.0                  | 0.24    |
| Urinary albumin excretion mg/g creatinine              | −34.9                  | 0.05    |
| Uric acid 1 mg/dL                                      | 223.2                  | 0.0004  |

Longitudinal brachial-ankle pulse wave velocity was analyzed with a linear mixed-effects model using time and lifestyle habits, and possible atherosclerotic risk factors at baseline as fixed effects, and patient as a random effect. % energy, the percentage of estimated daily total energy intake; BP, blood pressure; HDL, high-density lipoprotein; SFA, saturated fatty acid.
DISCUSSION

This might be the first prospective longitudinal study to investigate the intake of SFAs or their possible food sources in relation to arterial stiffness in type 2 diabetes patients. We showed that lower intake of SFAs, particularly in the form of dairy products, correlated with persistent higher baPWV in type 2 diabetes patients, in this exploratory study.

Higher intake of SFAs is considered to undermine cardiovascular health, because these compounds negatively affect cholesterol metabolism. Impaired health of the vascular wall is an important contributor to CVD. Indeed, published guidelines recommend limiting the intake of SFAs to delay or prevent CVD.

In contrast, Japanese type 2 diabetes patients appear to consume far lower amounts of SFAs than American patients. The National Health and Nutrition Examination Survey in 2017 reported that Japanese people aged ≥20 years consumed approximately 17.6 g of SFAs per day. The consumption of SFAs in the present study was slightly lower, at 12.8 g per day, but was very similar to that observed in patients with type 2 diabetes in other studies. The Fukuoka Diabetes Registry used the BDHQ questionnaire to determine that patients with type 2 diabetes consumed approximately 12.3 g per day. In addition, the Japan Diabetes Complications Study reported that SFA intake was likely to be approximately 8 g per day according to the Food Frequency Questionnaire. Thus, the amount of daily SFA intake in Japanese patients with type 2 diabetes is likely to be lower than that in the general population. The exact reason for this difference is currently unclear.

Table 4 | Correlation between saturated fatty acid and possible food sources of saturated fatty acids at baseline

| Food Source            | g/day (mean ± SD) | Correlation coefficient (95% confidence interval) | P-value |
|------------------------|-------------------|---------------------------------------------------|---------|
| Dairy products         | 118 ± 98          | 0.59 (0.54–0.63)                                  | <0.001  |
| Meats                  | 52.2 ± 39.9       | 0.54 (0.49–0.59)                                  | <0.001  |
| Processed meats        | 8.4 ± 8.8         | 0.47 (0.41–0.52)                                  | <0.001  |
| Eggs                   | 30.0 ± 24.4       | 0.41 (0.35–0.47)                                  | <0.001  |
| Sweets                 | 37.1 ± 35.8       | 0.47 (0.42–0.53)                                  | <0.001  |
| Butter                 | 0 ± 0             |                                                  |         |
| Nuts                   | 0 ± 0             |                                                  |         |

Total (n = 733). By Spearman’s rank correlation analysis. CI, confidence interval.

Table 5 | Predictors of longitudinal brachial-ankle pulse wave velocity during 5-year follow up

| Predictor                                      | Regression coefficient | P-value |
|------------------------------------------------|------------------------|---------|
| Intercept                                      | 179.1                  | 141.1   | 0.204  |
| Time 1 year                                    | 21.2                   | 2.48    | <0.0001|
| Dairy products intake g                        | −0.28                  | 0.10    | 0.006  |
| Time × dairy product intake                    | 0.02                   | 0.07    | 0.30   |
| Age 1 year                                     | 13.0                   | 1.13    | <0.0001|
| Sex Male vs female                             | 5.1                    | 220.0   | 0.83   |
| Physical activity 1 METs/h/week                | −0.07                  | 0.12    | 0.57   |
| Current smoker Former smoker vs current smoker | −5.75                  | 23.7    | 0.81   |
| Never-smoker vs current smoker                 | −8.60                  | 23.1    | 0.71   |
| Estimated duration of diabetes 1 year          | 3.11                   | 1.28    | 0.016  |
| Body mass index 1 kg/m²                        | −13.1                  | 2.51    | <0.0001|
| Systolic BP 1 mmHg                             | 5.70                   | 0.63    | <0.0001|
| HbA1c 1 mmol/mol                               | 1.84                   | 0.86    | 0.03   |
| Total cholesterol 1 mg/dL                      | 0.46                   | 0.35    | 0.19   |
| HDL cholesterol 1 mg/dL                       | −1.19                  | 0.73    | 0.10   |
| Triglyceride 1 mg/dL                           | 0.21                   | 0.12    | 0.07   |
| Insulin therapy Yes vs no                      | 9.77                   | 29.2    | 0.74   |
| Antihypertension drugs Yes vs no               | −2.55                  | 17.7    | 0.88   |
| Antihyperlipidemia drugs Yes vs no             | −24.8                  | 17.5    | 0.16   |
| Antiplatelet drugs Yes vs no                   | 112.7                  | 47.2    | 0.02   |
| Diabetic retinopathy Yes vs no                 | 14.8                   | 18.4    | 0.42   |
| Diabetic neuropathy Yes vs no                  | −24.25                 | 19.5    | 0.21   |
| Urinary albumin excretion mg/g creatinine      | −36.2                  | 18.1    | 0.045  |
| Uric acid 1 mg/dL                              | 22.3                   | 7.7     | 0.004  |
| Total caloric intake 1 kcal                    | −0.01                  | 0.02    | 0.55   |

Longitudinal brachial-ankle pulse wave velocity was analyzed with a linear mixed-effects model using time and dairy product intake at baseline as fixed effects, and patient as a random effect. BP, blood pressure; HDL, high-density lipoprotein.
possibility is that patients with type 2 diabetes pay more attention to their SFA intake as a result of their healthcare providers’ advice. Also, the diet histories of overweight individuals might underreport SFA intake39, as patients with type 2 diabetes have been shown to have a higher BMI than the general population in Japan.

Intriguingly, a cohort study carried out in Japan showed that SFA intake was inversely correlated with the incidence of lacunar infarction and deep intraparenchymal hemorrhage in a Japanese general population31. In that study, participants had a relatively low SFA intake averaging 16.3 g per day. The authors found that SFA intake of approximately 20 g per day was the threshold for the inverse association between SFA intake and stroke. Consistent with this finding, the SFA intake in the present study participants was 12.8 g per day, and we showed that a lower percentage of calories from SFAs was associated with a persistently higher baPWV. In addition, we found the linear relationships between saturated fatty acids intake and changes in baPWV (Table S1). This result might be due largely to relatively low SFA intake averaging 17.0 g per day, even in highest tertile group.

It is possible that the association between lower SFA intake and persistently higher baPWV partly depends on the food sources of SFAs. Interestingly, of the several food sources we examined, only dairy products were associated with persistently higher baPWV independently of other possible risk factors for atherosclerosis. The consumption of dairy products has been considered to be detrimental to cardiovascular health, because their SFA content might increase low-density lipoprotein cholesterol levels32. However, one of two recent studies showed an inverse relationship between dairy product intake and arterial stiffness in the general population33,34. Furthermore, recent studies, but not all, showed that dairy product intake was inversely associated with the risk of CVD and mortality in the general population35-37. In contrast, there are limited data on whether type 2 diabetes patients who are susceptible to CVD1 are more likely to develop atherosclerosis if they consume fewer dairy products, which is the case in the general population. In this regard, this is the first longitudinal study to show that lower intake of dairy products was correlated with persistent higher baPWV in type 2 diabetes patients. This association seemed to be affected by the basal intake of dairy products. Indeed, compared with no intake of dairy products, consumption of more than two servings per day was associated with a lower risk of mortality and CVD in low- and middle-income countries where dairy product consumption is low36. As dairy product consumption in Japan is much lower than that in Western countries38,39, increasing the amount of SFA intake from dairy products might not be detrimental in terms of preventing the progression of atherosclerosis in Japan.

Although it remains largely unknown how lower intake of dairy products induces arterial stiffness, we propose the following possibilities. First, bioactive peptides, such as casein-derived casokinins and whey-derived lactokinins, are capable of inhibiting the action of angiotensin-converting enzyme40, therefore reducing BP and increasing endothelium-dependent vasorelaxation. In addition, recent studies reported that intake of dairy products had a beneficial effect on BP41,42. This effect might explain why a higher intake of dairy products has a beneficial effect on arterial stiffness. In fact, lower intake of dairy products was modestly, but significantly, correlated with higher systolic BP in the present study (r = -0.10, P = 0.006). Second, dairy products are rich in magnesium, potassium and calcium. These minerals might have an impact on BP and arterial stiffness through their effects on vasodilator production43. Ultimately, the combined effects of a variety of biologically active components are likely to underlie the mechanism by which lower intake of dairy products induces arterial stiffness, as dairy products are recognized as providing a broad spectrum of essential nutrients for human health44.

There were certain limitations of the present study. First, the observational cohort study design made it impossible to evaluate whether dietary habits had a causal relationship with arterial stiffness. Second, we evaluated dietary intake by self-reported questionnaires, although this method has been successfully used in many studies. The results might have been influenced by social desirability and recall bias. Although weighed food records are more accurate for assessing an individual’s diet, they are not practical in studies with large sample sizes because of the need for extensive training of the participants. We evaluated just 56 food and beverage items, which might have led to an underestimation of energy intake. Thus, we used energy-adjusted nutrition intake to deal with this potential confounder44. In addition, we did not investigate whether specific dairy products, such as milk, cheese or yogurt, were associated with the progression of arterial stiffness, although we did find that lower intake of dairy products overall was associated with persistently higher baPWV. Third, we carried out analysis using dietary intake data collected only at baseline. In the future study, the changes in dietary intake when investigating the relationship between macronutrients and atherosclerosis should be considered. At least, there were no major changes in intake of each micronutrient at 2 years from baseline (Table S2). Fourth, although we adjusted for several atherosclerotic risk factors including BP, residual confounding factors, such as changes in BP and HbA1c over time, could not be ruled out. Finally, our findings could be applied to Japanese patients with type 2 diabetes, as there are regional and race differences in dietary consumption patterns and macronutrient intakes.

In conclusion, the present data showed that lower intake of SFAs, and particularly reduced consumption of dairy products, was correlated with persistent higher baPWV in Japanese type 2 diabetes patients. However, consuming very high amounts of SFAs should be avoided, as SFA intake was shown to be positively correlated with the onset of myocardial infarction31. The present data suggest that too great a restriction on the intake of SFAs might have a negative effect on CVD in Japanese type 2 diabetes patients. In addition, the present
results show that different food sources of SFAs might have varying effects on cardiovascular health.

ACKNOWLEDGMENTS
The authors thank all medical staff. This study was funded by the Manpei Suzuki Diabetes Foundation (to TM).

DISCLOSURE
The authors declare no conflict of interest.

REFERENCES
1. Haffner SM, Lehto S, Ronnemaa T, et al. Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. New Engl J Med 1998; 339: 229–234.
2. Look ARG, Wing RR, Bolin P, et al. Cardiovascular effects of intensive lifestyle intervention in type 2 diabetes. New Engl J Med 2013; 369: 145–154.
3. Evert AB, Dennison M, Gardner CD, et al. Nutrition therapy for adults with diabetes or prediabetes: a consensus report. Diabetes Care 2019; 42: 731–754.
4. Kinoshita M, Yokote K, Arai H, et al. Japan atherosclerosis society (JAS) guidelines for prevention of atherosclerotic cardiovascular diseases 2017. J Atheroscler Thromb 2018; 25: 846–984.
5. Hooper L, Martin N, Abdelhamid A, et al. Reduction in saturated fat intake for cardiovascular disease. Cochrane Database Syst Rev 2015; CD011737.
6. Mozaffarian D, Micha R, Wallace S. Effects on coronary heart disease of increasing polyunsaturated fat in place of saturated fat: a systematic review and meta-analysis of randomized controlled trials. PLoS Medicine 2010; 7: e1000252.
7. Guasch-Ferre M, Babio N, Martinez-Gonzalez MA, et al. Dietary fat intake and risk of cardiovascular disease and all-cause mortality in a population at high risk of cardiovascular disease. Am J Clin Nutr 2015; 102: 1563–1573.
8. Blekkenhorst LC, Prince RL, Hodgson JM, et al. Dietary saturated fat intake and atherosclerotic vascular disease mortality in elderly women: a prospective cohort study. Am J Clin Nutr 2015; 101: 1263–1268.
9. Siri-Tarino PW, Sun Q, Hu FB, et al. Meta-analysis of prospective cohort studies evaluating the association of saturated fat with cardiovascular disease. Am J Clin Nutr 2010; 91: 535–546.
10. Jiao J, Liu G, Shin HJ, et al. Dietary fats and mortality among patients with type 2 diabetes: analysis in two population based cohort studies. BMJ 2019; 366: i4009.
11. Taniwaki H, Kawagishi T, Emoto M, et al. Correlation between the intima-media thickness of the carotid artery and aortic pulse-wave velocity in patients with type 2 diabetes. Vessel wall properties in type 2 diabetes. Diabetes Care 1999; 22: 1851–1857.
12. Nakamura M, Yamashita T, Yajima J, et al. Brachial-ankle pulse wave velocity as a risk stratification index for the short-term prognosis of type 2 diabetic patients with coronary artery disease. Hypertension Res 2010; 33: 1018–1024.
13. Maeda Y, Inoguchi T, Etoh E, et al. Brachial-ankle pulse wave velocity predicts all-cause mortality and cardiovascular events in patients with diabetes: the kyushu prevention study of atherosclerosis. Diabetes Care 2014; 37: 2383–2390.
14. Ferreira MT, Leite NC, Cardoso CR, et al. Correlates of aortic stiffness progression in patients with type 2 diabetes: Importance of glycemic control: The rio de janeiro type 2 diabetes cohort study. Diabetes Care 2015; 38: 897–904.
15. Fukui M, Tanaka M, Shiraishi E, et al. Serum uric acid is associated with microalbuminuria and subclinical atherosclerosis in men with type 2 diabetes mellitus. Metab Clin Exp 2008; 57: 625–629.
16. Vaccaro JA, Huffman FG. Monounsaturated fatty acid, carbohydrate intake, and diabetes status are associated with arterial pulse pressure. Nutr J 2011; 10: 126.
17. Osonoi Y, Mita T, Osonoi T, et al. Morningness-eveningness questionnaire score and metabolic parameters in patients with type 2 diabetes mellitus. Chronobiol Int 2014; 31: 1017–1023.
18. Osonoi Y, Mita T, Osonoi T, et al. Poor sleep quality is associated with increased arterial stiffness in japanese patients with type 2 diabetes mellitus. BMC End Dis 2015; 15: 29.
19. Osonoi Y, Mita T, Osonoi T, et al. Relationship between dietary patterns and risk factors for cardiovascular disease in patients with type 2 diabetes mellitus: a cross-sectional study. Nutr J 2016; 15: 15.
20. Mita T, Osonoi Y, Osonoi T, et al. Breakfast skipping is associated with persistently increased arterial stiffness in patients with type 2 diabetes. BMJ Open Diabet Res Care 2020; 8: e001162.
21. Agency SaT. Standard tables of food composition in japan (in japanese). Tokyo. Printing Bureau of the Ministry of Finance. 2005:5th rev ed.
22. Kobayashi S, Murakami K, Sasaki S, et al. Comparison of relative validity of food group intakes estimated by comprehensive and brief-type self-administered diet history questionnaires against 16 d dietary records in japanese adults. Public Health Nut 2011; 14: 1200–1211.
23. Bellard S, Coudert M, Valero R, et al. Validation of a short food frequency questionnaire to evaluate nutritional lifestyles in hypercholesterolemic patients. Ann Endocrinol 2012; 73: 523–529.
24. Craig CL, Marshall AL, Sjostrom M, et al. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc 2003; 35: 1381–1395.
25. Yamashina A, Tomiyama H, Takeda K, et al. Validity, reproducibility, and clinical significance of noninvasive
brachial-ankle pulse wave velocity measurement. 

Hypertension Res 2002; 25: 359–364.

26. Lordan R, Tsoupras A, Mitra B, et al. Dairy fats and cardiovascular disease: Do we really need to be concerned? 
Foods 2018; 7: 29

27. Ministry of Health LaW. The national health and nutrition survey in japan 2017. 2017. (in japanese) https://www.mhlw.go.jp/stf/newpage_08789.html.

28. Fujii H, Iwase M, Ohkuma T, et al. Impact of dietary fiber intake on glycemic control, cardiovascular risk factors and chronic kidney disease in Japanese patients with type 2 diabetes mellitus: The fukuoka diabetes registry. 
Nutr J 2013; 12: 159.

29. Horikawa C, Kamada C, Tanaka S, et al. Meat intake and incidence of cardiovascular disease in Japanese patients with type 2 diabetes: Analysis of the Japan diabetes complications study (jdc). 
Eur J Nutr 2019; 58: 281–290.

30. Martin GS, Tapsell LC, Batterham MJ, et al. Relative bias in diet history measurements: A quality control technique for dietary intervention trials. 
Public Health Nutr 2002; 5: 537–545.

31. Yamagishi K, Iso H, Kokubo Y, et al. Dietary intake of saturated fatty acids and incident stroke and coronary heart disease in Japanese communities: The jphc study. 
Eur Heart J 2013; 34: 1225–1232.

32. Artaud-Wild SM, Connor SL, Sexton G, et al. Differences in coronary mortality can be explained by differences in cholesterol and saturated fat intakes in 40 countries but not in France and Finland. 
A paradox. Circulation 1993; 88: 2771–2779.

33. Ribeiro AG, Mill JG, Cade NV, et al. Associations of dairy intake with arterial stiffness in Brazilian adults: The Brazilian longitudinal study of adult health (ELSA-Brasil). 
Nutrients 2018; 10: 701

34. Diez-Fernandez A, Alvarez-Bueno C, Martinez-Vizcaino V, et al. Total dairy, cheese and milk intake and arterial stiffness: a systematic review and meta-analysis of cross-sectional studies. 
Nutrients 2019; 11: 741.

35. Wang H, Fox CS, Troy LM, et al. Longitudinal association of dairy consumption with the changes in blood pressure and the risk of incident hypertension: The framingham heart study. 
British J Nutr 2015; 114: 1887–1899.

36. Dehghan M, Mente A, Ranganrajan S, et al. Association of dairy intake with cardiovascular disease and mortality in 21 countries from five continents (pure): a prospective cohort study. 
Lancet 2018; 392: 2288–2297.

37. Tognon G, Nilsson LM, Shungin D, et al. Nonfermented milk and other dairy products: associations with all-cause mortality. 
Am J Clin Nutr 2017; 105: 1502–1511.

38. Nanni A, Mizoue T, Shimazu T, et al. Dietary patterns and all-causes, cancer, and cardiovascular disease mortality in Japanese men and women: The Japan public health center-based prospective study. 
PloS One 2017; 12: e0174848.

39. Tognon G, Rothenberg E, Petrolo M, et al. Dairy product intake and mortality in a cohort of 70-year-old swedes: a contribution to the nordic diet discussion. 
Eur J Nutr 2018; 57: 2869–2876.

40. FitzGerald RJ, Murray BA, Walsh DJ. Hypotensive peptides from milk proteins. 
J Nutr 2004; 134: 980S–988S.

41. Azadbakht L, Mirmiran P, Esmailizadeh A, et al. Beneficial effects of a dietary approach to stop hypertension eating plan on features of the metabolic syndrome. 
Diabetes Care 2005; 28: 2823–2831.

42. Kris-Etherton PM, Greiger JA, Hilpert KF, et al. Milk products, dietary patterns and blood pressure management. 
J Am Coll Nutr 2009; 28(Suppl 1): 103S–119S.

43. Lovegrove JA, Hobbs DA. New perspectives on dairy and cardiovascular health. 
Proc Nutr Soc 2016; 75: 247–258.

44. Wakai K. A review of food frequency questionnaires developed and validated in Japan. 
J Epidemiol 2009; 19: 1–11.

**SUPPORTING INFORMATION**

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

**Table S1 |** Predictors of longitudinal brachial-ankle pulse wave velocity during 5-year follow up.

**Table S2 |** Macronutrients intake of each group at baseline and 2-year follow up.