# Vascular function and atherosclerosis progression after 1 y of flavonoid intake in statin-treated postmenopausal women with type 2 diabetes: a double-blind randomized controlled trial[1–3]

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## ABSTRACT

**Background:** In healthy participants, short-term flavan-3-ol and isoflavone intakes improve vascular function; however, the potential combined benefit of these compounds on atherosclerosis progression remains unclear for those at elevated risk of cardiovascular disease.

**Objective:** The objective was to examine whether combined isoflavone and flavan-3-ol intake alters vascular function in postmenopausal women with type 2 diabetes mellitus (T2DM).

**Design:** A double-blind, parallel-design, placebo-controlled 1-y trial was conducted in postmenopausal T2DM patients randomly assigned to a split dose of 27 g flavonoid-enriched chocolate/d [850 mg flavan-3-ols (90 mg epicatechin) + 100 mg isoflavones (aglycone equivalents)/d] or matched placebo. Intima-media thickness of the common carotid artery (CCA-IMT), pulse wave velocity (PWV), augmentation index, blood pressure (BP), and vascular biomarkers were assessed.

**Results:** A total of 93 patients completed the trial. Overall, the flavonoid intervention did not significantly change CCA-IMT, augmentation index, or BP, but pulse pressure variability improved (flavonoid: $-0.11 \pm 0.07$ mm Hg/min; placebo: $0.10 \pm 0.11$ mm Hg/min; $P = 0.04$). In a subgroup with PWV data, net improvements were observed [flavonoid ($n = 18$): $-0.07 \pm 0.38$ m/s; placebo ($n = 17$): $0.68 \pm 0.25$ m/s; $P = 0.01$], which equated to a 10% CV risk reduction. Equol producers ($n = 17$) had larger reductions in diastolic BP, mean arterial pressure, and PWV ($-2.24 \pm 1.31$ mm Hg, $-1.24 \pm 1.30$ mm Hg, and $-0.68 \pm 0.40$ m/s, respectively; $P < 0.01$) compared with non–equol producers ($n = 30$).

**Conclusions:** Although the 1-y intervention did not change CCA-IMT or BP, clinically relevant improvements in arterial stiffness were observed; equol producers were particularly responsive. Flavonoids may augment existing therapeutic strategies to reduce cardiovascular disease risk in postmenopausal T2DM patients, and longer studies are needed to examine the effects on atherosclerosis progression. This trial was registered at clinicaltrials.gov as NCT00677599. *Am J Clin Nutr* doi: 10.3945/ajcn.112.043745.

## INTRODUCTION

Type 2 diabetes mellitus (T2DM)[4] accounts for 7% of global all-cause mortality (1), is associated with a greater risk of cardiovascular disease (CVD) (2), and is associated with a greater vascular burden for age in women than in men (2). Accelerated atherosclerosis progression in T2DM, as evidenced by increased intima-media thickness of the common carotid artery (CCA-IMT) (3), coronary plaque (4), endothelial dysfunction (5), and vascular function (3), is compounded by comorbidities of T2DM such as

hypertension (6), which doubles the CVD risk (7). Whereas standard T2DM therapies ameliorate CVD risk (8), additional strategies, including optimizing the intake of dietary constituents, are required to reduce CVD risk.

Higher intakes of dietary flavonoids, particularly flavan-3-ols from cocoa, have been inversely associated with atherosclerosis progression (9) or plaque development (10); however, evidence from randomized controlled trials is limited. One long-term isoflavone study (2.7 y) observed no effect on CCA-IMT in healthy postmenopausal subjects (11), and additional long-term studies, particularly for flavan-3-ols and in subjects at elevated CVD risk, are required. In vitro and animal data suggest favorable and potentially complimentary effects of flavan-3-ols and isoflavones on vascular function—including effects of flavan-3-ols on nitric oxide (NO) (12) and NADPH oxidase activity (13) and reduced vascular inflammation after isoflavone intervention, potentially mediated via the cyclic AMP/protein kinase A pathway (14). Despite this, no randomized controlled trial has assessed the combined effects of these compounds on hemodynamic function and vascular health.

In healthy subjects, short-term flavan-3-ol and isoflavone interventions improved endothelial function and peripheral blood pressure (BP) (15) and reduced arterial stiffness (16), and a longer-term isoflavone intervention improved BP in healthy populations (17). However, few studies have assessed the effects of flavonoids on central BP, which has greater predictive value for CVD risk than

does peripheral BP (18), and the effect of isoflavone and flavan-3-ol intake on vascular function in T2DM has been equivocal. Whereas recent studies have shown that isoflavones improve endothelial function and BP after 2 mo (19) and flavan-3-ols increase acute and chronic endothelial function after 1 mo (20), previous studies have observed no effect on BP in T2DM patients (after 1–3 mo of intervention; 20–22). These differences in vascular response to isoflavones may, in part, be attributable to interindividual differences in metabolism; specifically, in vitro data (23, 24) and retrospective analysis of randomized controlled trials (25) have identified equol, a metabolite of the isoflavone daidzein, present in ~20–30% of the population (25), as being important for vascular function. Despite this, the association between vascular function and the equol producer phenotype remains under studied.

We examined the effect of a 1-y intake of a combined flavan-3-ol and isoflavone enriched-chocolate on atherosclerosis progression and hemodynamic function in postmenopausal women with T2DM. These data follow on from our first published findings from this comprehensive long-term intervention (26), in which we reported improvements in lipoprotein status and insulin resistance (26).

## SUBJECTS AND METHODS

### Study design

A randomized, double-blind, placebo-controlled, parallel design study was conducted at the Clinical Trials Unit at the University of East Anglia (United Kingdom), as reported previously (26), with approval from the local Ethics Committee (www.clinicaltrials.gov; NCT00677599). Subjects provided written informed consent, and all investigations were conducted according to the principles expressed in the Declaration of Helsinki. One hundred eighteen postmenopausal women with T2DM, aged $\leq$75 y, and receiving established ($\geq$12 mo) therapy for T2DM [ie, receiving statins ($\geq$40 mg simvastatin or $\geq$10 mg atorvastatin)] were recruited after meeting the study inclusion criteria, which were described previously (26). Exclusion criteria included current or recent history of smoking (within 1 y), significant history of vascular disease or cancer, hormone replacement therapy use (within 6 mo), poor diabetes control (glycated hemoglobin $\geq$10%), and elevated systolic BP (SBP; $\geq$160 mm Hg).

After enrollment, the subjects were randomly allocated into 2 equal groups (1:1 ratio of allocation) with the use of randomization software (AR2007; Minsoo Kang & Jae-Hyeon Park) and were stratified by age, BMI, years since menopause, and insulin use at screening. The subjects were required to consume either placebo ($n = 59$ subjects) or flavonoid-enriched ($n = 59$ subjects) chocolate daily for 1 y, which was given as a split dose (2 $\times$ 13.5 g/d) to maintain circulating metabolite concentrations based on the known half-life of the dietary constituents (27). The daily dose of flavonoid-enriched chocolate provided 90 mg epicatechin (850 mg total flavan-3-ols) and 100 mg isoflavones (aglycone equivalents; from a daidzein-rich extract) as described in detail previously (26). Chocolate was matched for appearance, taste, and macronutrient content, and the subjects and researchers assessing the outcomes were blinded to the intervention. Standardized study assessment visits were conducted at baseline, 6 mo, and 12 mo.

### Dietary restrictions, body size assessment, and adherence to treatment

Throughout, and for 1 wk preceding the 1-y study, habitual diet and exercise behaviors were maintained, except for a limited intake of specific flavonoid-rich foods (eg, dark chocolate and soy foods; clear guidance was provided). Before each assessment, the subjects were instructed to avoid caffeine, alcohol, and strenuous exercise (for 24 h) and to consume the same low-flavonoid evening meal; an overnight fast followed ($>$10 h). Habitual dietary intake (baseline, 6 mo, and 12 mo) was assessed by 4-d diet diaries as previously described (26), and anthropometric measures were made in duplicate (height, weight, waist and hip circumferences, and bioimpedance). Adherence to the intervention was assessed at 6 and 12 mo by counting nonconsumed chocolate bars and returned labels and by quantification of 24-h urinary excretion concentrations of total epicatechin (parent compound and metabolites; epicatechin, 3′-methyl epicatechin, 4′-methyl epicatechin, epicatechin sulfates, and methyl epicatechin sulfates) and isoflavone (daidzein, genistein, and equol) by HPLC/mass spectrometry (described previously (26).

### Assessment of atherosclerosis progression and hemodynamic function

At baseline and 12 mo, CCA-IMT was measured via ultrasonography in the nonfasted state. Mean CCA-IMT was calculated across 10 images ($n = 5$ on both sides of the CCA) in patients while supine, by one consultant radiologist blinded to intervention group with the use of vessel recognition software (M'ATH version 3.1; Intelligence in Medical Technologies); pre- and postintervention scans were assessed consecutively to facilitate consistent CCA-IMT. Arterial and hemodynamic function were assessed at baseline, 6 mo, and 12 mo, and fasted blood samples were collected and stored at $-80°$C for subsequent analyses of markers of vascular function. An ambulatory BP monitor (model 90207; Spacelabs Health Care) recorded SBP, diastolic BP (DBP), mean arterial pressure (MAP), and pulse pressure (PP) every 10 min during the clinic assessment (excluding rest periods and arterial stiffness assessments) for 2 h. In a subanalysis, variability in the BP rate (mm Hg/min) during clinical assessment was calculated by using an adapted 24-h ambulatory BP method (28); briefly, the difference in BP (mm Hg) was divided by time elapsed between readings (ʳ) [ie, (BP$^{r2}$ $-$BP$^{r1}$)/(time$^{r2}$ $-$ time$^{r1}$)], and those with $<$10 valid BP measures were excluded. The augmentation index and aortic central pressures (aortic SBP, aortic DBP, aortic MAP, and aortic PP) were measured by assessing radial artery PP waveform (SphygmoCor CPM; AtCor Medical); waveform traces with a $<$80% quality index were excluded from the analysis. Because of technical problems, carotid to femoral pulse wave velocity (PWV) was completed by using an alternative system (Vicorder; Skidmore Medical Limited) in a subsample ($n = 18$ in the flavonoid group and $n = 17$ in the placebo group at baseline, 6 mo, and 12 mo) at standardized sites by using 3 assessments all within $\pm$10% of one another. Traces were quality assessed by 2 researchers, who were blinded to the intervention type.

Plasma total NO concentrations (determined by the summation of $NO_2^-$ and $NO_3^-$) were analyzed by using commercial colorimetric assays (product 780001, interassay CV of 6.6%; Cayman Chemical Company), and ELISA was used to assess angiotensin-converting

enzyme (product E90004Hu, interassay CV of 11.7%; USCN Life Science Inc) and endothelin-1 (ET1) (product QET00B, interassay CV of 3.3%; R&D Systems).

**Statistical analysis**

The results are expressed as means ± SEMs, with the exception of diabetes duration and years since menopause (**Table 1**), which were skewed and thus are presented as medians with 95% CIs. At baseline, between-group differences in patient characteristics (Student's independent $t$ tests, Pearson's chi-square test, and an independent-samples median test as appropriate) were assessed. Before analysis, outliers were identified (>3 SD) and excluded from the subsequent analyses (with few exclusions to our data set, except for SBP and PP variability in 4 subjects). The Shapiro-Wilk test was used to assess normality, and data that were not normally distributed were transformed before analyses; unadjusted mean values (0 mo, 12 mo, and change from 0 to 12 mo) are presented. A Mann-Whitney (2-samples) analysis was performed for non-normally distributed data.

The analysis of intervention effect excluded low compliers (returning ≤70% wrappers; $n = 3$ in the placebo group), and univariate ANCOVA was conducted as the primary test of effect [intervention group as fixed factor compared with change in variable (0 mo to 6 mo or 12 mo) and baseline value as covariate]; no random factors were included. Use of BP medication (yes or no) and change in BP medication (yes or no) were included as covariates in the BP and PWV analyses. Statistical significance was defined as $P < 0.05$ per SPSS, release 18.0.0 (IBM Corporation). The primary outcome was the change in CCA-IMT after the 12-mo intervention. Secondary outcomes included the effect of intervention on peripheral and central BP, PWV, augmentation index, and biological markers of CVD risk and hemodynamic function.

**RESULTS**

A total of 93 patients with T2DM completed the 1-y trial ($n = 47$ in the flavonoid group, $n = 46$ in the placebo group; **Figure 1**), and the baseline characteristics were similar in both groups (Table 1); BMI (in kg/m$^2$) ranged from 21.5 to 57.9, age from 51 to 74 y, and CCA-IMT from 0.54 to 1.05 mm. Patients who completed

the study had baseline characteristics ($P > 0.05$) similar to those of patients who withdrew ($n = 25$). At baseline, 57% of participants were medicated for hypertension ($n = 28$ in the flavonoid group, $n = 25$ in the placebo group; Table 1); during the year-long trial, 5 participants in the flavonoid group modified their BP medication, 1 participant in the flavonoid group commenced BP medication, and 2 participants in the placebo group commenced BP medication. Adherence to the study was high (flavonoid group, 91.3%; placebo group, 91.6%), calculated from wrapper returns, and 24-h urinary excretion of daidzein and total epicatechin at 6 and 12 mo further confirmed compliance in the flavonoid group (total epicatechin: 148.5 ± 12.3 and 154.1 ± 11.1 $\mu$mol/d, respectively; daidzein: 77.5 ± 4.9 and 75.2 ± 5.3 $\mu$mol/d, respectively). This compared with 24-h urinary excretion concentrations in the placebo group at 6 and 12 mo, respectively, of 18.7 ± 2.1 and 18.9 ± 2.2 $\mu$mol/d for total epicatechin and 0.9 ± 0.4 and 1.0 ± 0.4 $\mu$mol/d for daidzein.

After 1 y there was no significant effect of the intervention on CCA-IMT (flavonoid group: +0.006 ± 0.008 mm/y; placebo group: −0.006 ± 0.007 mm/y; $P = 0.19$) or augmentation index (flavonoid group: −0.57 ±1.18%; placebo group: −0.80 ± 1.40%; $P = 0.89$) (**Table 2**). However, in a subset of patients with arterial stiffness data ($n = 18$ in the flavonoid group, $n = 17$ in the placebo group), PWV was significantly reduced after the 12-mo flavonoid intervention (flavonoid group: −0.07 ± 0.38 m/s; placebo group: 0.68 ± 0.25 m/s; $P = 0.01$) (Table 2) but not after 6 mo (**Figure 2**). The baseline characteristics of those with PWV measures were similar to the whole study population (data not shown).

Although central aortic BP was reduced in the flavonoid group (Table 2), it was not statistically significant for aortic SBP (flavonoid group: −3.8 ± 2.2 mm Hg; placebo group: 0.8 ± 2.0 mm Hg; $P = 0.07$), aortic DBP (flavonoid group: −2.3 ± 1.1 mm Hg; placebo group: 1.4 ± 1.1 mm Hg; $P = 0.06$), or aortic MAP (flavonoid group: −2.7 ± 1.4 mm Hg; placebo group: 1.5 ± 1.4 mm Hg; $P = 0.06$). A significant decrease in PP variability (during ambulatory BP monitoring) was also observed after the flavonoid intervention (flavonoid group: −0.11 ± 0.07 mm Hg/min; placebo group: 0.10 ± 0.11 mm Hg/min; $P = 0.04$ ($n = 38$ in the flavonoid group, $n = 33$ in the placebo group); however, the intervention had no effect on mean ambulatory BP or biomarkers of hemodynamic function.

**TABLE 1**
Patient characteristics at baseline for the 93 postmenopausal women with type 2 diabetes who completed the 1-y flavonoid intervention trial[1]

|  | Flavonoid ($n = 47$) | Placebo ($n = 46$) |
|---|---|---|
| Age (y) | 62.1 ± 0.7[2] | 63.0 ± 0.8 |
| BMI (kg/m$^2$) | 32.7 ± 1.1 | 31.9 ± 0.9 |
| Waist-to-hip ratio | 0.90 ± 0.01 | 0.89 ± 0.01 |
| Duration of diabetes (y) | 5.0 (4.9, 9.2)[3] | 5.0 (4.4, 7.2) |
| Time since menopause (y) | 12.0 (11.0, 16.1) | 12.8 (11.1, 15.9) |
| Medicated for hypertension [$n$ (%)] | 28 (60) | 25 (54) |
| Medicated with oral hypoglycemic agents [$n$ (%)] | 38 (81) | 35 (76) |
| Medicated with insulin [$n$ (%)] | 9 (19) | 9 (20) |
| Glycated hemoglobin (%) | 7.13 ± 0.14 | 7.25 ± 0.15 |

[1] No significant differences ($P < 0.05$) were observed between groups (Student's independent $t$ tests, Pearson's chi-square test, or independent-samples median test, as appropriate).
[2] Mean ± SEM (all such values).
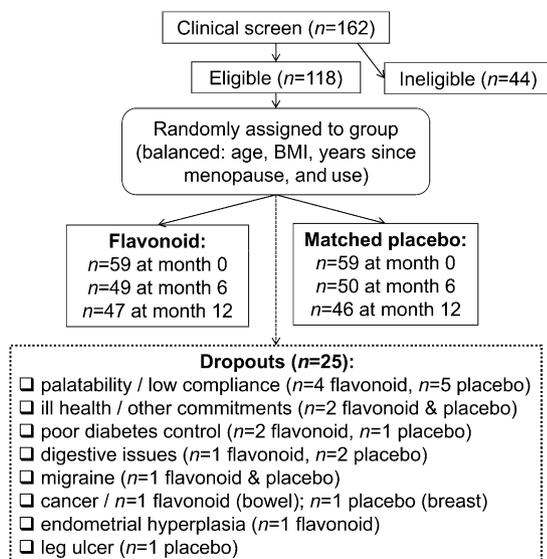[3] Median; 95% CI in parentheses (all such values).

**FIGURE 1.** Participant enrollment and retention through the assessment phases of the 1-y study.

In the exploratory analysis, after 1 y of exposure to isoflavones, equol producers had a significantly reduced PWV (equol producers: $-0.68 \pm 0.40$ m/s; non–equol producers: $0.32 \pm 0.55$ m/s; $P = 0.001$), DBP (equol producers: $-2.24 \pm 1.31$ mm Hg; non–equol producers: $1.00 \pm 0.89$ mm Hg; $P < 0.01$), and MAP (equol producers: $-1.24 \pm 1.30$ mm Hg; non–equol producers: $1.90 \pm 1.08$ mm Hg; $P = 0.01$) compared with non–equol producers (**Figure 3**). In the 17 equol producers, a moderate inverse correlation between DBP and urinary equol concentrations was observed ($r = -0.44$, $P = 0.08$). Whereas differential effects on biomarkers of hemodynamic function were not observed between the equol producers and non–equol producers after the intervention, equol producers had higher total plasma NO concentrations (ie, $NO_2^- + NO_3^-$) (equol producers: $61.1 \pm 6.5$ $\mu$mol/L; non–equol producers: $40.3 \pm 3.4$ $\mu$mol/L; $P < 0.01$) and a greater total plasma NO:ET1 ratio (equol producers: $46.6 \pm 7.5$; non–equol producers: $34.2 \pm 4.3$; $P = 0.04$) at baseline.

## DISCUSSION

To our knowledge, this was the first long-term study in patients with T2DM to assess the potential effects of a combined isoflavone and flavan-3-ol intervention on markers of atherosclerosis progression and hemodynamic function. Although 1-y daily intake of flavan-3-ols and isoflavones did not affect progression of CCA-IMT, an established surrogate measure of atherosclerosis and stroke (29), we did observe a significant improvement in PWV in a subgroup ($n = 18$ in the flavonoid group, $n = 17$ in the placebo

## TABLE 2
Effect of a 1-y combined flavan-3-ol and isoflavone intervention on arterial stiffness, hemodynamic function, and progression of intima-media thickness in 93 medicated postmenopausal patients with type 2 diabetes[1]

| | Flavonoid ($n = 47$) | | | Placebo ($n = 46$) | | | Net effect | $P$ value |
|---|---|---|---|---|---|---|---|---|
| | 0 mo | 12 mo | 0–12 mo | 0 mo | 12 mo | 0–12 mo | 0–12 mo | 0–12 mo |
| CCA-IMT (mm) | 0.75 ± 0.02 | 0.76 ± 0.02 | 0.01 ± 0.01 | 0.75 ± 0.02 | 0.74 ± 0.02 | −0.01 ± 0.01 | +0.02 | 0.19 |
| PWV (m/s)[2] | 8.9 ± 0.4 | 8.8 ± 0.2 | −0.1 ± 0.4 | 9.5 ± 0.4 | 10.1 ± 0.4 | 0.7 ± 0.3 | −0.8 | 0.01 |
| AIx at 75 HR (%) | 28.5 ± 1.2 | 27.9 ± 1.4 | −0.6 ± 1.2 | 29.5 ± 1.4 | 28.7 ± 1.3 | −0.8 ± 1.4 | +0.2 | 0.89 |
| Aortic central BP (mm Hg) | | | | | | | | |
| aSBP | 119.6 ± 1.9 | 115.8 ± 1.8 | −3.8 ± 2.2 | 119.5 ± 2.2 | 120.3 ± 2.4 | 0.8 ± 2.0 | −4.6 | 0.07 |
| aDBP | 74.4 ± 1.3 | 72.1 ± 0.9 | −2.3 ± 1.1 | 72.0 ± 1.0 | 73.4 ± 1.3 | 1.4 ± 1.1 | −3.7 | 0.06 |
| aMAP | 93.4 ± 1.4 | 90.8 ± 1.2 | −2.7 ± 1.4 | 91.8 ± 1.5 | 93.3 ± 1.7 | 1.5 ± 1.4 | −5.2 | 0.06 |
| aPP | 44.4 ± 1.3 | 44.0 ± 1.5 | −0.4 ± 1.3 | 48.7 ± 1.9 | 47.2 ± 1.8 | −1.6 ± 1.4 | +1.2 | 0.91 |
| Ambulatory BP | | | | | | | | |
| SBP (mm Hg) | 133.7 ± 1.6 | 135.6 ± 1.6 | 1.9 ± 1.3 | 138.1 ± 1.9 | 139.6 ± 1.8 | 1.5 ± 1.6 | +0.4 | 0.37 |
| SBP variability (mm Hg/min) | 0.90 ± 0.05 | 0.84 ± 0.04 | −0.06 ± 0.06 | 0.94 ± 0.06 | 1.00 ± 0.07 | 0.07 ± 0.09 | −0.13 | 0.09 |
| DBP (mm Hg) | 76.1 ± 1.3 | 76.0 ± 1.2 | −0.2 ± 0.8 | 76.6 ± 1.1 | 76.4 ± 1.0 | −0.1 ± 0.9 | −0.1 | 0.83 |
| DBP variability (mm Hg/min) | 0.69 ± 0.06 | 0.60 ± 0.04 | −0.10 ± 0.06 | 0.76 ± 0.06 | 0.73 ± 0.06 | −0.03 ± 0.08 | −0.07 | 0.09 |
| MAP (mm Hg) | 97.3 ± 1.4 | 98.0 ± 1.3 | 0.8 ± 0.9 | 99.5 ± 1.1 | 99.8 ± 0.9 | 0.3 ± 1.0 | +0.5 | 0.64 |
| MAP variability (mm Hg/min) | 0.79 ± 0.05 | 0.76 ± 0.04 | −0.02 ± 0.05 | 0.78 ± 0.05 | 0.80 ± 0.05 | 0.01 ± 0.08 | −0.03 | 0.60 |
| PP (mm Hg) | 57.3 ± 1.4 | 59.8 ± 1.4 | 2.5 ± 1.0 | 61.8 ± 1.8 | 63.2 ± 1.9 | 1.5 ± 1.1 | +1.0 | 0.92 |
| PP variability (mm Hg/min) | 0.92 ± 0.07 | 0.81 ± 0.05 | −0.11 ± 0.07 | 0.91 ± 0.07 | 1.01 ± 0.08 | 0.10 ± 0.11 | −0.21 | 0.04 |
| Hemodynamic biomarkers | | | | | | | | |
| ACE (ng/mL) | 125.9 ± 33.2 | 127.6 ± 31.9 | 1.7 ± 5.0 | 95.7 ± 19.2 | 94.3 ± 20.2 | −1.5 ± 5.1 | +3.2 | 0.80 |
| NO ($\mu$mol/L) | 49.5 ± 3.8 | 45.4 ± 3.0 | −4.2 ± 3.0 | 43.3 ± 2.9 | 41.1 ± 3.0 | −2.2 ± 2.1 | −2.0 | 0.80 |
| ET1 (pg/mL) | 1.6 ± 0.1 | 1.7 ± 0.1 | 0.1 ± 0.1 | 1.6 ± 0.1 | 1.5 ± 0.1 | −0.1 ± 1.0 | +0.2 | 0.19 |
| NO:ET1 | 38.8 ± 3.9 | 33.3 ± 2.9 | −5.4 ± 3.1 | 35.3 ± 3.8 | 32.7 ± 3.2 | −2.5 ± 2.4 | −2.9 | 0.37 |

[1] All values are means ± SEs. $P$ values were derived from a univariate ANCOVA with 0-mo values used as a covariate in all analyses. BP medication use and change in BP medication use were additional covariates in the BP and PWV analyses. No significant differences were found between groups at baseline ($P < 0.05$). ACE, angiotensin-converting enzyme; AIx, augmentation index; aDBP, aortic diastolic blood pressure; aMAP, aortic mean arterial pressure; aPP, aortic pulse pressure; aSBP, aortic systolic blood pressure; BP, blood pressure; CCA-IMT, intima-media thickness of the common carotid artery; DBP, diastolic blood pressure; ET1, endothelin-1; HR, heart rate; MAP, mean arterial pressure; NO, nitric oxide; PP, pulse pressure; PWV, pulse wave velocity; SBP, systolic blood pressure.

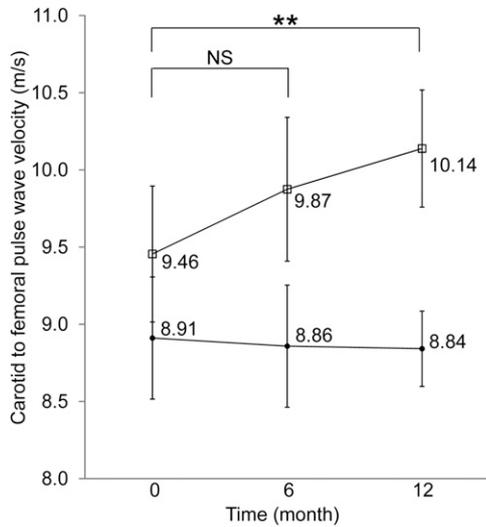[2] $n = 18$ in the flavonoid group and $n = 17$ in the placebo group.

**FIGURE 2.** Mean (±SEM) carotid to femoral artery pulse wave velocity after the 1-y flavonoid intervention in a subgroup of patients: flavonoid group (●, n = 18) and placebo group (□, n = 17). **P = 0.01 (univariate ANCOVA with 0-mo pulse wave velocity, blood pressure medication use, and change in blood pressure medication use as covariates).

group) who were representative of the study population group, and the magnitude of this effect may be of clinical significance in patients with T2DM. In a meta-analysis, arterial stiffness was shown to predict all-cause mortality and future CVD risk, especially in patients with established CVD risk (30); greater central artery stiffness (carotid – femoral PWV) was observed in T2DM patients than in matched control subjects (31), and the magnitude of the association between central PWV and total mortality was greater than for SBP or PP in elderly T2DM patients (32).

Our observed net change in PWV over 1 y (0.75 m/s), after flavonoid intervention, was similar to the effects observed in healthy men and women consuming soy isoflavones (33) (improvement of 0.7 m/s) and supports previous short-term data from T2DM patients, which suggested improvement in endothelial function after intake of flavan-3-ols (20) and isoflavones (19) for 1 and 2 mo, respectively. In a pooled analysis, it was shown that each 1-m/s increase in PWV corresponded to increases in cardiovascular events and all-cause mortality of 14% to 15% (28); on this basis, our change in PWV of −0.75 m/s in those consuming flavonoids corresponded to a 10–11% relative reduction in CVD risk, which is of potential clinical relevance. The results of our 1-y intervention suggest that improvements in arterial stiffness are sustainable in T2DM patients and, when considered alongside our previously reported improvements in lipoprotein status and insulin resistance (26), suggest that flavonoids may offer an additional strategy to reduce CVD risk profiles in this population.

Cross-sectional data have previously shown inverse associations between CCA-IMT and the habitual intake of total flavonoids (9), flavan-3-ols (9), and soy foods (34) in healthy subjects; however, our lack of effect on CCA-IMT agrees with a recent long-term (2.7-y) intervention trial with soy isoflavones, which found no significant effect on CCA-IMT progression in healthy postmenopausal women (11). In patients with T2DM, the effect of lifestyle intervention on longer-term CCA-IMT progression is limited. After 5 y of an intensive multifactorial intervention (diet, exercise, statin therapy, antihypertensive medication use,

aspirin use, and oral hypoglycemic intervention), a small attenuation in CCA-IMT progression (0.01 mm/y) was observed in patients with newly diagnosed T2DM (35)—a progression rate similar to our results. Indeed, in a recent 2-y statin trial in patients with coronary heart disease, coronary atheroma volume was reduced by only 0.99–1.22% (dependent on therapeutic regimen) (36).

Overall, we observed no effect of the intervention on peripheral BP, a result that is consistent with previous short-term flavan-3-ol and isoflavone studies in patients with T2DM (20–22). We did, however, observe a trend toward a reduction in central aortic BPs (DBP, SBP, and MAP; P = 0.06, P = 0.07, and P = 0.06, respectively) and a significant reduction in brachial PP variability (P = 0.04). Central pressures were previously identified as a key measure of CVD risk (37) and as having greater predictive capacity for vascular events than peripheral assessments of BP (18); likewise, variability in BP has been shown to affect vascular function and CVD risk (38). Taken together, the effect of our intervention on these endpoints may be of potential clinical importance.

Previous mechanistic insights support our observed in vivo effects, with evidence from both in vitro and animal data suggesting favorable and potentially complimentary effects of flavan-3-ols and isoflavones on aspects of vascular function. Isoflavones and flavan-3-ols exert antiinflammatory effects, modulate NO synthase activity/expression, inhibit endothelial NADPH oxidase activity (12–14), and inhibit angiotensin-converting enzyme activity both in vivo and in vitro (39). Interestingly, in vitro studies also suggest that a combination of isoflavones and statins exert potential synergistic effects on hydroxymethyl-glutaryl coenzyme A reductase or lipoprotein concentrations (40, 41).

In the subgroup analysis, the ability to produce equol, after isoflavone ingestion, was associated with an enhanced response to the intervention; compared with non–equol producers, equol producers had a greater reduction in PWV, DBP, and MAP (P < 0.0001, P < 0.01, and P = 0.01, respectively). Although the study was not designed to formally assess the biological
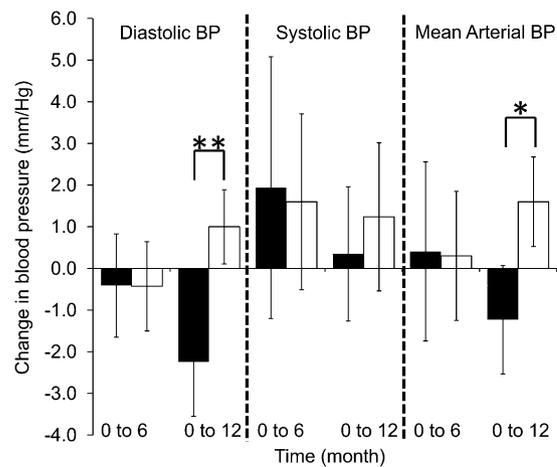


**FIGURE 3.** Mean (±SEM) change in BP after 6 and 12 mo of combined intake of flavan-3-ols and isoflavones in medicated postmenopausal women with type 2 diabetes, categorized by equol producer phenotype: equol producers (■, n = 17) and non–equol producers (□, n = 30). *P = 0.01 and **P < 0.01 (univariate ANCOVA with 0-mo pulse wave velocity, BP medication use, and change in BP medication use as covariates. BP, blood pressure.

efficacy of the equol producer phenotype, our findings are consistent with those of a previous retrospective analysis of short-term isoflavone intervention, which showed improvements in endothelial function in equol producers (25). Similarly, in vitro and animal data support the finding that equol exerts greater vascular bioactivity than daidzein does (24) and that, during the hypertensive state, equol retains a vasorelaxant potency, whereas daidzein's vasorelaxant properties are impaired (23). Interestingly, in our analysis, the improvements in PWV and BP parameters were only evident after 1 y of intervention, and not at the midpoint (*see* Figure 3 for BP changes; PWV data not shown), which suggests that longer-term exposure to isoflavones may be critical. Although we found no difference by equol-producer status in changes in the vascular and hemodynamic biomarkers assessed by commercial ELISAs [ET1, angiotensin-converting enzyme, and NO (ie, $NO_2^- + NO_3^-$)], it was apparent at baseline that equol producers had a significantly higher plasma NO concentration ($NO_2^- + NO_3^-$) and NO:ET1 ratio; however, these data require confirmation in studies principally designed to control for dietary $NO_3^-$ and using a systems-based approach to sample multiple compartments relevant to NO physiology simultaneously (42). Additional studies are now warranted to investigate the underlying mechanisms of potentially differential vascular responses between equol and non–equol producers, because emerging evidence suggests an association between equol-producer status, genetic factors, and vascular function (43), which may, in part, account for baseline differences in vascular function biomarkers.

As described previously (26), our pragmatic study had some limitations and further long-term research is now required to establish the relative influence of each flavonoid subclass (flavan-3-ols and isoflavones) on CVD risk. Our observed PWV effects are derived from a subgroup of patients, and additional studies are required to replicate these findings. Equally, the findings from our retrospective analysis of equol-producer status now require confirmation in studies that prospectively recruit subjects on the basis of equol-producer status. Future studies may also benefit from assessing 24-h ambulatory BP, as opposed to the in-clinic ambulatory assessment conducted in our trial. Given the number of endpoint measures included in our study, the potential for multiple testing cannot be excluded; it is also acknowledged that, despite similarities in key baseline characteristics and an equal number of dropouts in each group, our complete case analysis had less protection from bias than an intention-to-treat analysis. However, despite these limitations, to our knowledge our trial was the first long-term study to assess the effect of a combined flavan-3-ol and isoflavone intervention in a population at elevated risk of CVD and contributes to the understanding of longer-term flavonoid intake on clinically relevant surrogate markers of atherosclerosis progression and vascular function. In addition, this trial provides evidence that long-term flavan-3-ol and isoflavone intakes may improve markers of arterial stiffness and aspects of hemodynamic function in T2DM patients taking established medication, which may be of clinical importance.

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