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HABITUAL FLAVONOID INTAKE AND ENDOTHELIAL FUNCTION IN HEALTHY HUMANS

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Abstract

Objective—Endothelial function, as measured by non-invasive techniques, is known to vary widely within populations. Our study was designed to test the hypothesis that this variation is determined in large part by a person's habitual dietary intake of flavonoids.

Methods—This was an analytical study examining the relationship between endothelial function and dietary flavonoids in 19 healthy older adults (mean age 72 years). The study took place in the inpatient Clinical Research Center of the Brigham and Women's Hospital. Habitual flavonoid intake was assessed via a focused food frequency questionnaire. Endothelial function, measured as the reactive hyperemia response to one dose of flavonoid-rich cocoa, was recorded with a plethysmographic device via peripheral arterial tonometry (PAT).

Results—Background flavonoid intake and the reactive hyperemia (RH)-PAT response were significantly correlated ($r=0.7$, $p=0.001$); subjects with higher habitual flavonoid intake showed a significantly greater RH-PAT response than did lower consumers. PAT response to cocoa was also significantly correlated with simultaneous flavanol concentration in the blood ($r=0.5$, $p=0.03$).

Conclusion—Individual variation in endothelial function among healthy older people, measured as PAT response to flavonoid-rich cocoa, is highly dependent upon usual daily flavonoid consumption. These data raise the possibility that the consumption of fruits and vegetables dictates basal endothelial function, likely related to their flavonoid content and influence on nitric oxide.

Keywords

flavonoids; diet; endothelial function; aging

INTRODUCTION

Endothelial function, as measured by non-invasive techniques, is known to vary widely within populations [1;2]. This study was designed to test the hypothesis that this variation is determined in large part by a person's habitual dietary intake of flavonoids.

Most experts advocate a substantial intake of fruits and vegetables as an important part of a healthful diet. Remarkably, the precise mechanism involved in their protective effects remains obscure. One possibility is their rich content of flavonoids, a large group of phytochemicals. Possible health benefits of flavonoids elucidated from *in vitro* studies include scavenging of superoxide anions, singlet oxygen, and lipid peroxy-radicals [3]; and inhibiting atherogenicity and cytotoxicity of low-density lipoproteins [4]. More recently, we and others have shown that flavonoids activate nitric oxide (NO) [5]. These *in vitro* findings

are supported by observational studies suggesting that flavonoids reduce cardiovascular risk [6–8].

We undertook a prospective quantification of flavonoid intake in healthy older adults by focused food frequency questionnaire, and measured the baseline blood concentration of the subset of flavonoids called flavanols in blood. We then analyzed intake in relation to the vascular response to flavonoid-rich cocoa.

MATERIALS AND METHODS

Study Population

Nineteen healthy older people were studied (mean age 71.7 ± 6.8 ; Table). Subjects had normal routine laboratories, blood pressure and mean cholesterol and were mildly overweight. None smoked. The study was approved by the Brigham and Women's Hospital's institutional review committee, and informed consent was obtained prior to study. Vascular responses of some of these subjects to flavanol-rich cocoa have been previously reported [9;10].

PAT Technique

Each subject underwent repeated measurements of peripheral arterial tonometry (PAT) using a plethysmographic device on the fingertip (PAT 1000RD; Itamar Medical, Inc., Caesarea, Israel) designed to reflect only pulsatile arterial volume changes. Readings were taken twice, at baseline and following five minutes of radial artery occlusion at 30 mm Hg above systolic blood pressure. Flow-mediated vasodilation, measured as reactive hyperemia (RH-PAT), was assessed one minute post release. The procedure has been described in detail [5]. A single technician, blinded to subject, study day, time and treatment calculated pulsatile volume, employing area under the curve.

Protocols

On the study day, after an overnight fast, baseline blood pressure, basal PAT and RH-PAT readings were taken on the metabolic ward. Temperature was controlled between 24 and 25°C. Baseline blood was drawn for flavanol concentration. Each subject then drank a cocoa dose (one packet of Cocoapro™, Mars Inc., Hackettstown, NJ, mixed in one cup of water) in order to measure the highest PAT and RH-PAT response acutely. The cocoa contained 451 mg flavanols per packet as measured by high-performance liquid chromatography, including 84 mg epicatechin, 28 mg catechin, and 339 mg flavanol oligomers; also 19 mg caffeine, 204 mg theobromine and 119 calories. Measures were taken 2, 4, 6 and 8 hours after cocoa ingestion; peak (RH) PAT was marked at the time of highest absolute (RH) PAT reading; (RH) PAT response was the difference between peak and baseline.

Analyses

A focused food frequency questionnaire was based on the United States Department of Agriculture Databases for the Flavonoid Content of Selected Foods [11], and the Proanthocyanidin Content of Selected Foods [12]. Twenty-two foods highest in flavonoid content and readily available in the American diet were chosen. The flavonoid content for each food was normalized to typical serving size. Because flavanol monomers are quantified in both the flavonoid and the proanthocyanidin databases, the average value was used, similar to methods from Mink et al [13]. On the study day one technician administered the questionnaire; subjects based responses upon consumption during an average week. Total flavanol concentration in the blood was measured by reversed-phase HPLC and included the monomers catechin and epicatechin, as well as their glucuronide and methyl metabolites.

Subjects were divided into low, medium, and high habitual flavonoid consumption according to their weekly intake. Eight subjects were low consumers (<2000 mg/week), six were medium consumers (2000–4500 mg/week), and five were high consumers (>4500 mg/week). One subject in the high intake group and two in the low intake group were taking statins. None was taking any anti-hypertensive medication. In the high intake group there was one moderate exerciser (three or more 30-minute aerobic sessions/week); in the low intake group there were two. Data are described with means and standard deviations. An analysis of variance focused contrast was used to test for trends across the ordered groups. The Pearson correlation coefficient was used to assess correlations. SAS version 9.2 was used.

RESULTS

Median flavonoid intake among all subjects was 2428 mg/week (lower quartile 1242 mg; upper quartile 4789 mg). There was a highly significant correlation between habitual flavonoid intake and endothelial function as measured by the RH-PAT response to an acute dose of cocoa at baseline ($r=0.7$; $p=0.001$; Figure 1). Likewise habitual flavonoid intake was significantly correlated to the basal pulse wave PAT response prior to hyperemia ($r=0.56$, $p=0.01$). When analyzed categorically (Figure 2), there was a significantly increasing trend across the low, medium and high habitual flavonoid intake groups for RH-PAT response ($p=0.001$) and for PAT response ($p=0.01$). The main analyses were rerun excluding three subjects taking statins and correlations were virtually identical.

Total flavanol concentration in the blood was measured fasting, in the morning before baseline PAT response to cocoa was measured. The correlation between PAT response and flavanol concentration was positive and significant ($r=0.49$, $p=0.03$). Likewise, RH-PAT response with hyperemic stimulus was also positively correlated with total blood flavanol concentration ($r=0.44$, $p=0.06$).

DISCUSSION

This study demonstrated that spontaneous dietary flavonoid intake was associated with superior endothelial function in healthy older people, assessed as the magnitude of the vascular response to a cup of flavonoid-rich cocoa. Evidence was multi-layered. There was a significant correlation between peripheral vascular response and dietary flavonoids as assessed by questionnaire. There was also a correlation between vascular response and flavanols as measured in the blood. Our data indicate that basal endothelial function is largely a reflection of background dietary flavonoids. This study was prompted by the well-described substantial variation in the peripheral vasodilator response to acute dosing of flavonoid-rich cocoa, which cannot be attributed solely to imprecision in measurements [1;2].

A major role for NO in the vasodilator response to flavonoid-rich cocoa is supported by multiple lines of investigation. We previously demonstrated that a NO inhibitor, L-NAME (N^G -Nitro-L-arginine-methylester-HCl), reversed completely the vasodilator response to cocoa [5]. Multiple laboratories have since confirmed that observation [14]. Although we used flavonoid-rich cocoa as a convenient source of flavonoid, others have performed similar studies with grape juice, red wine, and tea [4;15–17].

The fact that purified epicatechin mimics the response to cocoa indicates at least one responsible chemical moiety for the vascular effects of cocoa [18]. The monomeric flavanols catechin and epicatechin, as well as their isomers, are absorbed and present in circulation for up to six hours [18]. In addition, however, while larger oligomeric procyanidins are not

absorbed and not present in human plasma, many metabolites of flavanols are [19]. Because we saw relationships between endothelial function and flavonoid intake even in the fasting state, our data support the potential importance of these metabolites.

Limitations

The study was small, making it impossible to control for comorbidities that might contribute to endothelial dysfunction and for lifestyle behaviors that might be beneficial. However, most existing conditions likely to result in endothelial dysfunction were excluded, including diabetes mellitus, hypertension, and smoking; also, there was not a disproportionate number of exercisers in the high intake group. Further, substantial correlations emerged despite the small sample size. Dietary recall is imperfect, and the questionnaire may not be completely precise. However, data suggest that older people exhibit a substantial reduction in food variety and food choices, so a diet consumed in one week is likely to be typical of habitual intake over a longer period of time [20]. Despite the small study size, the flavonoid distribution of these subjects generally reflected the distribution reported by Mink et al in a much larger cohort [13].

CONCLUSION

Our data indicate that flavonoid intake is likely a major determinant of endothelial function. If correct, this study may begin to shed light on practical preventive measures against vascular heart disease, stroke, and diabetes mellitus—among the most common causes of morbidity and mortality in the western world.

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Abbreviations

PAT	peripheral arterial tonometry
RH	reactive hyperemia
USDA	United States Department of Agriculture
NO	nitric oxide

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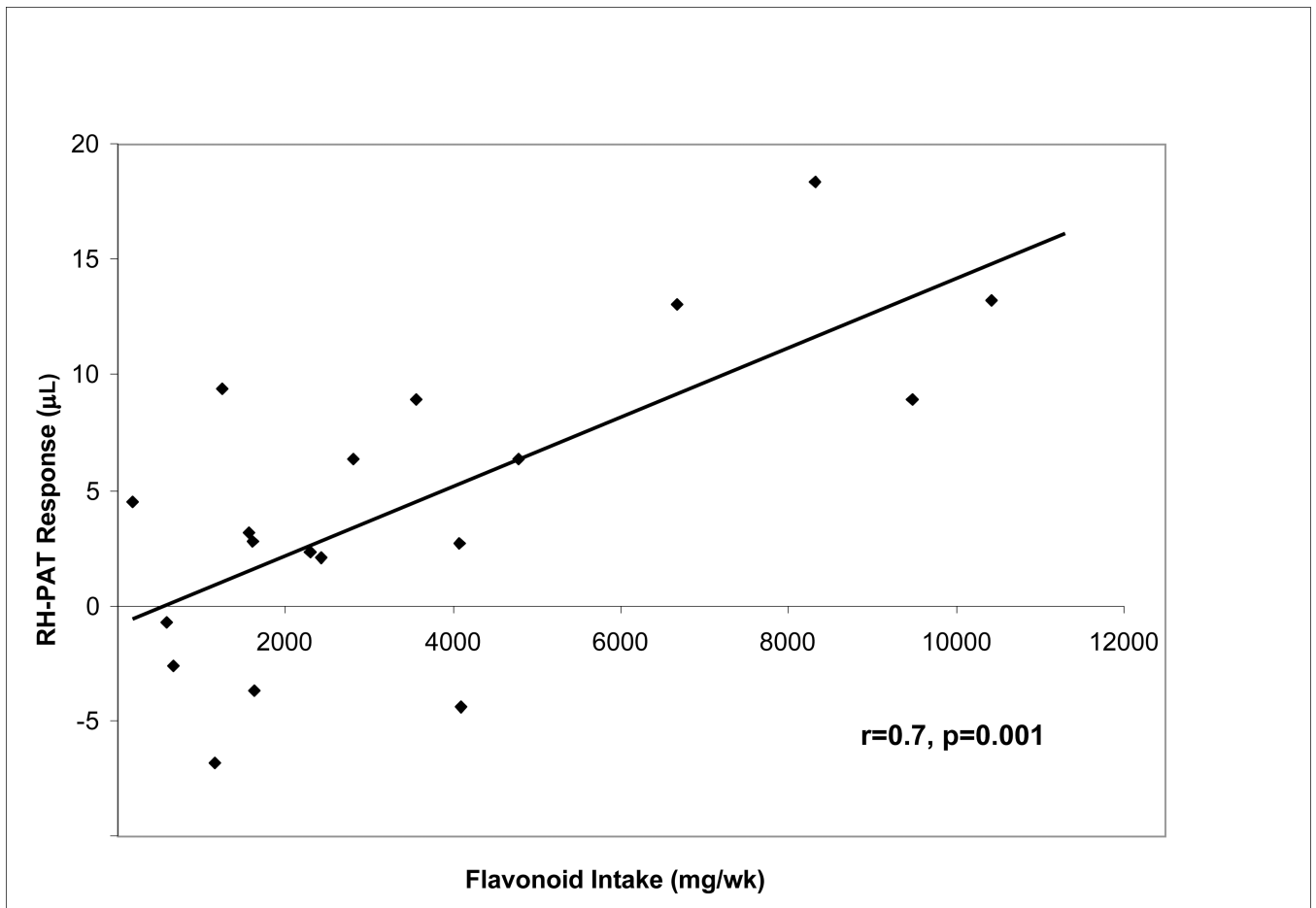
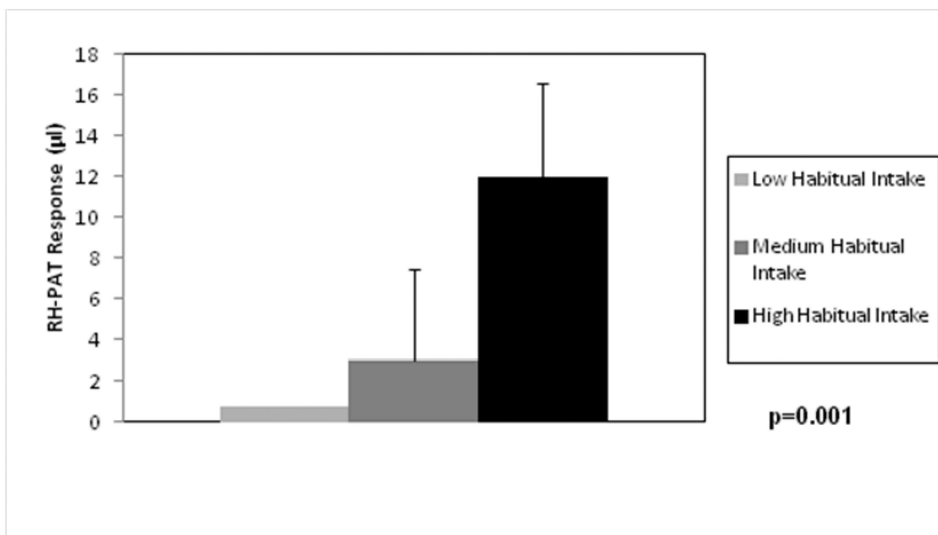


Figure 1.

There was a highly significant correlation between habitual flavonoid intake and endothelial function as measured by the continuous reactive-hyperemia peripheral arterial tonometry (RH- PAT) response to an acute dose of cocoa ($r=0.7$, $p=0.001$).

a



b

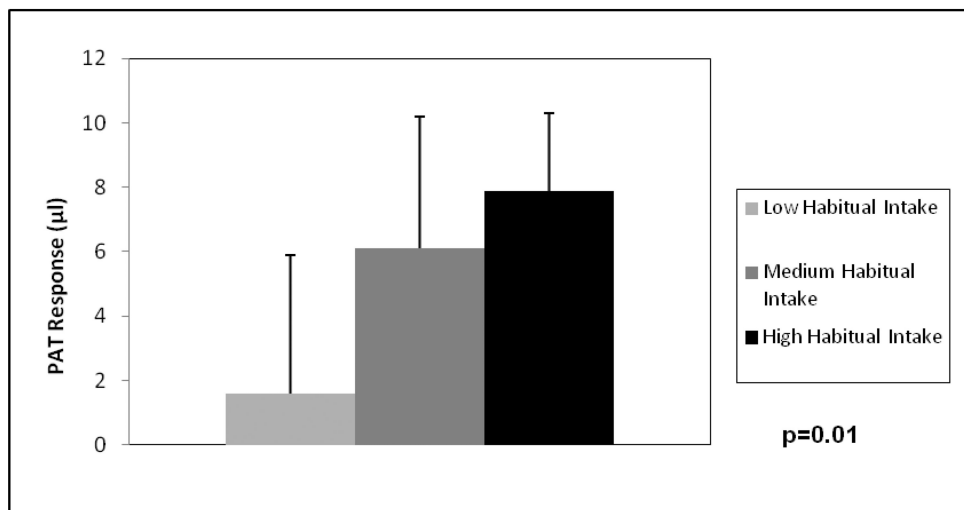


Figure 2. PAT responsiveness according to habitual flavonoid consumers at baseline, after initial cup of flavanol-rich cocoa. There was a significantly increasing trend across the low, medium and high habitual flavonoid intake groups for RH-PAT response (p=0.001; Figure 2a) and for PAT response (p=0.01; Figure 2b).

Table

Subject Characteristics (n=19)

Males	8
Caucasians	18
Age (yrs)	72 ± 7
SBP (mm Hg)	119 ± 11
DBP (mm Hg)	70 ± 8
BMI (kg/m ²)	25.6 ± 5
Cholesterol (mg/dL)	189±41
Median flavonoid intake (mg/day)	347