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A single intake of a resveratrol-arginine conjugate improves microvascular function compared to trans-resveratrol in postmenopausal women

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ABSTRACT

Resveratrol supplementation has been reported to improve markers of vascular function, but poor bioavailability of the parent compound is thought to limits its use. In this study, we compared the effects of a resveratrol-arginine conjugate (ResArg) to trans-resveratrol (trans-Res), the standard form used in supplements, on measures of microvascular function 1 h and 2 h after intake. Postmenopausal women were assigned to consume 90 mg of resveratrol as either ResArg or t-Res, in a double-blind, randomized, crossover design, at least one week apart. Microvascular function was assessed by peripheral arterial tonometry, and calculated as the reactive hyperemia index (RHI) and the Framingham RHI (fRHI); at 1 h, platelet reactivity, plasma resveratrol and nitrate/nitrite levels were also measured. A significant increase in fRHI was observed 1 h after intake of ResArg compared to trans-Res. Significant reductions in platelet reactivity were noted 1 h after ResArg intake, but not for trans-Res. Plasma resveratrol levels were increased 30 and 60 min after the consumption of both ResArg and t-Res. The results suggest a more rapid and pronounced response from ResArg than trans-Res on certain markers of vascular function.

1. Introduction

Epidemiological studies suggest that high intakes of plant-based foods rich in polyphenols are associated with a reduced risk for cardiovascular disease (CVD) [1,2]. Resveratrol, a polyphenolic stilbene found in grapes, red wine, purple grape juice, and some berries [3,4] was first isolated from plants used in traditional Chinese and Japanese medicines [5]. However, the compound was largely overlooked until 1992 when high intakes of resveratrol containing red wine was suggested as one explanation for the "French Paradox", which was the observation of lower CVD in French populations that regularly consume relatively high levels of saturated fat [5,6]. Subsequent results from numerous studies, both *in vitro* and in animal models, have supported the potential health benefits of resveratrol, albeit only when it is given in amounts higher than what occurs in the typical human diet [7], which are estimated to be 6–8 mg/day [8].

Of the two structural resveratrol isomers, trans-resveratrol (trans-Res) is the predominant form found in food [3] and dietary supplements [9] compared to the *cis*-isomer [3,5]. Supplemental trans-Res has been reported to improve a number of CVD-related biomarkers, including endothelial function, platelet reactivity, and blood lipids [9,10]. Improved vascular function has been shown in studies that provided 10–75 mg of trans-Res to volunteers from 6 weeks to 3 months [11,12]. Positive changes in total cholesterol was demonstrated after supplementation with 250 mg for three months [13], while a reduction in low density lipoprotein (LDL) cholesterol was reported after the intake of a mix of grape seed extract containing 8 mg of resveratrol for six months [14].

Defining the bioactivity of trans-Res is complicated by the fact that resveratrol is extensively biotransformed to a number of glucuronide and sulfate conjugates within 30 min to 2 h after intake; the parent aglycone is generally considered to have low bioavailability [9,15]. In light of the relatively low amounts of resveratrol in foods, and its limited bioavailability, novel

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resveratrol forms that have an increased bioavailability, and/or have increased bioactivity are of interest. In the current study, we compared the bioactivity of a novel resveratrol-arginine conjugate (ResArg; Gateway Health Alliances, Inc., Fairfield, CA) to that of trans-Res. The primary outcome measure was the reactive hyperemia index (RHI), with platelet reactivity, plasma nitrate/nitrite, and plasma resveratrol levels as secondary outcomes.

2. Materials and methods

2.1. Participants

Postmenopausal women, 50–70 years of age, were recruited from the greater Sacramento, CA area via email and newspaper advertisements. Postmenopausal status was defined as the cessation of menses for at least one year, and a level of follicular stimulating hormone [16] of 23–116.3 mIU/mL. The women did not smoke, had no history of chronic disease or allergies to fruits, and did not regularly use prescription drugs with the exception of thyroid medications. Use of dietary supplements other than standard multivitamin/mineral formulas (supplying up to 100% Daily Value) was exclusionary. Other inclusion criteria were a normal RHI measurement, normal values for comprehensive metabolic and lipid panels, and for Study 1 only, normal platelet function as determined by the platelet function analyzer-100 (PFA-100). The Institutional Review Board of the University of California, Davis approved the study protocol, and all participants provided written informed consent prior to enrollment.

2.2. Study design

The studies were designed to compare the bioactivity of ResArg to that of trans-Res. An initial study was conducted that examined the effects of the supplements at 30 min and 1 h post intake (Study 1). This initial study design was based on results from a pharmacokinetic study in rats that reported higher peak plasma ResArg levels within 1 h of intake compared to trans-res [17]. After examination of the vascular results from Study 1, a subsequent study was conducted to determine whether or not the trends observed at 1 h would persist 2 h after supplement consumption (Study 2). Both studies were randomized, double-blind, crossover trials that assessed the effects after an intake of a single dose of 90 mg of resveratrol as ResArg or trans-Res. Since the group assignments were random, some participants received ResArg first, while others received trans-Res first, and were then crossed over to the alternate treatment.

One day prior to each study visit, the participants were asked to refrain from consuming flavonoid-rich foods, including red wine, grape, tomato, tea or chocolate products. For the duration of the study, the participants were instructed to continue their normal dietary patterns while avoiding the intake of resveratrol-rich foods (red wine, red grapes, peanuts and berries).

For each study day visit, the participants were asked to arrive at the facility after an overnight fast. After a baseline PAT measurement and blood draw, the study participants were asked to consume their assigned treatment for the day. For Study 1, measurements were collected 30 min as well as 1 h post capsule intake, while for Study 2 measurements were conducted after 2 h of intake. Each study visit was separated by at least a one week washout period. The duration of the washout period was based on previous reports of improved vascular function after the intake of flavonoid-rich foods, and after the intake of resveratrol, using a washout period of three to seven days [12,18–20].

2.3. Outcome measures

Microvascular function was assessed using the Endo-PAT2000 (Itamar Medical Ltd., Caesarea, Israel) [21]. Briefly, prior to the PAT measurement, participants were asked to rest in a supine position in a temperature controlled room for 30 min. After a finger probe was placed on the middle finger of both hands, and a blood pressure cuff fitted on the proximal forearm of the non-dominant arm, 5–10 min of baseline pulse amplitude was recorded. Subsequently, the blood pressure cuff was inflated approximately 60 mmHg above the individual's systolic blood pressure. The pressure was maintained for 5 min, after which time the pressure was released and the resulting reactive hyperemia response was recorded for an additional three to five min. The system software automatically calculates the RHI. In addition a Framingham RHI (fRHI) was calculated as the natural logarithmic transformation of the RHI ratio, without the baseline correction factor and utilizing only the readings from 90 to 120 s following the reactive hyperemia, along with an augmentation index (AI) with and without normalization to a heart rate of 75 beats per min (AI@75). Participants with complete RHI data sets of sufficient quality (70% or greater validity during occlusion measurement) were included in the final data analysis.

Plasma resveratrol was determined in Study 1 only. Briefly, 1.2 M acetic acid, 80 μM naringinen in 25% HPLC methanol (recovery standard), and glucuronidase/sulfatase were added to plasma samples, flushed with argon, and subsequently incubated in a water bath at 37 °C for 40 min. 3.4% (w/v) phosphoric acid pH 1.2 was added to each sample and centrifuged at 1000 RPM, 4 °C, for 10 min in a Sorvall RC-5B, SM-24. Thereafter, solid phase extraction using a Visiprep SPE vacuum manifold was initiated by first conditioning the cartridge (waters, Oasis HLB 3cc (60 mg)) using DMF:methanol (7:3) and then 0.5% acetic acid in water. Samples were then loaded. The sample/cartridge was then washed with each of the following: 0.5% acetic acid in water, water: methanol:acetic acid (80:20:0.5), and 0.5% acetic acid in acetonitrile, respectively. Vacuum was used as needed, <5 mmHg. Analyte elution was achieved by lastly adding DMF:methanol (7:3) to the sample/cartridge which was collected by 5 mL culture test tubes containing 200 μL of 0.5% acetic acid in methanol and 5 μL 100 μg/μL L-Ascorbic Acid. Volume was reduced to roughly 50 μL by removing the solvents under vacuum with a 7 SpeedVac Concentrator (Thermo Electron Corp., Milford, USA). Samples were flushed with argon and stored at −80 °C until analysis using an Agilent high-pressure liquid chromatography (HPLC) 1100 series unit (Agilent Technologies, Santa Clara, USA). On the day of analysis, samples were thawed and 150 μL of 5 μM Carbamazepine in 0.5% HPLC methanol (internal standard) was added to each sample. Using the above methodologies, recovery of resveratrol was 87% at 2.5 uM and 86% at 20uM. Correspondingly, the recovery standard naringenin had a recovery rate 89% at 5uM and 88.9% at 40 uM.

Platelet reactivity was assessed in Study 1 only, using whole blood lumi-aggregometery (Chrono-log Model 700, Havertown, USA) [22,23]. Blood was diluted 1:1 in saline and 100 μL of luciferin-luciferase reagent were added to a plastic cuvette and stirred at 1000 rpm. After a 2 min incubation the following agonists was added: collagen (1 μM and 5 μM), or arachidonic acid (0.5 mM) or adenosine diphosphate (ADP) (10 μM), with aggregation response measured over a six min period.

Plasma nitrate and nitrite levels were assessed in both studies using a nitrate/nitrite colorimetric assay (Cayman Chemical Co., Ann Arbor, USA). Ethylene-diaminetetraacetic acid- treated plasma was separated from whole blood by centrifugation (15 min at 1800*g* and 4 °C) immediately after collection and stored at −80 °C until analysis. The samples were processed according to the manufacturer's protocol, and the absorbance was measured using