GRAPE SEED EXTRACT PLUS VITAMIN C IMPROVES INDICES OF VASCULAR HEALTH

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INTRODUCTION

Cardiovascular diseases (CVD) are the leading cause of hospitalization and death in industrialized societies (1). Deficits in vascular endothelial function appear to be a critical factor underlying the progression of nearly all types of CVD. Although the mechanism of endothelial dysfunction has yet to be fully defined, a decrease in nitric oxide (NO) production and/or bioavailability appears to be a common underlying factor.

There is increasing evidence that oxidative stress plays a role in the loss of NO signaling (2-6). In particular, increased vascular superoxide production is associated with inactivation of NO and loss of endothelial-dependent vasodilation. Because oxidants and oxidative stress appear to be central in CVD, there is a strong reason to believe antioxidants may provide significant protection. Previous studies showing benefits of antioxidants on cardiovascular health suggest that intakes should be well above the current RDA's (7-9). For example, Vita and coworkers have shown increased arterial vasodilation 2 hours following oral administration of 2 grams of ascorbic acid (10). In addition, diets rich in plant foods are also associated with a decreased risk for CVD (11). Plant foods are rich in flavonoids and may provide additional cardiovascular protection. Schroeter et al, for example, found that flavonoid intake (specifically epicatechin) was an independent predictor of the beneficial vascular effects following consumption of flavonoid-rich food (12).

Grape seed extract (GSE) is a common dietary supplement that is rich in flavonoids, including epicatechin. Thus, we hypothesized that the combination of GSE and ascorbic acid would improve endpoints of vascular health, including endothelium-dependent vasodilation. To test this hypothesis, we performed a double-blind, randomized, placebo-controlled crossover study to examine the effects of GSE (450 mg/day; USANA Health Sciences) in combination with ascorbic acid (1500 mg/day; USANA Health Sciences) for 4 weeks in subjects with clinically proven CVD.

TABLE 1. BASELINE CLINICAL CHARACTERISTICS

CHARACTERISTIC	PLACEBO FIRST (N=21)	ACTIVE FIRST (N=21)	P
Age (yrs)	59 ± 11	61± 9	0.50
Female gender, n (%)	5 (24%)	6 (28%)	0.73
Race, n (% Black)	5 (24%)	6 (28%)	0.73
Diabetes, n (%)	12 (57%)	9 (43%)	0.36
Hypertension, n (%)	17 (81%)	14 (67%)	0.29
Smoking, n (%)	13 (62%)	14 (67%)	0.75
Family History of CVD, n (%)	8 (40%)	13 (65%)	0.11
Waist (in)	40 ± 7	42 ± 5	0.35
Body Mass Index (kg/m^2)	31 ± 7	31 ± 5	0.99
Total Cholesterol (mg/dL)	167 ± 53	152 ± 33	0.28
LDL Cholesterol (mg/dL)	97 ± 46	86 ± 27	0.36
HDL Cholesterol (mg/dL)	45 ± 12	39 ± 11	0.07
Triglycerides (mg/dL)	123 ± 45	136 ± 53	0.42
Fasting Insulin (µUI/mL)	16 ± 17	13 ± 9	0.47
Fasting Glucose (g/dL)	120 ± 35	138 ± 41	0.15

Mean ± SD

MATERIALS AND METHODS

Subjects/Study Design

This was a double-blind, randomized, placebo-controlled crossover study. A total of 48 subjects were enrolled and underwent screening with 42 subjects completing the study. Subjects were treated with an acute dose of grape seed extract plus ascorbic acid (divided into 4 tablets) versus placebo. Subjects then took the same total dose (2 tablets in the morning and 2 tablets in the evening) daily for 4 weeks. After a 2-week washout period, subjects received the alternative treatment (active or placebo). The order of treatment was randomized. Blood samples were collected and vascular testing were performed 2 and 4 hours post treatment (acute), respectively. Tests were repeated after 4 weeks (chronic) with the last dose taken the morning of the final day of a particular treatment (acute on chronic). The study was completed in accordance with the approved protocol entitled "Chronic Study of the Effect of Grape Seed Extract Plus Ascorbic Acid on Endothelial Function in Patients with Coronary Artery Disease" (Boston Medical Center IRB Protocol #H-26356).

Determination of Serum Vitamin C

Serum samples were analyzed for ascorbic acid via HPLC according to the CDC's Laboratory Procedure Manual for vitamin C. Briefly, samples were mixed 1:1 with 10% w/v metaphosphoric acid (MPA), centrifuged at 10,000 x g for 10 min, and the resulting supernatant diluted 1:5 with MPA. Analyte concentrations were determined relative to authentic samples.

Determination of Plasma Epicatechin

Plasma samples were treated with β-glucuronidase and incubated at 37°C for 45 min. Liberated epicatechin was extracted with ethyl-acetate and centrifuged for 10 min at 3000 rpm. The ethyl-acetate layer was transferred into a glass flask and evaporated to dryness at 40°C. Samples were resuspended in methanol and analyzed via liquid chromatography-mass spectrometry (LC-MS). Analyte concentrations were determined relative to authentic samples.

Plasma Antioxidant Reserve

To determine SIN-1 induced isoprostanes and plasma antioxidant reserve (PAR) (13), plasma samples from each subject were treated with an enzyme mixture consisting of catalase (50 U/ml) and uricase (2.5 U/ml) in 0.15M NaCl and incubated at 25°C for 10 min. Samples were then treated with SIN-1 chloride (0.2 mmol) and incubated at 37°C for 4 hr with shaking. Concentrations of 8-isoprostane were measured using an ELISA kit according to the manufacturer's instructions (8-iso Prostaglandin F2α Kit. Cayman Chemical, Ann Arbor, MI).

Fingertip Peripheral Arterial Tonometry (PAT)

Fingertip peripheral arterial tonometry (PAT) was measured as described (14) and outlined in Figure 2. Patients reported to the clinic the morning of their appointment. At baseline or 4 hours following supplementation (for both acute and chronic time points), fingertip pulse amplitude was recorded in both a control finger and experimental finger during reactive hyperemia (EndoPAT, Itamar Medical, Ltd; Caesarea, Israel).

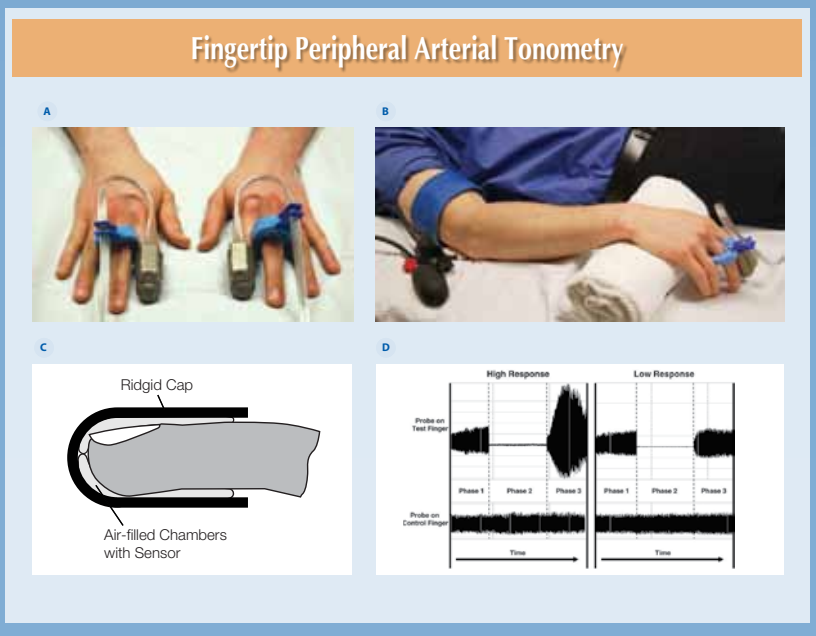

Figure 1. Fingertip peripheral arterial tonometry.

Peripheral arterial tonometry (PAT) is an emerging, non-invasive clinical test of endothelial function. Photo of a patient undergoing PAT analysis with a probe placed on both a control and experimental digit (A). An inflatable cuff is placed on the arm above the experimental digit and inflated to suprasystolic pressures to induce a localized ischemia, typically for 5 min (B). Cartoon of air-filled probe snuggly inflated around a single digit (C). As blood flows through the arterials and capillaries, the pressure exerted on the air-filled chambers is recorded. Phase 1 is the baseline reading; phase 2 is the readout during ischemia; phase 3 is recorded immediately following cuff release and subsequent hyperemia (D). A high response is recorded in the healthy volunteer representing greater endothelial-dependent arterial compliance relative to the low response in the unhealthy volunteer (D). Figure modified from Hamburg and Benjamin (14).

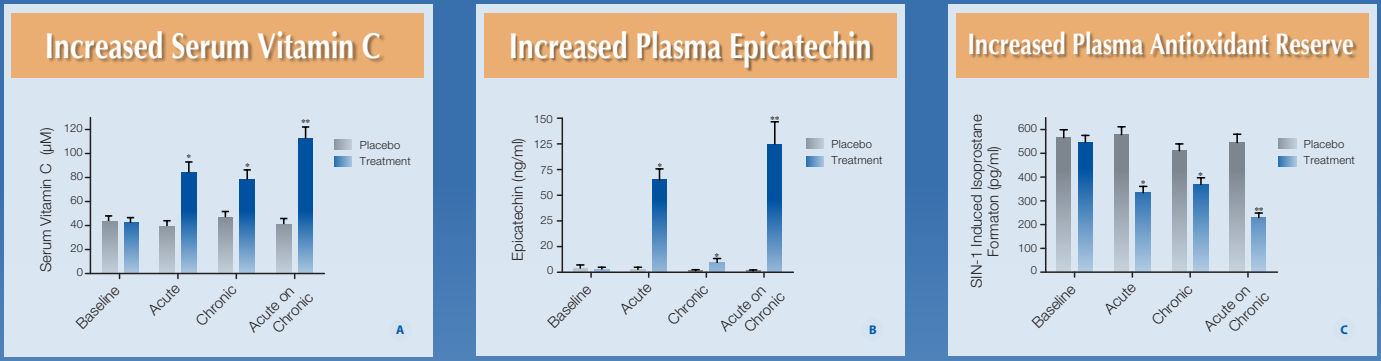

Figure 2. Supplementation with grape seed extract and ascorbic acid significantly improves vitamin C, epicatechin, and plasma antioxidant status. Results show a significant time-treatment interaction by repeated measures ANOVA for serum ascorbic acid (A), plasma epicatechin (B), and PAR (C) (P<0.001). On post hoc analysis, the changes from baseline in serum ascorbic acid (A), plasma epicatechin (B), and plasma antioxidant reserve (C) following acute (*P<0.001), chronic (*P<0.001), and acute-on-chronic (**P<0.001) treatment were different for active treatment compared to placebo treatment. Furthermore, the change in serum ascorbic acid (A), plasma epicatechin (B), and PAR (C) between chronic and acute-on-chronic treatment was also significantly different for active treatment compared to placebo treatments for all endpoints examined (**P<0.001).

Data are expressed as the natural logarithm of the ratio of hyperemic pulse amplitude to baseline divided by the ratio of hyperemic pulse amplitude to baseline in the opposite (control) finger (ln PAT ratio): ln ((Amplitude^{Hyperemia} /Amplitude^{Baseline})$^{Occlusion\ Finger}$/(Amplitude^{Hyperemia} /Amplitude^{Baseline}))$^{Control\ Finger}$

RESULTS

- **Treatment significantly increased serum vitamin C at all time points (Figure 2A).**
- **Treatment significantly increased plasma epicatechin at all time points (Figure 2B).**
- **Treatment significantly decreased SIN-1 induced 8-isoprostane formation indicating an increase in Plasma Antioxidant Reserve at all time points (Figure 2C).**
- **Treatment significantly improved endothelial function in the peripheral microvasculature (capillaries and arterials; Figure 3).**

CONCLUSIONS/DISCUSSION

This study showed that the combination of grape seed extract (GSE; 450 mg/d) plus vitamin C (1500 mg/d) improved antioxidant status and vascular function in patients with clinically proven cardiovascular disease (CVD). Specifically, we demonstrated that circulating levels of vitamin C increased at all time points following treatment (Figure 2A). Interestingly, Plasma Antioxidant Reserve (PAR), a measure of resistance to oxidative stress, mirrored exactly the vitamin C effect suggesting a causal link between vitamin C concentrations and PAR (compare Figures 2A and 2C). This finding is significant because oxidative stress is associated with a variety of chronic degenerative diseases including CVD. Thus, treatment with GSE plus vitamin C significantly increases the capacity to guard against the detrimental consequences of oxidative stress in the plasma. Epicatechin, a marker for absorbable bioflavonoids in GSE, was highly increased following treatment but only within a few hours of taking the supplement (Figure 2B; acute and acute-on-chronic). We also observed a significant improvement in endothe-

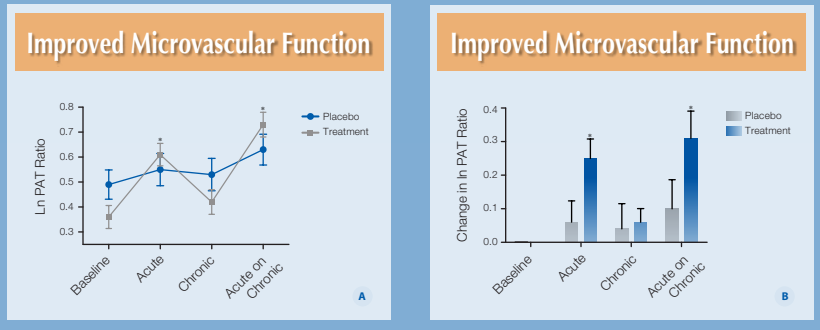

Figure 3. Supplementation with grape seed extract and ascorbic acid improves endothelial function in the fingertip vasculature.

There was a significant time-treatment interaction for the effect of active and placebo treatment on the ln PAT ratio over time (P=0.02). As shown, acute and acute-on-chronic active treatment were associated with significant improvements in ln PAT ratio. Data are expressed as both the ratio (A) and change in ln PAT ratio (B) over time (*P<0.05).

lial function as assessed by fingertip Peripheral Arterial Tonometry (PAT; Figure 1), a Federal Drug Administration (FDA) approved system to measure blood flow in the fingertip microvasculature (capillaries and arterials). Interestingly, we only saw significant improvements in PAT at those same time points where epicatechin levels were at their highest suggesting a causal link between circulating epicatechin and improved vascular function (compare Figure 2B and 3). The finding that GSE plus vitamin C improves endothelial function (increased blood flow) is significant because the role of endothelial dysfunction (decreased blood flow) in CVD is well established. Thus, treatment with GSE plus vitamin C may provide protection against the progression of CVD. Taken together, these results suggest that treatments with GSE plus vitamin C work in a complementary fashion to improve indices of vascular health by providing both antioxidant protection and improved microvascular endothelial function in patients with clinically proven CVD.

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