ACUTE RESVERATROL SUPPLEMENTATION IMPROVES FLOW-MEDIATED DILATATION IN OVERWEIGHT/OBESE INDIVIDUALS WITH MILDLY ELEVATED BLOOD PRESSURE

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Abstract

(1) Background and Aims – Flow mediated dilatation of the brachial artery (FMD) is a biomarker of endothelial function and cardiovascular health. Impaired FMD is associated with several cardiovascular risk factors including hypertension and obesity. Various food ingredients such as polyphenols have been shown to improve FMD. We investigated whether consuming resveratrol, a polyphenol found in red wine, can enhance FMD acutely and whether there is a dose-response relationship for this effect.

(2) Methods and Results – 19 overweight/obese (BMI 25-35 $kg.m^{-2}$) men or post-menopausal women with untreated borderline hypertension (systolic BP: 130-160 mmHg or diastolic BP: 85-100 mmHg) consumed three doses of resveratrol (resVida™ 30, 90 and 270 mg) and a placebo at weekly intervals in a double-blind, randomized cross-over comparison. One hour after consumption of the supplement, plasma resveratrol and FMD were measured. Data were analyzed by linear regression versus log_{10} dose of resveratrol. 14 men and 5 women (age 55 ± 2 years, BMI 28.7 ± 0.5 $kg.m^{-2}$, BP 141 ± 2 / 89 ± 1 mmHg) completed this study. There was a significant dose effect of resveratrol on plasma resveratrol concentration ($P<0.001$) and on FMD ($P<0.01$), which increased from 4.1 ± 0.8% (placebo) to 7.7 ± 1.5% after 270mg resveratrol. FMD was also linearly related to log_{10} plasma resveratrol concentration ($P<0.01$).

(3) Conclusion- Acute resveratrol consumption increased plasma resveratrol concentrations and FMD in a dose-related manner. This effect may contribute to the purported cardiovascular health benefits of grapes and red wine.

Key Words: Resveratrol, flow-mediated dilatation, blood pressure, cardiovascular risk factors, endothelial function

Introduction

Impaired flow-mediated dilatation (FMD) in the brachial artery is characterised by loss of endothelium-dependent vascular smooth muscle relaxation in response to vasodilator stimuli (1). The resultant effect of this impairment may be partly due to alterations in the nitric oxide (NO) pathway, notably a reduction in endothelium-derived NO bioavailability (2-3). Chronically reduced NO bioavailability could play a mechanistic role in the progression of cardiovascular disease (CVD) (2). In fact, impaired FMD is now recognised as an independent risk factor for the development of CVD (4-5). Several cardiovascular risk factors including hypertension (6) and overweight/obesity (7) are associated with impaired FMD, which may reflect structural and functional changes to the endothelium (8).

Consumption of various polyphenol-rich food ingredients such as cocoa (9), green and black tea (10-11), grape seed extract (12) and red wine extract (13) have been shown to acutely improve FMD in at-risk population groups. However, it is not known which specific polyphenolic compounds present in these foods are responsible for this beneficial effect. Resveratrol (3,5,4'-trihydroxystilbene) is a polyphenol predominately found in red wine and grapes that has attracted much interest due to its cardioprotective potential shown *in vivo* (14-16). Resveratrol exists in *cis*- and *trans*- isomeric forms, but only *trans*-isomer is mainly present in wine (17), which can be found at highest concentration in grape skin in ranges between 50 and 400µg/g of fresh weight (18). Hence, *trans*-resveratrol is often used in clinical trials due to its stability in absence of ultra-violet light (17). Depending on the variety of grape used in wine making, the concentration of *trans*-resveratrol in red wine is between 0.1 and 14.0mg/L (19). The estimated daily intake of resveratrol in the United State of

America from naturally occurring sources is 0.08mg with 90th percentile consuming no more than 0.26mg daily (20).

The purported cardiovascular benefits of resveratrol from experimental evidence include defence against ischaemic-reperfusion injury (21-23), suppression of platelet aggregation (16, 24), enhanced antioxidant status (16, 25) and increased NO bioavailability through enhanced endothelial NO synthase expression in cultured human endothelial cells (14-15). Recently, the progression of endothelial dysfunction and vascular remodelling in rats with pulmonary and cardiac abnormalities were slowed after three weeks of daily resveratrol administration (25mg/kg) (26). This cardioprotective effect was attributed to the actions of resveratrol in reducing vascular smooth muscle cell proliferation and enhancing endothelium-derived NO synthase and NO bioavailability.

Thus, resveratrol supplementation may be able to enhance FMD in overweight/obese and/or hypertensive individuals in whom FMD may be impaired. To date, no human studies on the effects of pure resveratrol on FMD have been reported. Therefore, the objective of this study was to investigate whether oral resveratrol supplementation could acutely improve FMD in a dose-dependent manner.

Methodology

Study Design

A randomised, double-blind, placebo-controlled, crossover human intervention trial comprising four visits at weekly intervals was undertaken. This study was approved

by the Human Research Ethics Committee of the University of South Australia. Each participant provided written and informed consent prior to participation.

Study Population

Overweight/obese (Body Mass Index [BMI] ≥ 25, <35 kg/m^2) adults aged 30-70 years with elevated blood pressure (BP) (systolic BP 130-160 mmHg and/or diastolic BP: 85-100 mmHg, determined at time of screening, were recruited for an acute dietary supplementation interventional trial. Sample size calculation indicated that 16 volunteers were required to complete the study in order to provide 80% power to detect a difference in FMD of 2.0% at α of 0.05, based on an estimated standard deviation of 2.64% (9).

Women were required to be post-menopausal (self reported cessation of menses for at least 12 months). An appropriately sized BP cuff was fitted to participants as they rested in a seated position for five minutes prior to BP assessment. Four readings were obtained with a SpaceLabs ambulatory BP monitor (Model 90217, SpaceLabs Medical, Florida, USA) at one-minute intervals with accordance to previously outlined procedures (27). Discarding the first reading, an average of the last three readings was used to determine BP eligibility. Volunteers had no history of CVD, diabetes or renal disease, were not taking diabetic or BP lowering medication and were not currently smoking or using nicotine replacement therapy.

Participants were advised to maintain their customary diet prior to each visit, but to limit their polyphenol intake (including peanuts, cranberries, mulberries, red grapes, red wine, green tea and dark chocolate and physical activity habits throughout the study.

Resveratrol Capsules

DNP-DSM Nutritional Products, Kaiseraugst, Switzerland, supplied all resveratrol (resVida™) and placebo capsules. Participants were allocated to consume each of three doses of resveratrol (30, 90 and 270mg) and a placebo at weekly intervals in a double-blind, randomized fashion. All doses consisted of six capsules and participants consumed a single dose once at each visit. The placebo capsules contained an inert filler consisting of Calcium Hydrogen Phosphate, microcrystalline cellulose of various particle sizes including Prosolv 50 and talcum powder (hydrated magnesium silicate), and were identical in appearance to the resveratrol capsules which contained 99% pure synthetic *trans*-resveratrol.

Each dose of resveratrol was assigned the letters A, B, C and D by an independent staff member. A random number generator was used to determine the allocation of each dose to participants.

Clinical Assessments

Participants arrived at the research centre having fasted for at least four hours before consuming the six capsules with *ad libitum* water in the presence of an investigator (to ensure compliance). Outcome measures were then assessed after participants rested in the research centre for 45 minutes. This protocol was repeated a total of four times at weekly intervals, once for each resveratrol dose or placebo. No baseline treatment was given.

FMD. Endothelial function was assessed using FMD by ultrasound (General Electric Logiq 5 Expert) with a two-dimensional B-mode 12 MHz transducer in accordance to published guidelines (28). Participants rested in a supine position for a further 15 minutes before FMD testing commenced. This timing was chosen based on pharmacokinetic results of peak plasma resveratrol concentrations, which occurred 0.8 to 1.5 hours after healthy subjects consumed a single dose of *trans*-resveratrol

(25-150mg) (29). Participants were fitted with an inflatable occlusion cuff, which was positioned on the right forearm abutting the cubital fossa to minimise movements in surrounding tissues when ultrasound images were collected. After obtaining an image of the brachial artery, 30 seconds of baseline data was recorded before the occlusion cuff was inflated to 200 mmHg for five minutes. Post-rapid deflation images of the brachial artery were recorded for a further three minutes. All recorded images were gated to end-diastole of the cardiac cycle for data analysis as determined by a single-lead electrocardiogram and digitally recorded onto a disc recorder hard drive (LG, LG Electronics, Eastern Creek, Australia).

The FMD video files were analysed using the edge-detection software, Brachial Analyzer (Medical Imaging Application LLC, Iowa, United States). Each digitally recorded FMD file was converted to .AVI format using iSofter DVD ripper Platinum (iSofter Inc. 2005, United States) and separated into two files (baseline and deflation) for analysis. For both baseline and deflation, a region of interest was carefully defined over a clear section of vessel with care to ensure that the region of interest was the same size and position for both baseline and deflation files. The automated edge-detection feature of the software then performed a frame-by-frame analysis to generate artery diameter (mm) values for both baseline and deflation. Baseline was defined as the average of the 30 seconds of pre-inflation diameter measures. A spline curve was fitted through the deflation values and from this curve the peak diameter was determined. Using baseline and peak diameters, the percent change in diameter was calculated, which will hereby be referred to as %FMD.

Plasma resveratrol concentration. Following FMD measurement, a venous blood sample was collected and centrifuged for 15 minutes at 1000g at 4ºC within 30 minutes of blood sampling. Approximately 1mL of cell free plasma supernatant was

immediately transferred into a polypropylene Eppendorf tube, treated with nitrogen and stored at -80ºC freezer until analysis of plasma resveratrol concentrations.

Liquid chromatography mass spectrometry (LC-MS) system was used to determine "free" *trans*-resveratrol, dihydroresveratrol (aglycone), "total" *trans*-resveratrol and dihydroresveratrol (aglycone plus glucuronide conjugates) in plasma samples. The samples were injected on a C18 column after adding internal standard and liquid-liquid extraction ("free" analyte) or pre-digested by ß-glucuronidase followed by liquid-liquid ("total" analyte). Detection was performed using MS in the SIM mode.

Statistical Analysis

Statistical analyses were performed using SPSS Version 17 (Chicago, Illinois, United States of America). The 30, 90 and 270mg resveratrol doses and their corresponding plasma resveratrol concentrations were log transformed prior to analysis to give a linear dose progression. Linear regression analyses were performed for each outcome measure to determine the significance of the (a) dose-response relationship and (b) the differences between each dose of resveratrol and placebo. Adjustment was made for clustering due to repeated measures within a participant. Baseline brachial artery diameters for all doses were compared using one-way ANOVA. All results are presented as mean ± SEM.

Results

Subjects

Participant flow is provided in Figure 1. Of the 20 participants who were enrolled, 19 (14 males and 5 females) completed the study. One withdrew after one visit due to bronchitis, which required medication but was unrelated to participation in the study. Baseline characteristics are shown in Table 1.

Plasma resveratrol concentration

With increasing doses of resveratrol, there were proportional increases in plasma resveratrol concentrations (ng/mL) (30mg: 181.31 ± 13.59, 90mg: 532.00 ± 86.38; 270mg: 1232.16 ± 147.57). Linear regression analysis revealed a significant relationship ($P<0.001$, $R^2=0.63$) between $\log_{10}$ of resveratrol dose and $\log_{10}$ of plasma resveratrol concentration. There was also a significant linear relationship ($P<0.01$, $R^2=0.08$) between $\log_{10}$ of plasma resveratrol concentration and acute FMD (Figure 2).

FMD

There was no significant difference ($P=0.21$) in the baseline brachial artery diameters measured following each dose (0mg: 4.25 ± 0.17mm; 30mg: 4.08 ± 0.15mm; 90mg: 4.24 ± 0.18mm; 270mg: 4.08 ± 0.16mm). However, there was a significant linear relationship ($P<0.01$, $R^2=0.06$) between $\log_{10}$ of resveratrol dose and acute FMD response. For each of the three doses of resveratrol (30, 90, 270mg), FMD was significantly increased ($P<0.05$) compared to placebo (Figure 3).

Discussion

This is the first study to evaluate the acute effects of resveratrol consumption on human circulatory function. Oral resveratrol supplementation elicited an acute dose-related improvement in endothelium-dependent vasodilatation, as demonstrated by significant increases in FMD at each dose relative to the placebo. Improvements in FMD were correlated with a dose-related increase in plasma resveratrol concentrations. Whilst the FMD assessment does not reveal whether resveratrol is acting on the endothelium or on vascular smooth muscle to enhance vasodilatation,

there is evidence to suggest that resveratrol can increase endothelium-derived NO bioavailability (14, 30-31). Compared to placebo, our lowest dose (30mg) was shown to significantly improve FMD. A recent *in vitro* study (25) has shown that NO production could be enhanced in platelet cells of healthy adults after 15 days of moderate red wine consumption (300mL/day), which was associated with only a small amount of resveratrol (0.5µmol/L) in the plasma. However, other polyphenols present in the red wine may have contributed to the increased NO production. Similarly in a study by Lekakis et al. (13), significant improvements in FMD were observed 60 minutes after acute oral supplementation with 600mg of red wine polyphenol extract. Although *trans*-resveratrol was present in their extract, its low concentration of less than 1mg was unlikely to contribute significantly to the observed improvement in FMD. Other polyphenolic compounds such as epicatechin, which were present in higher concentrations in this extract may have mediated this improvement. Thus, the present study is the first to demonstrate that synthetic *trans*-resveratrol can improve FMD acutely and in a dose-related manner in at-risk population groups. However, the lowest resveratrol dose (30mg) used in this study cannot be obtained from normal dietary habits (20).

Whilst the acute improvement in FMD after supplementation is encouraging, it is important to see if this improvement seen in FMD after acute oral resveratrol supplementation is sustainable, given that little is known about the long-term effects of *trans*-resveratrol in human subjects following chronic supplementation.

In conclusion, results of this acute study demonstrated a dose-related improvement in FMD that correlated with increased plasma resveratrol concentrations. FMD was significantly increased by each dose of resveratrol compared with placebo. These findings suggest that resveratrol may contribute to the purported cardiovascular

health benefits of grapes and red wine. Further research is warranted to confirm the sustainability of the effect of resveratrol on FMD.

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Table

Table 1 - Baseline characteristics of participants who completed the study.

Number (Male/Female)	19 (14/5)
Height (m)	1.76 ± 0.03
Weight (kg)	88.7 ± 2.9
BMI ($kg.m^{-2}$)	28.7 ± 0.5
Age (years)	55 ± 2
SBP (mmHg)	141 ± 2
DBP (mmHg)	89 ± 1
HR (bpm)	69 ± 2

Figure legends

Figure 1 - Consort diagram of participants who were screened, enrolled, completed the study and included in the analysis.

Figure 2 – Individual FMD responses relative to $\log_{10}$ of plasma resveratrol concentration ($P<0.01$, $R^2=0.06$). The solid line represents the mean of the slope.

Figure 3 – FMD responses following consumption of each dose of resveratrol (mean ± SEM). * = significant difference ($P<0.05$) relative to placebo.

Figures

Figure 1 - Consort diagram of participants who were screened, enrolled, completed the study and included in the analysis.

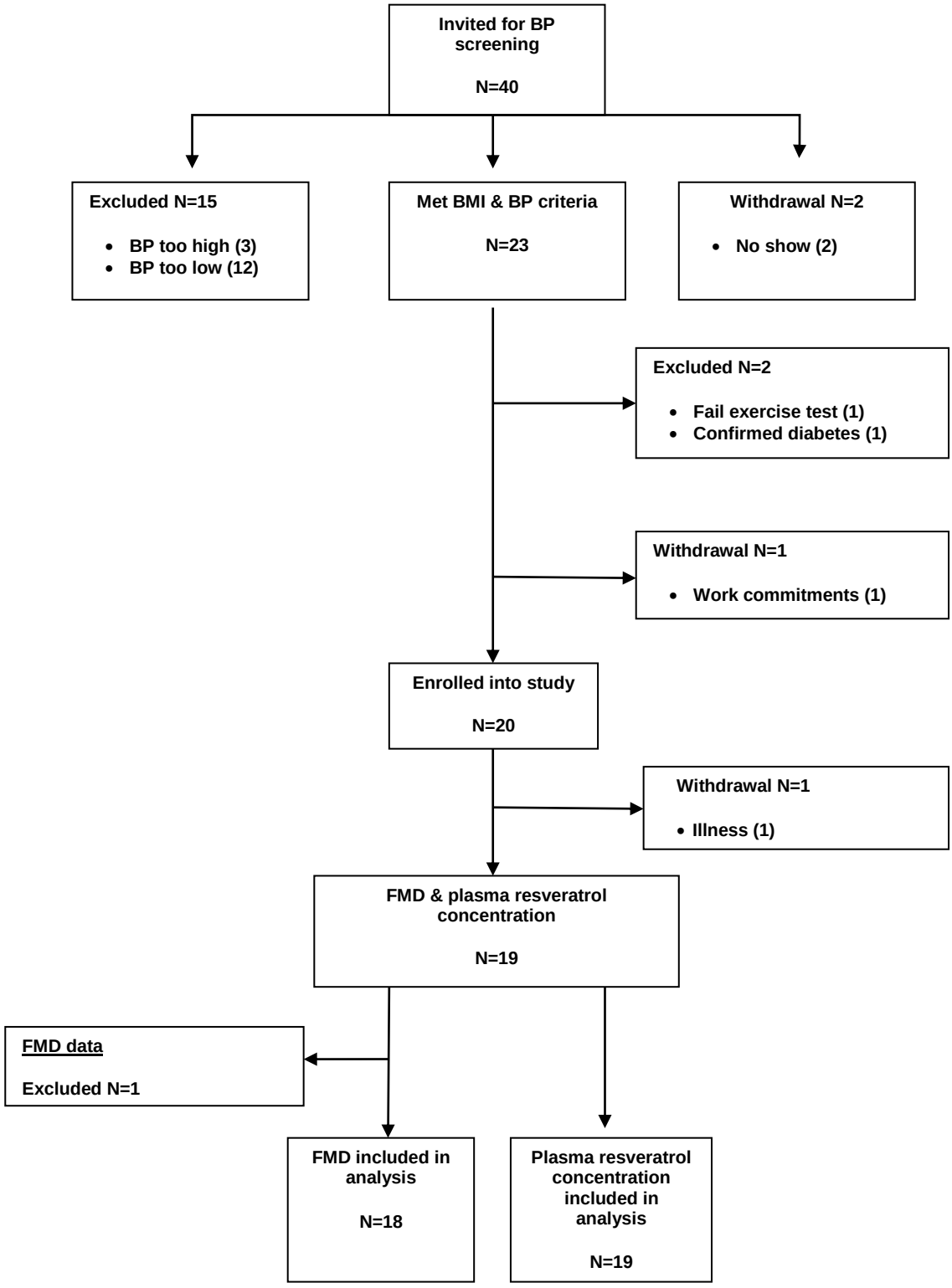

Figure 2 – Individual FMD responses relative to log_{10} of plasma resveratrol concentration ($P<0.01$, $R^2=0.08$). The solid line represents the mean of the slope.

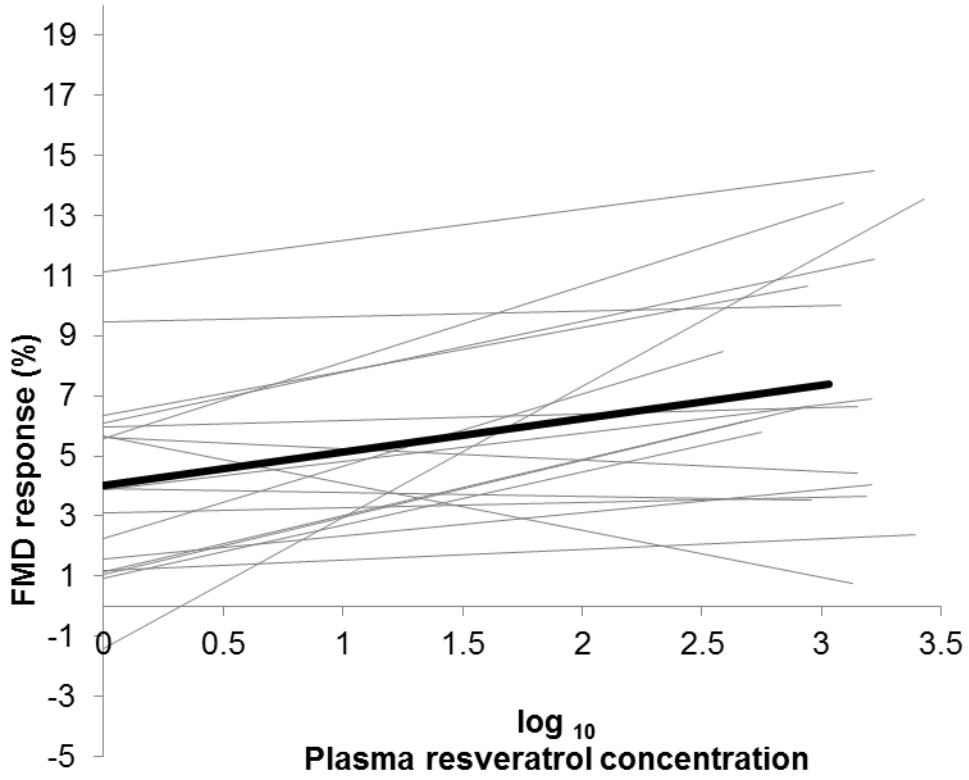

Figure 3 – FMD responses following consumption of each dose of resveratrol (mean ± SEM). * = significant difference (P<0.05) relative to placebo.