Effects of Acute Grape Seed Extract Supplementation on Hemodynamics in Normal Body Weight and Obese Males

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Summary Recently, it has been reported that dietary supplementation with grape seed extract (GSE) ameliorates endothelial function and increase nitric oxide (NO) bioavailability. Thus, we investigated if elevated blood pressure and aortic stiffness (AoS) characterized in obese individuals are attenuated following acute GSE supplementation. Twenty men (obese=10; normal body weight (NBW)=10) participated in this study. Systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), heart rate (HR), stroke volume (SV), cardiac output (CO), total peripheral resistance (TPR), and AoS were compared 2 h after ingestion of GSE or placebo (PL) on different days, 1 wk apart. Compared with the PL, GSE supplementation significantly decreased SBP (NBW: 103±4 vs. 99±3 mmHg; obese: 118±3 vs. 112±5 mmHg) and MAP (NBW: 75±2 vs. 72±2 mmHg; obese: 86±3 vs. 84±3 mmHg) in both groups, while there were no differences in HR, SV, DBP, TPR, and AoS. GSE supplementation significantly decreased CO in only obese group. In NBW group, TPR tended to be decreased, but there was no significant difference. Our study suggests that acute supplementation with GSE reduced both SBP and MAP via a reduction in CO in obese individuals and decreased peripheral vasoconstriction in NBW group.

Key Words nitric oxide, endothelial function, vasoconstriction, grape seed extract, blood pressure

According to the report published by the National Health and Nutrition Examination Survey, using a body mass index (BMI) of 25 to 29.9 kg·m⁻² and 30 kg·m⁻² or greater, the prevalence of overweight and obesity is 31.6% and 39.6% for adults in the United States, respectively (1, 2). Obesity can lead to serious health risks (3) such as aortic stiffness (AoS), hypertension, and coronary artery disease (4, 5) compared with normal weight individuals. Therefore, functional food materials which improve obesity or obesity-related disorders have been investigated by a number of nutritionists.

Excessive baseline sympathetic activity and central arterial stiffening are associated with the future development of hypertension and cardiovascular disease (6, 7). Particularly, arterial stiffness may predispose obese individuals to induce hypertension (8). Evidence shows that increased production of nitric oxide (NO) lead to improved AoS in both humans and animals (9, 10). NO is a molecule that is endogenously synthesized in the vascular endothelium by an enzyme called the endothelial nitric oxide synthase (eNOS) and plays a key role in regulating vascular tone, blood flow, and reducing AoS (10–12). However, it has been reported that NO bioavailability is decreased in obese individuals due to impaired endothelial function and reactive oxidative species (ROS)-induced reduction in the production of NO (13). Taken together, these findings may imply that endothelial dysfunction contributes to increased AoS and develop into hypertension for obese individuals. Accordingly, the endothelium may be a target for preventive intervention in adults with obesity.

As an alternative pathway of NO synthesis, dietary therapy using nutritional supplements appears to be a potential intervention for improving hemodynamics by increasing NO bioavailability, as some obese individuals have low exercise tolerance and musculoskeletal pain (14, 15). It has been suggested that consumption of food rich in polyphenols decreases the risk of cardiovascular diseases (16). Grapes can be a source of dietary supplementation to increase the production of NO since they are known to be rich in polyphenols which are mostly found in seeds and contain anthocyanins, flavonoids, and resveratrol phenolic compounds (17). Due to the fact that grape seed extract (GSE) contains flavonoids which consists primarily of (+)-catechin, (−)-epicatechin, and procyanidin polymers (17), the phenolic compounds have a beneficial effect on cardiovascular diseases (18, 19). In this regard, previous studies have proven that acute dietary GSE supplementation improves endothelial function in prehypertensive individuals (20, 21) and postmenopausal women (22). However, it still remains unknown whether dietary supplementation with GSE acutely can reduce AoS and arterial pressure in obese individuals. We hypothesized that acute dietary supplementation with GSE attenuates AoS, systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), and total
peripheral resistance (TPR). These effects are partially due to reductions in peripheral vasoconstriction and AoS.

MATERIALS AND METHODS

Study population. The study consisted of obese (n=10) and normal body weight (NBW) (n=10) men aged 18–29 y old who were recruited from California Baptist University campus. The study was approved by the Institutional Review Board (081-1819-EXP) at the university prior to testing. Subjects were informed of pretesting guidelines and informed of risks and benefits prior to participating in the study. Then they reviewed and signed a consent form. This current study complied with the code of ethics of the World Medical Association (Declaration of Helsinki). Subjects were excluded if they had any of the following as determined by a Physical Activity Readiness Questionnaire (PAR-Q): cardiovascular diseases or consume any antihypertensive medication or supplementation that can affect arterial pressure or AoS. All participants were asked to abstain from drinking alcohol and caffeine for 24 h prior to each trial. This study was registered in Clinicaltrials.gov (NCT04389060).

Study design. The study was designed as a double-blind, cross-over test. Subjects were asked to come to the laboratory on three different occasions. During the first visit, height and weight were measured to calculate BMI to determine if they were eligible for this study. At least two measurements of blood pressure (BP) were taken from the brachial artery, 2 min apart, using a sphygmomanometer and pressure cuff. During the second visit, subjects were asked to take either GSE or placebo (PL) capsules 2 h prior to coming into the laboratory. Subjects were instrumented for 5 min prior to measuring the cardiovascular responses via PhysioFlow (non-invasive device). Hemodynamics were monitored for 5 min. Following each trial, a 1-wk washout period separated the supplementation periods. During this time, subjects were asked to maintain a similar diet and normal routine of daily activity. After the washout period, cross-over treatment with the other supplement (either PL or GSE) was administered. The same procedure was repeated.

Supplementation. Subjects were randomly assigned via a double-blind, cross-over design to evaluate the effects of acute GSE supplementation (300 mg, two capsules: Meganatural®-BP. Polyphenolics Inc., Madera, CA, USA) on AoS compared to a PL treatment (starch; 300 mg, two capsules). The PL treatment was made to look identical to the GSE supplementation, so both the participant and investigator were blinded to the treatment being received. It has been known that the total phenol ingredients in GSE (300 mg) determined by the Folin-Ciocalteu method was 95.3% (grams gallic acid equivalent/100 g) and contained 2.8% gallic acid, 5.1% catechin plus epicatechin, and 87.4% total oligomers as measured by high-performance liquid chromatography (23). Subjects were instructed to take the supplementation 2 h prior to testing. Our acute intervention was selected since a previous finding showed that a single dose of GSE could reduce resting BP in individuals with prehypertension (20).

Data collection. Impedance cardiography (PhysioFlow, Manatec Biomedical, Paris, France) was continuously used to measure stroke volume (SV), heart rate (HR), and cardiac output (CO). This device consists of six different electrodes to measure real-time HR and SV and provide CO data. Two of the electrodes were placed right above the carotid artery located on the neck, two other electrodes were placed by the xiphoid process, and the last two were used for ECG. To measure SV, the bioimpedance detects changes in transthoracic impedance during each cardiac cycle. The PhysioFlow has been found to be a reliable method used in calculating hemodynamic variables and AoS (24–27).

Resting BP was measured on the brachial artery in a resting position using a sphygmomanometer by the same investigator throughout the study. MAP was calculated by using the formula: (SBP – DBP)/3 + DBP. TPR was calculated by the formula: MAP divided by CO.

Statistical analysis. Average values of all variables across conditions, two-way repeated-measures ANOVA and Tukey’s post hoc test were used. Means were considered to be statistically different at p<0.05. In order to detect a statistically significant change (p<0.05) of 10% in marker of BP between before and after supplementation, a power test (power=0.80) was 10.

RESULTS

Table 1 indicated physical characteristics of subjects. NBW and obese individuals were age and height matched, however, due to the purpose of this study obese group had a significantly higher BMI compared to NBW group. Obese individuals had a significantly higher resting HR, SBP, and DBP than the NBW individuals.

Table 2 shows the averaged SBP, DBP, MAP, HR, SV, CO, TPR, and AoS after PL and GSE supplementation in
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In this regard, our findings may have clinical implications leading to reductions in BP in obese individuals. Similar to what was observed in previous studies, our study demonstrated that obese individuals had elevated CO and arterial pressure compared to the NBW individuals. However, following the ingestion of GSE, CO was significantly reduced in obese individuals, whereas there was no difference in NBW individuals. The mechanisms mediating this reduction in CO are not well understood. One possible explanation for the potential influence of GSE is that an increase in NO bioavailability into the renal artery increases sodium and water excretion (33, 34). These results were supported by previous studies that urinary volume and sodium excretion were decreased with inhibitors of endogenous NO production (35, 36). Obesity is related to abnormal sympathetic activity, renin-angiotensin system activity, and blunted response of atrial natriuretic peptide (ANP), which contributes to sodium retention and elevated arterial pressure (37, 38). If this hypothesis is true, increased water excretion elicited by an increase in production of NO would decrease venous return, stroke volume, and CO as demonstrated in this study. However, our study is limited that the effects of GSE supplementation on natriuretic and diuretic were not assessed.

**Effect of GSE on BP, AoS, and TPR**

In the current study, compared with the PL, GSE significantly reduced MAP in both obese individuals without changes in peripheral vasodilation. It is generally accepted that obesity has high BP mainly due to an increase in CO at rest. TPR is calculated by dividing MAP by CO. Accordingly, TPR remains similar by a reduction in CO along with a decrease in MAP compared the NBW individuals (39, 40). Our study indicated that acute dietary supplementation with GSE had reduction in BP in obese individuals with no change in TPR. The reduction in MAP is only accountable to decrease in CO. On the other hand, despite an increase in CO the fall in MAP occurred via a reduction in TPR in NBW individuals even though it was not significantly different (p=0.1). These findings indicate that in NBW the reduction in arterial pressure mediated by GSE sup-

### Discusssion

The major finding of this study is that acute supplementation with dietary GSE reduced SBP and MAP at rest in both groups, while there are no significant differences in HR, SV, TPR, DBP, and AoS. These reductions in BP occurred without any changes in AoS. As observed previously, our study indicated that obese individuals had higher CO, AoS, and arterial pressure compared to the NBW individuals. The decrease in BP occurred mainly via a greater reduction in CO with minimal if any net change in peripheral vasoconstriction in respect to the control values after the dietary GSE supplementation. In contrast, the reduced BP was likely due to a decrease in peripheral vasoconstriction in NBW group.

**Effect of GSE on cardiac output**

Obesity is characterized by the abnormal cardiovascular hemodynamics such as elevated intravascular volume, CO, arterial pressure, sympathetic nerve activity, and peripheral resistance (28–30). Increasing evidence suggests that excessive sympathetic activity and the abnormal hemodynamic changes may contribute to the development of hypertension and cardiovascular diseases (31, 32). In this regard, our findings may have

### Table 2. Hemodynamics after PL and GSE supplementation in both normal body weight and obese individuals.

|                | NBW                  | Obesity               | p values | Body weight | Supplementation | Interaction |
|----------------|----------------------|-----------------------|----------|-------------|-----------------|-------------|
|                | PL                   | GSE                   | PL       | GSE         |                 |             |
| SBP (mmHg)     | 103±4                | 99±3                  | 118±3    | 112±4       | p=0.035         | p=0.001     | p=0.491     |
| DBP (mmHg)     | 61±2                 | 59±2                  | 71±3     | 70±3        | p=0.022         | p=0.167     | p=0.405     |
| MAP (mmHg)     | 75±2                 | 72±2                  | 86±3     | 84±3        | p=0.021         | p=0.002     | p=0.865     |
| HR (bpm)       | 70±4                 | 73±4                  | 78±4     | 77±4        | p=0.357         | p=0.380     | p=0.248     |
| SV (mL)        | 91.0±4.3             | 93.3±7.5              | 98.5±4.3 | 92.7±7.7   | p=0.642         | p=0.721     | p=0.332     |
| CO (L/min)     | 6.4±0.5a             | 6.8±0.6a              | 7.6±0.3b | 6.9±0.3a   | p=0.383         | p=0.575     | p=0.040     |
| TPR (mmHg/mL/min) | 0.02±0.0            | 0.01±0.0              | 0.01±0.0 | 0.01±0.0   | p=0.811         | p=0.998     | p=0.075     |
| AoS (AU)       | 8.6±0.53             | 10.3±0.79             | 14.0±1.61| 12.9±2.41  | p=0.082         | p=0.781     | p=0.292     |

Values are expressed as means±standard error. PL, placebo; GSE, grape seed extract; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; HR, heart rate; SV, stroke volume; CO, cardiac output; TPR, total peripheral resistance; AoS, aortic stiffness. Groups with different superscripts are significantly different (p<0.05).
plementation is attributed to a decrease in peripheral vasoconstriction. In obesity, the reduction in BP may depend primarily on a decrease in CO.

Limitations of the study
A limitation to this study was the fact that endothelial function before and after each supplement was not measured to determine effect of GSE on peripheral vasodilation, since no biological markers of NO bioavailability (e.g., nitrates and nitrite concentrations) were assessed. These measurements would reinforce our conclusions concerning the contribution of GSE-induced NO bioavailability to an improvement in endothelial function. We studied only men because resting BP fluctuates during the phases of the menstrual cycle (41). In consequence, detection of effects of dietary supplementation with GSE on resting hemodynamics due to sex difference will likely result in potential compounding if the phase of the menstrual cycle is not held constant. Despite the fact that acute/single dose of GSE reduced arterial pressure without change in AoS, it is further needed to confirm that this effect is maintained over time. Thus, investigation of chronic effects of GSE supplementation on AoS, arterial pressure, and endothelial function should be determined in obese subjects.

Perspectives and significance
It is well documented that obesity is associated with pathological conditions such as hypertension, diabetes, dyslipidemia, and coronary artery diseases. Our findings suggest that GSE supplementation can act as a dietary nutraceutical that is capable of reducing arterial pressure and CO in obese individuals. This supplement additionally may be a non-pharmacological intervention to prevent future hypertension via enhanced endothelial function. Since this GSE product has been known to a highly concentrated, purified source of polyphenolic flavan-3-ols, even small dosages that are taken daily can be beneficial to cardiovascular health.

CONCLUSIONS
To our knowledge, this study is the first to investigate that dietary supplementation with GSE can have the potential of reducing AoS and arterial pressure in obese individuals. Our study demonstrated that acute dietary supplementation with GSE decreased arterial pressure without any changes in AoS in both groups. GSE decreases CO which contributes to decrease in peripheral vasodilation and reducing arterial pressure in NBW. This effect is not likely to be caused by the reduction in AoS.

Authorship
Conception and design of research: KND and JKK; experiments: KND, BS, BT, and RS; statistical analysis of the data: JKK; interpretation of the data: KND, BS, and BT; writing of the manuscript: KND and JKK.

Disclosure of state of COI
None of the authors declares a conflict of interest.

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