Grape Powder Lowers Serum Triglycerides in Postmenopausal Women

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ABSTRACT

Objective: The purpose of this study was to determine if consuming dehydrated grape powder can alter cardiovascular disease markers in healthy postmenopausal women.

Methods: Dehydrated grape powder (10 g/day) was administered to healthy postmenopausal women for a period of 21 days. Study subjects consumed a standard American diet; none had diabetes or were taking hormone replacement therapy. Blood was tested for triglycerides, total cholesterol (TC), low-density lipoprotein (LDL) cholesterol, and high-density lipoprotein (HDL) cholesterol, apolipoprotein A-I and apolipoprotein B, C-reactive protein, and oxygen radical absorbance capacity (ORAC). Urine was tested for F_2-isoprostanes to gauge free radical damage.

Results: In support of the study hypothesis, triglycerides (P=0.005) and the ratio of TC:HDL cholesterol declined (P=0.038). Neither isoprostanes nor ORAC changed significantly.

Conclusions: Grape powder may improve the lipid profile of postmenopausal women. Grape powder did not change inflammation markers.

INTRODUCTION

Ancient Biblical texts contain the first written record of organized agricultural operations, indicating that grapes were the first crop cultivated by humans (Genesis 9:20). It was not until the 1980s that the positive health effects of grapes drew attention from researchers. The impetus for this investigation was the "French Paradox": the observation that many French eat a diet considered dangerous for heart health, and yet have a low prevalence of cardiovascular disease

relative to other nations in the western hemisphere.[1] Red wine contains alcohol and phenolic compounds, both of which have been associated with a lower risk of coronary heart disease. The phenolic substances in grapes may explain the French paradox.[2] In fact, phenols have been associated with a lower risk of coronary heart disease,[3-5] stroke, arthritis, and asthma[6,7] chronic obstructive pulmonary disease,[8] neurological degeneration,[9] and cancer of the stomach,[10] upper digestive tract,[11] lungs,[12-14] and breast.[15]

While these studies make a persuasive argument that phenols have health benefits, cardiovascular disease does not strike all genders or ages equally. A higher percentage of women compared with men die within 1 year of experiencing a heart attack, and postmenopausal women have a 2-3 times greater risk of coronary heart disease compared with premenopausal women.[16] Few studies have investigated the effect of phenols in older women. In one study, the ovariectomized animal model was used to mimic the effects of menopause.[17] Guinea pigs were fed a dehydrated grape powder, similar to the one used in the present study, for 12 weeks. Plasma triglyceride (TG) and very low-density lipoprotein cholesterol concentrations were significantly lowered ($P<0.05$). The postprandial effect of water, red wine, and dealcoholized red wine was tested on serum lipids in 17 postmenopausal women with dyslipidemia.[18] No changes in the lipid profile [apolipoprotein B-48, TG, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c) and low-density lipoprotein cholesterol (LDL-c)] occurred.

Epidemiological evidence on the effect of phenols on postmenopausal women was derived from data collected as part of The Iowa Women's Health Study, which began in 1986.[19] Researchers asked 34,492 postmenopausal women to fill out food frequency questionnaires and used the data to assess flavonoid intake. Prior to adjustment for possible confounders, there was a significant ($P=0.04$) inverse association between flavonoid intake and coronary heart disease mortality. The purpose of this study is to evaluate the effects of a dried whole-grape product on the cardiovascular health of postmenopausal women. Many of the studies used to build the foundation for the protective effects of grapes have utilized red wine and/or grape juice as the source of phenols. This study is distinctive because it uses a grape product that differs from the whole fruit only in water content. By using this whole, dried grape source, we can examine the effect of micronutrients and phytochemicals in all parts of the grape (skin, seeds and flesh).

SUBJECTS

Ten women were recruited from the Minneapolis-St. Paul metropolitan area via flyers posted on the University of Minnesota-Twin Cities campus. All subjects had been postmenopausal for at least 2 years. Other inclusion criteria included consumption of a standard American diet (less than 15 g of fiber and low in fruits, vegetables, and whole grains), normal blood pressure (systolic and diastolic blood pressure less than 140 and 90 mmHg, respectively) and TC less than 200 mg/dL. Exclusion criteria included smoking, diabetes mellitus (fasting blood sugar greater than 120 mg/dL), cardiovascular disease, cancer, chronic inflammatory disease, recent bacterial infection, alcohol abuse in the preceding 6 months, use of certain medications (hormone replacement therapy, lipid-lowering, anti-inflammatory, or antihypertensive medications), recent weight loss (intentional or unintentional, of greater than 5 kg in 3 months) or participation in another intervention study

Table 1. Baseline characteristics of 10 postmenopausal women enrolled in study on effects of grape powder on lipid profile and inflammatory markers

Characteristics	Measurement (mean ± SD)
Age (years)	55.9 ± 2.9
Height (cm)	165 ± 5
Weight (kg)	73.5 ± 14.8
Body mass index (kg/height in meters^2)	27.1 ± 6.4
Systolic blood pressure (mmHg)	114 ± 11
Diastolic blood pressure (mmHg)	63 ± 12
Fasting plasma glucose (mmol/L)*	5.2 ± 0.3

*To convert mmol/L glucose to mg/dL, multiply mmol/L by 18.0.

in the past 30 days. If subjects were consuming any dietary supplements, their intake was stopped 2 weeks prior to the commencement of participation and through the conclusion of this study. All inclusion and exclusion criteria were screened over the telephone by the study coordinator. Subjects received and signed a consent form prior to beginning the study. Information about the study protocol was conveyed orally and through a newsletter mailed to each participant's home prior to the first visit.

MATERIALS AND METHODS

This study was approved by the University of Minnesota Institutional Review Board Human Subjects Committee. A grant for this research was provided by the California Table Grape Commission. The General Clinical Research Center (GCRC), funded by M01-RR00400 of the National Center for Research Resources, National Institutes of Health, and located on the University of Minnesota-Twin Cities campus, assisted with the administration of study protocol.

Grape Powder Composition

The California Table Grape Commission (CTGC, Fresno, CA) provided the dehydrated grape powder used in this study. The powder was manufactured by freezing, grinding with dry ice, freeze-drying, and regrinding fresh red, blue-black, and green seeded and seedless California grapes. The mixture was stored in moisture-proof containers at -70ºC to maintain the integrity of active components. Analysis by the CTGC indicates the grape powder contained 375 kilocalories and 89.1 g carbohydrate per 100 g of powder. In addition, the phytochemical profile was as follows: 5.8 mg total phenols/g powder, 4.1 mg flavans/g powder, 770 mg anthocyanins/kg powder, and 102, 8, 11, and 7 mmoles/kg powder of quercetin, myricetin, kaempferol, and resveratrol, respectively. It is estimated that 18.2 g of grape powder is the equivalent of 100 g of fresh grapes.

Food Records

Subjects completed two 3-day food records. One record was completed in the first several days of the study and the other in the final days of the study to confirm that dietary intake remained static. A registered dietitian taught each woman how to accurately complete the food record. Participants were instructed to consume their normal diet, while paying close attention to maintaining consistent levels of caffeine, alcohol, tea, and grape-derived products. Of the three days recorded, one was a weekend day

Table 2. Dietary intake of postmenopausal women participating in a 21-day grape powder intervention*

	Baseline	Final	*P*-value†
Energy (kcal)	1955.9 ± 310.5	1883.2 ± 327.5	0.610
Total fat (g)	64.7 ± 19.5	66.3 ± 17.2	0.796
Saturated fatty acids (g)	19.4 ± 7.1	21.9 ± 9.1	0.398
Monounsaturated fatty acids (g)	19.9 ± 8.9	21.6 ± 6.8	0.517
Polyunsaturated fatty acids (g)	14.1 ± 3.5	14.2 ± 4.0	0.946
Carbohydrate (g)	272.1 ± 85.9	261.1 ± 78.9	0.689
Protein (g)	79.3 ± 18.9	66.6 ± 13.8	0.080
Total fiber (g)	25.1 ± 13.0	25.0 ± 13.6 0.946	
Vitamin A (RE) (μg)	1242.2 ± 881.2	1063.4 ± 633.8	0.292
Vitamin E (α-TE) (mg)	2.9 ± 3.4	3.9 ± 6.1	0.547
Vitamin C (mg)	167.5 ± 164.7	106.9 ± 77.2	0.181
Beta-carotene (μg)	580.9 ± 652.2	486.0 ± 605.3	0.586
Alcohol (g)	3.8 ± 4.5	4.4 ± 5.5	0.697
Cholesterol (mg)	165.7 ± 83.6	157.5 ± 62.5	0.808
Caffeine (mg)	175.8 ± 202.7	105.4 ± 106.4	0.404

Data reported as mean ± standard deviation.

*Excludes 2 study subjects in whom energy intake reported <1000 kcal (n=8).

†*P*-value of two-tailed matched-pairs Student's *t*-test with α=0.05.

and the remaining two days fell during the week. Food records were analyzed using the First DataBank Nutritionist Five software (San Bruno, CA).

Powder Administration

Each of the 10 participants in the study consumed a 10-g dose of grape powder daily for 21 days. The dose was divided into a 5-g portion in the morning and another 5-g portion in the evening. Powder was consumed by mixing it with a liquid beverage. An electric hand blender was provided to ensure that the powder completely dissolved and that the entire dose was ingested.

Blood Pressure

Subjects were admitted to a private room in the GCRC and instructed to sit quietly for 5 minutes. No reading, conversation, or physical activity was allowed. The subject's blood pressure was taken 3 times using a standard sphygmomanometer. The average systolic and diastolic readings were used for analysis.

Height and Weight

Subjects wore light indoor clothing without shoes during weight measurement. Weight was accurate within one-tenth kilogram and height to the nearest centimeter.

Blood Samples

For each visit requiring a blood sample, patients were instructed to fast for 12 hours. Three separate samples of blood (10 mL each) were drawn into ethylenediamine tetraacetic acid (EDTA)-coated tubes. Two of the samples were immediately centrifuged (1500 x *g* for 15 minutes at 4ºC). The plasma layers were aliquoted into vials and refrigerated for transport to Quest Diagnostics (Teterboro, NJ) via courier for analysis. Quest Diagnostics performed a compre-

Table 3. Lipid panel, oxygen radical absorbance capacity (ORAC), urinary isoprostane, and C-reactive protein (CRP) results for 10 postmenopausal women enrolled in 21-day grape powder supplementation study

	Units	Baseline	Day 21	*P*-value*
Triglycerides†	mmol/L	1.13 ± 0.36	0.87 ± 0.24	0.005
Total Cholesterol‡	mmol/L	5.42 ± 0.73	5.21 ± 0.53	0.156
HDL-c‡	mmol/L	1.92 ± 0.4	1.90 ± 0.3	0.678
LDL-c‡	mmol/L	2.98 ± 0.5	2.90 ± 0.5	0.517
Chol/HDL-c	ratio	3.0 ± 0.78	2.8 ± 0.66	0.038*
ApoA-I	mg/dL	185.1 ± 25.0	175.1 ± 20.4	0.050*
ApoB	mg/dL	93.5 ± 15.3	89.5 ± 12.9	0.176
HDL-c:ApoA-I	ratio	0.395 ± 0.048	0.416 ± 0.039	0.031*
Whole ORAC	ORAC units	3156.5 ± 280.1	3051.0 ± 367.7	0.331
Fast ORAC	ORAC units	465.5 ± 55.0	445.7 ± 73.9	0.489
Slow ORAC	ORAC units	1766.6 ± 152.9	1693.6 ± 247.6	0.327
Isoprostanes	nG/mg creatinine	0.814 ± 0.503	0.773 ± 0.474	0.217
CRP	mg/dL	0.186 ± 0.122	0.292 ± 0.229	0.053

All values are mean ± standard deviation.
**P*-value of two-tailed matched-pairs Student's *t*-test with α=0.05.
†To convert mmol/L triglycerides to mg/dL, multiply mmol/L by 88.6.
‡To convert mmol/L total cholesterol, HDL-c, or LDL-c to mg/dL, multiply mmol/L by 38.7.
HDL-c=high-density lipoprotein cholesterol; LDL-c=low-density lipoprotein cholesterol; Chol=cholesterol; Apo=apolipoprotein

hensive metabolic panel in addition to measuring TG, TC, HDL-c, LDL-c (via the Friedewald equation), C-reactive protein (CRP), and apolipoprotein A-I (apoA-I) and apolipoprotein B. The remaining 10-mL sample was allowed to clot for 30 minutes, centrifuged (1500 x *g* for 15 minutes at 4ºC), aliquoted and stored at -20ºC until analysis by Genox Corporation (Baltimore, MD) for oxygen radical absorbance capacity (ORAC).

The ORAC assay was first developed in 1993 and has been validated as an accurate measure of total antioxidant capacity.[20] The first step in the assay is peroxyl radical production via 2,2l-azobis (2-amidinopropane) dihydrochloride (AAPH). The free radical reaction is allowed to proceed to completion. Then the damage to a marker protein (beta-phycoerythrin) is measured and compared to a Trolox equivalent. One ORAC unit is equal to one µM Trolox equivalent.

Urine Specimen Collection

Each subject was required to complete two 24-hour urine collections. The first collection was completed before grape powder treatment began. Participants were provided with instructions for urine collection protocol both orally from GCRC staff and as written instructions. The first void of the morning was discarded by the subjects, but the time was recorded. Each subsequent void was collected for the next 24 hours. The final collection occurred exactly 24 hours from the time of the initial void discarded the prior day. Samples were stored at -20ºC until analysis for F_2-isoprostanes.

Urinary Isoprostane Analysis

Isoprostanes are compounds similar to prostaglandins. They are generated as a

direct result of the peroxidation of arachidonic acid.[21] Their production is independent of the cyclooxygenase (COX) pathway, in which leukotrienes and prostaglandins are made from arachidonic acid. Therefore, the levels of isoprostanes in urine can be used to gauge oxidative damage. In this study, a specific isoprostane, 8-epi prostaglandin F_2, was used as a biomarker.

Urinary isoprostane analysis was performed using a standard mass spectrometric procedure.[22] First, the urine samples were purified through a C18 Sep Pak and Silica Sep Pak. (Waters, Milford, MA) Following evaporation under nitrogen gas, samples were brought to 1 mL using a dilution buffer and 100 µL of either standard or sample was then pipetted onto a microtiter plate and subjected to a competitive immunoassay specific for 8-epi prostaglandin F_2 (Bioxytech, enzyme immunoassay for urinary isoprostane, Oxis International, Portland, OR). The plates were washed four times with 400-µL wash buffer. The directly proportional relationship between color development and isoprostane concentration was utilized to calculate the presence of isoprostanes. Final urinary isoprostane measurements were standardized using urinary creatinine concentration from the same sample.

RESULTS

Statistical Analysis

Data were analyzed using Microsoft Excel 2000 (Microsoft Corporation, Redmond, WA). All calculations are presented as mean ± standard deviation. A two-tailed matched pairs Student's *t*-test with α=0.05 was used to determine statistical significance.

All 10 subjects completed the study. Baseline characteristics are summarized in Table 1. Body mass, body mass index (BMI), blood pressure, and fasting plasma glucose were not changed by the protocol (data not shown). Dietary intake data are presented in Table 2. None of the dietary factors analyzed changed throughout the study.

Main outcome measures are shown in Table 3. TG concentration decreased significantly (P=0.005) from baseline. Average decline was 22.4 mg/dL. TC did not change significantly from baseline (P=0.156). Neither HDL-c (P=0.678) nor LDL-c (P=0.517) was altered significantly. However, the TC-to-HDL-c ratio was lowered (P=0.038) significantly. Apo B did not change (P=0.176) over the course of the grape powder treatment. No components of the metabolic profile changed with statistical significance except albumin, which declined (P=0.029) during the course of the study (data not shown). In administering this dietary treatment, we expected to see an attenuation of inflammation, as measured by CRP. However, there was a statistically insignificant (P=0.053) increase in this marker. There was no significant change in whole ORAC (P=0.331), slow ORAC (P=0.327), or fast ORAC (P=0.489). There was no significant change in urinary F_2 isoprostanes (P=0.217).

DISCUSSION

This study demonstrated that a 10-g dose of dehydrated grape powder administered daily to postmenopausal women for 3 weeks decreased TG concentration. Results of other measures of cardiovascular disease risk were generally neutral.

TG levels have been inversely associated with the risk of experiencing a coronary event.[23] The predictive value is even stronger in women than in men.[24] Therefore, the highly significant decline in TG concentrations observed in this study is the most important indicator of the success of the intervention. Another improvement in the lipid profile was observed indirectly. The ratio of TC-to-

HDL-c declined, although those parameters were not altered individually. A recent placebo-controlled study by Zern et al also found an improvement in triglyceride levels in postmenopausal women given grape powder.[25] Cardiovascular health may have been improved in other respects not measured by this study protocol.

Other results did not support cardioprotection. CRP, a marker of systemic inflammation, trended upward, rather than downward (P=0.053). CRP predicts cardiovascular events more strongly in women than does LDL-c.[27] However, there has been speculation that CRP is too sensitive to provide meaningful data about cardiovascular risk assessment.[28] Indeed, the American Heart Association and the Centers for Disease Control and Prevention oppose the use of CRP as a routine cardiovascular assessment measure in healthy persons.[29] CRP was unaffected in an analogous grape powder study conducted on pre- and postmenopausal women.[25]

ORAC and isoprostanes did not change. Perhaps the dose of grape powder (10 g/day) was insufficient to achieve a larger effect. It would take approximately 23 g of grape powder to equal a standard $^3/_4$-cup serving of fresh grapes. Zern et al observed positive changes in triglycerides, LDL-c, cholesteryl ester transfer protein (CETP) activity, a plasma protein involved in HDL metabolism, and isoprostanes when grape powder was administered to postmenopausal women at a dose of 36 g/d for 4 weeks.[25] It is suggested that grape powder dosage be increased in future studies.

CONCLUSION

In conclusion, this study provides data that fresh grapes contain components that could potentially improve the cardiovascular health of postmenopausal women. Future studies using whole fruits are essential in order to promote a healthy diet among this at-risk population. Such endeavors should seek to enroll a larger study population and administer greater doses of fruit in order to discern the precise effects of the dietary treatment.

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