

Available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/jff

A randomized, double-blind, placebo-controlled, pilot study to evaluate the effect of whole grape extract on antioxidant status and lipid profile ☆☆☆☆☆



Malkanthi Evans*, Dale Wilson, Najla Guthrie

KGK Synergize Inc., 255 Queens Ave., Suite 1440, London, ON, Canada

ARTICLE INFO

Article history:

Received 28 May 2013

Received in revised form

3 December 2013

Accepted 9 December 2013

Available online 11 January 2014

Keywords:

Whole grape extract

Cardiovascular risk

Antioxidant status

8-Isoprostane

HDL-C

ABSTRACT

The health benefits of grapes, which contain polyphenols, are well documented. The anti-oxidative and cardioprotective effects of a whole grape extract (WGE) were studied in a single-centre, randomized, double-blind, placebo-controlled, 6-week pilot study conducted on 24 pre-hypertensive, overweight, and/or pre-diabetic subjects. Blood and urine biomarkers of antioxidant status were assessed at the beginning and end of the study. WGE subjects had significantly lower superoxide dismutase concentrations ($P = 0.032$) and total cholesterol/HDL-C ratios ($P = 0.037$), and significantly higher HDL-C levels ($P = 0.001$) compared to the placebo subjects after 6 weeks. The concentration of 8-isoprostane and oxidized LDL decreased by 5% and 0.5%, respectively, for WGE subjects, but increased by 50% and 5%, respectively, for the placebo subjects. This is the first North American study to report efficacy of WGE on antioxidant status and lipid profile.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

Oxidative stress is associated with a variety of chronic degenerative diseases, including cancer, diabetes and cardiovascular diseases (Belli et al., 2005; Davi et al., 1997; Gopaul et al., 1995). Free radicals, such as reactive oxygen species (ROS),

are produced from metabolic processes in the human body or from exposure to ozone, X-rays, environmental and industrial pollutants, and cigarette smoke (Dean, Fu, Stocker, & Davies, 1997). Under normal physiological conditions, enzymes such as superoxide dismutases, catalases, lactoperoxidases and glutathione peroxidases act as antioxidants, protecting

☆ This study was sponsored by Ethical Naturals Inc., 1167 North Fair Oaks Ave., Sunnyvale, CA.

☆☆ Author disclosures: M. Evans, D. Wilson and N. Guthrie, no conflicts of interest.

☆☆☆ The ClinicalTrials.gov registry number is NCT01551706.

* Corresponding author. Address: KGK Synergize Inc., 255 Queens Avenue, London, ON N6A 5R8, Canada. Tel.: +1 519 438 9374x239; fax: +1 519 438 8314.

E-mail address: mevans@kgksynergize.com (M. Evans).

Abbreviations: ALT, alanine transaminase; AST, aspartate aminotransferase; CBC, complete blood count; eGFR, estimated glomerular filtration rate; GGT, gamma glutamyltransferase; GSPE, grape seed proanthocyanidin extract; LGP, lyophilized grape powder; NHPD, Natural Health Products Directorate; oxLDL, oxidized low density lipoprotein; WGE, Ethical Naturals Incorporated patented whole grape extract; ROS, reactive oxygen species; SOD, superoxide dismutase; TBARS, thiobarbituric acid-reactive substances; 8-OHdG, 8-hydroxydeoxyguanosine

1756-4646/\$ - see front matter © 2014 Elsevier Ltd. All rights reserved.

<http://dx.doi.org/10.1016/j.jff.2013.12.017>

cells from ROS damage. An imbalance between ROS production and the natural antioxidant system leads to a state of oxidative stress (Rabovsky, Cuomo, & Eich, 2006).

Grape products made from grape seed, grape skin and grape juice, including red wine, contain a wide variety of powerful antioxidants in the form of polyphenols, which include phenolic acids (e.g., gallic acid), resveratrol, proanthocyanidins and flavonoids such as anthocyanins, flavonols and quercetin (Tsao, 2010). Grape skin, seeds and stems are known to contain the highest concentration of polyphenols (Sanchez-Moreno, Cao, Ou, & Prior, 2003). Several studies have investigated the beneficial mechanisms of grape polyphenols focusing on their antioxidant properties (Rho & Kim, 2006; Stein, Keevil, Wiebe, Aeschlimann, & Folts, 1999; Zern et al., 2005). Polyphenols prevent ROS damage by scavenging free radicals, thereby reducing oxidative stress (Rice-Evans, Miller, & Paganga, 1996).

Products of oxidative damage to macromolecules have been identified in biological materials such as plasma, urine and blood cells, and serve as biomarkers of oxidative damage. When oxidative damage occurs in DNA, the resulting products are usually eliminated by repair enzymes and can be detected as nucleoside derivatives. Urinary 8-hydroxydeoxyguanosine (8-OHdG) is one adduct of this reaction and has been proposed as a sensitive biomarker of oxidative DNA damage and repair (Loft, Fischer-Nielsen, Jeding, Vistisen, & Poulsen, 1993; Shigenaga, Gimeno, & Ames, 1989). Measuring 8-OHdG in urine is a more accurate assessment of oxidative stress in humans because of the lack of artifact formation in urine, unlike in DNA. This results in a more reproducible measurement (Kasai, 2002).

8-Isoprostane is a prostaglandin (PG)-F₂-like compound belonging to the F₂ isoprostane class that is produced *in vivo* by the free radical-catalyzed peroxidation of arachidonic acid (Longmire et al., 1994; Roberts & Morrow, 2000). The amounts of 8-isoprostane in the plasma and urine are regarded as the best indices of lipid peroxidation and oxidative stress currently available, and the concentration of 8-isoprostane in urine is associated with the urinary excretion concentration of 8-OHdG (Harman et al., 2003). Sources of free radicals that may contribute to isoprostane formation include mitochondrial electron transport chain, oxidases, uncoupled nitric oxide synthetase, and transition metal ion catalyzed reactions (Morrow, 2005). Isoprostanes are bioactive and are present in atherosclerotic lesions, with its formation increasing in persons with coronary artery disease independent of other risk factors (Schwedhelm et al., 2004). Therefore, a decrease in isoprostane production may attenuate risk of developing cardiovascular disease.

In vivo studies have demonstrated the antioxidant capabilities of grapes. Whole grape, pomace, or juice has shown positive effects on antioxidant capacity in Sprague–Dawley rats (Rho & Kim, 2006). Grape diets in rats promoted antioxidative enzyme activities such as superoxide dismutase (SOD), catalase, and glutathione peroxidase (Rho & Kim, 2006). SOD converts superoxide anion into hydrogen peroxide (Turrens, 2003), which detoxifies into water either by glutathione peroxidase or catalase. Excess superoxide anion reduces transition metal ions such as ferric and cupric ions, the reduced forms of which react with hydrogen peroxide to produce hydroxyl

radicals (Reddy, 2006). *In vivo* studies have also demonstrated that flavonoids and resveratrol derived from grape limit low-density lipoprotein (LDL) oxidation. Hypercholesterolaemic mice had markedly less atherosclerosis than control animals when consuming wine polyphenols for 6 weeks (Hayek et al., 1997). Intake of a lyophilized grape preparation led to a reduction in cholesterol accumulation in guinea pigs (Zern, West, & Fernandez, 2003). Clinically, urinary isoprostane concentrations in pre- and postmenopausal women decreased when on lyophilized grape powder for 4 weeks (Zern et al., 2005). Other clinical studies have reported that red wine consumption was associated with the reduction of urinary concentrations of prostaglandin F₂- α , a marker of lipid peroxidation (Pignatelli et al., 2006). Clinical studies also have shown that consumption of grape juice or polyphenols derived from grapes produced a reduction in the susceptibility of LDL to oxidation (Frankel, Waterhouse, & Kinsella, 1993; Stein et al., 1999). A recent study showed that 2 weeks of apple and grape juice consumption increased the plasma total antioxidant capacity in healthy participants (Yuan et al., 2011).

Interventions that improve cellular redox status may reduce health risks such as cardiovascular diseases, and are thus interesting alternative avenues to pursue. This study investigated the effect of a whole grape extract (WGE) versus placebo on antioxidant status. WGE is derived from seed, skin and pulp of the grape *Vitis vinifera*.

The primary objective of this study was to determine antioxidant status prior to and after 6 weeks of supplementation with WGE via total antioxidant capacity and SOD, 8-OHdG and 8-isoprostane. The secondary objective was to assess the effect of WGE on oxidized LDL, and lipid profiles.

2. Materials and methods

2.1. Subjects and study design

This was a 6-week single-centre, randomized, double-blind, placebo-controlled pilot study with two treatment arms and conducted in London, ON, Canada. This study was reviewed by the Natural Health Products Directorate (NHPD), Health Canada, Ottawa, ON and a research ethics board. Notice of authorization was granted on January 23, 2012 by the NHPD and ethics approval was received on January 31, 2012 from Institutional Review Board Services, Aurora, ON. The study was conducted in accordance with the ethical principles that have their origins in the Declaration of Helsinki and its subsequent amendments. Recruitment and screening began in February 2012 with the last subject exiting the study in April 2012. Written informed consent was obtained from each participant at the screening visit prior to any study related activities.

Twenty-six subjects, 18–65 years of age were enrolled in the study. Fourteen participants (six males and eight females) were randomized to the WGE treatment arm and 12 (six males and six females) to the placebo arm. Participants were included if they had pre-hypertension, defined as diastolic blood pressure of 80–89 mmHg and systolic blood pressure of 120–139 mmHg at screening, and/or BMI from 25.0 to 34.9 kg/m² and/or pre-diabetes defined as a fasting blood

glucose from 5.2 to 6.9 mmol/L. Participants who smoked agreed to report smoking habits at each visit and did not plan on changing their smoking habits during the study. Female, participants were included if they were not of child bearing potential or agreed to use a medically approved method of birth control and had a negative urine pregnancy test. Exclusion criteria included women who were pregnant, breastfeeding, or planning to become pregnant during the course of the trial; any clinically significant medical conditions including cardiovascular disease, hypertension (systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg), diabetes, liver or kidney disease; allergy or sensitivity to test product ingredients; clinically significant abnormal laboratory results at screening; any other condition which in the investigator's opinion might adversely affect the participant's ability to complete the study or its measures or which may pose significant risk to the participant. Use of medication for the treatment any of the following conditions: hypertension, vasodilation, erectile dysfunction, weight loss, and hypercholesterolaemia, use of anticoagulants and use of NHPs/dietary supplements known to have significant antioxidant activity were contraindicated for this study. The planned sample size for this study was 26 subjects. A formal sample size calculation was not performed as this was a pilot study.

2.2. Intervention and randomization

At the baseline visit, current conditions, smoking status, concomitant therapies and inclusion/exclusion criteria were reviewed and physical examination (excluding breast, rectal/vaginal examination) was performed. Each eligible participant was assigned a randomization code according to the order of the randomization list. Two computer generated randomization lists, one for each gender, were prepared with participants randomized to product in blocks of two. Participants were stratified by their gender and smoking status. Non-smokers were randomized starting at the top of the respective randomization list and smokers were randomized from the bottom of the respective randomization list. The investigator was provided with one envelope for each randomization number. These envelopes were sealed and contained the identity of the test product corresponding to the randomization number. These envelopes were to be opened only in an emergency where it was necessary to unblind a subject. This was not necessary during this study and all envelopes remained sealed. Subjects were given bottles of product; each bottle contained the name and batch number of both products, differing only in randomization number, in order to ensure maintenance of the blind. The test product WGE capsules and placebo were identical in shape, colour and size. Subjects, research staff and the investigator were blinded to product allocation. Statistical analysis was conducted on blinded data and the study was not unblinded until all analyses were complete. Participants were instructed to begin taking the study product the day following the baseline visit (Day 1), with a dose regimen of one capsule a day at the same time daily, with or without a meal, preferably right before bedtime, for 6 weeks. Paper diaries were provided to participants for recording study product use. Compliance was

assessed by counting the returned capsules at each study visit.

2.3. Polyphenol profile of whole grape extract

The whole grape extract (Ethical Naturals Inc., 1167 North Fair Oaks Ave., Sunnyvale, CA) capsule was a gelatin capsule containing 350 mg of whole grape extract (60–70% proanthocyanidins, consisting of flavan-3-ol units [catechin, epicatechin and epicatechin-3-O-gallate], characterized by C4–C8 or C4–C6 linkage. Epicatechin is the major component in the extended chain. The simplest and most common procyanidins are dimmers, primarily proanthocyanidins B1–B4, also known as oligomeric proanthocyanidins (OPCs). 15–25% flavan-3-ols: catechin (10–15%), epicatechin (0–2%), epigallocatechin (3–8%) and epigallocatechin gallate (0–2%); 0–1% minor flavonoids: quercetin, myricetin and kaempferol; 0–1% non-flavonoid polyphenols: gallic acid (0–1%), ellagic acid, caffeic acid, chlorogenic acid (0–1%), resveratrol (0–0.5%) and others (0–1%) and microcrystalline cellulose as filler.

The placebo capsule was a gelatin capsule containing microcrystalline cellulose and looked identical in size, shape and colour to the product under investigation. The capsules were provided to participants in polyethylene bottles and participants instructed in detail about the dosing regimen.

2.4. Blood pressure, heart rate and weight measurements

At each visit (screening, baseline and week 6), seated resting blood pressure, heart rate and weight were measured. Height was measured at screening. Body weight was measured to the nearest 0.1 kg and height measured to the nearest 0.1 cm on a calibrated beam scale equipped with a stadiometer. Two recordings of body weight were made at each visit and the mean value was used. Systolic and diastolic blood pressure was measured and heart rate determined from 3 measurements obtained at least 1 min apart. Blood pressure was checked in both arms at the first examination. If a consistent interarm difference existed, the arm with the higher pressure was used throughout the study. The arm selected for use at the initial visit was documented and the same arm used for the duration of the study. The same recording method and equipment were used for each participant throughout the study.

2.5. Laboratory analysis

At screening and week 6, fasting blood (12 h) samples were collected from participants via venipuncture into ethylenediaminetetraacetic acid (EDTA) tubes (Becton, Dickinson & company, Franklin Lakes, NJ, USA) and whole blood analyzed for complete blood count. Serum was generated from blood collected into SST tubes (Becton, Dickinson & company, Franklin Lakes, NJ, USA) which were allowed to clot at room temperature for 30 min followed by centrifugation for 10 min at 3200 rpm (1763g), 25 °C. Serum was analyzed for electrolytes (Na, K, Cl), fasting glucose, lipid profile (total cholesterol, LDL-C, HDL-C, triacylglycerol), creatinine, estimated glomerular filtration rate (eGFR), aspartate aminotransferase (AST),

alanine aminotransferase (ALT), gamma glutamyltransferase (GGT) and bilirubin by LifeLabs (London, ON, Canada).

Fasting blood (12 h) samples were collected at baseline and week 6 into SST tubes and serum generated for analysis of SOD, total antioxidant capacity and oxidized LDL (oxLDL). Serum was frozen at -40°C until analyzed. oxLDL, SOD and total antioxidant capacity were measured using commercially available kits (oxidized low density lipoprotein ELISA, Northwest Life Science Specialties, LLC, Vancouver, WA, USA; Superoxide Dismutase Assay Kit and Antioxidant Assay Kit, Cayman Chemical, Ann Arbor, MI, USA).

Urine samples were collected at baseline and end of study and analyzed for 8-OHdG, 8-isoprostane and creatinine. In order to reduce the day-to-day variation in excretion of urinary antioxidants, two first-morning void urine samples were collected on the day before and the day of the study visits. These two samples were pooled and analyzed. An aliquot was transferred into an amber tube and analyzed for urinary creatinine by LifeLabs (London, ON, Canada). Additional aliquots were made and frozen at -40°C until analyzed for 8-OHdG and 8-isoprostane. Analysis of 8-OHdG and 8-isoprostane were performed using commercially available kits (Urinary 8-OHdG ELISA and Urinary 8-Isoprostane Assay, Northwest Life Science Specialties, LLC, Vancouver, WA, USA). Urinary concentrations of 8-isoprostane and 8-OHdG were calculated by correcting for urinary creatinine.

2.6. Dietary compliance

Participants agreed to avoid antioxidant and polyphenol rich foods and beverages for at least 1 week prior to baseline and during the study. At screening, participants received a 3-day food record and were instructed to complete it for any two weekdays and one weekend day prior to the baseline visit. At the baseline visit, the 3-day food records dispensed during screening were returned for review to ensure that participants avoided antioxidant and polyphenol rich foods and beverages. At week 3, participants received a scheduled phone call and were questioned about their dietary intake to ensure they continued to avoid the prohibited foods and beverages as instructed. Participants were also questioned about their dietary intake at week 6 to ensure prohibited foods were avoided during the study period.

2.7. Adverse events and changes in concomitant therapies

Participants were instructed to record changes in concomitant therapies and any side effects/changes in diaries which were provided to them at the baseline visit and reviewed at week 6.

2.8. Statistical methods

Statistical analyses were performed using SAS software (version 9.1; SAS Institute). The data are represented as mean $\pm$ SD and the percentage difference from baseline to week 6 was calculated for each participant and averaged for a group mean. For efficacy and safety analyses, between-group comparisons were made by ANCOVA adjusting for baseline as a covariate. Between-group comparisons of the

within-group change in efficacy parameters were made using an unpaired *t*-test. Within-group comparisons were made using a *t*-test. For antioxidant analyses, data were first examined for normality. Where data were not normally distributed, statistical comparison was made after appropriate transformation of the data. More specifically, for SOD, statistical comparisons were made using square root of the log of the log of SOD and for urinary antioxidants, statistical comparisons were made after log-transforming the data. Probability values less than 0.05 were considered statistically significant.

3. Results

3.1. Participant disposition

A total of 41 participants were screened and 26 eligible participants (12 males and 14 females) were randomized. Of the 26 participants randomized, 25 participants completed the study (Fig. 1). The proportion of participants withdrawing from the study was not significant between groups. All participants randomized to the study were included in the analysis of safety. One participant randomized to placebo was lost to follow up from the study and there was no post baseline data available for analysis. One participant randomized to WGE had ongoing rheumatoid arthritis. This information was not provided by the participant at enrollment and there was no evidence in the blood work at the time to associate the participant with a disease. However, subsequently, the participant revealed that she had ongoing rheumatoid arthritis; therefore the participant was excluded from the efficacy analysis. As such statistical comparisons of efficacy analysis at week 6 were based on $n = 24$.

The mean age was 38 ± 12 years for the placebo group, and 46 ± 11 years for the WGE group (Table 1). A total of four participants were hypertensive (3 in WGE and 1 in placebo), 15 participants were prediabetic (8 in WGE and 7 in placebo) and 20 participants were of a BMI range between 25.0 and 34.9 kg/m^2 (11 in WGE and 9 in placebo). Over 90% of the participants were white and of Western-European origin. Treatment compliance was high in this study and similar between groups. Participants in both groups demonstrated a mean compliance rate greater than 96%.

3.2. Antioxidant status

After 6 weeks of WGE or placebo supplementation, total antioxidant capacity and SOD increased from baseline however, the increase was only significant ($P < 0.001$; $P = 0.008$ respectively) for the placebo group (Table 2). At week 6, SOD concentrations were significantly ($P = 0.032$) higher for participants on placebo compared to WGE participants (Table 2). However, the difference from baseline to week 6 was not significantly different between the two groups. The placebo group had a high standard deviation for SOD measurement which was attributed to a high SOD value of one subject during baseline and week 6. Participants on WGE showed a decrease in serum oxLDL concentrations from baseline to week 6 while participants on placebo showed an increase in oxLDL concentrations from baseline to week

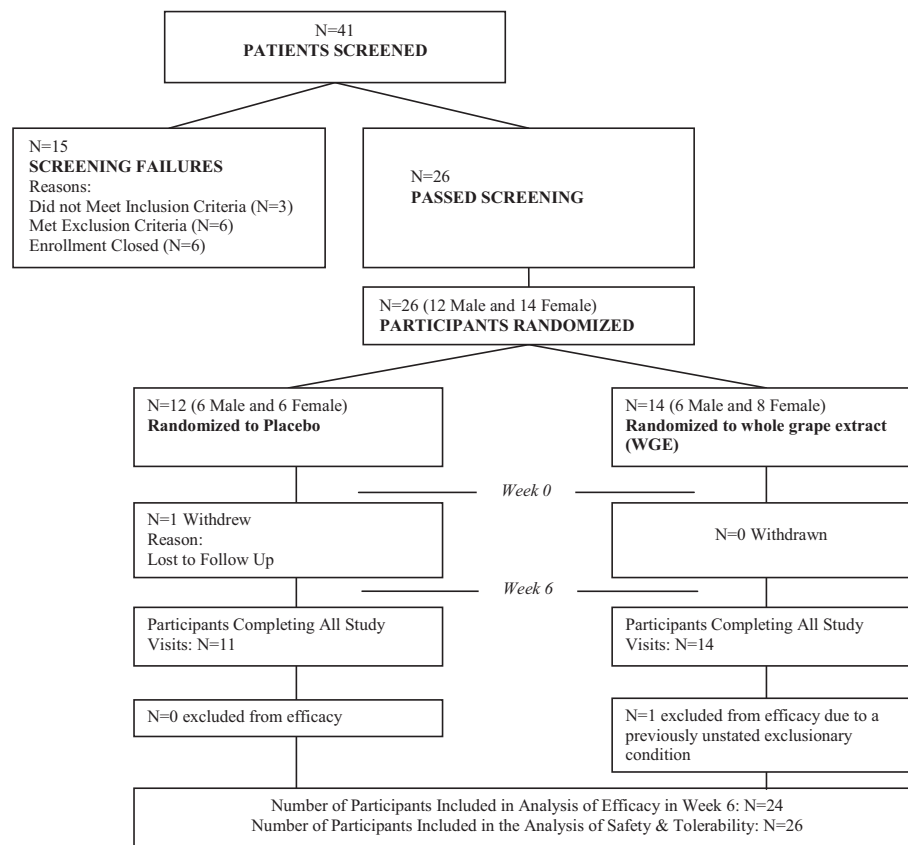


Fig. 1 – Illustration of the disposition of all participants screened and enrolled into the study.

Table 1 – Demographics and characteristics of all participants (n = 26) randomized at screening to whole grape extract (WGE) or placebo.

	WGE (n = 14)	Placebo (n = 12)	P Value
Age (years) ^a	46.1 ± 11.1	38.0 ± 12.3	0.09 ^c
Gender ^b			
Female	8	6	0.72 ^d
Male	6	6	
Ethnicity ^b			
Not Hispanic or Latino	14	11	0.46 ^e
Hispanic or Latino	0	1	
Race ^b			
Western European white	13	10	0.72 ^e
Eastern European white	1	1	
Other	0	1	
Alcohol use ^b			
None	1	3	0.59 ^e
Occasional	9	6	
Weekly	3	3	
Daily	1	0	
Smoker ^b			
Yes	1	1	>0.99 ^e
Ex-smoker	2	1	
No	11	10	

^a Data are mean ± SD.

^b Number of participants.

^c Between-group statistical comparisons conducted using a t-test.

^d Between-group statistical comparisons conducted using a Chi-square test.

^e Between-group statistical comparisons conducted using a Fisher's exact test.

Table 2 – Plasma total antioxidant status, superoxide dismutase and oxidized LDL concentrations of participants at baseline (week 0) and after 6 weeks of supplementation with whole grape extract (WGE) or placebo.

	WGE (n = 13)		Placebo (n = 12) ^a		P Value
	Mean ± SD	P Value	Mean ± SD	P Value	
<i>Total antioxidant capacity (mmol/L)</i>					
Baseline (week 0)	1.73 ± 1.14	–	1.24 ± 0.80	–	–
Week 6	2.20 ± 0.40	–	2.34 ± 0.53	–	0.67 ^b
Difference from baseline to week 6	0.47 ± 1.32	0.22 ^c	1.29 ± 0.67	<0.001 ^c	0.07 ^d
<i>SOD (U/mL)</i>					
Baseline (week 0)	4.60 ± 0.76	–	5.24 ± 2.59	–	–
Week 6	4.67 ± 0.77	–	6.90 ± 4.02	–	0.032 ^b
Difference from baseline to week 6	0.07 ± 1.21	0.93 ^e	1.59 ± 1.65	0.008 ^e	0.09 ^d
<i>Oxidized LDL (U/L)</i>					
Baseline (week 0)	62.7 ± 17.7	–	55.0 ± 16.1	–	–
Week 6	61.5 ± 17.2	–	52.9 ± 15.5	–	0.79 ^b
Difference from baseline to week 6	–1.15 ± 12.7	0.75 ^c	1.23 ± 16.2	0.81 ^c	0.69 ^d

SOD is superoxide dismutase; U is unit.

^a n = 11 for week 6 and difference from baseline to week 6 assessments as one participant was lost to follow up subsequent to the baseline visit.

^b Between group comparisons of log transformed data were made using ANCOVA.

^c Within group comparisons were made using a t-test.

^d Between group comparisons of log transformed data were made using a t-test.

^e Within group comparisons of the square root of the log of the log of SOD were made using a t-test.

6. There was an approximate 0.5% decrease and 5% increase in oxLDL concentrations for participants on WGE and the placebo, respectively. The oxLDL changes were not statistically significant between groups or within groups (Table 2).

The concentrations of 8-isoprostane from baseline to week 6 had an approximate 5% decrease for participants on WGE and an approximately 50% increase for those on placebo. This increase in urinary 8-isoprostane concentrations in the placebo group from baseline to week 6 was significant ($P = 0.034$, Table 3). The difference in 8-isoprostane concentra-

tions from baseline to week 6 of participants on WGE compared to those on placebo approached but did not reach statistical significance ($P = 0.06$).

Urinary 8-OHdG concentrations from baseline to week 6 showed an increase of approximately 17% and 26% for participants on WGE and placebo, respectively. The difference did not reach statistical significance for either group. However, the placebo group showed a trend towards significance ($P = 0.07$, Table 3). There was no statistical difference for the changes in urinary 8-OHdG concentrations from baseline to week 6 between WGE group and the placebo group (Table 3).

Table 3 – Urinary 8-isoprostane, 8-hydroxydeoxyguanosine (8-OHdG) and creatinine concentrations of participants at baseline (week 0) and after 6 weeks of supplementation with whole grape extract (WGE) or placebo.

	WGE (n = 13)		Placebo (n = 12) ^a		P Value
	Mean ± SD	P Value ^b	Mean ± SD	P Value ^b	
8-Isoprostane (pmol/mmol creatinine)					
Baseline (week 0)	686 ± 403	–	536 ± 179	–	–
Week 6	623 ± 319	–	722 ± 276	–	0.12 ^c
Difference from baseline to week 6	–62.1 ± 370	0.55	197 ± 302	0.034	0.06 ^d
8-OHdG (mmol/mol creatinine)					
Baseline (week 0)	3.28 ± 2.70	–	2.58 ± 0.65	–	–
Week 6	3.59 ± 2.41	–	3.34 ± 1.62	–	0.52 ^c
Difference from baseline to week 6	0.32 ± 1.96	0.36	0.73 ± 1.20	0.07	0.49 ^d
Urine creatinine (mmol/L)					
Baseline (week 0)	10.7 ± 4.44	–	13.4 ± 6.54	–	–
Week 6	10.0 ± 5.13	–	10.9 ± 4.13	–	0.70 ^c
Difference from baseline to week 6	0.30 ± 3.90	0.86	–1.67 ± 5.03	0.41	0.47 ^d

8-OHdG is 8-hydroxydeoxyguanosine.

^a n = 11 for week 6 and difference from baseline to week 6 assessments as one participant was lost to follow up subsequent to the baseline visit.

^b Within group comparisons were made using a t-test.

^c Between group comparisons of log transformed data were made using ANCOVA.

^d Between group comparisons of log transformed data were made using a t-test.

3.3. Lipid profile

There was no significant difference between WGE group and the placebo group or within groups for total cholesterol, LDL-C and triacylglycerol concentrations (Table 4). However, the difference in HDL-C concentrations from screening to week 6 showed an increasing trend for participants on WGE ($P = 0.08$, Table 4). For participants on placebo, HDL-C concentrations from screening to week 6 dropped significantly ($P = 0.003$, Table 4). This change in HDL-C concentrations from screening to week 6 in WGE was significantly different than the placebo group ($P = 0.001$, Table 4).

The total cholesterol to HDL-C ratio from screening to week 6 decreased for participants on WGE and increased for those on placebo. However, this change was only significant for the placebo group ($P = 0.019$, Table 4). This change in total cholesterol to HDL-C ratio from screening to week 6 in the WGE group was significantly different from the placebo group ($P = 0.037$, Table 4).

3.4. Safety

There were no statistically significant differences between groups with respect to biometrics, vital signs, haematology (complete blood count), liver function, kidney function and electrolytes after 6 weeks of supplementation (Table 5).

A total of nine adverse events were reported during the study by eight participants (31%). None of the adverse events were classified as being related to the test product by the prin-

cipal investigator. There was no significant difference in the number of participants reporting any adverse event between treatment groups ($P = 0.32$). One serious unrelated adverse event (shoulder dislocation) was reported.

4. Discussion

Numerous studies illustrate the ability of various forms of extracts from different parts of the grape to attenuate the effects of oxidative stress. Most of the clinical trials on grape studied extracts from a single source, such as seeds, skin, pulp, juice or wine. Wine and grape juice increased plasma antioxidant capability, improved glycaemic control and improved hepatic function values on diabetic North American subjects (Banini, Boyd, Allen, Allen, & Sauls, 2006). Grape seed extract decreased serum total cholesterol and LDL-C in Dutch smokers (Weseler et al., 2011).

There are only a few reports on the effects of extracts obtained from the whole fruit of the grape. A significant decrease in CVD risk markers was observed in a statin-treated, Spanish population treated with resveratrol-enriched grape extract (Tome-Carneiro et al., 2012). Others have reported on the effects of grape skin and raisins in Japanese subjects and found that oxLDL was significantly inhibited (Kamiyama et al., 2009). The effects of raisins on overweight but otherwise healthy, North American subjects showed that antioxidant capacity was modestly improved (Rankin, Andreae, Oliver Chen & O'Keefe, 2008). There are no other studies to date on the effects of WGE in North American populations

Table 4 – Lipid profile (total cholesterol, LDL-C, HDL-C, total cholesterol/HDL-C ratio and triacylglycerol) of participants at screening and after 6 weeks of supplementation with whole grape extract (WGE) or placebo.

	WGE (n = 13)		Placebo (n = 12) ^a		P Value
	Mean ± SD	P Value ^b	Mean ± SD	P Value ^b	
Total cholesterol (mmol/L)					
Screening	4.94 ± 1.09	–	4.67 ± 0.50	–	–
Week 6	5.10 ± 1.06	–	4.60 ± 0.75	–	0.39 ^c
Difference from baseline to week 6	0.16 ± 0.57	0.35	0.00 ± 0.47	0.98	0.47 ^d
LDL-C (mmol/L)					
Screening	3.06 ± 0.83	–	2.74 ± 0.60	–	–
Week 6	3.14 ± 0.83	–	2.72 ± 0.70	–	0.80 ^c
Difference from baseline to week 6	0.09 ± 0.51		0.08 ± 0.37	0.503	0.97 ^d
HDL-C (mmol/L)					
Screening	1.32 ± 0.37	–	1.42 ± 0.29	–	–
Week 6	1.39 ± 0.39	–	1.34 ± 0.29	–	0.001 ^c
Difference from baseline to week 6	0.06 ± 0.12	0.08	–0.12 ± 0.10	0.003	0.001 ^d
Total cholesterol/HDL-C ratio					
Screening	3.93 ± 1.05	–	3.43 ± 0.86	–	–
Week 6	3.88 ± 1.00	–	3.55 ± 0.82	–	0.09 ^c
Difference from baseline to week 6	–0.05 ± 0.41	0.64	0.29 ± 0.34	0.019	0.037 ^d
Triacylglycerol (mmol/L)					
Screening	1.23 ± 0.53	–	1.12 ± 0.35	–	–
Week 6	1.24 ± 0.58	–	1.18 ± 0.48	–	0.92 ^c
Difference from baseline to week 6	0.02 ± 0.32	0.85	0.08 ± 0.61		0.74 ^d

^a n = 11 for week 6 and difference from baseline to week 6 assessments as one participant was lost to follow up subsequent to the baseline visit.

^b Within group comparisons were made using a t-test.

^c Between group comparisons made using ANCOVA.

^d Between group comparisons made using a t-test.

Table 5 – Clinical chemistry parameters of subjects at screening and after 6 weeks of supplementation with whole grape extract (WGE) or placebo.

	WGE (n = 14) Mean ± SD	Placebo (n = 12) ^a Mean ± SD	P Value
Sodium (mmol/L)			
Screening	142.21 ± 1.31	142.42 ± 1.73	–
Week 6	142.00 ± 1.47	143.27 ± 2.76	0.229
Potassium (mmol/L)			
Screening	4.65 ± 0.41	4.76 ± 0.41	–
Week 6	4.61 ± 0.37	4.69 ± 0.41	0.808
Chloride (mmol/L)			
Screening	107.21 ± 2.19	106.83 ± 2.37	–
Week 6	107.21 ± 1.19	107.45 ± 1.63	0.561
Glucose (mmol/L)			
Screening	5.10 ± 0.48	5.33 ± 0.46	–
Week 6	5.22 ± 0.47	5.41 ± 0.56	0.370
Creatinine (μmol/L)			
Screening	64.86 ± 12.27	70.33 ± 14.47	–
Week 6	67.07 ± 13.25	71.36 ± 15.15	0.618
eGFR (mL/min/1.73 m²)			
Screening	99.71 ± 13.25	95.50 ± 16.25	–
Week 6	96.57 ± 14.29	94.45 ± 12.87	0.480
AST (U/L)			
Screening	23.21 ± 5.16	23.42 ± 4.38	–
Week 6	24.14 ± 6.87	20.91 ± 3.33	0.156
ALT (U/L)			
Screening	22.64 ± 9.07	27.92 ± 13.98	–
Week 6	23.57 ± 11.21	21.09 ± 6.04	0.235
GGT (U/L)			
Screening	30.29 ± 35.02	19.42 ± 8.47	–
Week 6	26.64 ± 17.64	18.91 ± 8.34	0.532
Bilirubin (μmol/L)			
Screening	10.36 ± 3.34	10.33 ± 3.06	–
Week 6	10.43 ± 3.65	10.55 ± 3.83	0.845

Between group comparisons were made using Analysis of Covariance (ANCOVA). Probability values $P < 0.05$ are statistically significant.

^a One subject excluded as lost to follow up subsequent to baseline visit in placebo group. Statistical comparisons were based on $n = 11$ in week 6.

and as far as we are aware, this is the first randomized controlled clinical trial for a whole grape extract conducted in North America.

The results of the current study showed that SOD concentrations were significantly lower for subjects on WGE compared to placebo after 6 weeks of supplementation. The placebo group had a high standard deviation for SOD measurement which was attributed to a high SOD value of one subject during baseline and week 6. The higher value in the placebo group may have contributed to the within group significance detected at week 6. However, the increase from baseline to week 6 was not significant between placebo and WGE supplementation. Catechin, the main polyphenol constituent in a grape pomace enzymatic extraction was reported to be responsible for achieving antioxidant protection in an *in vivo* SOD pathway model (Perez-Ternero et al., 2013). Catechin is also a major constituent of WGE (15–25%) and therefore may be a major contributor to the antioxidant activity observed in the current study. Furthermore, phenolics from sun dried white (Peinado et al., 2013) and red grape skins

(Lopez de Lerma, Peinado, & Peinado, 2013) including resveratrol and quercetin are implicated in the antioxidant properties of grapes and therefore may also be active in WGE (Peinado et al., 2013). The main phenolic constituent of WGE, proanthocyanidins (60–70%), have been demonstrated in a number of recent grape product studies to possess antioxidant and cytoprotective effects; likely acting as the main contributor to the antioxidative activity observed in this study (Ding et al., 2013; Wang et al., 2013).

Serum HDL-C was significantly increased in subjects on WGE compared to placebo after 6 weeks of supplementation. Furthermore, the subjects on placebo showed a significantly higher total cholesterol/HDL-C ratio compared to those on WGE. Grape seed polyphenols such as gallic acid, catechin and epicatechin have been reported to inhibit pancreatic cholesterol esterase, bind to bile acids, and reduce the solubility of cholesterol in micelles (Ngamukote, Makynen, Thilawech, & Adisakwattana, 2011). Since the principal steps in cholesterol absorption are emulsification, hydrolysis of the ester bond by pancreatic esterase, micellar solubilization and

absorption in the proximal jejunum, it is possible to suggest that grape seed polyphenols affect the absorption of dietary cholesterol (Hui & Howles, 2005).

In an *in vitro* study, grape seed extract reduced antioxidant enzymes (glutathione peroxidase, superoxide dismutases, and catalase) in human platelets treated with hydrogen peroxide (Kedzierska et al., 2011). Animal studies have also demonstrated improvements in antioxidant status with grape extracts (Chidambara Murthy, Singh, & Jayaprakasha, 2002). Pretreatment of rats with a methanolic extract of grape pomace restored catalase, SOD and peroxidase activities (Chidambara Murthy et al., 2002). Grape seed proanthocyanidin extract (GSPE) increased Cu/Zn-SOD activity in both diabetic and non-diabetic rats (Puiggros et al., 2009). Another study showed that thiobarbituric acid-reactive substances (TBARS) concentrations were significantly elevated and plasma SOD activity decreased in diabetic rats, but supplementation with GSPE attenuated the elevated TBARS concentrations and increased plasma SOD activity. This suggests that supplementation with GSPE may attenuate oxidative stress through the inhibition of lipid peroxidation, restore endothelial function and reduce the risk of vascular disease in diabetics (Okudan et al., 2011).

The current literature on the impact of grape extract on the total antioxidant capacity and SOD concentrations, in human studies, are mixed. In a small study, participants who consumed red grape juice concentrate showed an increase in serum total antioxidant capacity after first ingestion (Day, Kemp, Bolton, Hartog, & Stansbie, 1997). However, in male human smokers grape seed extract reduced both adenosine-diphosphate and epinephrine-stimulated platelet activity, but plasma antioxidant capacity (total radical trapping antioxidant potential), lipid oxidation, (TBARS) and serum uric acid concentrations were not affected by grape seed extract (Polagruto et al., 2007). This discrepancy may be the result of inherent differences in pro- and antioxidative capacities of the populations being studied, which may vary with age, health status and life-style choices. In the current study, the difference in mean ages of the two groups may have impacted the antioxidant results with SOD. Of the 14 participants on WGE, 3 were less than 40 years old and 11 were 40 years or older. In contrast, of the 12 participants on placebo, 6 were less than 40 years old and 6 were 40 years or older. The mean age of the group on placebo (38 years) was lower than the mean age of the group on WGE (46 years). Various studies have reported an increase in pro-oxidative capacities and a decrease of antioxidative capacities during aging (Erden-Inal, Sunal, & Kanbak, 2002; Gil et al., 2006; Jones, Mody, Carlson, Lynn, & Sternberg, 2002; Junqueira et al., 2004; Ozbay & Dülger, 2002). In this study, the younger ages of participants on placebo compared to those on WGE may explain the observation that though both groups had increases in plasma total antioxidant capacity from baseline to week 6, only the increase for participants on placebo was significant. Furthermore, the population of subjects studied was pre-hypertensive, overweight to obese and/or pre-diabetic, and were expected to be oxidatively stressed. It would therefore be expected that there would be individual variation in the parameters that were investigated. It is noteworthy that in spite of a small sample size, as well as the different

indications that comprised this sample size, major movement in oxLDL, 8-OHdG, 8-isoprostane, total cholesterol/HDL-C ratio and HDL-C were demonstrated.

Elevated total cholesterol, triacylglycerol and LDL-C concentrations have been identified as coronary heart disease risk factors (Schaefer, 2002). Prevention of coronary artery disease has focused on modifying risk factors, such as lipid concentrations, obesity, hypertension, smoking and diet (Feringa, Laskey, Dickson, & Coleman, 2011). A growing body of research reports that grape seed extract may benefit the cardiovascular system by modifying risk factors.

Several studies have demonstrated that grape polyphenols decreased LDL-C concentrations (Stein et al., 1999; Vinson, Teufel, & Wu, 2001); however, much of the evidence of the effects of polyphenols has been derived from *in vitro* or animal experiments (Feringa et al., 2011). The results of randomized controlled trials conducted in humans to evaluate effects of grape seed extract on different cardiovascular risk markers have been mixed, with a meta-analysis of such studies showing no statistically significant effects on any blood lipid concentration, including total cholesterol, triacylglycerol, LDL-C or HDL-C concentrations (Feringa et al., 2011). However, Feringa et al. (2011), also noted that many grape seed extract studies were often performed with grape seed extract doses much higher than those to which humans were exposed through intervention, and suggested that higher doses of grape seed extract may be needed to significantly reduce lipid concentrations in human (Feringa et al., 2011). It is possible that the primary benefit is exerted through mechanisms other than lipid reduction, such as antioxidant activities, including scavenging of hydroxyl and peroxy radicals and inhibition of the oxidation of LDL (Leifert & Abeywardena, 2008). The intake of lyophilized grape powder (LGP) was found to have no effect on total cholesterol or HDL-C concentrations and decreased triacylglycerol and LDL-C concentrations in pre- or post-menopausal women (Zern et al., 2005). On the other hand, red grape juice supplementation led to a significant increase in plasma HDL-C levels and a significant decrease in total cholesterol levels in patients undergoing hemodialysis. In this study, the significant improvements of HDL-C levels and total cholesterol/HDL-C ratios in subjects on WGE compared to subjects on placebo suggest that WGE may have beneficial effects on lipid profiles.

Oxidized LDL is an independent risk factor for cardiovascular disease and oxidative modification of LDL plays an important role in the pathogenesis of atherosclerosis and coronary heart disease (Steinberg, Parthasarathy, Carew, Khoo, & Witztum, 1989). Proanthocyanidins from grape seed extract are capable of inactivating superoxide anions and inhibiting the formation of low density lipoprotein oxidation (Bagchi et al., 2000). However, data on the association of grape extract and LDL oxidation are inconclusive. An *in vitro* study found that phenolic compounds in grapes inhibited LDL oxidation, (Teissedre, Frankel, Waterhouse, Peleg, & German, 1996) while a clinical study on red grape juice concentrate suggested that there was reduced susceptibility of LDL to oxidation (Day et al., 1997). In contrast, others have reported that polyphenols did not have a measurable effect on LDL oxidation (Loest, Noh, & Koo, 2002; Stein et al., 1999). This may be due in part to the partitioning of polyphenols in plasma, in which only

10–15% appear to be associated with lipoprotein (van het Hof, Wiseman, Yang, & Tijburg, 1999). The direct effect of polyphenols on protecting circulating LDL from oxidation appears to be minimal (Zern et al., 2005). The current study showed oxLDL decreased by 0.5% in subjects on WGE but increased by 5% in subjects on placebo, suggesting that WGE may have a positive influence on oxLDL. The lack of significant efficacy of various parameters in this study such as oxLDL may have been a consequence of short study duration. A randomized clinical trial of healthy subjects reported that grape seed extract significantly decreased oxLDL at 6 weeks only in the 400 mg dose of grape seed extract and not in the 200 mg dose; however, the lower dosage was efficacious at 12 weeks post supplementation (Sano et al., 2007). In this study, subjects were supplemented with 350 mg grape extract for 6 weeks. As in the Sano et al. (2007) study, it is possible that either greater dosage or longer supplementation duration was required in order to detect changes in oxLDL.

The compound 8-isoprostane is a biomarker for oxidative stress and lipid peroxidation (Zern et al., 2005). LGP was found to decrease isoprostane concentrations in both pre and post-menopausal women, with menopausal status having no effect on isoprostane concentrations (Zern et al., 2005). This demonstrated that LGP had significant cardioprotective effects in both pre and post-menopausal women. In the current study, 8-isoprostane increased significantly in subjects on placebo but decreased for participants on WGE, and the between group differences approached significance. There was an increase of 8-OHdG, a biomarker of oxidative damage to DNA, in subjects on placebo and on WGE; but only subjects on placebo approached statistical significance ($P = 0.07$). These results suggest that WGE had a positive influence in reducing oxidative stress in participants.

WGE is generally recognized as safe (GRAS) in the United States, supported by the safety profiles of seed, skin and pulp of grapes. Studies on related GSE and GSKE products that are currently on the market have demonstrated that GSE and GSKE are non-toxic (Bentivegna & Whitney, 2002; Wren, Cleary, Frantz, Melton, & Norris, 2002; Yamakoshi, Saito, Kataoka, & Kikuchi, 2002) and non-mutagenic (Aiub et al., 2004; Yamakoshi et al., 2002). The results of the current pilot study provide evidence for the safety of the whole grape extract.

In conclusion, in spite of a small sample size, high individual variation and short study duration, a significant increase in HDL-C and a significant decrease in total cholesterol/HDL-C ratio were seen in subjects supplemented with WGE compared to placebo. Though not significant several important positive trends were found in oxLDL, 8-OHdG and 8-isoprostane. Even though changes in oxLDL and 8-isoprostane were not significant between groups, WGE decreased oxLDL and 8-isoprostane whereas placebo increased oxLDL. Although there was an increase in 8-OHdG in both groups, the increase observed in subjects on placebo was twice as much as those on WGE. The results seen in subjects on the placebo demonstrate the movement of parameters associated with oxidative stress in hypertensive, pre-diabetic, and/or overweight/obese subjects not given an intervention. These results are of clinical significance in this population of

subjects as the role of HDL-C in reverse cholesterol transport is well documented (Barter & Rye, 2006) and is believed to be cardioprotective, with each 1 mg/dL increase in HDL-C being associated with a 2–4% reduction in the risk of coronary heart disease (Gordon et al., 1989; McGrowder, Riley, Morrison, & Gordon, 2011). It is also known that raising HDL-C concentration reduces cardiovascular disease risk independent of LDL-C lowering (Brewer, 2004). In addition, the lowering of total cholesterol/HDL-C ratios is of clinical significance as changes in ratios have been shown to be better indicators of successful CHD risk reduction than changes in absolute concentrations of lipids or lipoproteins alone (Kannel, 2005). Furthermore, a 5% decrease in 8-isoprostane concentrations in participants on WGE versus a 50% increase in participants on placebo, coupled with an attenuated increase in 8-OHdG (17% in supplemented subjects vs. 26% in placebo), suggests a positive influence of WGE in reducing oxidative stress. Further studies with larger subject numbers are warranted.

Acknowledgements

We wish to thank the volunteers who took part in this study for their willingness and diligence in complying with the study protocol. The authors wish to thank Sonya Barss for overseeing the conduct of the study, Larry Stitt, Biostatistician, University of Western Ontario for statistical analysis and Eh-Sanus Fahim for overseeing data management. We thank Joshua Baisley for regulatory and quality activities and for his technical support. We thank Hui Jun Chew for reviewing and editing the manuscript. This study was sponsored by Ethical Naturals Inc. M.E. and D.W. designed and conducted research; M.E. and N.G. wrote the paper. M.E. had primary responsibility for final content. All authors have read and approved the final manuscript.

REFERENCES

- Aiub, C., Stankevics, L., da Costa, V., Ferreira, F., Mazzei, J., Ribeiro da Silva, A., Soares de Moura, R., & Felzenszwalb, I. (2004). Genotoxic evaluation of a vinifera skin extract that present pharmacological activities. *Food and Chemical Toxicology*, 42(6), 969–973.
- Bagchi, D., Bagchi, M., Stohs, S. J., Das, D. K., Ray, S. D., Kuszynski, C. A., Joshi, S. S., & Pruess, H. G. (2000). Free radicals and grape seed proanthocyanidin extract: Importance in human health and disease prevention. *Toxicology*, 148(2–3), 187–197.
- Banini, A. E., Boyd, L. C., Allen, J. C., Allen, H. G., & Sauls, D. L. (2006). Muscadine grape products intake, diet and blood constituents of non-diabetic and type 2 diabetic subjects. *Nutrition*, 22(11–12), 1137–1145.
- Barter, P. J., & Rye, K. A. (2006). The rationale for using apoA-I as a clinical marker of cardiovascular risk. *Journal of Internal Medicine*, 259(5), 447–454.
- Belli, R., Amerio, P., Brunetti, L., Orlando, G., Toto, P., Proietto, G., Vacca, M., & Tulli, A. (2005). Elevated 8-isoprostane levels in basal cell carcinoma and in UVA irradiated skin. *International Journal of Immunopathology and Pharmacology*, 18(3), 497–502.
- Bentivegna, S. S., & Whitney, K. M. (2002). Subchronic 3-month oral toxicity study of grape seed and grape skin extracts. *Food and Chemical Toxicology*, 40(12), 1731–1743.

- Brewer, H. B. Jr., (2004). Focus on high-density lipoproteins in reducing cardiovascular risk. *American Heart Journal*, 148(Suppl. 1), S14–S18.
- Chidambara Murthy, K. N., Singh, R. P., & Jayaprakasha, G. K. (2002). Antioxidant activities of grape (*Vitis vinifera*) pomace extracts. *Journal of Agricultural and Food Chemistry*, 50(21), 5909–5914.
- Davi, G., Alessandrini, P., Mezzetti, A., Minotti, G., Bucciarelli, T., Costantini, F., Cipollone, F., Bon, G. B., Ciabattini, G., & Patrono, C. (1997). In vivo formation of 8-epi-prostaglandin F₂ alpha is increased in hypercholesterolemia. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 17(11), 3230–3235.
- Day, A. P., Kemp, H. J., Bolton, C., Hartog, M., & Stansbie, D. (1997). Effect of concentrated red grape juice consumption on serum antioxidant capacity and low-density lipoprotein oxidation. *Annals of Nutrition and Metabolism*, 41(6), 353–357.
- Dean, R. T., Fu, S., Stocker, R., & Davies, M. J. (1997). Biochemistry and pathology of radical-mediated protein oxidation. *Biochemical Journal*, 324(Pt 1), 1–18.
- Ding, Y., Dai, X., Jiang, Y., Zhang, Z., Bao, L., Li, Y., Zhang, F., Ma, X., Cai, X., Jing, L., Gu, J., & Li, Y. (2013). Grape seed proanthocyanidin extracts alleviate oxidative stress and ER stress in skeletal muscle of low-dose streptozotocin- and high-carbohydrate/high-fat diet-induced diabetic rats. *Molecular Nutrition & Food Research*, 57(2), 365–369.
- Erden-Inal, M., Sunal, E., & Kanbak, G. (2002). Age-related changes in the glutathione redox system. *Cell Biochemistry and Function*, 20(1), 61–66.
- Feringa, H. H., Laskey, D. A., Dickson, J. E., & Coleman, C. I. (2011). The effect of grape seed extract on cardiovascular risk markers: A meta-analysis of randomized controlled trials. *Journal of the American Dietetic Association*, 111(8), 1173–1181.
- Frankel, E. N., Waterhouse, A. L., & Kinsella, J. E. (1993). Inhibition of human LDL oxidation by resveratrol. *Lancet*, 341(8852), 1103–1104.
- Gil, L., Siems, W., Mazurek, B., Gross, J., Schroeder, P., Voss, P., & Grune, T. (2006). Age-associated analysis of oxidative stress parameters in human plasma and erythrocytes. *Free Radical Research*, 40(5), 495–505.
- Gopaul, N. K., Anggard, E. E., Mallet, A. I., Betteridge, D. J., Wolff, S. P., & Nourooz-Zadeh, J. (1995). Plasma 8-epi-PGF₂ alpha levels are elevated in individuals with non-insulin dependent diabetes mellitus. *FEBS Letters*, 368(2), 225–229.
- Gordon, D. J., Probstfield, J. L., Garrison, R. J., Neaton, J. D., Castelli, W. P., Knoke, J. D., Jacobs, D. R., Jr., Bangdiwala, S., & Tyroler, H. A. (1989). High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. *Circulation*, 79(1), 8–15.
- Harman, S. M., Liang, L., Tsitouras, P. D., Gucciardo, F., Heward, C. B., Reaven, P. D., Ping, W., Ahmed, A., & Cutler, R. G. (2003). Urinary excretion of three nucleic acid oxidation adducts and isoprostane F(2)alpha measured by liquid chromatography-mass spectrometry in smokers, ex-smokers, and nonsmokers. *Free Radical Biology & Medicine*, 35(10), 1301–1309.
- Hayek, T., Fuhrman, B., Vaya, J., Rosenblatt, M., Belinky, P., Coleman, R., Elis, A., & Aviram, M. (1997). Reduced progression of atherosclerosis in apolipoprotein E-deficient mice following consumption of red wine, or its polyphenols quercetin or catechin, is associated with reduced susceptibility of LDL to oxidation and aggregation. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 17(11), 2744–2752.
- Hui, D. Y., & Howles, P. N. (2005). Molecular mechanisms of cholesterol absorption and transport in the intestine. *Seminars in Cell & Developmental Biology*, 16(2), 183–192.
- Jones, D. P., Mody, V. C., Jr., Carlson, J. L., Lynn, M. J., & Sternberg, P. Jr., (2002). Redox analysis of human plasma allows separation of pro-oxidant events of aging from decline in antioxidant defenses. *Free Radical Biology & Medicine*, 33(9), 1290–1300.
- Junqueira, V. B., Barros, S. B., Chan, S. S., Rodrigues, L., Giavarotti, L., Abud, R. L., & Deucher, G. P. (2004). Aging and oxidative stress. *Molecular Aspects of Medicine*, 25(1–2), 5–16.
- Kamiyama, M., Kishimoto, Y., Tani, M., Andoh, K., Utsunomiya, K., & Kondo, K. (2009). Inhibition of low-density lipoprotein oxidation by Nagano purple grape (*Vitis vinifera* × *Vitis labrusca*). *Journal of Nutritional Science and Vitaminology (Tokyo)*, 55(6), 471–478.
- Kannel, W. B. (2005). Risk stratification of dyslipidemia: Insights from the Framingham study. *Current Medicinal Chemistry – Cardiovascular & Hematological Agents*, 3(3), 187–193.
- Kasai, H. (2002). Chemistry-based studies on oxidative DNA damage: Formation, repair, and mutagenesis. *Free Radical Biology & Medicine*, 33(4), 450–456.
- Kedzierska, M., Olas, B., Wachowicz, B., Stochmal, A., Oleszek, W., & Erler, J. (2011). Changes of platelet antioxidative enzymes during oxidative stress: The protective effect of polyphenol-rich extract from berries of *Aronia melanocarpa* and grape seeds. *Platelets*, 22(5), 385–389.
- Leifert, W. R., & Abeywardena, M. Y. (2008). Cardioprotective actions of grape polyphenols. *Nutrition Research*, 28(11), 729–737.
- Loest, H. B., Noh, S. K., & Koo, S. I. (2002). Green tea extract inhibits the lymphatic absorption of cholesterol and alpha-tocopherol in ovariectomized rats. *Journal of Nutrition*, 132(6), 1282–1288.
- Loft, S., Fischer-Nielsen, A., Jeding, I. B., Vistisen, K., & Poulsen, H. E. (1993). 8-Hydroxydeoxyguanosine as a urinary biomarker of oxidative DNA damage. *Journal of Toxicology and Environmental Health*, 40(2–3), 391–404.
- Longmire, A. W., Swift, L. L., Roberts, L. J., Awad, J. A., Burk, R. F., & Morrow, J. D. (1994). Effect of oxygen tension on the generation of F₂-isoprostanes and malondialdehyde in peroxidizing rat liver microsomes. *Biochemical Pharmacology*, 47(7), 1173–1177.
- Lopez de Lerma, N., Peinado, J., & Peinado, R. A. (2013). In vitro and in vivo antioxidant activity of musts and skin extracts from off-vine dried *Vitis vinifera* cv. “Tempranillo” grapes. *Journal of Functional Foods*, 5(2), 914–922.
- McGrowder, D., Riley, C., Morrison, E. Y., & Gordon, L. (2011). The role of high-density lipoproteins in reducing the risk of vascular diseases, neurodegenerative disorders, and cancer. *Cholesterol*, 2011, 496925.
- Morrow, J. D. (2005). Quantification of isoprostanes as indices of oxidant stress and the risk of atherosclerosis in humans. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 25(2), 279–286.
- Ngamukote, S., Makynen, K., Thilawech, T., & Adisakwattana, S. (2011). Cholesterol-lowering activity of the major polyphenols in grape seed. *Molecules*, 16(6), 5054–5061.
- Okudan, N., Bariskaner, H., Gokbel, H., Sahin, A. S., Belviranli, M., & Baysal, H. (2011). The effect of supplementation of grape seed proanthocyanidin extract on vascular dysfunction in experimental diabetes. *Journal of Medicinal Food*, 14(11), 1298–1302.
- Ozbay, B., & Dulger, H. (2002). Lipid peroxidation and antioxidant enzymes in Turkish population: Relation to age, gender, exercise, and smoking. *Tohoku Journal of Experimental Medicine*, 197(2), 119–124.
- Peinado, J., Lopez de Lerma, N., Peralbo-Molina, A., Priego-Capote, F., de Castro, C., & McDonagh, B. (2013). Sunlight exposure increases the phenolic content in postharvested white grapes. An evaluation of their antioxidant activity in *Saccharomyces cerevisiae*. *Journal of Functional Foods*, 5(4), 1566–1575.
- Perez-Ternero, C., Rodriguez-Rodriguez, R., Parrado, J., & Alvarez de Sotomayor, M. (2013). Grape pomace enzymatic extract restores vascular dysfunction evoked by endothelin-1 and DETCA via NADPH oxidase downregulation and SOD activation. *Journal of Functional Foods*, 5(4), 1673–1683.

- Pignatelli, P., Ghiselli, A., Buchetti, B., Carnevale, R., Natella, F., Germano, G., Fimognari, F., Di Santo, S., Lenti, L., & Violi, F. (2006). Polyphenols synergistically inhibit oxidative stress in subjects given red and white wine. *Atherosclerosis*, 188(1), 77–83.
- Polagruto, J. A., Gross, H. B., Kamangar, F., Kosuna, K., Sun, B., Fujii, H., Keen, C. L., & Hackman, R. M. (2007). Platelet reactivity in male smokers following the acute consumption of a flavanol-rich grape seed extract. *Journal of Medicinal Food*, 10(4), 725–730.
- Puiggros, F., Sala, E., Vaque, M., Ardevol, A., Blay, M., Fernandez-Larrea, J., Arola, L., Blade, C., Pujadas, G., & Salvado, M. J. (2009). In vivo, in vitro, and in silico studies of Cu/Zn-superoxide dismutase regulation by molecules in grape seed procyanidin extract. *Journal of Agricultural and Food Chemistry*, 57(9), 3934–3942.
- Rabovsky, A., Cuomo, J., & Eich, N. (2006). Measurement of plasma antioxidant reserve after supplementation with various antioxidants in healthy subjects. *Clinica Chimica Acta*, 371(1–2), 55–60.
- Rankin, J. W., Andreae, M. C., Oliver Chen, C. Y., & O'Keefe, S. F. (2008). Effect of raisin consumption on oxidative stress and inflammation in obesity. *Diabetes, Obesity and Metabolism*, 10(11), 1086–1096.
- Reddy, P. H. (2006). Amyloid precursor protein-mediated free radicals and oxidative damage: Implications for the development and progression of Alzheimer's disease. *Journal of Neurochemistry*, 96(1), 1–13.
- Rho, K. A., & Kim, M. K. (2006). Effects of different grape formulations on antioxidative capacity, lipid peroxidation and oxidative DNA damage in aged rats. *Journal of Nutritional Science and Vitaminology (Tokyo)*, 52(1), 33–46.
- Rice-Evans, C. A., Miller, N. J., & Paganga, G. (1996). Structure–antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology & Medicine*, 20(7), 933–956.
- Roberts, L. J., & Morrow, J. D. (2000). Measurement of F(2)-isoprostanes as an index of oxidative stress in vivo. *Free Radical Biology & Medicine*, 28(4), 505–513.
- Sanchez-Moreno, C., Cao, G., Ou, B., & Prior, R. L. (2003). Anthocyanin and proanthocyanidin content in selected white and red wines. Oxygen radical absorbance capacity comparison with nontraditional wines obtained from highbush blueberry. *Journal of Agricultural and Food Chemistry*, 51(17), 4889–4896.
- Sano, A., Uchida, R., Saito, M., Shioya, N., Komori, Y., Tho, Y., & Hashizume, N. (2007). Beneficial effects of grape seed extract on malondialdehyde-modified LDL. *Journal of Nutritional Science and Vitaminology (Tokyo)*, 53(2), 174–182.
- Schaefer, E. J. (2002). Lipoproteins, nutrition, and heart disease. *American Journal of Clinical Nutrition*, 75(2), 191–212.
- Schwedhelm, E., Bartling, A., Lenzen, H., Tsikas, D., Maas, R., Brummer, J., Gutzki, F. M., Berger, J., Frolich, J. C., & Boger, R. H. (2004). Urinary 8-iso-prostaglandin F2alpha as a risk marker in patients with coronary heart disease: A matched case-control study. *Circulation*, 109(7), 843–848.
- Shigenaga, M. K., Gimeno, C. J., & Ames, B. N. (1989). Urinary 8-hydroxy-2'-deoxyguanosine as a biological marker of in vivo oxidative DNA damage. *Proceedings of the National Academy of Sciences of the United States of America*, 86(24), 9697–9701.
- Stein, J. H., Keevil, J. G., Wiebe, D. A., Aeschlimann, S., & Folts, J. D. (1999). Purple grape juice improves endothelial function and reduces the susceptibility of LDL cholesterol to oxidation in patients with coronary artery disease. *Circulation*, 100(10), 1050–1055.
- Steinberg, D., Parthasarathy, S., Carew, T. E., Khoo, J. C., & Witztum, J. L. (1989). Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. *New England Journal of Medicine*, 320(14), 915–924.
- Teissedre, P. L., Frankel, E. N., Waterhouse, A. L., Peleg, H., & German, J. B. (1996). Inhibition of in vitro human LDL oxidation by phenolic antioxidants from grapes and wines. *Journal of the Science of Food and Agriculture*, 70(1), 55–63.
- Tome-Carneiro, J., Gonzalez, M., Larrosa, M., Yanez-Gascon, M. J., Garcia-Almagro, F. J., Ruiz-Ros, J. A., Garcia-Conesa, M. T., Tomas-Barberan, F. A., & Espin, J. C. (2012). One-year consumption of a grape nutraceutical containing resveratrol improves the inflammatory and fibrinolytic status of patients in primary prevention of cardiovascular disease. *American Journal of Cardiology*, 110(3), 356–363.
- Tsao, R. (2010). Chemistry and biochemistry of dietary polyphenols. *Nutrients*, 2(12), 1231–1246.
- Turrens, J. F. (2003). Mitochondrial formation of reactive oxygen species. *Journal of Physiology*, 552(Pt 2), 335–344.
- van het Hof, K. H., Wiseman, S. A., Yang, C. S., & Tijburg, L. B. (1999). Plasma and lipoprotein levels of tea catechins following repeated tea consumption. *Proceedings of the Society for Experimental Biology and Medicine*, 220(4), 203–209.
- Vinson, J. A., Teufel, K., & Wu, N. (2001). Red wine, dealcoholized red wine, and especially grape juice, inhibit atherosclerosis in a hamster model. *Atherosclerosis*, 156(1), 67–72.
- Wang, X. H., Huang, L. L., Yu, T. T., Zhu, J. H., Shen, B., Zhang, Y., Wang, H. Z., & Gao, S. (2013). Effects of oligomeric grape seed proanthocyanidins on heart, aorta, kidney in DOCA-salt mice: Role of oxidative stress. *Phytotherapy Research*, 27(6), 869–876.
- Weseler, A. R., Ruijters, E. J., Driessens-Reijnders, M. J., Reesink, K. D., Haenen, G. R., & Bast, A. (2011). Pleiotropic benefit of monomeric and oligomeric flavanols on vascular health—A randomized controlled clinical pilot study. *PLoS One*, 6(12), e28460.
- Wren, A. F., Cleary, M., Frantz, C., Melton, S., & Norris, L. (2002). 90-Day oral toxicity study of a grape seed extract (IH636) in rats. *Journal of Agriculture and Food Chemistry*, 50(7), 2180–2192.
- Yamakoshi, J., Saito, M., Kataoka, S., & Kikuchi, M. (2002). Safety evaluation of proanthocyanidin-rich extract from grape seeds. *Food and Chemical Toxicology*, 40(5), 599–607.
- Yuan, L., Meng, L., Ma, W., Xiao, Z., Zhu, X., Feng, J. F., Yu, H., & Xiao, R. (2011). Impact of apple and grape juice consumption on the antioxidant status in healthy subjects. *International Journal of Food Sciences and Nutrition*, 62(8), 844–850.
- Zern, T. L., West, K. L., & Fernandez, M. L. (2003). Grape polyphenols decrease plasma triglycerides and cholesterol accumulation in the aorta of ovariectomized guinea pigs. *Journal of Nutrition*, 133(7), 2268–2272.
- Zern, T. L., Wood, R. J., Greene, C., West, K. L., Liu, Y., Aggarwal, D., Shachter, N. S., & Fernandez, M. L. (2005). Grape polyphenols exert a cardioprotective effect in pre- and postmenopausal women by lowering plasma lipids and reducing oxidative stress. *Journal of Nutrition*, 135(8), 1911–1917.