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# Antioxidant Capacity and Phenolic Content of Grapes, Sun-Dried Raisins, and Golden Raisins and Their Effect on ex Vivo Serum Antioxidant Capacity

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Grapes and raisins provide phenolic antioxidants, which contribute to their potential health benefits. The objectives of this study were to compare the antioxidant capacity and phenolic content of green Thompson seedless grapes (the most common variety of grapes consumed in the United States), sun-dried raisins, and golden raisins (both produced from Thompson seedless grapes) and to observe the effects of their consumption over 4 weeks in 15 healthy human males with a cross-over design. The oxygen radical absorbance capacity (ORAC) (positive statistical significance for grapes after 2 weeks and golden raisins after 3 weeks), serum oxidation (positive statistical significance for golden raisin lag time after 4 weeks), total phenolics (no significant effects), and C-reactive protein (no significant effects) were monitored. Immediately postconsumption, there were some significant oxidation, a known effect after carbohydrate consumption. Golden raisins had the highest antioxidant capacity and phenolic content. The consumption of a serving of grapes or raisins each day, in addition to a typical diet, may not be sufficient to overcome postprandial oxidation when consumed with other high carbohydrate foods but may have beneficial antioxidant effects over time.

KEYWORDS: Thompson seedless; grapes; raisins; ORAC; serum; antioxidant capacity; phenolics; postprandial; C-reactive protein

## INTRODUCTION

Grapes have been cultivated for thousands of years and were dried into raisins as early as 1000 BC. Raisins continue as an important grape product; today, the United States is the largest raisin producer and the fifth largest grape producer (1, 2). Thompson seedless grapes, first introduced in 1876, account for 95% of the California crop used for raisin production; they are the source of both sun-dried and golden raisins (3).

Antioxidant properties of grapes are attributed at least partly to their phenolic content. Grapes have been shown to be good sources of phenolic antioxidants (between 115 and 361 mg/kg total phenolics) (4–6), and the phenolic composition has also been widely studied (7–12). The phenolic content of fruits like grapes is also an important contributor to color, stability, and sensory characteristics (13). The human consumption of grape juice was linked to inhibition of platelet aggregation (14) and low-density lipoprotein (LDL) oxidation (15). Grape seed extracts have also led to decreased oxidative stress in plasma

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lipoproteins (16, 17). A conspicuous gap remains to be filled with regard to daily human consumption of typical serving sizes of grapes or raisins, as one may expect in the human population.

The objectives of this study were to (i) compare the antioxidant capacities of Thompson seedless grapes with two raisin products produced from them, namely, sun-dried and golden raisins; (ii) examine their phenolic contents; and (iii) measure blood parameters of antioxidant capacity [oxygen radical absorbance capacity (ORAC) and serum lipoprotein oxidation] as well as total phenolics and C-reactive protein in a 15 subject human study. Subjects consumed a daily serving of grapes or raisins each day over a 4 week period, followed by a washout and cross-over 4 week period.

#### MATERIALS AND METHODS

**Materials.** Thompson seedless grapes were purchased from a local grocery store where a consistent supply from a South American crop was available throughout the study period. A portion of each week's grapes that were given to participants was frozen (-20 °C) for later analysis. Sun-dried and golden raisins were kindly provided by the California Raisin Marketing Board. Grapes were stored at 4 °C, and raisins were stored at room temperature (RT, controlled at 23 °C).

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**Standards and Kits.** 2,2'-Azobis(2-amidino-propane)dihydrochloride (AAPH) was purchased from Wako Chemical (Richmond, VA). Common buffer ingredients, fluorescein, quercetin dihydrate, kaempferol, and Trolox, were purchased from Fisher Scientific (Fair Lawn, NJ). All other phenolic standards, as well as *meta*-phosphoric acid, Folin– Ciolcalteu's phenol reagent, and cupric sulfate pentahydrate, were purchased from Sigma Chemical (St. Louis, MO). A human C-reactive protein instant enzyme-linked immunosorbent assay (ELISA) kit was purchased from Bender MedSystems Inc. (Burlingame, CA).

**Collection of Serum and Plasma Samples.** Blood (10 mL) was drawn into sterile serum Vacutainer tubes and into sterile plasma ethylenediaminetetraacetic acid Vacutainer tubes (7 mL) (Fisher Scientific) by a certified phlebotomist. Serum blood samples were allowed to clot in the dark (RT, 30 min), centrifuged (1500g, 10 min), aliquoted, nitrogen flushed, and stored at -80 °C. Plasma was kept on ice, centrifuged (1000g, 10 min), aliquoted, nitrogen flushed, and stored at -80 °C as quickly as possible after collection.

Human Study. Fifteen healthy [average BMI, 25.8 (range, 20.1–31.7); no history of major disease], nonsmoking subjects between the ages of 18 and 45 (average age, 29.5) from the Champaign-Urbana, Illinois, area were recruited. A brief health questionnaire, description of the study, liability waiver, and right to opt out of the study were provided to each participant. The study was approved by the University of Illinois Institutional Review Board before recruitment began. No detailed dietary questions were asked, nor were food recalls requested before or during the study. We were making no attempt to change the diet other than the addition of grapes or raisins; thus, we relied on statistical analyses to determine if, on average, a significant difference could be seen in a free-living environment, whatever it may be for the individuals in the study. For 4 weeks, baseline 12 h fasted blood samples were collected each week from each participant, the average of which would serve as each individual's control for subsequent treatment groups. Subjects were instructed to continue with their usual diet (including fruit normally consumed, other than grapes or raisins). They were also asked to discontinue any supplements containing phenolic compounds or fruit or vegetable extracts at least 1 week prior to the beginning of the baseline period. For the following 4 weeks, five participants randomly received either 250 g of fresh Thompson seedless grapes, 50 g of sun-dried raisins, or 50 g of golden raisins. Sample sizes were designed to match a typical raisin serving size (small snack size box of raisins) and approximately the same number of fruit for the fresh grape group. Participants were provided, in weekly allotments, sufficient bagged samples to consume each day for 4 weeks. Participants visited each week for a 12 h fasting blood draw and consumed that day's sample (with a plain bagel and water), and serum and plasma samples were drawn 1 and 2 h postconsumption. This was followed by a 3 week washout period with weekly fasting blood samples collected, followed by a cross-over 4 week testing period with each subject switching to a different sample. A similar sample collection was done as in the first 4 week testing period. Overall, each participant received two of the three treatments for 28 days each.

Serum Antioxidant Capacity. ORAC-PCA assays were performed with human serum on a BioTek FL600 fluorometer (BioTek Instruments Inc., Winooski, VT) using 96 well black side with clear bottom plates (Corning Inc., Corning, NY), as derived from Davalos et al. (18). One part serum was mixed with one part 0.5 M perchloric acid (150  $\mu$ L each), vortexed briefly, and centrifuged (10000g, 10 min). The supernatant was removed and diluted 1:19 in phosphate buffer (PB). Each well contained 120  $\mu$ L of 70.3 nM fluorescein (prepared in 75 mM PB, final concentration), 20 µL of PB (blank), Trolox (prepared in PB, standard curve) or sample, and 60 µL of 12 mM AAPH (final concentration), added immediately prior to beginning measurement. For each run, one row consisted of a blank well followed by a Trolox standard curve of 1, 2, 3, and 4  $\mu$ M Trolox (final concentration), repeated in reverse order, and a second blank. Subsequent rows contained a similar symmetrically matched blank, 1  $\mu$ M Trolox (internal standard), and samples. Thus, each sample was measured in duplicate and values were averaged. Measurement was made at an emission wavelength of 515 nm and an excitation wavelength of 493 nm every minute for 80 min. Results were expressed in  $\mu$ mol Trolox equivalents (TE)/L using the Trolox standard curve run with each group of samples.

Fruit Antioxidant Capacity. Extraction was performed according to Wu et al. (19). Ten to 20 frozen grapes from each week of the study (n = 8), an equal quantity of fresh grapes, or a 1:1 w/w mixture of sun-dried or golden raisins and water (30-50 g each) were blended with a common blender. One gram was weighed and rinsed into a 15 mL screw cap tube with 10 mL of 70:29.5:0.5 acetone:water:acetic acid (AWA). Samples were vortexed (30 s), sonicated at 37 °C (5 min) (shaken twice during the sonication to diminish clumping), held at RT for 5 min, vortexed for 30 s, and held for 5 min at RT. Tubes were centrifuged (2000g, 10 min), and the supernatant was transferred to a 25 mL screw cap tube. A second aliquot (10 mL) of AWA was added to the pellet, and the extraction was repeated. AWA (~5 mL) was added to the combined aliquots for a final volume of 25 mL. Grape sample extracts were further diluted with AWA (1:10), sun-dried raisin extracts (1:20), and golden raisin extracts (1:40). Trolox was prepared in AWA. ORAC measurement and calculation were performed as above, except that results were expressed as  $\mu$ mol TE/g.

Assessment of ex Vivo Serum Lipoprotein Oxidation. Copperinduced (12 µM cupric sulfate, final concentration) serum oxidation (0.67% serum diluted in phosphate-buffered saline buffer) was assayed based on the method of Regnström et al. (20), as previously conducted in our laboratory (21, 22). Oxidation of lipoproteins was measured in the serum without specific isolation of LDL, allowing for a more realistic analysis of lipoprotein oxidation in the presence of other components in serum. Disposable UV cuvettes containing the reaction mixture were covered with parafilm to prevent evaporation and mixed, and the reaction was monitored in a Spectronic Genesys 2 spectrophotometer (Spectronic Instruments, Inc., Rochester, NY) every 10 min for 4 h (37 °C) at 234 nm using WinSpec software (Spectronic Instruments, Inc.) kinetics. All samples were analyzed in duplicate. The first reading was eliminated to allow samples to reach 37 °C, and the second reading served as a baseline for calculations. The area under the curve (AUC) and lag time (intercept between the baseline and the tangent of the absorbance curve during the propagation phase) were calculated using Microsoft Excel.

**Total Plasma Phenolics.** Total phenolics were determined using the Folin–Ciocalteu method (23) according to Serafini et al. (24) with the following modifications. The procedure was carried out in disposable 13 mm × 100 mm tubes and filtered with 0.45  $\mu$ m GHP Acrodisc 25 mm syringe filters (Pall Life Sciences, Ann Arbor, MI). The final extract (100  $\mu$ L) was combined with 0.5 mL of 10% (v/v) Folin–Ciocalteu phenol reagent (3 min), followed by addition of 0.4 mL of 75 g/L sodium carbonate, and incubated at 45 °C (25 min). Absorbance was determined (Spectronic Genesys 2) at 765 nm. Standard curves were prepared from 0 to 75 mg/L gallic acid.

**C-reactive Protein Assay.** The C-reactive protein was assayed according to the C-reactive protein ELISA kit instructions, using a BioTek Instruments Inc. ELx808 plate reader, with an ELx50 washer.

**Fruit Phenolic Profiles.** Phenolic extraction was conducted similarly to Wrolstad et al. (25), as modified by Karadeniz et al. (26). Sixty to 70 g of fresh or frozen grapes or 1:1 raisin:water mixtures were blended. The slurry (50 g) was accurately weighed, and 70 mL of acetone was added (vortex, 30 s). The mixture was filtered through Whatmann (no. 1) paper, and the slurry was transferred back to the container and revortexed with 30 mL of 70:30 acetone:water. This re-extraction was repeated. Acetone was removed from the filtrate using rotary evaporation (40 °C, 60 min). The final volume was adjusted to 25 mL with double deionized water, filtered with a 0.45  $\mu$ m GHP syringe filter, and injected onto the HPLC. Samples were analyzed in triplicate.

A Hewlett-Packard 1050 HPLC pump, 50  $\mu$ L sample loop, and photodiode array detector were used with simultaneous detection at 260, 280, and 320 nm using Chemstation software (Agilent Technologies, Palo Alto, CA). Absorption spectra were recorded from 220 to 600 nm for all peaks. Peak separation was accomplished with a Waters Xterra RP18 (5  $\mu$ m) 3.9 mm × 150 mm column. Solvent A, 0.1% H<sub>3</sub>PO<sub>4</sub> in water. Solvent B, 0.1% H<sub>3</sub>PO<sub>4</sub> in 95% acetonitrile. At a flow rate of 1 mL/min, the elution program followed a linear gradient from 5 to 60% B over 60 min. All phenolic peaks eluted within 30 min. Sample peak areas were calculated with external standard curves using three different concentrations. Caftaric and coutaric acids were

Table 1. ORAC Values ( $\mu$ mol TE/g  $\pm$  SD) of Raisins and Grapes<sup>a</sup>

golden raisin sun-dried raisin	$\begin{array}{c} 104.5\pm8.7c\\ 37.4\pm3.7b\end{array}$
fresh grape	10.8 ± 0.49 a
frozen grape	11.5 ± 1.2 a

<sup>*a*</sup> Mean values not sharing letters are significantly different (p < 0.0001).

quantitated as cholorogenic acid. Quercetin and kaempferol glycosides were quantitated as rutin. Rutin obtained from Sigma Chemical Co. is quercetin diglycoside containing mannose and glucose.

**Statistical Analysis.** All samples were evaluated in duplicate or triplicate. Significant differences were determined using analysis of variance at the 95% level using SAS statistical software (SAS Institute, Inc., Cary, NC). For statistical analysis of the human study, n = 9 in some cases due to participants being unable to attend a scheduled blood draw. Each subject served as his own control. The average of the 4 week baseline blood draw values for each individual was subtracted from values obtained during the study periods. Results were averaged into treatment groups. For short-term analyses, the fasting blood draw value for each individual, followed by pooling of treatment groups.

#### **RESULTS AND DISCUSSION**

**Grape and Raisin ORAC Analysis.** Grapes and raisins distributed to study participants were analyzed for antioxidant capacity (**Table 1**). Weekly samples were frozen for analysis, and fresh grapes were monitored for comparison. The ORAC values are similar to those reported by Wu et al. (19) for grapes and Wu et al. (27) for sun-dried raisins. To our knowledge, this is the first report of golden raisin ORAC, and it is interesting to note that its value is much higher per gram than sun-dried raisins. Golden raisins are treated with hot water and SO<sub>2</sub> to inactivate polyphenol oxidase and to inhibit nonenzymatic browning, allowing the raisin to retain the phenolic antioxidants of the original grape, which are then concentrated during drying.

Grape and Raisin Phenolic Analyses. Tyrosine and HMF were detected in some frozen grape, golden raisin, and sundried raisin samples, although quantities were highly variable and not well-resolved, so values are not reported (Table 2). Only two quercetin glycosides are reported for both grape samples. Although other phenolic glycosides were present and somewhat similar to the raisin sample phenolic glycosides, the amounts were very small and the spectra were difficult to confirm with certainty and are thus reported only as a trace. For golden and sun-dried raisins, quercetin glycosides (including rutin) and kaempferol glycosides were better resolved, although they had nearly identical spectra. The elution order reported was based on solubility and the work of Cheynier and Rigaud (8) and Karadeniz et al. (26). There is not a way to differentiate the specific glycoside of quercetin or kaempferol without mass spectrometry, and that was not available for this study; thus, we designated A and B based upon the elution order as by Karadeniz et al.

No effort was made to inhibit oxidation during sample phenolic analysis, although samples were extracted and analyzed as quickly as possible. This was done to simulate the actual consumption of grapes or raisins and the likely phenolic profile that would be consumed and presented to the digestive tract after chewing. Singleton et al. (28) reported that caftaric and coutaric acids are rapidly converted to a reaction product upon processing. Some of this degradation is apparent in the grape samples, as the levels are much lower than those reported by Karadeniz et al. (26), who took steps to minimize oxidation in sample preparation. Interestingly, our raisin samples did not

appear to lose caftaric or coutaric acids after blending. Additionally, 2-S-glutathionyl caftaric acid forms when glutathione and caftaric acids are decompartmentalized upon processing (29). Evidence of such reactions is seen in **Table 2** for the grape samples; once again, Karadeniz et al. (26) reported that they did not detect 2-S-glutathionyl caftaric acid in their grape samples. Note that freezing the grapes resulted in additional decompartmentalization, as 2-S-glutathionyl caftaric acid levels are higher in the frozen samples.

Previous reports of analyses of phenolic components of grapes and raisins (26) did not include antioxidant capacity estimation by ORAC assay. This will be the first literature report comparing the ORAC antioxidant capacity of grapes and raisins to phenolic profiles. Almost all phenolic compounds measured in golden raisins were significantly higher than those present in grapes or sun-dried raisins suggesting that preservation of the phenolic compounds in golden raisins contributes significantly to the antioxidant activity of this processed fruit, although the contribution of other reactions or compounds cannot be entirely ruled out.

Antioxidant Capacity, Long-Term. There is an apparent trend toward increasing the serum antioxidant capacity by the second and third weeks of sample consumption, although the values fall again in the fourth week (Figure 1). Two instances of statistically significant increases in serum antioxidant capacity occurred at 2 weeks of grape consumption and 3 weeks of golden raisin consumption. We speculate that there may be a physiological plateau or peak approximately 2 or 3 weeks after consistent consumption, although a varied diet over the study period means that other explanations are possible. However, these results are similar to those of Cao et al. (30), who noted an apparent decrease in serum ORAC values between days 11 and 16. Controlled diets consisting of 10 servings of fruits and vegetables a day for 15 days were provided in a metabolic research unit. Although measurements by Cao et al. did not continue beyond day 16, they also noted an apparent plateau; this requires further study.

Serum Oxidation, Long-Term. Serum oxidation analyses (Figures 2 and 3) do not directly agree with ORAC analyses. None of the values were significant over the 4 week period when AUC was assessed, nor was an apparent peak observed at 2 or 3 weeks, as was the case with the ORAC assay. However, when the lag time was analyzed, a trend was noted. Values increased, on average, over the 4 week period, and reached statistical significance by week 4 for golden raisin consumption. Again, the differences may be explained by uncontrolled factors in the diets of the participants. In addition, the ORAC and lipoprotein oxidation assays may be influenced by and thus reflect different components in the serum. One possible contributing factor may have been stress (and thus potentially increased oxidation) or dietary changes over the 15 week period, as a majority of the study participants were graduate students and this study took place over the course of a 16 week semester.

**Short-Term Serum Oxidation and Antioxidant Capacity.** For short-term analysis (**Figures 4–6**), blood was drawn upon arrival (fasting), and 1 and 2 h after grape/raisin and bagel consumption weekly during each of the 4 week consumption periods. For both the ORAC assay as well as the serum oxidation assays, values indicated increased oxidation. Statistically significant changes in ORAC values occurred for golden raisins in week 1 after 2 h and for grapes in weeks 3 and 4 after both 1 and 2 h. For the serum oxidation assay AUC, sun-dried raisins were significantly negative during week 1 after 2 h. Lastly, for serum oxidation lag time, grapes

Table 2. Tryptophan, Flavonoid, and Phenolic Composition of Grapes and Raisins (mg/kg of Sample  $\pm$  SD)<sup>a</sup>

	raisin		grape	
	golden ( $n = 4$ )	sun-dried ( $n = 3$ )	fresh ( $n = 3$ )	frozen ( $n = 3$ )
tryptophan	ND	ND	40.4 ± 4.1 a	$16.7\pm3.9$ b
trans-caftaric acid <sup>b</sup> , <sup>d</sup>	$130.4 \pm 7.0 \ a$	$41.4\pm5.8$ b	$7.9\pm1.5$ c	$7.9\pm0.19$ c
trans-coutaric acid <sup>b</sup>	$34.1\pm8.8$ A	ND	$14.8\pm1.0~\text{B}$	ND
2-S-glutathionyl caftaric acid <sup>b, d</sup>	ND	ND	$8.8\pm0.9$ a	$12.1 \pm 4.4$ a
protocatechuic acid	ND	$4.4 \pm 1.2$	ND	ND
rutin	$14.4 \pm 0.9 \ { m a}$	$8.3\pm1.6$ b	trace	trace
quercetin glycoside A <sup>c</sup>	$65.7 \pm 13.7  \mathrm{a}$	$15.6\pm2.8$ b	$15.2\pm3.1$ b	$21.5\pm4.7$ b
quercetin glycoside B <sup>c</sup>	$43.4\pm5.2~\mathrm{aA}$	$6.5\pm0.3$ b	$25.6\pm3.7~\mathrm{c}$	$27.7\pm6.5\mathrm{acB}$
kaempferol glycoside A <sup>c</sup>	$9.8\pm0.5$ a	$7.0\pm3.1a$	trace	trace
kaempferol glycoside B <sup>c</sup>	$14.3\pm2.9$ a	$9.3\pm2.6~a$	trace	trace

<sup>a</sup> Mean values not sharing letters in each row are significantly different (lowercase, $p < 0.01$ ; uppercase, $p < 0.05$ ).	<sup>b</sup> Quantitated as chlorogenic acid. <sup>c</sup> Quantitated as
rutin (see Materials and Methods). <sup>d</sup> Frozen grape value for trans-caftaric acid represent the average of two samples;	none was detected in the third sample, although
increased levels of 2-S-glutathionyl caftaric acid (16.8 mg/kg) in that sample suggest exidation. ND. not detected.	



**Figure 1.** Long-term serum ORAC (oygen radical absorbance capacity) change over 4 weeks of daily consumption of golden raisins, sun-dried raisins, or grapes. Higher values indicate a higher serum antioxidant capacity. Baseline is zero. Baseline raw values: golden, 1113; sun-dried, 1155; and grape, 1095  $\mu$ mol TE/L. \*p < 0.05. Shown as means  $\pm$  SEM ( $n \ge 9$ ).

were significantly negative during week 1 after 1 and 2 h, and golden raisins were significantly negative during week 4 after 2 h. We hypothesized that the antioxidant components in grapes or raisins would have a positive effect on serum antioxidant capacity, as seen in other grape-related studies of this nature (15, 17). Thus, when some of the analyses were significantly impacted by treatment in the negative direction, these results were unexpected. We speculate that the high sugar content of the grapes or raisins and carbohydrate content of the bagels may have affected blood antioxidant capacity by causing postprandial oxidative stress (31-33). The metabolic effects may have resulted in an increase in free radical activity in the serum, resulting in the apparent drop in antioxidant capacity, short-term. Although the antioxidants present in the grape or raisin sample theoretically should have ameliorated the effects, the dosage may have been insufficient. However, as our study did not include a postprandial control group (subjects who consumed only bagels after the fasting blood draw), we are unable to determine if the grapes or raisins would ameliorate the effects of bagels alone. Other researchers have used a much more concentrated grape seed extract to observe a decrease in postprandial oxidative stress (16).



**Figure 2.** Serum lipoprotein oxidation AUC over 4 weeks of daily consumption of golden raisins, sun-dried raisins, or grapes. Lower values indicate less oxidation. Baseline is zero. Baseline raw values {in  $\Sigma$ [ $\Delta A$ (234 nm)]\*min}: golden, 20.2; sun-dried, 19.1; and grapes, 19.3. No values were significant. Shown as means  $\pm$  SEM ( $n \ge 9$ ).

Our short-term ORAC assays and serum oxidation results agree in direction, although significant values varied by week and fruit. Oxidation consistently increased in both assays over the 2 h period, although there does not appear to be any pattern for significant values.

Total Phenolics and C-Reactive Protein. Plasma samples were analyzed for total phenolics and C-reactive protein (data not shown) to evaluate the positive impact of long-term feeding intervention with grapes and raisins. No significant changes or trends were found for total phenolics between control samples and weeks 1-4. Variation between subjects was great and inconsistent within subjects over the 4 week period for Creactive protein analysis, making it impossible to obtain meaningful results. Despite the use of controlled isocaloric diets (including vegetables, berries, and apples) providing 90% of calories to males and females 19-52 years over 6 weeks, Freese et al. (34) were also unable to obtain significant C-reactive protein changes as compared to habitual diet controls. The age range, level of dietary intervention, and dietary history of participants and controls may be contributing factors to our results and those of Freese et al.

In conclusion, we report the first comparison of grape and raisin ORAC values with their phenolic composition, showing that there is a correlation between increased phenolic content



**Figure 3.** Serum oxidation lag time over 4 weeks of daily consumption of golden raisins, sun-dried raisins, or grapes. Higher values represent less rapid onset of oxidation. Baseline is zero. Baseline raw values (in min): golden, 91; sun-dried, 95.5; and grapes, 91. \*p < 0.05. Shown as means  $\pm$  SEM ( $n \ge 9$ ).



**Figure 4.** ORAC measured 1 and 2 h after grape/raisin (and bagel) consumption. Higher ORAC values indicate greater serum antioxidant capacity. Baseline is zero. Baseline raw values (golden raisins, sun-dried raisins, and grapes, respectively, in  $\mu$ mol TE/L): week 1, 1104, 1211, and 1046; week 2, 1188, 1207, and 1192; week 3, 1233, 1211, and 1148; and week 4, 1197, 1119, and 1122. \*p < 0.05. Shown as means  $\pm$  SEM ( $n \geq 9$ ).

and increased antioxidant activity in vitro. There appear to be positive benefits to consumption of golden raisins or grapes each day over a month-long period, despite uncontrolled variation of the diets of healthy males and lifestyle changes that may occur over a 15 week period. Data for short-term grape/raisin consumption (i.e., 2 h after consumption), while apparently negative, are valuable to better understanding postprandial oxidation after a high carbohydrate meal. This will improve our understanding of the link between postprandial oxidative stress and atherogenesis (ref (35), originally proposed by ref (36)) and possibly lead to improved food preparation techniques by manufacturers (i.e., improved



**Figure 5.** Serum oxidation AUC 1 and 2 h postconsumption. Lower values indicate less serum oxidation. Baseline is zero. Baseline raw values {golden raisins, sun-dried raisins, and grapes, respectively, in  $\Sigma[\Delta A(234 \text{ nm})]^*$ min}: week 1, 20.8, 18.9, and 20.1; week 2, 21.3, 20.3, and 20.3; week 3, 21.4, 17.8, and 20.8; and week 4, 20.2, 19.0, and 18.6. \*p < 0.05. Shown as means  $\pm$  SEM ( $n \geq 9$ ).



**Figure 6.** Serum oxidation lag time for 1 and 2 h postconsumption. Higher values indicate a longer time to the onset of oxidation. Baseline is zero. Baseline raw values (golden raisins, sun-dried raisins, and grapes, respectively, in min): week 1, 85, 93, and 90.5; week 2, 89.5, 91.7, and 88; week 3, 86.5, 100, and 90.5; and week 4, 100, 97, and 99.5. \*p < 0.05. Shown as means  $\pm$  SEM ( $n \ge 9$ ).

antioxidant activity of common carbohydrate foods) and/or better food choices by consumers.

### ABBREVIATIONS USED

AUC, area under the curve; AAPH, 2,2'-azobis(2-amidinopropane)dihydrochloride; LDL, low-density lipoprotein; ORAC, oxygen radical absorbance capacity; RT, room temperature; TE, Trolox equivalents.

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#### LITERATURE CITED

- (1) http://www.fas.usda.gov/htp/Presentations/2007/2007%20Raisins.pdf.
- (2) http://www.fas.usda.gov/htp/Hort\_Circular/2006/04-06/ Table%20Grape%20Situation%20and%20Outlook%202006.pdf.
- (3) http://www.raisins.org.
- (4) Teissedre, P. L.; Frankel, E. N.; Waterhouse, A. L.; Peleg, H.; German, J. B. Inhibition of in vitro human LDL oxidation by phenolic antioxidants from grapes and wines. *J. Sci. Food Agric.* **1996**, *70*, 55–61.
- (5) Cantos, E.; Espin, J. C.; Tomas-Barberan, F. A. Varietal differences among the polyphenol profiles of seven table grape cultivars studied by LC-DAD-MS-MS. J. Agric. Food Chem. 2002, 50, 5691–5696.
- (6) Pastrana-Bonilla, E.; Akoh, C. C.; Sellappan, S.; Krewer, G. Phenolic content and antioxidant capacity of muscadine grapes. *J. Agric. Food. Chem.* **2003**, *51*, 5497–5503.
- (7) Lea, A. G. H.; Bridle, P.; Timberlake, C. F.; Singleton, V. L. The procyanidins of white grapes and wines. *Am. J. Enol. Vitic.* **1979**, *30*, 289–300.
- (8) Cheynier, V.; Rigaud, J. HPLC separation and characterization of flavonols in the skins of *Vitis vinifera* var. Cincault. Am. J. Enol. Vitic. **1986**, 37, 248–262.
- (9) Jaworski, A. W.; Lee, C. Y. Fractionation and HPLC determination of grape phenolics. J. Agric. Food Chem. 1987, 35, 257–259.
- (10) Merida, J.; Moyana, L.; Millan, C.; Medina, M. Extraction of phenolic compounds in controlled macerations of Pedro Ximenez grapes. *Vitis* **1991**, *30*, 117–127.
- (11) Souquet, J. M.; Cheynier, V.; Brossaud, F.; Moutounet, M. Polymeric proanthocyanidins from grape skins. *Phytochemistry* **1996**, *43*, 509–512.
- (12) Alonso Borbalan, A. M.; Zorro, L.; Guillen, D. A.; Barroso, C. G. Study of the polyphenol content of red and white grape varieties by liquid chromatography-mass spectroscopy and its relationship to antioxidant power. J. Chromatogr. A 2003, 1012, 31–38.
- (13) Macheix, J. J.; Fleuriet, A.; Billot, J. *Fruit Phenolics*; CRC Press: Boca Raton, FL, 1990.
- (14) Keevil, J. G.; Osman, H. E.; Reed, J. D.; Folts, J. D. Grape juice, but not orange juice or grapefruit juice, inhibits human platelet aggregation. J. Nutr. 2000, 130, 53–56.
- (15) Miyagi, Y.; Miwa, K.; Inoue, H. Inhibition of human low-density lipoprotein oxidation by flavonoids in red wine and grape juice. *Am. J. Cardiol.* **1997**, *80*, 1627–1631.
- (16) Natella, F.; Belelli, F.; Gentili, V.; Ursini, F.; Scaccani, C. Grape seed proanthocyanidins prevent plasma postprandial oxidative stress in humans. J. Agric. Food Chem. 2002, 50, 7720–7725.
- (17) Vigna, G. B.; Costantini, F.; Aldini, G.; Carini, M.; Catapano, A.; Schena, F.; Tangerini, A.; Zanca, R.; Bombardelli, E.; Morazzoni, P.; Mezzetti, A.; Fellin, R.; Maffei Facino, R. Effect of a standardized grape seed extract on low-density lipoprotein susceptibility to oxidation in heavy smokers. *Metabolism* 2003, 52, 1250–1257.
- (18) Davalos, A.; Gomez-Cordoves, C.; Bartolome, B. Extending applicability of the oxygen radical absorbance capacity (ORACfluorescein) assay. J. Agric. Food Chem. 2004, 52, 48–54.
- (19) Wu, X.; Beecher, G. R.; Holden, J. M.; Haytowitz, D. B.; Gebhardt, S. E.; Prior, R. L. Lipophilic and hydrophilic antioxidant capacities of common foods in the Unites States. *J. Agric. Food Chem.* **2004**, *52*, 4026–4037.

- (20) Regnström, J.; Strom, K.; Moldeus, P.; Nilsson, J. Analysis of lipoprotein diene formation in human serum exposed to copper. *Free Radical Res. Commun.* **1993**, *19*, 267–278.
- (21) Gheldof, N.; Engeseth, N. J. Antioxidant capacity of honeys from various floral sources based on the determination of oxygen radical absorbance capacity and inhibition of in vitro lipoprotein oxidation in human serum samples. J. Agric. Food Chem. 2002, 50, 3050– 3055.
- (22) Gheldof, N.; Wang, X.-H.; Engeseth, N. J. Buckwheat honey increases serum antioxidant capacity in humans. J. Agric. Food Chem. 2003, 51, 1500–1505.
- (23) Swain, T.; Hillis, E. The penolic constituents of *Prunus domestica*. The quantitative analysis of phenolic constituents. J. Sci. Food Agric. **1959**, 10, 63–68.
- (24) Serafini, M.; Maiani, G.; Ferro-Luzzi, A. Alcohol-free red wine enhances plasma antioxidant capacity in humans. J. Nutr. 1998, 128, 1003–1007.
- (25) Wrolstad, R. E.; Skrede, G.; Lea, P.; Enersen, G. Influence of sugar on anthocyanin pigment stability in frozen strawberries. J. Food Sci. 1990, 55, 1064–1065, 1072.
- (26) Karadeniz, F.; Durst, R. W.; Wrolstad, R. E. Polyphenolic composition of raisins. J. Agric. Food Chem. 2000, 48, 5343– 5350.
- (27) Wu, X.; Gu, L.; Holden, J. M.; Haytowitz, D. B.; Gebhardt, S. E.; Beecher, G. R.; Prior, R. L. Development of a database of total antioxidant capacity in foods: A preliminary study. *J. Food Compos. Anal.* **2004**, *17*, 407–422.
- (28) Singleton, V. L.; Zaya, J.; Trousdale, E.; Salgues, M. Caftaric acid in grapes and conversion to a reaction product during processing. *Vitis* **1984**, *23*, 113–120.
- (29) Singleton, V. L.; Trousdale, E. White wine phenolics: Varietal and processing differences as shown by HPLC. *Am. J. Enol. Vitic.* **1983**, *34*, 27–34.
- (30) Cao, G.; Booth, S. L.; Sadowski, J. A.; Prior, R. L. Increases in human plasma antioxidant capacity after consumption of controlled diets high in fruit and vegetables. *Am. J. Clin. Nutr.* **1998**, *68*, 1081–1087.
- (31) Ursini, F.; Sevanian, A. Postprandial oxidative stress. *Biol. Chem.* 2002, 383, 599–605.
- (32) Rebolledo, O. R.; Actis Dato, S. M. Postprandial hyperglycemia and hyperlipidemia generated glycoxidative stress: Its contribution to the pathogenesis of diabetes complications. *Eur. Rev. Med. Pharmacol. Sci.* 2005, *9*, 191–208.
- (33) Sies, H.; Stahl, W.; Sevanian, A. Nutritional, dietary, and postprandial oxidative stress. J. Nutr. 2005, 135, 969–972.
- (34) Freese, R.; Vaaralo, O.; Turpeinen, A. M.; Mutanen, M. No difference in platelet activation or imflammation markers after diets rich or poor in vegetables, berries and apple in healthy subjects. *Eur. J. Nutr.* 2004, *43*, 175–182.
- (35) Bowen, P. E.; Borthakur, G. Postprandial lipid oxidation and cardiovascular disease risk. *Curr. Atheroscler. Rep.* 2004, 6, 477– 484.
- (36) Zilversmit, D. B. Atherogenesis: A postprandial phenomenon. *Circulation* **1979**, *60*, 473–485.

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