Effect of raisin consumption on oxidative stress and inflammation in obesity

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Aim: Oxidative stress can initiate increased inflammation that elevates risk for cardiovascular disease. The objective of this study was to determine the effects of daily consumption of raisins on markers of oxidative stress, inflammation and endothelial activation in response to an acute high-fat meal in overweight individuals.

Methods: Seventeen overweight men and women consumed 90 g raisins or isocaloric placebo (264 kcal/day) for 14 days in a randomized, crossover design while following a low-flavonoid diet. The oxidative [urinary 8-iso-prostaglandin-$F_{2\alpha}$ (8-epi $PGF_{2\alpha}$) and serum oxygen radical absorbance capacity (ORAC)], inflammatory (serum C-reactive protein and interleukin-6), endothelial (serum soluble intercellular adhesion molecule-1 and soluble vascular cell adhesion molecule-1, sVCAM-1) and metabolic [free fatty acids (FFAs), triacylglycerol, glucose and insulin] response to four high-fat (53%) meals was tested pre- and postintervention.

Results: Urinary 8-epi $PGF_{2\alpha}$ decreased (-22%) and fasting ORAC increased ($+3\%$) after both interventions combined. Fasting protein-free ORAC was modestly ($+3.5\%$) higher during the raisin than the placebo intervention. Neither the meals nor the raisins consistently induced fasted markers of inflammation or endothelial dysfunction. Gender influenced postprandial metabolic responses in that males responded with higher serum FFAs, sVCAM-1 and glucose compared with females.

Conclusions: Serum antioxidant capacity was modestly increased by daily raisin consumption, but this did not alter fasted or postprandial inflammatory response in these relatively healthy but overweight individuals. Providing all food in regular pattern reduced measures of oxidative stress.

Keywords: antioxidants, cytokines, endothelial activation, ORAC, postprandial response

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Introduction

Chronically elevated inflammation in obese or overweight individuals, reflected by increases in serum cytokines, acute-phase proteins and markers of endothelial activation, predict higher risk of cardiovascular disease (CVD) [1–4]. The cause of the higher inflammatory status in obesity has been hypothesized to be related to higher oxidative stress [3,5], which has also been implicated as a link to impaired endothelial function [6].

Some studies have examined the association between consumption of antioxidant and flavonoid-rich foods and risk factors for heart disease. In epidemiological studies, investigators demonstrated that individuals with the highest flavonoid consumption had reduced risk of CVD [7,8]. The mechanism for this association has not been clarified but could involve reduction in oxidative stress and inflammation. Cao et al. [9] found significant positive correlations between fasting oxygen radical absorbance capacity (ORAC) of plasma and the reported daily

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consumption of antioxidants from fruits and vegetables during the previous year. Increase in daily consumption of one high-flavonoid food, Concord grape juice, reduced LDL susceptibility to oxidation [10], while another grape-derived product, lyophilized grape powder supplement, failed to reduce LDL oxidation but lowered a marker of lipid oxidation and plasma tumour necrosis factor-α [11]. Similar studies have not been reported using dried grapes, raisins.

As much of the day is spent in the postprandial state, responses to meals can influence chronic disease risk [12,13]. High-fat meals can acutely increase inflammatory and oxidative stress markers while reducing endothelial function. For example, Nappo *et al.* [14] found that a high-fat meal increased plasma concentrations of a variety of inflammatory and endothelial activation markers in type 2 diabetics as well as normal controls. The addition of antioxidants E and C ameliorated these effects, suggesting that this response is linked to the ability of the body to quench free radicals [14]. Other studies provide further evidence for a link between dietary components, oxidative state and endothelial function. Esposito *et al.* [15] confirmed deterioration in vascular function following a high-fat meal (59% fat) that was improved with simultaneous consumption of vitamins E and C, suggesting an oxidative mechanism for the disruption of endothelial function. While many of these studies were performed using an antioxidant supplement, it is of interest to determine whether addition of whole foods containing antioxidant compounds to the daily diet of overweight individuals could reduce the postprandial inflammatory response.

This study investigated the effects of acute and chronic consumption of seedless raisins on markers of oxidative stress, inflammation and endothelial activation in the fasted state and in response to an acute high–fat meal challenge in overweight and obese subjects. The purpose of this study was to help determine whether a dietary intervention utilizing a whole food source of phenolic compounds would be capable of diminishing the magnitude of inflammation in overweight individuals.

Methods

Subjects

Twenty overweight men and women [body mass index (BMI) $\geq$ 26] between the ages of 18 and 40 years gave their written agreement to participate after being informed of all risks associated with the investigation. All investigative procedures were reviewed and approved by the Virginia Tech Human Subjects Institutional Review Board.

Subjects were asked to maintain their weight and physical activity level as well as refrain from taking any dietary supplements or anti-inflammatory medications 2 weeks prior to and for the duration of the study. Individuals with diagnosed CVD, diabetes or hypertension, tobacco use, lack of stable weight (±0.5 kg) over the last 3 months, participation in heavy exercise (>120 min/week of moderate to intense activity) and individuals with pertinent food aversions or allergies were excluded. Subjects were instructed on completion of a 4-day food record (3 weekdays and 1 weekend day) prior to study commencement in order to estimate subjects' habitual macronutrient and micronutrient intake. Food records and menus were analysed by NUTRITION DATA SYSTEM FOR RESEARCH (NDS-R version 5.0, Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN, USA).

Qualifying subjects had fasting blood samples taken for analysis of C-reactive protein (CRP) 2 weeks prior to starting the study. Any subjects with values greater than 10 mg/l were interviewed in order to dismiss any evidence of acute infection or illness. Any subjects with values greater than 20 mg/dl were prohibited from participating in the investigation. One subject was excluded based on these exclusion criteria.

Study Design

A randomized, counterbalanced, crossover design was used in order to have subjects undergo raisin and isoenergetic placebo treatments. The duration of the study for each subject totalled 56 days: days 1–14 consisted of a lead-in low-flavonoid diet modelled after diets by O'Byrne *et al.* [10], followed by 14 days (15–28) of low-flavonoid diet plus the first intervention, 14 days (29–42) of washout between interventions and finally 14 days (43–56) of the alternate intervention (figure 1).

During the controlled feeding period of each intervention (days 22–28 and 50–56), subjects were provided with all their food. All subjects were provided with a low-flavonoid/antioxidant breakfast and dinner meals in our facility (foods Laboratory in the department of Human Nutrition, Foods, and Exercise at Virginia Tech) and were given a take-away lunch. Foods were weighed before and after serving to estimate actual consumption. Three days of rotating menus were designed with 50% of energy from carbohydrate, 30% from fat, 15% from protein and low vitamins E and C. Menus were adjusted to accommodate multiple calorie levels: 1700, 2000, 2500, 3000 and 3500 kcal/day with subjects matched to energy level corresponding to energy need estimates using the modified Harris Benedict equation for sedentary overweight adults (American Dietetic Association). Table 1 provides an
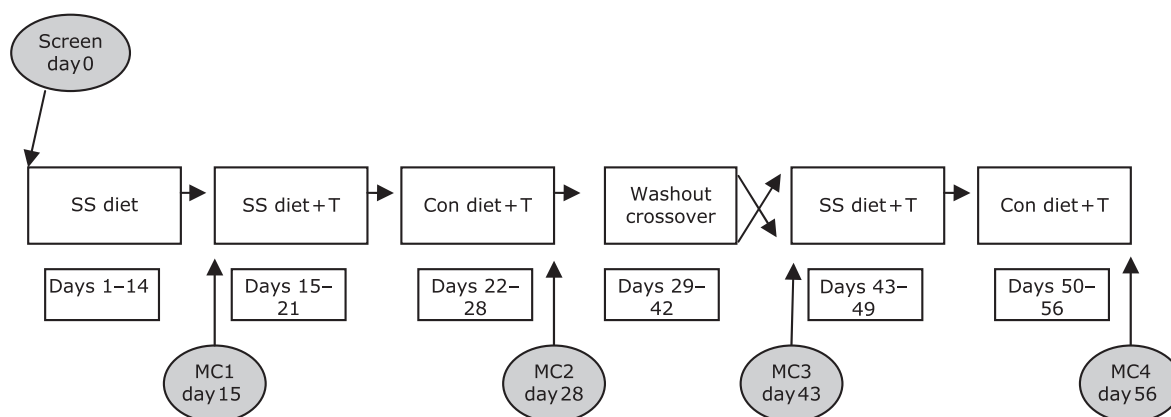
Fig. 1 Study design. MC, meal challenge; Con, controlled-feeding diet; SS, self-selected diet; T, treatment.

example menu for the controlled feeding period for a subject in the R group (54.7% carbohydrate, 15.1% protein, 31.3% fat, 8 IU vitamin E and 9.4 mg vitamin C). Adjustments for weight loss were made, as needed, using 100 kcal low-fat carbohydrate snacks.

Following arrival to the laboratory each day of the controlled feeding period and on all four meal challenge days prior to testing (days 15, 28, 43 and 56), fasted body mass was measured. The meal challenge consisted of a commercially purchased breakfast (slightly modified McDonald's Big Breakfast®: scrambled eggs, two sausage patties, one buttermilk and one biscuit) as well as 132 kcal either of Thompson sun-dried raisins (R) (45-g serving) or jelly candy (35 g, Starburst® Jelly Beans, P). The meal with the intervention contained 900 kcal. The proportion of macronutrients was very slightly different between the meal challenges because of the low but measurable protein and fat content of raisins (52.9, 33.4 and 14.8% and

53.0, 35.1 and 14.2% proportion of total energy from fat, carbohydrate and protein in meal with raisins and meal with placebo respectively).

Dietary fibre was 2.7 and 1.0 g for raisin and placebo meals respectively. Blood draws were taken at times 0 and 2, 3 and 4 h postprandial.

Blood Collection

Blood was taken from the arm by venipuncture with minimum stasis. After collection, blood was allowed to clot for 15 min, centrifuged at 400 g for 15 min at 4°C and the serum was frozen at −80°C until later analysis.

Time 0 serum samples were measured for total ORAC (ORACtotal), protein-free ORAC (ORACpca), CRP, interleukin-6 (IL-6), soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1), free fatty acids (FFAs), triacylglycerol, glucose and insulin. All measures except ORACtotal and ORACpca were measured in the blood samples taken 2, 3 and 4 h after the meal challenge.

Sample Analysis

Fasting serum samples designated for ORACpca were prepared immediately according to Prior and Cao [16] and frozen at −80°C until later analysis. The ORAC assay was conducted at the Antioxidants Research Lab of the Jean Mayer United States Department of Agriculture Human Nutrition Research Center on Aging according to the method of Cao and Prior with modifications for application to an automated plate reader [17] and use of fluorescein as the fluorescence oxidizing probe [18]. The ORAC assay provides an integrated and quantitative determination of 'total antioxidant capacity' by employing the area under the curve of the magnitude and time to

Table 1 Sample 2500 kcal daily menu during controlled feeding period

Meal	Food
Breakfast	75 g scrambled eggs
	28 g biscuit
	227 g canned pears
	22 g jelly
	45 g raisins
Snack	Four peanut butter crackers
Lunch	357 g chicken noodle soup
	Eight saltine crackers
	One and a half grilled cheese sandwich
Dinner	140 g ground beef patty with bun, 21 g American cheese slice, 9 g mayonnaise, 23 g yellow mustard and 24 g iceberg lettuce
	45 g raisins
Snack	56 g pretzels
	600 g pudding

the oxidation of fluorescein because of peroxyl radicals generated by the addition of 2,2′-azo-bis(2-amidinopropane) dihydrochloride. Fluorescence intensity derived from fluorescein was monitored at 485/520 (ex/em) every 2 min for 60 min (FLUOstar OPTIMA; BMG Labtech, Offenburg, Germany). Final results were expressed as mmol/l trolox equivalent. Intra-assay variation was 3.9%.

Serum samples were analysed for CRP, IL-6, sICAM-1 and sVCAM-1 in duplicate through enzyme-linked immunosorbent assay (ELISA) using commercially available kits (United Biotech, Mountain View, CA, USA). Intra-assay variations were 8.4, 8.4, 2.7 and 2.4% respectively.

Serum samples were analysed for FFA, triacylglycerol and glucose in duplicate through spectrophotometry using commercially available enzymatic colorimetric kits (Wako Chemicals USA, Richmond, VA, USA and Stanbio, Boerne, TX, USA) respectively. The adapted microplate procedure was employed for FFA and triacylglycerol (Wako Chemicals USA). Intra-assay variations were 2.6, 4.1 and 2.0% respectively. Insulin was analysed through radioimmunoassay (Diagnostic Products, Los Angeles, CA, USA). Intra-assay variation was 7.1%.

Urine Collection

Subjects provided first-void urine samples after an overnight 12-h fast on the mornings of the CRP screening (day 1) and all four meal challenges (days 15, 28, 43 and 56). Urine samples were collected in 100-ml polypropylene containers and were stored at 5°C prior to being aliquoted into cryovials for storage at −80°C until analysis of creatinine and urinary 8-epi-prostaglandin-$F_{2\alpha}$ (8-epi $PGF_{2\alpha}$). Urinary 8-epi $PGF_{2\alpha}$ concentrations were quantified in duplicate through a commercial ELISA (Neogen, Lexington, KY, USA) and expressed as per milligram creatinine. Intra-assay variation was 5.0%. Urinary creatinine was analysed in duplicate through a quantitative colorimetric assay (Stanbio). Intra-assay variation was 0.7%.

Raisin Analysis

Raisins were obtained from the California Raisin Marketing Board in multiple batches at the start of the study. All batches were combined, mixed and aliquoted into five large bags and placed in opaque plastic sealable containers and stored in a freezer at 2.5°C until needed. In order to quantify phenolic content of the raisins and to determine whether this changed during storage, 45-g randomized samples of raisins were taken at intervals over the course of the study and frozen at −80°C for later analysis. For extraction of pigments, a modified procedure was used as outlined by Hong and Wrolstad [19] and Karadeniz

et al. [20], and the total phenolic compounds in resulting extracts were analysed by the Folin–Ciocalteu method by Spanos and Wrolstad [21]. Intra-assay variation was 5.6%. Of the three raisin samples analysed, the total phenolic compounds extracted were 755, 828 and 1050 mg/kg gallic acid equivalents.

Statistical Analysis

Student's *t*-tests were used to identify differences between groups for initial subject characteristics and measures. Repeated measures (RM) analysis of variance (ANOVA) was used on fasting and postprandial data. Statistical analysis for all measures except glucose, sICAM-1 and sVCAM-1 was based on log (ln) or square root transformation of the per cent change data: analyses for CRP, IL-6 and insulin used the log response and the square root response for FFA and triacylglycerol. Log and square root transformations were performed in order to accommodate non-normal distributions of the data. sVCAM-1 required no data transformation. Insulin RM ANOVA–fasted comparisons were based on differences computed by subtracting post- from pretreatment values, and the significance was based on the difference from 0. A separate analysis was required for insulin because ANOVA assumptions were not upheld under the standard analysis because of a high number of identical values. For postprandial response of dependent measures, each measurement was expressed as a per cent change of the initial time 0 value (100%), and a three-factor mixed model ANOVA was used to determine the main effects of either treatment (raisin or placebo), time (2, 3 and 4 h postprandial), intervention (pre and post), gender (male or female), order (period) and the interaction among these main factors. All analyses were computed through SAS software (version 9.1.3; SAS Institute, Cary, NC, USA). A mixed model was used in order to circumvent case-wise deletion of subjects with missing datapoints. *Post hoc* analyses were performed using Tukey–Kramer test to identify the source of significant effects. Data were expressed as mean ± s.d. unless otherwise stated. A p value of <0.05 was considered significant.

Results

Compliance

Seventeen subjects, eight males and nine females, successfully completed the investigation. One of the original subjects dropped out because of personal reasons, while two were asked to discontinue participation because of

a self-report of non-compliance to study requirements. According to self-reported information in the exit survey for completing subjects, there was almost full compliance with daily treatment consumption for the remaining 17 subjects: 98.7 and 98.8% of R and P doses respectively. No subjects reported regular consumption of the prohibited foods during the intervention with low-flavonoid diet period. Admitted one-time consumption of prohibited foods ranging from a single hamburger to a glass of iced tea or lemonade were reported by several subjects but was not considered sufficient to affect results. The statistical test for order effect showed that the order of treatment assignment did not influence most dependent measures. Only fasting serum FFA and glucose were lower for the second part of the crossover regardless of treatment.

Baseline characteristics and fasting serum analytes for subjects are provided in table 2. There were significant gender differences for fasting baseline values for insulin and urinary 8-iso PGF$_{2\alpha}$ as well as a trend for difference in CRP (p = 0.060) with females higher than males for all factors. Baseline dietary intake was 2481 $\pm$ 579 kcal/day and 41 $\pm$ 7, 44 $\pm$ 9 and 16 $\pm$ 3 for percentage of energy from fat, carbohydrate and protein, respectively, for pre-experimental period records.

Body weight was maintained within an average standard deviation of 0.7 and 0.6 kg from initial body weight during the two controlled feeding weeks (days 22–28 and 50–56 respectively).

Fasting Blood

In order to more clearly describe the subject population, those with elevated fasting serum glucose ($\geq$100 mg/dl) [22], triacylglycerol ($\geq$150 mg/dl) and CRP (>3.0 mg/l) [2] were quantified (table 3). About 40% of the subjects had elevated CRP prior to each of the four meals. None of the subjects were hyperglycaemic before each meal, and only one was hypertriglyceridaemic at each fasted measurement.

The treatment did not have a significant main effect or interact with any other factor on fasting serum levels of CRP, sICAM-1, triacylglycerol or insulin (table 4). There was no effect of treatment but a significant effect of pre/post on IL-6 (11% lower post), sVCAM-1 (3.7% higher post), glucose (5% lower post) and urinary 8-iso PGF$_{2\alpha}$ (22% lower post). There was a significant main effect of treatment for both fasting serum ORACtotal (8001 and 8171 μmol/l for P and R, respectively) and ORACpca (958 and 992 μmol/l for P and R respectively). A significant interaction between gender and treatment was observed for ORACpca in that this value was higher for R than P in men but the opposite pattern for female subjects. FFA concentration was ~9% higher for all R measures than P. A significant interaction between treatment and pre/post was also observed in that there was a reduction in concentration of FFA after treatment for R but an increase in this metabolite after the P

Table 2 Initial characteristics of the subjects

	Total	Males	Females
n	17	8	9
Age (years)*	26.5 $\pm$ 7.6	30.1 $\pm$ 7.3	23.2 $\pm$ 6.6
Weight (kg)	97.2 $\pm$ 19.8	100.4 $\pm$ 17.5	94.3 $\pm$ 22.2
BMI (kg/m^2)	33.5 $\pm$ 6.7	31.4 $\pm$ 5.1	35.3 $\pm$ 7.5
Waist circumference (cm)	107.4 $\pm$ 12.7	106.8 $\pm$ 11.4	108.0 $\pm$ 14.5
Waist-to-hip ratio	0.91 $\pm$ 0.08	0.95 $\pm$ 0.10	0.87 $\pm$ 0.03
Free fatty acids (μmol/l)	345 $\pm$ 189	331 $\pm$ 154	355 $\pm$ 214
Triacylglycerol (mg/dl)	125.8 $\pm$ 100.1	159.1 $\pm$ 131.9	96.2 $\pm$ 52.2
Glucose (mg/dl)	97.4 $\pm$ 11.8	95.6 $\pm$ 12.7	98.9 $\pm$ 11.5
Insulin (μIU/ml)	13.2 $\pm$ 10.5	12.5 $\pm$ 11.4	18.1 $\pm$ 12.4$\dagger$
CRP (mg/l)	5.5 $\pm$ 5.8	2.8 $\pm$ 4.2	8.3 $\pm$ 6.2
IL-6 (pg/ml)	1.5 $\pm$ 1.1	1.2 $\pm$ 1.2	1.7 $\pm$ 1.1
sICAM-1 (ng/ml)	224.5 $\pm$ 50.7	206.8 $\pm$ 49.6	240.3 $\pm$ 48.9
sVCAM-1 (ng/mL)	695.8 $\pm$ 136.8	717.6 $\pm$ 140.4	676.3 $\pm$ 138.9
ORACtotal (μmol/l TE)	8392 $\pm$ 1273.6	8192 $\pm$ 1118	8570 $\pm$ 1441
ORACpca (μmol/l TE)	1011.8 $\pm$ 163.4	1033 $\pm$ 204	993 $\pm$ 127
Urinary 8-epi PGF$_{2\alpha}$ (pg/mg CR)	4271.9 $\pm$ 1203.7	4101.9 $\pm$ 1172.1	4423.0 $\pm$ 1281.0$\dagger$

8-epi PGF$_{2\alpha}$, 8-epi-prostaglandin-F$_{2\alpha}$; BMI, body mass index; CR, creatinine; CRP, C-reactive protein; IL-6, interleukin-6; ORACpca, protein-free oxygen radical absorbance capacity; ORACtotal, total ORAC; sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, soluble vascular cell adhesion molecule-1; TE, trolox equivalent.

n = 17; Values are mean $\pm$ s.d.

*,$\dagger$Significant difference between genders.

Table 3 Frequency of elevated fasted values for glucose, triacylglycerol and CRP

Per cent of Subjects	Never	One meal	Two meals	Three meals	Four meals
Glucose ≥100 mg/dl	47% (n = 8)	35% (n = 6)	12% (n = 2)	6% (n = 1)	0% (n = 0)
Triacylglycerol ≥150 mg/dl	59% (n = 10)	23% (n = 4)	12% (n = 2)	0% (n = 0)	6% (n = 1)
C reactive protein > 3.0 mg/l (high risk)	47% (n = 8)	6% (n = 1)	6% (n = 1)	0% (n = 0)	41% (n = 7)

All data were based on fasting levels (time 0) prior to consuming the meals.

treatment. Male subjects had higher fasting glucose in R, while women had higher concentration in P.

Postprandial Blood

Postprandial serum CRP, sVCAM-1, sICAM-1 and IL-6 did not significantly change in response to the meal challenge or treatments (table 5). However, there was a trend for reduction in IL-6 when results from all four meals were combined (p = 0.0546) with the greatest decrease at 2 h (−20.9%) and a return to baseline by 4 h. sVCAM-1 fell by 5% at 3 h postmeal challenge for males but had little change for females in response to the meal (figure 2).

Serum FFA significantly decreased in response to the meal but responded differently by treatment in that this metabolite was reduced more following R than P. Gender also influenced FFA response; serum FFA fell initially at 2 h but then increased above baseline for men but were lower than baseline for each time point during the 4-h postprandial period for female subjects (figure 3). Serum triacylglycerol increased following the meals

with no difference by treatment. There was a trend for higher serum triacylglycerol for men compared with women after the meal (p = 0.072).

Serum glucose modestly increased in response to the meals with a greater increase in glycaemia for P than R over the postprandial period. Males had a greater overall increase in serum glucose than females with the difference widening over the postprandial period (figure 4). Serum insulin increased for all meals with the peak at 2 h after the meal and decreased to baseline by 4 h with no difference by treatment or gender.

Discussion

The most unexpected but consistent effect of the intervention was an improvement in several health indicators (lower oxidative stress, glycaemia and a marker of inflammatory status) following the controlled feeding period regardless of treatment. These unintentional benefits may have been secondary to improved nutritional content or chronological pattern of food consumption relative to the subjects' *ad libitum* intake. Examination of the literature

Table 4 Fasting values for blood measures before and after raisin and placebo intervention

	Preraisin	Postraisin	Preplacebo	Postplacebo
CRP (mg/l)	2.18 (1.03–4.66)	2.24 (1.05–4.80)	2.20 (1.03–4.70)	2.15 (1.01–4.58)
IL-6 (pg/ml)*	1.23 (0.78–1.78)	1.08 (0.66–1.60)	1.23 (0.78–1.78)	1.02 (0.61–1.52)
sVCAM-1 (ng/ml)*	657.9 (585.8–730.1)	699.0 (626.8–771.2)	671.9 (599.7–744.1)	678.9 (606.8–751.1)
sICAM-1 (ng/ml)	208.3 (189.1–228.4)	210.0 (190.8–230.2)	209.6 (190.4–229.7)	199.2 (180.5–218.9)
ORACtotal (µmol/L TE)*,†	7163.0 (6479.3–7918.9)	7614.0 (6887.1–8417.4)	8335.7 (7540.0–9215.4)	8403.1 (7601.0–9289.9)
ORACpca (µmol/l TE)*,†	886.0 (822.9–954.1)	932.3 (865.8–1003.9)	980.1 (910.2–1055.3)	1014.8 (942.4–1092.7)
Urinary 8-epi PGF$_{2\alpha}$ (pg/mg CR)*	4164.1 (3538.3–4900.5)	3107.6 (2640.6–3657.2)	4298.2 (3665.3–5040.4)	3485.0 (2971.8–4086.7)
FFA (µmol/l)*,†	371 (309–439)	346 (286–412)	256 (205–314)	338 (278–403)‡
Triacylglycerol (mmol/l)	1.03 (0.75–1.40)	1.05 (0.77–1.42)	1.12 (0.82–1.52)	1.04 (0.76–1.41)
Glucose (mmol/l)*	5.26 (5.07–5.46)	4.93 (4.75–5.12)	5.13 (4.95–5.32)	4.93 (4.75–5.11)
Insulin (µIU/ml)	12.5 ± 10.5	9.4 ± 6.5	10.5 ± 8.5	10.4 ± 9.0

8-epi PGF$_{2\alpha}$, 8-epi-prostaglandin-F$_{2\alpha}$; CR, creatinine; CRP, C-reactive protein; FFA, free fatty acids; IL-6, interleukin-6; ORACpca, protein-free oxygen radical absorbance capacity; ORACtotal, total ORAC; sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, soluble vascular cell adhesion molecule-1.
Values are means with 95% CI for time 0 blood draws prior to meals.
*Significant effect of pre/post when interventions combined.
†Significant difference for R and P.
‡Significant interaction between treatment and pre/post.

Table 5 Postprandial changes in blood measures with raisin and placebo treatments

	Treatment	2 h	3 h	4 h
CRP (mg/l)	P	16.8 (79.51)	66.9 (288.74)	55.9 (272.90)
	R	23.04 (76.67)	72.2 (294.93)	65.7 (257.02)
IL-6 (pg/ml)	P	−15.9 (35.2)	−1.7 (56.6)	36.7 (170.6)
	R	−25.8 (38.8)	−5.6 (65.4)	−14.5 (25.4)
sVCAM-1 (ng/ml)*	P	−1.5 (5.9)	−0.4 (7.15)	−0.04 (7.04)
	R	−1.8 (6.51)	−3.7 (8.01)	−3.2 (7.51)
sICAM-1 (ng/ml)	P	−1.3 (5.4)	−1.3 (8.8)	−1.2 (9.1)
	R	−1.0 (11.0)	−2.3 (14.7)	0.8 (13.5)
FFA (µmol/l)*,†,‡,§	P	−58.4 (23.96)	−32.03 (35.07)	−6.8 (39.40)
	R	−54.2 (43.30)	−12.6 (71.60)	−21.1 (84.15)
Triacylglycerol (mg/dl)*,†,§	P	35.1 (34.9)	66.7 (57.3)	74.5 (72.9)
	R	50.3 (39.7)	77.4 (50.9)	94.9 (52.8)
Glucose (mg/dl)*,†,§	P	2.5 (15.6)	3.0 (10.10)	6.0 (12.41)
	R	4.7 (15.34)	7.8 (13.39)	10.9 (12.91)
Insulin (µIU/ml)*	P	289.9 (217.5)	173.8 (162.0)	80.0 (91.7)
	R	310.8 (209.8)	193.2 (146.1)	106.2 (99.3)

CRP, C-reactive protein; FFA, free fatty acids; IL-6, interleukin-6; P, placebo treatment. R, raisin treatment; sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, soluble vascular cell adhesion molecule-1.
Values are percentage change from time 0 with standard deviation in parenthesis.
*Significant interaction of gender and time.
†Significant effect of time.
‡Significant effect of treatment.
§Significant effect of gender.

shows that others have observed a beneficial effect of a controlled feeding period on oxidative stress markers [23,24] as well as blood lipids [25]. Two weeks of a reduced fruit and vegetable run-in diet was paradoxically associated with a 33% reduction in urinary 8-epi PGF$_{2\alpha}$ [24], while a 3-week controlled feeding period in another study caused an increase in blood ORAC and glutathione from baseline regardless of whether the test food diet was consumed [23]. These benefits may be secondary to consistent spacing of food consumption.
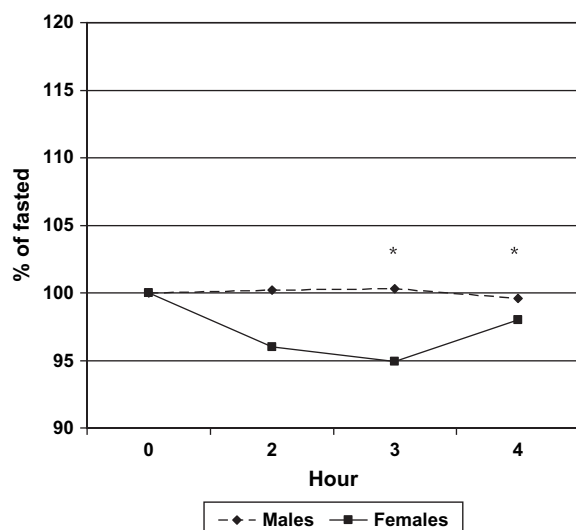

Fig. 2 Interaction of gender with time for postprandial sVCAM-1. *Significant differences between genders at time point.
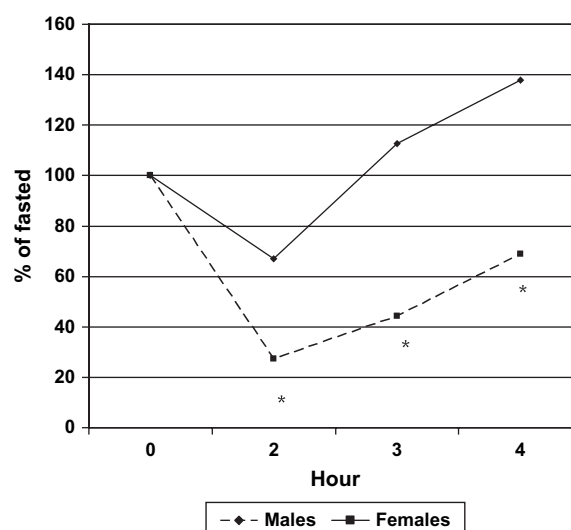

Fig. 3 Interaction of gender with time for postprandial serum FFA. *Significant differences between genders at time point.
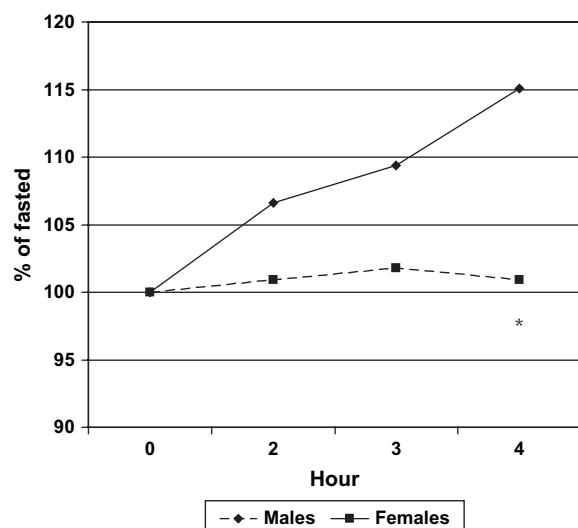
Fig. 4 Interaction of gender with time for postprandial serum glucose. *Significant difference between genders.

Farshchi *et al.* [25] noted that consumption of food in a 'regular' pattern by obese women was associated with lower LDL cholesterol and postprandial insulin compared with a 'variable' pattern with three to nine meals each day. Thus, a dietary pattern that included less binging and eating out and more regularly spaced meals may be responsible for the improvement in some health markers in our study. This is an important consideration for experimental design in studies evaluating the effect of dietary components on biomarkers. A placebo/control arm is critical to confirm that any changes in dependent measures are a consequence of the treatment rather than the controlled background diet. For example, Cao *et al.* [9] concluded that both their treatments, 10 servings per day of fruits and vegetables or that diet plus two servings per day of broccoli, were equally effective in improving antioxidant capacity because plasma ORAC increased for both diets compared with baseline. However, because there was no control group, it is not possible to exclude the possibility that the residential, controlled feeding alone rather than either treatment altered the ORAC.

The raisin treatment in this study had only one effect on the measured oxidative or inflammatory factors: a higher fasted ORAC was observed for subjects during the raisin intervention period. ORAC is a measure of the collective ability of the various water-soluble antioxidant compounds in serum, particularly water-soluble antioxidants, such as vitamin C and uric acid, to reduce oxidants that may damage susceptible molecules. It can be influenced by vitamins such as vitamin C but also

chemicals such as uric acid and flavonoids. The increased ORAC observed in the raisin consumption period suggests that antioxidant constituents in raisins, such as phenolic compounds and flavonoids, might enhance magnitude of antioxidant defence in blood either through direct radical scavenging activity or through modulation of other antioxidant mechanisms. Further, Lotito and Frei [26] suggested that enhancement in total antioxidant capacity in blood after consumption of fruits or related products might be attributed to an increase in uric acid metabolically generated from fructose.

Other investigations have reported an increase in ORAC with daily ingestion of grapes or grape products. O'Byrne *et al.* [10] observed increased fasted ORAC after 14 days of daily Concord grape juice ingestion. However, as the same increase was noted for the other treatment and there was no control group, the increase in ORAC for both groups in that study cannot clearly be ascribed to the grape juice rather than being secondary to the implemented controlled feeding. Another study reported that daily consumption of grape juice for 14 days in haemodialysis patients increased another measure of total plasma antioxidant capacity (Trolox equivalent antioxidant capacity, TEAC) concurrently with a reduction in oxidized LDL compared with a control group [27]. Thus, these studies as well as our own provide limited, albeit inconclusive, evidence that consumption of grapes or grape-derived products might increase the antioxidant capacity of the blood.

The measure of oxidative stress we included, urinary 8-epi $PGF_{2\alpha}$, was not altered by the raisin consumption. This lack of consistency between changes in markers of antioxidant capacity and markers of oxidative stress has been noted by others [28]. Consistent with our results, Devaraj *et al.* [29] observed that supplementation with a pine bark extract rich in polyphenols increased ORAC within 3 weeks but did not alter plasma lipid peroxides or LDL susceptibility to oxidation in the normal weight, healthy subjects. More relevant to our study was the similar response observed in a population known to have high oxidative stress, smokers. Van den Berg *et al.* [30] reported that daily consumption of a vegetable/fruit combination treatment for 3 weeks increased serum vitamin C, β carotene, and TEAC in male smokers but had no effect on lipid, protein or DNA biomarkers of oxidative stress. Thus, clearly, there is a complex relationship between blood total antioxidant capacity and markers of oxidative stress. Future studies should include a variety of oxidative stress markers to fully explore the potential beneficial effects of consumption of raisins or other plant foods on oxidative status.

Although it is tempting to assume that the effects of raisin ingestion should mimic those of grape or grape juice ingestion, the drying process alters the phytonutrient profile of the fruit. Several laboratories have compared the flavonol content of grapes with raisins. Karadeniz *et al.* [20] demonstrated that the grape drying process reduced two major phenolic compounds, caftaric and coutaric acid, by ~90%. Flavonols were less influenced by processing such that quercetin glycosides, rutin and kaempferol glycosides were reduced ~67% compared with fresh grapes. It is also important to clarify that content of antioxidant compounds in the fruit does not necessarily translate to an increase in *in vivo* antioxidant capacity. Bioaccessibility issues as well as metabolic conversions to less powerful antioxidants limit the ability to predict the ability of specific foods to boost *in vivo* antioxidant capacity [31]. Polyphenolic compounds in raisins likely have lower bioavailability relative to grapes, secondary to enzymatic degradation, oxidation reactions and polymerization of polyphenolic compounds that occurs in the drying process [20] and thus may be less likely to alter oxidative state [32].

The higher ORAC during the raisin period did not translate to favourable changes in fasted or postprandial inflammatory markers. This suggests a lack of connection between biomarkers of total plasma antioxidant capacity and inflammatory status in this cohort or that the magnitude of increase in ORAC from raisin consumption was inadequate to alter inflammation. It has been hypothesized that overconsumption of energy in obesity increases production of reactive oxygen species that subsequently increases production of various inflammatory factors [3]. However, some studies report a lack of association between changes in oxidative stress and inflammation consequent to dietary modification. For example, daily grape juice consumption for 14 days increased TEAC in haemodialysis patients but did not change sICAM-1, sVCAM-1 or CRP [27]. Vegetable soup consumption each of 14 days reduced an oxidative stress marker but not any of the inflammatory indicators measured [33]. It is important to note that neither of these studies used obese subjects. An alternative explanation for lack of change in inflammatory markers in our study could be the apparent good health of the subjects. Although all subjects in our study were overweight or obese, few had evidence of insulin insensitivity. Several studies demonstrate a more robust inflammatory and lipaemic response to a meal in subjects with insulin resistance or diabetes compared with non-insulin-resistant individuals [14,34,35].

The postprandial response to the meal was influenced by gender in our study in that men had higher and a more prolonged response to the meal for postprandial FFA, sVCAM-1 and glucose (and a trend for triacylglycerol) concentrations than females. Some other investigators have observed a higher lipaemic [36] and glycaemic [37] response to a meal in men than women, while others see no difference [38] in these responses by gender. A higher postprandial glycaemia and reduced drop in FFA is consistent with worse insulin sensitivity in male subjects in our study.

For exploratory purposes, we examined the postprandial response of the one individual who was at highest risk of CVD based on the fasted metabolic measures. This male subject with a BMI of 35 (eighth rank for subject BMI but had the highest waist-to-hip ratio) was the only subject with elevated fasting values for serum CRP, glucose, triacylglycerol and insulin (as defined previously and in table 3) at both baseline measures prior to each intervention. Interestingly, his serum CRP increased 29 and 15% after the two meals that included placebo ingestion (pre- and postplacebo period) but either did not change or fell for both the meal challenges that included raisins. Although obviously very limited, this differential response when raisin was part of the meal is provocative and suggests that future research should be performed using dietary interventions in subjects with higher initial risk because of insulin insensitivity or inflammation.

It is possible that the quantity of raisins provided in our study was inadequate to sufficiently alter oxidative stress and inflammation. The dose we chose was based on provision of two additional servings of fruit per day. Our analysis demonstrated that the dose of raisins we used resulted in consumption of 68–94.5 mg/day of phenolics, close to the lowest end of the range for average total daily intake of polyphenols estimated in postmenopausal women (95.8–603.3 mg/day) [39]. Although this dose is within the average intake of polyphenols, it should be noted that this dose is substantially lower than studies using fresh grapes, grape extract or grape powder when total phenolics provided by the treatment ranged from 208 to 600 mg/day [10,40]. However, the practicality of ingestion of grape juice in the dose used by some studies [10] could be questioned (e.g. 10 ml/kg/day would translate to 1.225 l for one of our subjects).

We do not believe that the lack of effect on oxidative or inflammatory markers was the result of inadequate duration of the intervention. Others have observed changes in antioxidant capacity of plasma or oxidative stress markers after consumption of grape juice for 14 days [10,27]. Fifteen days of multiple antioxidant consumption reduced the fasting as well as postprandial increase in oxidatively damaged biomarkers as well as sVCAM-1 in normal, diabetic and glucose-intolerant individuals [34].

Summary/Conclusions

In summary, fasted serum total antioxidant capacity was modestly higher over the raisin intervention but this did not alter oxidative stress or inflammatory markers or affect postprandial response to a high-fat meal. The controlled feeding improved a number of biomarkers of oxidative stress and inflammation regardless of treatment, demonstrating the importance of inclusion of a placebo and/or control group in studies evaluating dietary manipulations.

Acknowledgements

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