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ScienceDirect
Metabolism Clinical and Experimental 58 (2009) 120 – 128
Metabolism
Clinical and Experimental
www.metabolismjournal.com

Raisins and walking alter appetite hormones and plasma lipids by modifications in lipoprotein metabolism and up-regulation of the low-density lipoprotein receptor

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Received 7 June 2008; accepted 13 August 2008

Abstract

The purpose of this study was to determine the effects of consuming raisins, increasing steps walked, or a combination of these interventions on lipoprotein metabolism and appetite hormones by assessing plasma apolipoprotein concentrations, cholesterol ester transfer protein activity, low-density lipoprotein (LDL) receptor messenger RNA (mRNA) abundance, and plasma ghrelin and leptin concentrations. Thirty-four subjects (17 men and 17 postmenopausal women) were matched for weight and sex and randomly assigned to consume 1 cup raisins per day (RAISIN), increase the amount of steps walked per day (WALK), or a combination of both interventions (RAISIN + WALK). The subjects completed a 2-week run-in period, followed by a 6-week intervention. Ribonucleic acid was extracted from mononuclear cells, and LDL receptor mRNA abundance was quantified by use of reverse transcriptase polymerase chain reaction. Plasma apolipoproteins were measured by Luminex (Austin, TX) technology. Apoproteins A-1, B, C-II, and E and cholesterol ester transfer protein activity were not altered for any of the groups. In contrast, apolipoprotein C-III was significantly decreased by 12.3% only in the WALK group ($P < .05$). Low-density lipoprotein receptor mRNA abundance was increased for all groups after the intervention ($P < .001$). There was a significant group effect for plasma leptin ($P = .026$). Plasma concentrations increased for RAISIN and RAISIN + WALK. Similarly, plasma ghrelin concentrations were elevated postintervention for both groups consuming raisins ($P < .05$). These data suggest that walking and raisin consumption decrease plasma LDL cholesterol by up-regulating the LDL receptor and that raisin consumption may reduce hunger and affect dietary intake by altering hormones influencing satiety.

1. Introduction

Cardiovascular disease (CVD) is the number 1 killer in the United States [1]. Lifestyle modifications that decrease risk for CVD include increasing fruit intake [2] and increasing physical activity. In addition, the prevalence of obesity, a risk factor for developing CVD, in the United States continues to rise despite the efforts of health professionals to combat this problem. Investigation of how diet and exercise alter satiety hormones to affect appetite may help researchers to determine appropriate lifestyle modifications to promote weight loss and lower CVD risk. Adding fruit to the diet increases intake of dietary fiber and polyphenols. Viscous fiber has the potential to reduce plasma low-density lipoprotein cholesterol (LDL-C) via up-regulation of expression of the LDL receptor [3]. In the guinea pig, the depletion of hepatic cholesterol to replace bile acid losses causes an increase in expression of the hepatic LDL receptor to take up LDL-C, reducing plasma concentrations [4]. Dietary fiber may also affect satiety and reduce energy intake through the addition of bulk and viscosity to foods, promoting gastric distention [5] and slowing the rate of nutrient absorption, which prolongs the time for nutrients to stimulate factors that affect satiety [6].

Polyphenols provided by raisins may interfere with cholesterol absorption [7], decreasing hepatic cholesterol

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0026-0495/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2008.08.014

concentrations, as reported with guinea pigs after supplementation with lyophilized grape powder [8]. As a result of lower hepatic cholesterol concentrations, hepatic LDL receptor expression increases to enhance cholesterol uptake from LDL particles, thus lowering plasma LDL-C. Aerobic exercise has been reported to increase LDL receptor expression in mice [9] and in humans [10].

Leptin is secreted by adipocytes in proportion to adipose tissue mass [11]. Although adipose tissue mass is a major driver of leptin secretion, glucose metabolism is also important for influencing leptin secretion [12]. Glucose uptake stimulates leptin secretion in rat adipocytes [12], whereas reduction in serum leptin was correlated with grams of carbohydrate, but not dietary fat, protein, or energy in a short-term dietary restriction in humans [13].

Ghrelin, an orexigenic hormone secreted primarily by the stomach and the proximal small intestine [14], is a signal of increased need for energy [14,15]. If a diet or exercise intervention results in weight loss, plasma ghrelin is usually increased [16]. Exercise does not normally alter plasma ghrelin when it is acute or when weight is stable [17-19]. The macronutrient composition of a diet may alter plasma ghrelin concentrations, as a high-carbohydrate diet was found to induce weight loss without elevating circulating ghrelin [16].

Glucagon-like peptide 1 (GLP-1) is a hormone that is secreted by the gastrointestinal tract in response to glucose and fat, and also soluble and fermentable fibers, as reported in animal [20,21] and human studies [22,23]. Intravenous administration of GLP-1 in healthy male subjects significantly decreased hunger and food intake [24], potentially via slowing of gastric emptying [25,26]. Peptide YY (PYY) is produced in the ileum and colon, as well as in the pancreatic islet cells [27-29]. Peptide YY increases synthesis of proopiomelanocortin-derived peptides and inhibits neuropeptide Y in the hypothalamus to reduce appetite [30,31]. Peptide YY inhibits gastric emptying, which may also contribute to its role in inducing satiety [29,32].

The purpose of this study was to determine the effects of consuming raisins, increasing steps walked, or a combination of these interventions on lipoprotein metabolism by assessing plasma apolipoprotein concentrations, cholesterol ester transfer protein (CETP) activity, and LDL receptor expression in mononuclear cells. In addition, the effects of these interventions on hormones related to satiety were assessed by measuring plasma leptin, ghrelin, PYY, and GLP-1.

2. Methods

2.1. Materials

Enzymatic cholesterol and triglyceride (TG) kits were purchased from Roche Diagnostics (Indianapolis, IN); aprotinin, sodium azide, and phenylmethylsulfonyl fluoride were purchased from Sigma (St Louis, MO); raisins were provided by the California Raisin Marketing Board (Fresno, CA); and the pedometers were purchased at Omron (Vernon Hills, IL). The CETP kit was purchased from BioVision (Mountain View, CA), the TRIzol reagent was from Invitrogen (Carlsbad, CA), and the DNA-Free kit was from Ambion (Austin, TX). The kits for plasma apolipoproteins, leptin, ghrelin, PPY, and GLP-1 were purchased from LINCO (St Charles, MO).

2.2. Subjects

Men and postmenopausal women between the ages of 50 and 70 years were recruited from the university community. Subjects provided informed consent and completed a medical history during recruitment. Exclusion criteria included taking blood-thinning medications, cigarette smoking, diabetes, CVD, or renal disease. Subjects with a body mass index (BMI) greater than 37 kg/m^2 were also excluded from the study. A total of 17 men and 17 postmenopausal women volunteered to participate in the study. Study protocols were approved by the Institutional Review Board.

2.3. Study design and randomization

The subjects in the study were matched according to sex and body mass, then randomly assigned to 1 of 3 groups: (1) a group that consumed 1 cup raisins per day (RAISIN, n = 12), (2) a group that increased the amount of steps taken each day (WALK, n = 12), or (3) a group that consumed 1 cup raisins per day and increased the amount of steps taken (RAISIN + WALK, n = 10). The subjects completed a 2-week washout period, followed by a 6-week intervention. There were no differences in BMI or waist circumference among groups. Body mass index ranged from 21 to 31.5 kg/m^2, and waist circumference was from 80 to 103 cm. Mean values for BMI were 24.9 ± 2.3, 27.9 ± 3.9, and 27.5 ± 3.8 kg/m^2 for subjects in the RAISIN, WALK, and RAISIN + WALK, respectively.

2.4. Diet and exercise description

All subjects completed a 2-week run-in period to standardize exercise and dietary habits. Subjects were asked to maintain their normal level of activity and abstain from polyphenol-rich foods, including grapes, berries, wine, chocolate, raisins, tea, vitamins, and any other supplements. These restrictions were continued during the 6-week intervention to isolate the antioxidant effects of the raisins. Diet was verified by dietary records, and restrictions were reinforced by a Registered Dietitian during continuous counseling. The subjects who were asked to increase the amount of steps walked per day during the intervention were issued an Omron pedometer. The subjects maintained their normal daily activity during the 2-week period to obtain an estimate of the amount of steps per day. The subjects in RAISIN were asked to maintain their normal level of activity during the 6-week intervention.

The raisins consumed by the subjects in this study were provided by the researchers weekly with a checklist to

determine compliance. The raisins were California Thompson raisins, which provided an additional 10 g of dietary fiber and approximately 3 g of soluble fiber each day. The macronutrient composition of the raisins is as follows: 96% carbohydrate, 3.5% protein, and 0.5% fat. Although some polyphenols are lost in the drying process and concentrations of some of these compounds are small, these raisins provide various polyphenols. These include quercetin and kaempferol glycosides, caftaric and coutaric acid [33], chlorogenic acid, caffeic acid, quinic acid, gallic acid, catechin, and epicatechin. The total amount of polyphenols present in raisins is approximately 850 mg per cup [34]. A Registered Dietitian provided dietary instruction for substituting the raisins for other foods to ensure weight maintenance and provided written materials to reinforce the information. The dietitian also counseled WALK subjects to ensure weight maintenance during the intervention. The subjects were encouraged to consume the raisins with other foods and space their intake throughout the day to make consumption easier. Subjects were also instructed on how to accurately complete a food frequency questionnaire and a detailed food record.

The WALK participants were instructed to increase their steps by walking an additional 10 min/d (above their normal activity) every 2 weeks in an attempt to increase their walking by approximately 1 km/d every 2 weeks. Therefore, the subjects were walking an additional 10 min/d for the first 2 weeks, 20 min/d for the second 2 weeks, and 30 min/d for the last 2 weeks (or the equivalent of an additional 3 km/d). The subjects logged their steps daily and noted any difficulties with the pedometer or unusual physical activity.

2.5. Data collection

Subjects reported to the laboratory after an overnight fast (~12 hours) at the beginning of the 2-week washout period (prebaseline). A phlebotomist collected 10 mL of blood from the antecubital vein into a tube containing EDTA to assess lipids for screening purposes.

The subjects returned to the laboratory twice: at the start of the intervention (baseline) and at the end of the intervention (week 6). Blood was drawn as before: 60 mL in the first visit and 10 mL in the second visit (just for assessment of lipids). Plasma was separated by centrifugation at 2200*g* for 20 minutes at 4°C; and sodium azide (1 μL/mL), phenylmethylsulfonyl fluoride (1 μL/mL), and aprotinin (5 μL/mL) were added to the samples for preservation. Approximately 1 mL of plasma was aliquoted at both time points to assess plasma lipids. The remainder of the plasma was aliquoted into microcentrifuge tubes and stored at −80°C for later analysis.

Subjects completed a 5-day diet record, including 3 weekdays and 2 weekend days, during the study to assess normal dietary consumption. Subjects in the RAISIN and RAISIN + WALK groups received sheets to record raisin consumption, and subjects in the WALK and RAISIN + WALK groups received forms to record daily step totals. These forms were distributed and reviewed for each subject weekly.

2.6. Plasma CETP activity

Plasma CETP activity was determined with a CETP activity assay kit following the manufacturer's instruction. The reaction mixture contained 3 μL of plasma sample, a fluorescent self-quenched neutral lipid as the donor molecule, and an acceptor molecule. All samples were completed in duplicate. The CETP activity was assessed by determining the amount of fluorescent neutral lipid transferred to the acceptor molecule. This was measured as an increase in fluorescence, which was read in a fluorescence plate reader at excitation 456 nm and emission 535 nm. The CETP activity was expressed as millimoles of neutral lipid transferred per liter of plasma per hour.

2.7. Mononuclear cell isolation and RNA extraction and purification

Mononuclear cells were isolated from whole blood according to the method developed by Boyum [35] as previously described. Total RNA was extracted from mononuclear cells according to a method based on that developed by Chomczynski and Sacchi [36]. The TRIzol reagent was used according to the manufacturer's instructions, using isopropyl alcohol for RNA precipitation. The DNA-Free kit allowed for removal of trace contaminating genomic DNA. Ribonucleic acid was extracted by precipitation using 100% ethanol and 3 mol/L sodium acetate (pH 5.2). The RNA pellet was washed with 70% ethanol and dissolved in diethyl pyrocarbonate–treated water.

2.8. Complementary DNA synthesis and real-time polymerase chain reaction

Complementary DNA was synthesized using iScript complementary DNA synthesis kit following the manufacturer's instructions (Bio-Rad Laboratories, Hercules, CA). A 20-μL reaction volume was prepared with 1 μg purified RNA, 5 μL 5× iScript reaction mix, 1 μL iScript reverse transcriptase, and nuclease-free water for the remainder of the volume. This mixture was incubated for 5 minutes at 25°C, 30 minutes at 42°C, and 5 minutes at 85°C. Primers for the LDL receptor and the reference gene glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) [37] were designed using LightCycler Probe Design software 2.0 (Roche Diagnostics). The forward and reverse primer sequences for LDL receptor were AAGAACATCAACAGCATCAACT and TGGCAAATGTGGACCTCA, respectively. Similarly, for *GAPDH*, the sequences were ATCCATGACAACTTTGGTATCG and TCTTCTGGGTGGCAGTG, respectively. The estimated product size of each reaction product was 74 base pairs for LDL receptor and 73 base pairs for *GAPDH*.

Real-time polymerase chain reaction (PCR) was performed in duplicate using the LightCycler FastStart DNA

Master[plus] SYBR Green I (Roche Diagnostics) following the manufacturer's instruction. The reactions were performed in LightCycler 2.0 instrument under the following reaction condition: polymerase activation at 95°C for 5 minutes followed by 45 cycles of denaturing at 95°C for 10 seconds, annealing at 54°C for 10 seconds, and extension at 72°C for 10 seconds. After amplification, a melting curve was obtained to determine the optimal PCR conditions. Quantification was done by analyzing the fluorescence curves and detecting the crossing point of samples using LightCycler Software 4.0 (Roche Diagnostics).

2.9. Plasma apolipoproteins

Plasma apolipoproteins, including apolipoprotein (apo) A-1, B, C-II, C-III, and E, were measured using ×MAP technology on a Luminex (Austin, TX) IS 200 system with antibodies to these analytes [38]. Assays were completed according to manufacturer's instructions.

2.10. Plasma leptin, ghrelin, PYY, and GLP-1

Plasma leptin, ghrelin, PYY, and GLP-1 were measured using xMAP technology on a Luminex IS 200 system with antibodies to these analytes [38]. Assays were completed according to manufacturer's instructions.

2.11. Statistical analyses

All statistical analyses were performed with SPSS 12.0 for Windows (SPSS, Chicago, IL). Repeated-measures analysis of variance was used to determine changes in variables over time. If a significant main effect was found, Tukey post hoc analyses were completed. Significance was set at P less than or equal to .05.

3. Results

3.1. Weight and steps walked and diet

We have previously reported that subjects did not have a change in weight from baseline to postintervention [39]. The number of steps walked were significantly increased from 6084 to 11 198 steps per day (addition of ~3.26 km) for WALK and from 9482 to 12 547 steps per day for RAISIN + WALK (addition of ~2.04 km) [39].

Although the macronutrient energy distribution differed among groups, total energy intake was not different. Total kilocalories were 2061 ± 167, 2178 ± 179, and 2050 ± 719 for the RAISIN, WALK, and RAISIN + WALK groups, respectively. Macronutrient and fiber intakes for the 3 groups are shown in Fig. 1. Dietary records indicated

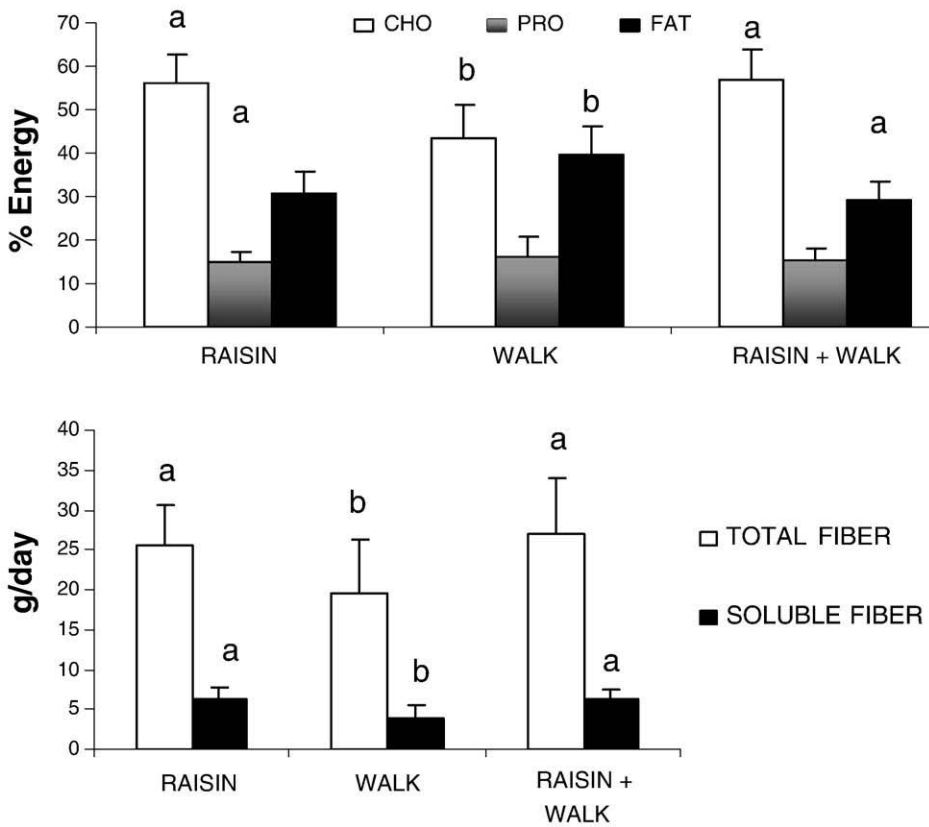

Fig. 1. Macronutrient intake as a percentage of total kilocalories (A), total dietary fiber (B), and soluble fiber (C) for RAISIN, WALK, and RAISIN + WALK. Values represent mean ± SD for the number of subjects indicated in parentheses. Values for each macronutrient with different superscripts are significantly different as determined by 1-way analysis of variance and the least significance test.

that subjects given raisins consumed a greater percentage of their kilocalories as carbohydrate as well as more total dietary fiber and soluble fiber, resulting from raisin intake, whereas WALK subjects consumed a larger percentage of their kilocalories as fat. Protein intake did not differ among groups.

3.2. Plasma lipids and CETP activity

As previously reported [39], LDL-C was significantly reduced for all subjects by 13.7% from baseline (beginning of the study). Plasma TGs were only decreased in subjects from the WALK group, with a 19.5% reduction for this group [39]. Plasma CETP activity was not affected for any of the groups as a result of the intervention. Values were 9.50 mmol/L of plasma per hour at baseline and 9.87 mmol/L of plasma per hour postintervention for all 3 groups.

3.3. Plasma apolipoproteins

Plasma apo A-1, B, C-II, and E were not altered by treatment for any of the groups. Apolipoprotein C-III was significantly decreased by 12.3% for WALK from pre- to postintervention ($P < .05$), but was unchanged for RAISIN and RAISIN + WALK (Fig. 2).

3.4. LDL receptor messenger RNA abundance

As indicated in Fig. 3, LDL receptor messenger RNA (mRNA) abundance calculated using reverse transcriptase PCR was significantly increased for all subjects as a result of the intervention ($P < .001$). The Cp values were decreased for RAISIN, WALK, and RAISIN + WALK to a similar extent as a result of the intervention.

3.5. Plasma leptin, ghrelin, PYY, and GLP-1

There were a time effect and a group effect for plasma leptin. The time effect ($P < .005$) was due to the decrease in leptin for both groups consuming raisins, which also explains the group effect ($P < .05$). Leptin concentrations increased 37% for RAISIN and 42% for RAISIN + WALK as a result of the intervention (Table 1). A significant time effect was present for plasma ghrelin, as plasma concentrations were significantly elevated at postintervention for RAISIN and RAISIN + WALK ($P < .05$) (Table 1). The interventions did not significantly affect PYY or GLP-1.

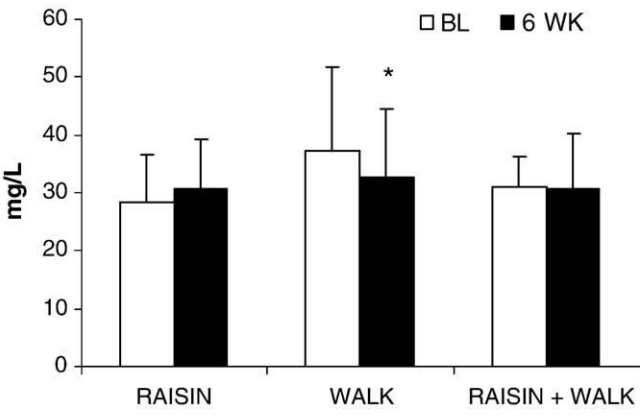

Fig. 2. Plasma apo C-III concentrations in RAISIN, WALK, and RAISIN + WALK. ∗Significantly different from baseline.

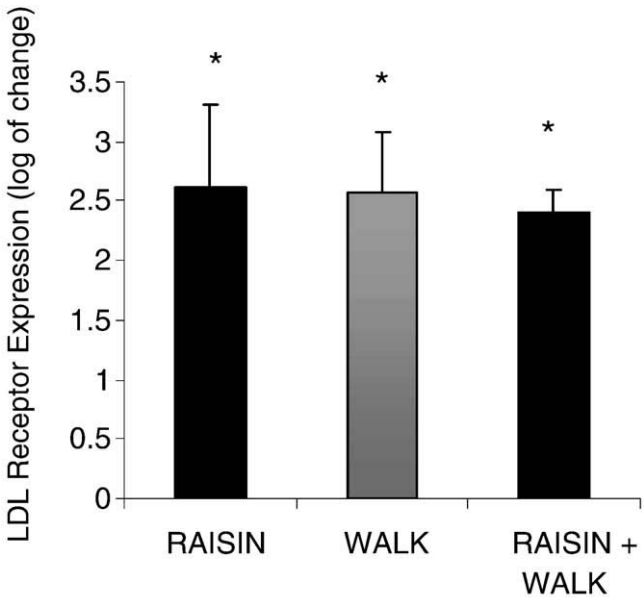

Fig. 3. Low-density lipoprotein receptor mRNA abundance ratio increase in RAISIN, WALK, and RAISIN + WALK. The initial values were arbitrarily set to 1, and the post values are log increases in expression. ∗Significantly different from baseline.

4. Discussion

Raisin intake and walking significantly affected lipoprotein metabolism, whereas the consumption of raisins affected plasma concentrations of hormones related to satiety to

Table 1
Plasma leptin, ghrelin, PYY, and GLP-1 of RAISIN, WALK, and RAISIN + WALK

Variable	Leptin (pg/mL)	Ghrelin (pg/mL)	PYY (pg/mL)	GLP-1 (pg/mL)
RAISIN (n = 12)				
Baseline	6045 ± 4124	28.9 ± 20.9	114.7 ± 50.6	69.2 ± 26.2
6 wk	8330 ± 6630	35.8 ± 24.6	114.9 ± 51.9	72.2 ± 33.6
WALK (n = 12)				
Baseline	11 950 ± 12 299	25.16 ± 7.7	147.7 ± 118.3	63.2 ± 30.4
6 wk	11 845 ± 10 536	28.3 ± 15.1	142.1 ± 79.1	58.7 ± 47.8
RAISIN + WALK (n = 10)				
Baseline	16 606 ± 16 587	25.68 ± 11.2	111.0 ± 43.1	53.2 ± 31.6
6 wk	23 578 ± 21 958	33.7 ± 19.2	103.6 ± 51.4	51.8 ± 21.7
Time effect	$P < .005$	$P < .05$	NS	NS
Group effect	$P < .05$	NS	NS	NS
Interaction	NS	NS	NS	NS

Values represent mean ± SD for the number of subjects indicated in parentheses. NS indicates not significant.

potentially improve risk for CVD. Mononuclear cells were used to estimate gene expression of the LDL receptor in the liver; LDL receptor gene expression in human mononuclear cells were previously shown to mimic gene expression in the liver [40,41]. The findings that the LDL receptor was up-regulated for all the subjects in the study makes sense, given the decrease in plasma LDL-C that occurred [39]. The increase in LDL receptor expression may be in response to a decrease in hepatic cholesterol concentrations for RAISIN and RAISIN + WALK that resulted from an increase in dietary fiber. Roughly 30% of the total fiber is soluble fiber [42]. The additional soluble fiber in the raisins allowed for an overall increase in soluble fiber intake for the raisin-consuming subjects. As demonstrated in animal models, soluble fiber increases bile acid excretion [43], necessitating the utilization of hepatic cholesterol to replace bile acid losses. The lower hepatic cholesterol concentrations trigger an increase in expression of the hepatic LDL receptor, reducing plasma LDL-C [4].

A mechanism through which exercise training for WALK and RAISIN + WALK may have up-regulated the LDL receptor is via an increase in interleukin (IL)-6. Interleukin-6 increases to a large degree with exercise; even exercise that is mild in nature elevates plasma IL-6 20-fold acutely in humans [44]. Gierens et al [45] clearly found greater transcription of the LDL receptor and enhanced LDL receptor activity when hepatocytes were treated with IL-6. Repeated bouts of exercise may provide adequate stimulus to promote an increase in LDL receptor expression and subsequent lowering of plasma LDL-C.

The reduction in apo C-III for WALK most likely contributed to the decrease in plasma TGs for this group [39]. Apolipoprotein C-III is an inhibitor of lipoprotein lipase [46], the primary enzyme for removal of TG fatty acids from the circulation [47]; and overexpression of apo C-III results in hypertriglyceridemia in mice [48] and humans [49]. An increase in peroxisome proliferator–activated receptor (PPAR) α may have contributed to the lowering of apo C-III for WALK; previous research has been published displaying the up-regulation of PPARα expression in humans [50] and PPARα DNA binding activity in rats [51] as a result of aerobic exercise training. Peroxisome proliferator–activated receptor α not only increases transcription of genes involved in fatty acid catabolism, including carnitine palmitoyl transferase–1 and 3-hydroxyacyl coenzyme A dehydrogenase [51], but also inhibits transcription of apo C-III [52]. This inhibition of apo C-III allows for greater lipoprotein lipase expression and clearance of plasma TGs [53]. Previous research assessing the effects of aerobic training on apo C-III is limited. However, Gill et al [54] has reported a reduction in apo C-III postprandially after acute walking exercise in humans, which contributed to more efficient removal of large very low-density lipoprotein particles from the plasma.

Apolipoprotein C-III was unchanged for RAISIN + WALK during the intervention despite an increase in steps walked. This lack of change may have been the result of the subjects' activity levels at baseline. The subjects in WALK were more sedentary at baseline (less steps walked per day) than the subjects in RAISIN + WALK [39], making a change in PPARα and apo C-III more likely for WALK. In addition, the subjects in WALK increased their steps walked by a greater amount than those in RAISIN + WALK [39], providing greater stimulus for an increase in PPARα.

The increase in leptin is most likely a result of a greater intake of carbohydrate for RAISIN and RAISIN + WALK. Previous research has shown a correlation between serum leptin and carbohydrate intake, but not dietary fat, protein, or energy in a short-term dietary restriction in humans [13]. Glucose uptake by adipocytes is an important factor for stimulation of leptin secretion [55], and inhibition of glucose transport in adipocytes has been shown to reduce leptin production [12]. A larger amount of carbohydrate consumed by RAISIN and RAISIN + WALK may have significantly elevated glucose uptake by adipocytes, stimulating leptin production and secretion via increased activity of the hexosamine biosynthetic pathway [56]. A portion of the glucose that enters the adipocytes and is phosphorylated to form glucose-6-phosphate is converted to fructose-6-phosphate for entry into the tricarboxylic acid pathway [57]. However, some of this fructose-6-phosphate is converted to glucosamine-6-phosphate by glucosamine:fructose 6-phosphate amidotransferase, the initial and rate-limiting enzyme in the hexosamine pathway [57].

The hexosamine pathway has been shown to regulate serum leptin in rats [58]. Greater biosynthesis of uridine diphosphate *N*-acetylglucosamine, an end product in the hexosamine pathway, stimulated by treatment of adipocytes with glucosamine, increased *Lep* gene expression as well as leptin release [59]. A potential mechanism for leptin production with increased hexosamine biosynthesis involves *O*-linked glycosylation of transcription factors by the end product uridine diphosphate *N*-acetylglucosamine [11]. Glycosylation of the transcription factor Sp1 affects its transcriptional activity [11]. Sp1 binds in the promoter region for the *Lep* gene [60]; therefore, increased hexosamine biosynthesis may promote leptin production by altering Sp1 transcriptional activity.

The increase in plasma leptin for RAISIN and RAISIN + WALK may suppress appetite, as leptin inhibits synthesis of the orexigenic neuropeptides agouti gene-related protein and neuropeptide Y in the hypothalamus and increases expression of the anorexigenic neuropeptides propiomelanocortin and cocaine- and amphetamine-regulated transcript, reducing appetite [29,61,62]. An increase in circulating leptin is not always viewed as a positive outcome because leptin resistance can occur with elevated circulating concentrations, preventing suppression of appetite [63]. However, leptin resistance is prevalent in obese individuals; it is unlikely that resistance to leptin's effects occurred in this study because the subjects were at healthy weights.

The increase in plasma ghrelin for RAISIN and RAISIN + WALK is paradoxical given the increase in satiety reported previously with greater fiber intake [5,6]. However, previous research involving intake of soluble fiber in the form of arabinoxylan [64] and insoluble wheat fiber [65] have increased postprandial circulating ghrelin in humans. However, the increase in ghrelin resulting from wheat fiber consumption was not associated with satiety [65]. The rise in plasma ghrelin for RAISIN and RAISIN + WALK were not expected given the increase in plasma leptin, as an inverse relationship between these variables usually exists [66]. However, it is possible that the elevation in circulating leptin decreased appetite for the subjects who consumed raisins, causing a reduction in food intake. This lowered food intake would then increase plasma ghrelin. This mechanism has been displayed in animal studies involving administration of leptin gene therapy [67,68]. Dube et al [67] reported a reduction in energy intake and an increase in serum ghrelin in rats with leptin gene therapy. Similar to the subjects in our study, weight was unchanged for the rats receiving leptin gene therapy [67]. The subjects in the present study may have decreased intake enough to elevate plasma ghrelin without affecting their body weight.

Plasma leptin and ghrelin were unchanged for WALK. Exercise reduces plasma leptin when it is exhaustive or energy demanding [69]. Walking was low in intensity and relatively short in duration; therefore, it was not an adequate stimulus to elicit a reduction in plasma leptin for WALK. The lack of change in ghrelin with exercise is consistent with the literature, as many studies have reported no change in circulating ghrelin after exercise of various intensities [2,17-19,70].

In conclusion, raisins, walking, or a combination of these interventions will alter lipoprotein metabolism, which may reduce the risk for CVD in men and women aged 50 to 70 years. In addition, whereas walking had no effect on hormones related to satiety, raisin consumption increased plasma leptin, which could promote satiety and decrease caloric intake. Low-density lipoprotein receptor expression in mononuclear cells was increased for all subjects, promoting a reduction in plasma LDL-C. Walking decreased apo C-III to contribute to a reduction in plasma TGs. The increase in plasma ghrelin observed by consumption of raisins may have been caused by a decrease in intake with greater circulating leptin. As a source of soluble and fermentable fiber, raisins may reduce hunger and affect intake by altering hormones that influence satiety. These findings indicate that easily implemented lifestyle changes such as increasing raisin consumption or walking additional steps each day may serve as effective interventions to promote weight control and lower CVD risk.

Acknowledgment

These studies were supported by the California Raisin Marketing Board.

References

[1] Prevalence of heart disease—United States, 2005. MMWR Morb Mortal Wkly Rep 2007;56:113-8.
[2] Dauchet L, Amouyel P, Hercberg S, Dallongeville J. Fruit and vegetable consumption and risk of coronary heart disease: a meta-analysis of cohort studies. J Nutr 2006;136:2588-93.
[3] Shrestha S, Freake HC, McGrane MM, Volek JS, Fernandez ML. A combination of psyllium and plant sterols alters lipoprotein metabolism in hypercholesterolemic subjects by modifying the intravascular processing of lipoproteins and increasing LDL uptake. J Nutr 2007; 137:1165-70.
[4] Fernandez ML. Distinct mechanisms of plasma LDL lowering by dietary fiber in the guinea pig: specific effects of pectin, guar gum, and psyllium. J Lipid Res 1995;36:2394-404.
[5] Duncan KH, Bacon JA, Weinsier RL. The effects of high and low energy density diets on satiety, energy intake, and eating time of obese and nonobese subjects. Am J Clin Nutr 1983;37:763-7.
[6] Burton-Freeman B. Dietary fiber and energy regulation. J Nutr 2000; 130:272S-5S.
[7] Zern TL, Fernandez ML. Cardioprotective effects of dietary polyphenols. J Nutr 2005;135:2291-4.
[8] Zern TL, West KL, Fernandez ML. Grape polyphenols decrease plasma triglycerides and cholesterol accumulation in the aorta of ovariectomized guinea pigs. J Nutr 2003;133:2268-72.
[9] Wei C, Penumetcha M, Santanam N, Liu YG, Garelnabi M, Parthasarathy S. Exercise might favor reverse cholesterol transport and lipoprotein clearance: potential mechanism for its anti-atherosclerotic effects. Biochim Biophys Acta 2005;1723:124-7.
[10] Vinagre CG, Ficker ES, Finazzo C, Alves MJ, de Angelis K, Irigoyen MC, et al. Enhanced removal from the plasma of LDL-like nanoemulsion cholesteryl ester in trained men compared with sedentary healthy men. J Appl Physiol 2007;103:1166-71.
[11] Considine RV. Regulation of leptin production. Rev Endocr Metab Disord 2001;2:357-63.
[12] Mueller WM, Gregoire FM, Stanhope KL, Mobbs CV, Mizuno TM, Warden CH, et al. Evidence that glucose metabolism regulates leptin secretion from cultured rat adipocytes. Endocrinology 1998;139:551-8.
[13] Jenkins AB, Markovic TP, Fleury A, Campbell LV. Carbohydrate intake and short-term regulation of leptin in humans. Diabetologia 1997;40:348-51.
[14] Kojima M, Kangawa K. Ghrelin, an orexigenic signaling molecule from the gastrointestinal tract. Curr Opin Pharmacol 2002;2:665-8.
[15] Gruendel S, Garcia AL, Otto B, Mueller C, Steiniger J, Weickert MO, et al. Carob pulp preparation rich in insoluble dietary fiber and polyphenols enhances lipid oxidation and lowers postprandial acylated ghrelin in humans. J Nutr 2006;136:1533-8.
[16] Weigle DS, Cummings DE, Newby PD, Breen PA, Frayo RS, Matthys CC, et al. Roles of leptin and ghrelin in the loss of body weight caused by a low fat, high carbohydrate diet. J Clin Endocrinol Metab 2003;88: 1577-86.
[17] Martins C, Morgan LM, Bloom SR, Robertson MD. Effects of exercise on gut peptides, energy intake and appetite. J Endocrinol 2007;193: 251-8.
[18] Schmidt A, Maier C, Schaller G, Nowotny P, Bayerle-Eder M, Buranyi B, et al. Acute exercise has no effect on ghrelin plasma concentrations. Horm Metab Res 2004;36:174-7.
[19] Burns SF, Broom DR, Miyashita M, Mundy C, Stensel DJ. A single session of treadmill running has no effect on plasma total ghrelin concentrations. J Sports Sci 2007;25:635-42.
[20] Gee JM, Lee-Finglas W, Wortley GW, Johnson IT. Fermentable carbohydrates elevate plasma enteroglucagon but high viscosity is also necessary to stimulate small bowel mucosal cell proliferation in rats. J Nutr 1996;126:373-9.
[21] Reimer RA, McBurney MI. Dietary fiber modulates intestinal proglucagon messenger ribonucleic acid and postprandial secretion

of glucagon-like peptide–1 and insulin in rats. Endocrinology 1996; 137:3948-56.
[22] Adam TC, Westerterp-Plantenga MS. Glucagon-like peptide–1 release and satiety after a nutrient challenge in normal-weight and obese subjects. Br J Nutr 2005;93:845-51.
[23] Adam TC, Westerterp-Plantenga MS. Nutrient-stimulated GLP-1 release in normal-weight men and women. Horm Metab Res 2005; 37:111-7.
[24] Gutzwiller JP, Goke B, Drewe J, Hildebrand P, Ketterer S, Handschin D, et al. Glucagon-like peptide–1: a potent regulator of food intake in humans. Gut 1999;44:81-6.
[25] Schirra J, Katschinski M, Weidmann C, Schafer T, Wank U, Arnold R, et al. Gastric emptying and release of incretin hormones after glucose ingestion in humans. J Clin Invest 1996;97:92-103.
[26] Schirra J, Kuwert P, Wank U, Leicht P, Arnold R, Goke B, et al. Differential effects of subcutaneous GLP-1 on gastric emptying, antroduodenal motility, and pancreatic function in men. Proc Assoc Am Physicians 1997;109:84-97.
[27] Ali-Rachedi A, Varndell IM, Adrian TE, Gapp DA, Van Noorden S, Bloom SR, et al. Peptide YY (PYY) immunoreactivity is co-stored with glucagon-related immunoreactants in endocrine cells of the gut and pancreas. Histochemistry 1984;80:487-91.
[28] El-Salhy M, Grimelius L, Wilander E, Ryberg B, Terenius L, Lundberg JM, et al. Immunocytochemical identification of polypeptide YY (PYY) cells in the human gastrointestinal tract. Histochemistry 1983;77:15-23.
[29] Perez-Tilve D, Nogueiras R, Mallo F, Benoit SC, Tschoep M. Gut hormones ghrelin, PYY, and GLP-1 in the regulation of energy balance [corrected] and metabolism. Endocrine 2006;29:61-71.
[30] Challis BG, Pinnock SB, Coll AP, Carter RN, Dickson SL, O'Rahilly S. Acute effects of PYY3-36 on food intake and hypothalamic neuropeptide expression in the mouse. Biochem Biophys Res Commun 2003; 311:915-9.
[31] Halatchev IG, Cone RD. Peripheral administration of PYY(3-36) produces conditioned taste aversion in mice. Cell Metab 2005;1: 159-68.
[32] Pappas TN, Debas HT, Taylor IL. Peptide YY: metabolism and effect on pancreatic secretion in dogs. Gastroenterology 1985;89:1387-92.
[33] Parker TL, Wang XH, Pazmino J, Engeseth NJ. Antioxidant capacity and phenolic content of grapes, sun-dried raisins, and golden raisins and their effect on ex vivo serum antioxidant capacity. J Agric Food Chem 2007;55:8472-7.
[34] Loaiza J, Murano E, Cisneros-Zavallos L. Analysis of raisin extracts to determine phenolic content and antimicrobial activity. New Orleans: IFT; 2001.
[35] Boyum A. Isolation of mononuclear cells and granulocytes from human blood. Isolation of monuclear cells by one centrifugation, and of granulocytes by combining centrifugation and sedimentation at 1 g. Scand J Clin Lab Invest Suppl 1968;97:77-89.
[36] Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 1987;162:156-9.
[37] Barber RD, Harmer DW, Coleman RA, Clark BJ. *GAPDH* as a housekeeping gene: analysis of *GAPDH* mRNA expression in a panel of 72 human tissues. Physiol Genomics 2005;21:389-95.
[38] Liu MY, Xydakis AM, Hoogeveen RC, Jones PH, Smith EO, Nelson KW, et al. Multiplexed analysis of biomarkers related to obesity and the metabolic syndrome in human plasma, using the Luminex-100 system. Clin Chem 2005;51:1102-9.
[39] Puglisi MJ, Vaishnav U, Shrestha S, Torres-Gonzalez M, Wood RJ, Volek JS, et al. Raisins and additional walking have distinct effects on plasma lipids and inflammatory cytokines. Lipids Health Dis 2008;7: 14.
[40] Powell EE, Kroon PA. Low density lipoprotein receptor and 3-hydroxy-3-methylglutaryl coenzyme A reductase gene expression in human mononuclear leukocytes is regulated coordinately and parallels gene expression in human liver. J Clin Invest 1994;93:2168-74.
[41] Roach PD, Kerry NL, Whiting MJ, Nestel PJ. Coordinate changes in the low density lipoprotein receptor activity of liver and mononuclear cells in the rabbit. Atherosclerosis 1993;101:157-64.
[42] Camire ME, Dougherty MP. Raisin dietary fiber composition and in vitro bile acid binding. J Agric Food Chem 2003;51:834-7.
[43] Trautwein EA, Kunath-Rau A, Erbersdobler HF. Increased fecal bile acid excretion and changes in the circulating bile acid pool are involved in the hypocholesterolemic and gallstone-preventive actions of psyllium in hamsters. J Nutr 1999;129:896-902.
[44] Fischer CP, Hiscock NJ, Penkowa M, Basu S, Vessby B, Kallner A, et al. Supplementation with vitamins C and E inhibits the release of interleukin-6 from contracting human skeletal muscle. J Physiol 2004;558:633-45.
[45] Gierens H, Nauck M, Roth M, Schinker R, Schurmann C, Scharnagl H, et al. Interleukin-6 stimulates LDL receptor gene expression via activation of sterol-responsive and Sp1 binding elements. Arterioscler Thromb Vasc Biol 2000;20:1777-83.
[46] Kawakami A, Aikawa M, Alcaide P, Luscinskas FW, Libby P, Sacks FM. Apolipoprotein CIII induces expression of vascular cell adhesion molecule–1 in vascular endothelial cells and increases adhesion of monocytic cells. Circulation 2006;114:681-7.
[47] Otarod JK, Goldberg IJ. Lipoprotein lipase and its role in regulation of plasma lipoproteins and cardiac risk. Curr Atheroscler Rep 2004;6:335-42.
[48] Ito Y, Azrolan N, O'Connell A, Walsh A, Breslow JL. Hypertriglyceridemia as a result of human apo CIII gene expression in transgenic mice. Science 1990;249:790-3.
[49] Lemieux I, Salomon H, Despres JP. Contribution of apo CIII reduction to the greater effect of 12-week micronized fenofibrate than atorvastatin therapy on triglyceride levels and LDL size in dyslipidemic patients. Ann Med 2003;35:442-8.
[50] Horowitz JF, Leone TC, Feng W, Kelly DP, Klein S. Effect of endurance training on lipid metabolism in women: a potential role for PPARalpha in the metabolic response to training. Am J Physiol Endocrinol Metab 2000;279:E348-55.
[51] Iemitsu M, Miyauchi T, Maeda S, Tanabe T, Takanashi M, Irukayama-Tomobe Y, et al. Aging-induced decrease in the PPAR-alpha level in hearts is improved by exercise training. Am J Physiol Heart Circ Physiol 2002;283:H1750-60.
[52] Olivieri O, Martinelli N, Sandri M, Bassi A, Guarini P, Trabetti E, et al. Apolipoprotein C-III, n-3 polyunsaturated fatty acids, and "insulin-resistant" T-455C APOC3 gene polymorphism in heart disease patients: example of gene-diet interaction. Clin Chem 2005;51:360-7.
[53] Fruchart JC, Duriez P, Staels B. Peroxisome proliferator–activated receptor–alpha activators regulate genes governing lipoprotein metabolism, vascular inflammation and atherosclerosis. Curr Opin Lipidol 1999;10:245-57.
[54] Gill JM, Al-Mamari A, Ferrell WR, Cleland SJ, Sattar N, Packard CJ, et al. Effects of a moderate exercise session on postprandial lipoproteins, apolipoproteins and lipoprotein remnants in middle-aged men. Atherosclerosis 2006;185:87-96.
[55] Wellhoener P, Fruehwald-Schultes B, Kern W, Dantz D, Kerner W, Born J, et al. Glucose metabolism rather than insulin is a main determinant of leptin secretion in humans. J Clin Endocrinol Metab 2000;85:1267-71.
[56] Marshall S. Role of insulin, adipocyte hormones, and nutrient-sensing pathways in regulating fuel metabolism and energy homeostasis: a nutritional perspective of diabetes, obesity, and cancer. Sci STKE 2006;2006:re7.
[57] Marshall S, Bacote V, Traxinger RR. Discovery of a metabolic pathway mediating glucose-induced desensitization of the glucose transport system. Role of hexosamine biosynthesis in the induction of insulin resistance. J Biol Chem 1991;266:4706-12.
[58] Wang J, Liu R, Hawkins M, Barzilai N, Rossetti L. A nutrient-sensing pathway regulates leptin gene expression in muscle and fat. Nature 1998;393:684-8.
[59] Considine RV, Cooksey RC, Williams LB, Fawcett RL, Zhang P, Ambrosius WT, et al. Hexosamines regulate leptin production in

human subcutaneous adipocytes. J Clin Endocrinol Metab 2000;85: 3551-6.

[60] Mason MM, He Y, Chen H, Quon MJ, Reitman M. Regulation of leptin promoter function by Sp1, C/EBP, and a novel factor. Endocrinology 1998;139:1013-22.

[61] Stephens TW, Basinski M, Bristow PK, Bue-Valleskey JM, Burgett SG, Craft L, et al. The role of neuropeptide Y in the antiobesity action of the obese gene product. Nature 1995;377:530-2.

[62] Schwartz MW, Seeley RJ, Campfield LA, Burn P, Baskin DG. Identification of targets of leptin action in rat hypothalamus. J Clin Invest 1996;98:1101-6.

[63] El-Haschimi K, Lehnert H. Leptin resistance-or why leptin fails to work in obesity. Exp Clin Endocrinol Diabetes 2003;111:2-7.

[64] Mohlig M, Koebnick C, Weickert MO, Lueder W, Otto B, Steiniger J, et al. Arabinoxylan-enriched meal increases serum ghrelin levels in healthy humans. Horm Metab Res 2005;37:303-8.

[65] Weickert MO, Spranger J, Holst JJ, Otto B, Koebnick C, Mohlig M, et al. Wheat-fibre–induced changes of postprandial peptide YY and ghrelin responses are not associated with acute alterations of satiety. Br J Nutr 2006;96:795-8.

[66] Schmitz KH, Warren M, Rundle AG, Williams NI, Gross MD, Kurzer MS. Exercise effect on oxidative stress is independent of change in estrogen metabolism. Cancer Epidemiol Biomarkers Prev 2008;17: 220-3.

[67] Dube MG, Beretta E, Dhillon H, Ueno N, Kalra PS, Kalra SP. Central leptin gene therapy blocks high-fat diet-induced weight gain, hyperleptinemia, and hyperinsulinemia: increase in serum ghrelin levels. Diabetes 2002;51:1729-36.

[68] Beretta E, Dube MG, Kalra PS, Kalra SP. Long-term suppression of weight gain, adiposity, and serum insulin by central leptin gene therapy in prepubertal rats: effects on serum ghrelin and appetite-regulating genes. Pediatr Res 2002;52:189-98.

[69] Keller P, Keller C, Steensberg A, Robinson LE, Pedersen BK. Leptin gene expression and systemic levels in healthy men: effect of exercise, carbohydrate, interleukin-6, and epinephrine. J Appl Physiol 2005;98: 1805-12.

[70] Kraemer RR, Durand RJ, Acevedo EO, Johnson LG, Kraemer GR, Hebert EP, et al. Rigorous running increases growth hormone and insulin-like growth factor-I without altering ghrelin. Exp Biol Med (Maywood) 2004;229:240-6.