Glycemic regulation, appetite, and ex-vivo oxidative stress in young adults following consumption of high carbohydrate cereal bars fortified with polyphenol-rich berries

Consumption of certain berries appears to slow postprandial glucose absorption, attributable to polyphenols, which may benefit exercise and cognition, reduce appetite and/or oxidative stress. This randomized, cross-over, placebo-controlled study determined whether polyphenol-rich fruits added to carbohydrate-based foods produce a dose-dependent moderation of postprandial glycemic, glucoregulatory hormone, appetite, and ex-vivo oxidative stress responses. Twenty participants (18M/2F; 24±5 yr; BMI: 27±3 kg/m2) consumed one of five cereal bars (~88% carbohydrate) containing no fruit ingredients (reference), freeze-dried black raspberries (10% or 20% total weight; LO-R and HI-R, respectively), and cranberry extract (0.5% or 1% total weight; LO-C and HI-C), on trials separated by 5 d. Postprandial peak/nadir from baseline (max) and incremental postprandial area-under-the-curve (AUC) over 60- and 180-min, for glucose and other biochemistries were measured. Fortification with freeze-dried black-raspberries (~25 g, containing 1.2 g of polyphenols) seems to slightly improve the glucoregulatory hormone and glycemic responses to a high-carbohydrate food item in young adults, but did not affect appetite or oxidative stress responses.

polyphenols, tannins, glycemia, appetite, oxidative stress, young adults
GLYCEMIC REGULATION, APPETITE, AND EX-VIVO OXIDATIVE STRESS IN YOUNG ADULTS FOLLOWING CONSUMPTION OF HIGH CARBOHYDRATE CEREAL BARS FORTIFIED WITH POLYPHENOL-RICH BERRIES
ABSTRACT

Consumption of certain berries appears to slow postprandial glucose absorption, attributable to polyphenols, which may benefit exercise and cognition, reduce appetite and/or oxidative stress. This randomized, cross-over, placebo-controlled study determined whether polyphenol-rich fruits added to carbohydrate-based foods produce a dose-dependent moderation of postprandial glycemic, glucoregulatory hormone, appetite, and ex-vivo oxidative stress responses. Twenty participants (18M/2F; 24±5 yr; BMI: 27±3 kg/m2) consumed one of five cereal bars (~88% carbohydrate) containing no fruit ingredients (reference), freeze-dried black raspberries (10% or 20% total weight; LO-R and HI-R, respectively), and cranberry extract (0.5% or 1% total weight; LO-C and HI-C), on trials separated by ≥5 d. Postprandial peak/nadir from baseline (Δmax) and incremental postprandial area-under-the-curve (AUC) over 60- and 180-min, for glucose and other biochemistries were measured to examine dose-dependent effects. Glucose AUC0-180mins trended towards being higher (43%) after HI-R versus LO-R (P=0.06), with no glucose differences between the raspberry and reference bars. Relative to reference, HI-R resulted in a 17% lower Δmax insulin, 3% lower C-peptide (AUC0-60mins and 3% lower GIP (AUC0-180mins) p<0.05. No treatment effects were observed for the cranberry bars regarding glucose and glucoregulatory hormones, nor were any treatment effects noted for either berry-type regarding ex-vivo oxidation, appetite mediating hormones, or appetite. Fortification with freeze-dried black-raspberries (~25 g, containing 1.2 g of polyphenols) seems to slightly improve the glucoregulatory hormone and glycemic responses to a high-carbohydrate food item in young adults, but did not affect appetite or oxidative stress responses at doses or with methods studied herein. **Clinical Trials Registration Number:** NCT02763020

**Key words:** polyphenols, tannins, glycemia, appetite, oxidative stress, young adults
A sustained postprandial glycemic response is advantageous for facilitating physical and
cognitive performance. Working memory and selective attention, for example, were
improved in the later postprandial period following consumption of a food item (bread enriched
with guar gum) that elicited higher net glucose availability in the postprandial period compared
to a reference food item (bread without guar gum). Additionally, evidence suggests that
consuming moderate glycemic index foods 1-2 hours prior to exercise better maintains
euglycemia and maximizes carbohydrate oxidation during endurance exercise versus high
glycemic index foods which result in lower net glucose availability during the postprandial
period. Additionally, slowing glucose absorption prevents postprandial hyperinsulinemia,
which in turn causes a rapid drop in blood glucose to below fasting concentrations. The resulting
relative hypoglycemia initiates a counter-regulatory hormone response that promotes
gluconeogenesis, glycogenolysis, free fatty acid release, and oxidative stress. Given the
popularity of foods high in rapidly-digested carbohydrate and the advantages of promoting a
sustained glycemic response to promote physical and cognitive performance, there is substantial
interest in developing food products that moderate postprandial glycemic responses.

Polyphenols are a heterogeneous group of phytochemicals found in plant-based foods,
many of which display antioxidant and anti-inflammatory properties, but which are also thought
to modulate carbohydrate metabolism. In support, some studies have shown that whole
berries, berry extracts, apple extract or juice, and a mixture of polyphenol and fibre-rich foods
(e.g., green tea, apple peel and freeze-dried berry powders) modulate the glycemic response
following consumption of sugar water or starch-based food items. For example,
Torronen et al. demonstrated that consuming polyphenol-rich berry nectars or berry purees
with a high-carbohydrate food favorably modulated postprandial glycemia in healthy adults by
slowing glucose absorption, and enhancing insulin and glucagon-like peptide-1 (GLP-1) secretion. However, an acknowledged limitation of those studies was a higher dietary fiber content and viscosity of the berry interventions relative to control, as both fiber and viscosity influence postprandial glycemia and endocrine responses. In contrast, studies by Castro-Acosta (2016 and 2017) observed a blunted glycemic response when a starchy meal was provided with polyphenol-rich apple and blackcurrant extracts (i.e., devoid of fiber). With regard to mechanisms of the glycemic and glucoregulatory hormone modulation, in vitro and animal studies suggest that polyphenols, including those found in berries and in extracts of polyphenol-rich foods, can inhibit the carbohydrate digestive enzymes \( \alpha \)-amylase and \( \alpha \)-glucosidase, slow glucose absorption, modulate secretion of insulin and/or the incretin hormones glucose-dependent insulinotropic polypeptide (GIP) and GLP-1\(^{(9; 14; 15)}\), and stimulate glucose uptake into insulin-sensitive tissues through increased activation of insulin receptors \(^{(7)}\). As such, polyphenol fortification of high carbohydrate foods may help improve postprandial glycemic control. This could have the added benefits of reducing free fatty acid release and oxidative stress \(^{(16)}\), and preventing increases in appetite that may result from rapid drops in blood glucose concentrations \(^{(7)}\).

Our group and others have previously reported that cranberry and black raspberry polyphenols inhibit \( \alpha \)-amylase and \( \alpha \)-glucosidase activities in vitro \(^{(17; 18; 19)}\). The primary objective of this study was to translate those findings to human metabolism by determining dose-response effects of fortifying a high carbohydrate food with freeze-dried black raspberries or with cranberry extract on postprandial glycemia (i.e., glucose incremental area under the curve with respect to baseline, AUC, 0-180 mins) in healthy adults. Fortified and non-fortified cereal bars were created that were approximately matched for fiber, macronutrients, and
physicochemical characteristics. Secondary objectives were to determine effects on postprandial
glucoregulatory hormone responses, appetite, and ex-vivo oxidative stress. We hypothesized
that polyphenol fortification would result in a dose-dependent improvement in postprandial
metabolic profiles, and reduce appetite and ex-vivo oxidative stress.

Materials and methods

Participants

Participants were military and civilian personnel assigned to Natick Soldier Systems Center,
Natick, MA. Twenty of the 21 participants who were enrolled and began the study completed data
collection and were included in the data analyses. One participant was withdrawn from the study
prior to consuming any of the cereal bars due to multiple failed catheter placements. Data collection
occurred from January to November 2016 at the U.S. Army Research Institute of Environmental
Medicine (Natick, MA). Each participant gave their written, informed consent after an oral
explanation of the study. Men and women were included if they were 18-39 yr, were generally
healthy, and had no history of liver disease, alcoholism, impaired glucose metabolism, thyroid
disease, bleeding disorders, or GI-related conditions that may impact glucose absorption, and had no
allergy or aversion to any of the test foods. The study was approved by the Institutional Review
Board, U.S. Army Research Institute of Environmental Medicine. Investigators adhered to the
policies for protection of human subjects as prescribed DOD Instruction 3216.02 and the research
was conducted in adherence with the provisions of 32 CFR Part 219. The Clinicaltrials.gov
identifier is NCT02763020.

Design
This was a randomized, placebo-controlled, cross-over trial, conducted over five experimental sessions each separated by ≥5d (9.5 ± 5.2 days). Participants were assigned to experimental conditions using on-line software, Research Randomizer (www.randomizer.org).

Flavor profile and color of the bars provided some indication of the fruit contents, however, participants were unaware of which bars contained high versus low doses of the fruit ingredients.

Study participants received written and verbal instructions to consume a low-polyphenol diet for two consecutive days prior to each session. Participants also consumed a provided dinner meeting approximately 1/3 of their estimated weight maintenance energy requirements the evening before testing.

On test days, participants arrived following a ≥12hr overnight fast. Adherence to pre-trial dietary restrictions and consumption of the standardized dinner meal was verified using food records which were reviewed by research dietitians during each session. Following IV catheter placement, participants consumed one of five cereal bars in ≤15min. After bar consumption, overall acceptability was rated on a Likert scale ranging from 1 (dislike extremely) to 9 (like extremely).

Blood samples were collected and appetite was rated before and periodically for 180min after bar consumption, in order to detect both the early and late postprandial responses of the outcome measures and to facilitate the ad libitum lunch test by providing a more realistic time frame between the “breakfast” and lunch meals. During the 180min postprandial period participants remained seated and supervised, and were not provided additional food or beverages other than 360 g of water.

After 180min, energy intake was measured during an ad libitum lunch.

Description of high-carbohydrate snack bars

Five different fiber and macronutrient-matched high-carbohydrate cereal bars were tested (Table 1). The placebo bar contained no freeze-dried fruit or fruit extract. Two bars contained
freeze-dried black raspberries [10% (LOW-Rasp) or 20% (HIGH-Rasp) total weight], and two bars contained cranberry extract [0.5% (LOW-Cran) or 1.0% (HIGH-Cran) total weight]. The base bar consisted of rice crisp cereal, marshmallows, butter, and vanilla extract. The bar was loosely modeled after a Rice Krispie Treat (Kellogg Company, Battle Creek, MI), in an effort to promote palatability and to provoke marked glycemia, which was necessary for testing the efficacy of polyphenol supplementation to moderate the glycemic response.

Fructose powder, glucose powder or wheat bran was added to the bars, in order to approximately match sugar and fiber content between bars. The cranberry extract and raspberry powder were chosen based on their polyphenolic content and previous work by members of our group and others suggesting that the polyphenol components (e.g., anthocyanins and proanthocyanidines) in these fruits effectively inhibited α-amylase and glucoamylase activity in vitro (17; 18; 19). The LOW-Rasp and HI-Rasp bars contained approximately 0.6 and 1.2 g of total polyphenols, respectively, based on gram weight of the bars (Table 1) and data indicating that black raspberries contain 0.98 g of polyphenols per 100 g of whole fruit (20) (i.e., or ~5.1 g polyphenols per 100 g of freeze-dried black raspberry powder). The LOW-Cran and HI-Cran bars contained 0.3 and 0.6 g of polyphenols, respectively, based on gram weight of the bars (Table 1) and data indicating that the cranberry extract contains 45 g of polyphenols per 100 g of extract (21). The polyphenols contained within HI-R and LO-R were mostly anthocyanins, ellagitannins, ellagic acid and quercitin (20; 22), while the HI-C and LO-C mainly consisted of flavanols (e.g., epicatechin), flavonols (e.g., quercetin) and phenolic acids (e.g., benzoic acid and chlorogenic acid), in addition to other polyphenolic compounds (21). The highest dose of each fruit was based on the maximum dose that could be incorporated without compromising organoleptic properties of the bars. The lower doses were included to assess dose-response effects.
Blood sampling

An indwelling catheter was placed in the participants’ forearm or antecubital space upon arrival to the testing site. Blood samples were taken after catheter placement and every 15 mins for the first hour and every 30-min thereafter (up to 180 minutes) following bar consumption. Whole blood was collected into serum tubes for measurement of glucose, insulin, and C-peptide, and chilled EDTA tubes for measurement of GIP, GLP-1, and acylated ghrelin. EDTA tubes contained 4-(2-aminoethyl)-benzenesulfonyl fluoride, hydrochloride (100 mM; 50 µL/mL whole blood), dipeptidyl peptidase inhibitor IV (10 µL/mL whole blood), and aprotinin (500 KIU/mL whole blood). Following serum and plasma separation, samples were stored at -80°C until analysis.

Glucose was measured on a Siemens Dimension Xpand Plus clinical chemistry analyzer, while insulin and C-peptide were measured on a Siemens Immulite 2000 immunoassay system. GIP, active GLP-1, and acylated ghrelin were measured using the Milliplex MAP human metabolic hormone panel (Millipore; Billerica, MA) according to manufacturer instructions. Assay sensitivity was 0.6 pg/mL for GIP, 1.2 pg/mL for GLP-1, and 13 pg/mL for acylated ghrelin.

Ex-vivo LDL resistance against Cu²⁺-induced oxidation

Postprandial oxidative stress is regarded as a secondary response to postprandial hyperglycemia and hypertriglyceridemia (23). While there are many markers for assessment of oxidative stress, LDL oxidation was selected as a biomarker because of the involvement of oxidized LDL in the development of atherosclerosis. For example, Natella et al. reported that postprandial LDL was more susceptible to metal-catalyzed oxidation than the homologous baseline LDL after an ethanol meal (24). It was anticipated that postprandial hyperglycemia may
have the same impact on LDL susceptibility to oxidation as acute hyperglycemia-induced oxidative stress in healthy people\textsuperscript{(25)}. Plasma was mixed with sucrose (0.6% final concentration), aliquoted, and stored at -80°C. An ex-vivo LDL oxidation assay was performed within 2 months of the sample collection. LDL (1.019-1.063 g/mL) was collected from the frozen plasma according to Chung et al. using a Beckman NVT-90 rotor (or similar) in a Beckman L8-M centrifuge (or similar) (Palo Alto, CA)\textsuperscript{(26)}. After salt removal using a desalting PD-10 column (Bio-Rad, Hercules, CA), the concentration of LDL was determined using a BCA protein assay kit (Pierce; Rockford, IL or similar). Ex-vivo LDL oxidation induced by Cu\textsuperscript{2+} was performed according to the method described by Chen et al.\textsuperscript{(16)}. Formation of conjugated dienes was monitored by absorbance at 234 nm at 37°C for 6-h using a Shimadzu UV1800 spectrophotometer (Japan) equipped with a 6-position automated sample changer. The results of the assay are expressed as lag time, the intercept at the abscissa in the diene-time plot.

**Plasma flavonoids and phenolic acids**

Flavonoids (including the flavanols catechin and epicatechin and the flavonols quercetin, myricetin, and isorhamnetin) and phenolic acids (including protocatechuic, phenylacetic, gentisic acid, benzoic acid, sinapic, caffèic, ferulic, vanillic, and p-coumaric acids) in plasma were determined according to Chen et al. (2005), in order to provide insight into oxidative stress results\textsuperscript{(16)}. Briefly, plasma was incubated with vitamin C-EDTA and β-glucuronidase/sulfatase at 37°C for 45 min. Phenolic acids and flavonoids in the resulting mixture were extracted with acetonitrile, dried under purified N2 gas, and reconstituted with mobile phase A for HPLC analysis using a ESA CoulArray System (ESA, Inc. Chelmsford, MA). Analyte separation was achieved using a Zorbax ODS C18 column (4.6 x 250 mm, 3.5 μm). Quantification of phenolic
acids and flavonoids in unknown samples were calculated based on standard curves constructed using authentic standards with adjustment for the internal standard (4’-hydroxy-3’-methoxyacetophenone).

**Appetite testing**

Two separate visual analog scales were administered before each blood sample collection to measure self-perceived appetite. Participants rated their levels of hunger and fullness by marking anywhere on a 10-cm scale anchored by phrases representing opposite extremes of a spectrum (e.g., “not at all hungry” and “extremely hungry”).

An *ad libitum* lunch was served within 10 min after the final blood sample to provide an objective measure of appetite. The meal consisted of Stouffer’s lasagna (41% carbohydrate, 36% fat, 23% protein) and 240 g water. Participants were served 1653 ± 57 g and instructed to eat until “comfortably full”. The amount of uneaten lasagna was weighed to calculate energy intake.

**Statistical analysis**

Sample size estimates based on peak postprandial glucose concentrations, and using mean and variance data from Torronen et al. indicated that 20 participants would allow detection of a 0.9 mmol/L (~15 mg/dL) difference in peak glucose between trials with power = 0.8 and α = 0.01 to account for multiple comparisons.

Statistical analyses were conducted using the IBM SPSS statistical package version 24.0 (IBM Inc., Armonk, New York). Data were examined for outliers both quantitatively and graphically, and normal distribution of data was examined via the Shapiro-Wilk test. All data, except glucose, appetite ratings, energy intake, and LDL lag were log_{10}-transformed for analysis to normalize distributions. Values that were below the assay limits of detection (13% of values
for GLP-1, 2% for ghrelin, 4% for insulin) were replaced with the lowest detectable limit for that
assay prior to analysis.

Time to peak (i.e., for glucose, insulin, GLP-1, GIP, C-peptide) or nadir (i.e., for ghrelin)
concentrations, change from baseline (time 0) to peak (i.e., for glucose, insulin, GLP-1, GIP, C-
peptide, fullness) or nadir (i.e., for ghrelin, hunger) concentrations (Δmax), and incremental area
under the curve with respect to baseline (AUC) from 0-60min and 0-180min were computed for
all outcomes to standardize the results and used in the analyses to detect any differences between
bars with regards to initial (AUC 0-60) and overall (AUC 0-180) postprandial responses.

Analyses were run separately for the raspberry and cranberry interventions because the study
objective was to assess dose-response effects within each intervention type and not to compare
interventions. Data were analyzed using marginal models to test for main effects of treatment.
Baseline (i.e., time 0min) values were entered as covariates in the models, and carry-over effects
were assessed by including terms for treatment order and its interaction with treatment. These
terms were removed from the model if not significant. When significant main effects of
treatment were observed all possible t-tests were conducted using the Bonferroni correction to
adjust for multiple comparisons. The Kruskall-Wallis test was used to test for main effects of
treatment on plasma flavonoid/phenolic acid content, because transformations did not normalize
the data distribution. When significant main effects of treatment were observed, Mann-Whitney
tests were conducted using Bonferroni corrections to adjust for multiple comparisons. Results are
presented as mean ± SD, unless otherwise noted. Two-tailed p-values ≤ 0.05 were considered
statistically significant, and p-values ≤ 0.10 were considered trends.

Results

Participant characteristics
Eighteen men and two women completed the study (age: 24 ± 5 years; BMI: 26.8 ± 3.5 kg/m²) (Figure 1). Energy and macronutrient intake in the two days before each trial, and in the evening meal prior to each trial, did not differ across trials (P > 0.6) (Table 1). Body weight, and fasting glucose and insulin concentrations did not differ across trials (P > 0.3) (Table 2). Relative to the reference, overall acceptability of the bars was rated lower for HI-Rasp, and both cranberry bars (main effect of treatment, P ≤ 0.02 (Table 3).

**Glucose**

Within the raspberry treatments, there was a significant treatment effect on the overall glucose response (P = 0.04) wherein a trend for a higher glucose AUC₀₋₁₈₀min was observed during HI-Rasp versus LO-Rasp (P = 0.06; Figure 2A). Glucose Δmax, time to peak and AUC₀₋₆₀min was not affected by treatment (Table 4).

The cranberry treatments had no effect on the postprandial glucose response (Table 4 and Figure 2B).

**Insulin**

Within the raspberry treatments, there was a significant treatment effect on insulin Δmax (P = 0.02), wherein the HI-Rasp response was lower relative to the reference (Table 4 & Figure 3). There were no effects of the raspberry treatments on time to peak insulin or insulin AUC₀₋₆₀min or AUC₀₋₁₈₀min (Table 4 and Figure 2C).

The cranberry treatment had no effects on the postprandial insulin response (Table 4 Figure 2D).

**C-peptide**

Within the raspberry treatments (Figure 2E), there was a significant treatment effect (P = 0.01), wherein participants C-peptide AUC₀₋₆₀ was lower during HI-Rasp (247 ± 117 ng/dL)
compared to the reference (279 ± 243 ng/dL), but there was no effect on AUC\textsubscript{0-180} or time to peak C-peptide (Table 4).

The cranberry treatments had no effects on the postprandial C-peptide response (Table 4 and Figure 2F).

**Glucagon-like peptide-1 (GLP-1)**

Within the raspberry treatments, there was a trend for a treatment effect (P = 0.09), wherein GLP-1 \(\Delta\text{max} \) trended towards being lower during HI-Rasp relative to the reference (Table 4 & Figure 3A). No differences in time to peak GLP-1, AUC\textsubscript{0-60min} or AUC\textsubscript{0-180min} were observed (Figure 3A).

Within the cranberry treatment, a carryover effect was noted for GLP-1 AUC\textsubscript{0-180min}.

After removing the treatment sequence responsible for the carryover effect, no differences across treatments were observed. No differences in time to peak GLP-1 or GLP-1\(\Delta\text{max} \) were observed (Table 4).

**Glucose-dependent insulinotropic polypeptide (GIP)**

Within the raspberry treatments, there were significant treatment effects on the overall GIP response (p < 0.05), wherein AUC\textsubscript{0-60} and AUC\textsubscript{0-180} for GIP was significantly lower following consumption of the HI-Rasp versus LO-Rasp and/or reference bar (Figure 3C). There was also a significant treatment effect (P = 0.01), wherein GIP \(\Delta\text{max} \) was lower after participants consumed HI-Rasp versus LO-Rasp, but no effect on time to peak GIP was observed (Table 4).

The cranberry treatment had no effect on postprandial GIP response (Table 4 and Figure 3D).

**Ghrelin**

The raspberry treatments had no effects on the postprandial acylated ghrelin response (Table 4 and Figure 3E).
Within the cranberry treatment, there was a trend (P = 0.07) for a treatment effect wherein the acylated ghrelin AUC\(_{0-180\text{min}}\) trended towards being more negative (i.e., larger decrease) during HI-Cran relative to LO-Cran (P = 0.08). There was no effect of cranberry treatment on the maximal decrease in acylated ghrelin from baseline or time to nadir (Table 4 & Figure 3F).

*Ex-vivo LDL resistance against Cu\(^2+\)-induced oxidation*

There was no treatment effect when the raspberry bars were compared to one another and/or the reference bar (Figure 4A).

There were no treatment, time or treatment x time interactions when the cranberry bars were compared to one another and/or the reference bar (Figure 4B).

*Flavonoids and phenolic acids*

The raspberry treatment had no effects on plasma concentrations of measured flavonoids or phenolic acids (select phenolic acids shown in Figures 5 & 6).

Within the cranberry treatment, there were significant treatment effects on the plasma concentrations of phenolic acids (i.e., gentisic, vanillic, caffeic, coumaric, ferulic and sinapic acids, p<0.05), but not flavonoids. Most notably, post-hoc testing indicated that gentisic acid was significantly higher after HI-cran versus LO-cran and/or the reference bar; coumaric, ferulic and sinapic acids were significantly higher after consumption of HI-cran and LO-cran compared to the reference bar; and, vanillic acid and caffeic acid were significantly higher after LO-cran or HI-cran, respectively, versus the reference bar (p<0.02) (Figures 5 & 6).

*Appetite*

Within the raspberry treatments, there was a trend for a main effect of treatment on *ad libitum* energy intake (P = 0.10), but post hoc testing did not indicate significant differences
between treatments. The raspberry treatments did not impact postprandial hunger or fullness ratings (Table 5).

Within the cranberry treatments, there was a trend for a main effect of treatment on fullness \( AUC_{0-180\text{min}} \) with post hoc comparisons indicating a trend for lower fullness during HI-Cran relative to the reference bar \( (P = 0.06) \). A trend for a main effect of treatment on the peak change in fullness \( (P = 0.08) \) was also observed, but post hoc testing did not indicate significant differences between treatments. The cranberry treatments did not impact hunger ratings or \textit{ad libitum} energy intake (Table 5).

**Discussion**

The main findings of this study were that fortifying a high-carbohydrate cereal bar with a high-dose, but not a smaller-dose, of freeze-dried raspberries blunted postprandial peak insulin and incretin hormone responses compared to an unfortified bar, and tended to increase postprandial glucose AUC, but not peak concentrations. Together, these findings suggest that fortifying a high-carbohydrate bar with a high dose, but not a smaller dose, of freeze-dried black raspberry powder attenuated postprandial insulinemia and slowed glucose absorption. These results are supported by reduced GIP concentrations following consumption of the high-dose raspberry bar, as GIP is a sensitive marker of intestinal glucose uptake and no differences in plasma glucose concentrations were observed in the 60-min postprandial period when the predominant influence on blood glucose is absorption rate). These effects did not result in differences in appetite or ex-vivo LDL resistance against oxidation, and fortification with a polyphenol-rich cranberry extract did not elicit similar effects.

Our findings demonstrate that the high-dose raspberry bar favorably modulated postprandial glucose and glucoregulatory hormone responses. The 43% higher postprandial
glucose AUC, but no difference in peak glucose concentrations, following consumption of the high-dose compared to the low-dose raspberry bar suggests delayed glucose absorption or uptake into peripheral tissues. The former response could reduce the postprandial insulin demand \(^{(7;31)}\), while the latter could be driven by lower postprandial insulin concentrations. Although diminished postprandial insulinemia was not observed when comparing the high-dose to low-dose raspberry bar, the high-dose bar did show a modest blunting of postprandial insulin, C-peptide and glucoregulatory hormones responses when compared to the reference bar which is consistent with other studies \(^{(14;15)}\). The insulinemic response to the high dose black raspberry bar, compared to the reference, is consistent with the observation that C-peptide was lower in the 60 minutes following consumption of the high-dose black raspberry bar compared to the reference. C-peptide is not used by the liver and other organs, thus it is a more sensitive biomarker for endogenous insulin secretion compared to insulin itself due to unknown variability in tissue clearance of insulin\(^{(32)}\). *In-vitro* studies demonstrating that polyphenols extracted from a variety of flavanol-rich foods inhibit digestive enzymes—specifically \(\alpha\)-amylase, \(\alpha\)-glycosidase, and glucoamylase—during the breakdown of dietary carbohydrates into glucose \(^{(17)}\) provide a plausible mechanism. For example, recent *in vitro* studies demonstrate the inhibitory effects of different plants/extracts (e.g., grapeseed extract and African pear fruit) on \(\alpha\)-amylase and \(\alpha\)-glycosidase \(^{(11;33;34;35;36)}\). Additionally, the raspberry powder used in the current study has exhibited similar effects *in vitro*, and these actions would be expected to inhibit starch digestion and slow glucose absorption \(^{(7;13;14;19)}\). Alternately, the blunted GIP and GLP-1 responses following the high-dose fortification could underpin the lower postprandial C-peptide and insulin concentrations which could slow glucose uptake into peripheral tissues. Regardless of the mechanism, previous studies suggest that one possible advantage of slowed glucose absorption
and/or uptake is improved physical and cognitive performance, especially during exercise (1; 2; 3; 4; 5).

We acknowledge the inconsistency of not finding evidence for delayed glucose absorption when comparing the high-dose raspberry and reference bar despite the blunted insulin and glucoregulatory hormone responses. However, consistent with our results three prior studies reported that the initial insulin response to a glucose beverage or starch-based food (i.e., bread) was attenuated by berries with little or no appreciable effect on glycemic response (37; 38; 39; 40). These findings suggest an alternative mechanism whereby postprandial glucose metabolism may require less insulin when polyphenol-rich foods are consumed with high carbohydrate foods, compared to high carbohydrate foods alone (37; 39; 40; 41; 42). Although the underpinning mechanisms have not been clearly defined, polyphenols may possibly act acutely to improve insulin sensitivity in peripheral tissue (7; 31; 39; 40). We also acknowledge the inconsistency in the observation that the overall glucose response (AUC0-180 min) was higher following consumption of HI-R versus LO-R, in the absence of a higher overall insulin response. This suggest a lack of compensatory insulin secretion, possibly secondary to a lower overall GIP response given that GIP is an incretin hormone. Indeed, inhibition of GIP has been observed in response to anthocyanin-rich black-currents (8). As suggested by others, this effect may be attributable to delayed glucose uptake in response to polyphenols as GIP is secreted from the proximal region of the small intestine, whereas glucose absorption may be occurring more distally because of the polyphenols (8; 10).

The observed effects of fortifying high-carbohydrate foods with polyphenol-rich foods on postprandial glycemia were similar in magnitude (with regard to AUC) to a recent study that supplemented starch (i.e., white bread) with a mixture of polyphenol and fibre-rich foods (e.g.,
green tea powder, apple peel, blackberry, blackcurrent, and strawberry freeze-dried powders\(^{(11)}\), but less pronounced than those reported in other studies \(^{(13; 14; 30)}\). While the estimated amounts of total polyphenols were similar between this study and those of Torronen et al, it is possible that the type of polyphenols provided, as well as the fiber content and viscosity of the test meals, may be partially responsible for this discrepancy. For example, prior trials demonstrating a substantially altered glycemic response used whole berries or berry purees consisting mainly of anthocyanins and proanthoyanidins \(^{(13; 14; 30)}\), whereas the current study used freeze-dried raspberry powder which contained mostly anthocyanins, ellagitannins, ellagic acid and quercitin. Further, the polyphenol-rich foods used in those studies contained more soluble fiber and were more viscous than their control foods. Authors of those studies asserted that the modified glucose response they observed was not solely attributed to differences in the soluble fiber content of the berry meals (up to 1.5 g), however, it may partially explain their results since soluble fiber increases viscosity and mitigates postprandial glycemic response to high carbohydrate foods \(^{(43)}\). In contrast, the polyphenol-rich and reference bars used in the current study contained no soluble fiber and did not differ in viscosity. Indeed, similar to the current study, Castro-Acosta (2016 and 2017) removed fiber as a potential confounding variable (i.e., by testing apple and blackcurrant extracts), and observed that the fruit extract modulated the glycemic response to fruit juice and white bread with apricot jam\(^{(8; 9)}\). Differential findings between the aforementioned and current study may be attributable to the type of fruits, and their polyphenolic constituents, that were tested.

Contrary to our hypothesis, polyphenol fortification did not suppress appetite, and did not dose-dependently potentiate postprandial increases in GLP-1 or postprandial decreases in acylated ghrelin, both appetite-mediating hormones. Although relatively few studies have
evaluated the acute effects of polyphenol-rich foods on appetite and associated hormones (44), several have reported that consuming polyphenol-rich foods may alter appetite-mediating hormones in a direction that would be expected to suppress appetite. For example, in separate studies, adding a polyphenol-rich berry puree to sugar water potentiated postprandial increases in the appetite-suppressing hormone GLP-1 (14), while adding polyphenol-rich soluble carob fiber to a liquid meal potentiated the postprandial suppression of the appetite-stimulating hormone acylated ghrelin (45). *In vitro* and animal studies suggest that certain polyphenols may directly modulate GLP-1 and ghrelin secretion, and interact with hormones known to influence their biological activity (44). However, the effects of polyphenols on GLP-1 and acylated ghrelin appear to vary by polyphenol type (46; 47), and the food matrix in which polyphenols are consumed (45; 48; 49). Importantly, studies demonstrating effects of polyphenol-rich foods on GLP-1 and/or ghrelin in a direction that would be expected to suppress appetite could not separate effects of polyphenols from the fiber and viscosity of the test meals (14; 45), which are both factors also thought to influence appetite-mediating hormone responses (50). Additionally, a recent study found no acute effect of consuming 0.5-1.5g of polyphenol-rich grape seed extract on appetite (51) despite the extract having been shown to inhibit α-amylase and α-glucosidase *in vitro* (52), similar to the polyphenols used in the present study. Collectively, these findings do not support an appetite suppressing effect of berry polyphenols within the doses studied when fiber intake and viscosity are matched across fortified and non-fortified food products. Nonetheless, an impact of polyphenols on appetite is biologically plausible given evidence for roles of various polyphenols in modulating glucose metabolism, and the concentrations of appetite-regulating neuropeptides and enteroendocrine hormones (7; 44; 46; 53). As such, these results may not pertain to other polyphenol forms or sources administered in different doses or other food matrices.
Findings from the current study indicate that neither of the fruit ingredients, in the doses provided, enhanced the ex-vivo resistance of LDL lipids to oxidation following consumption of a high carbohydrate snack bar. This is not surprising, given there was little effect of the reference bar on LDL oxidation. Postprandial oxidative stress, as a sub-form of nutritional oxidative stress, ensues from sustained postprandial hyperlipidemia and/or hyperglycemia and is associated with a higher risk for cardiometabolic diseases\(^{(54)}\). Even though absorbed polyphenols, including flavonoids and phenolic acids, may confer anti-oxidative protection to LDL against oxidation during the postprandial state, we speculated that the protection of LDL against oxidation might be mainly attributed to diminished postprandial glucose response derived from lower glucose absorption in the GI tract. Thus, the interpretation of LDL oxidation data cannot solely focus on polyphenol bioavailability. Additionally, this study only measured one biomarker to evaluate the effect of antioxidants within cranberry and black-raspberry on oxidative stress, while a complete assessment of oxidative stress status from antioxidant interventions requires application of an array of biomarkers, including antioxidants (e.g., enzymes and small molecular antioxidants) and free radical-derived oxidized products.

Altogether, future studies should consider assessing the effect of fortifying a high fat food item with raspberry or cranberry polyphenols on LDL oxidation, using ex-vivo or more robust in-vivo methods, or in populations with increased susceptibility to oxidative stress such as the obese and elderly.

The raspberry and cranberry treatments produced differential effects on plasma concentrations of flavonoids and phenolic acids. Specifically, the raspberry treatment had no effect on circulating flavonoids or phenolic acid concentrations, whereas the cranberry treatment increased postprandial concentrations of several phenolic acids. These differences are likely
attributable to the type of polyphenolic constituents within the berries and the methods of
detection used in the current study. For example, anthocyanins are a main polyphenolic
c constituent of cranberries and black raspberries which are not quantified using the method
described herein. Further, the bioavailability of polyphenols is also dependent on a number of
factors which may have limited their detection in plasma, e.g., chemical structure of the food
matrix, intestinal absorption, interaction with gut microbiota and inter-subject differences in
physiological, genetic, and biochemical conditions \(^{(55)}\).

**Limitations**

While the cereal bars were formulated to contain similar sugar and fiber composition,
post-production chemical analysis revealed slight differences in fructose, sucrose and insoluble
fiber content between the bars. However, these discrepancies are minor and unlikely to explain
the differences we observed in glycemic, insulinemic and glucoregulatory hormone responses
between the bars. Further, while polyphenol types within the bars were not measured, this data is
reported in Phenol-Explorer \(^{(56)}\) by Wada et al (2002), and a detailed characterization of the
cranberry extract was published by Martín et al, 2015. Additionally, the cyclical reproductive
hormones for the two female participants were not considered and the study was not powered to
assess differences between male and females. Lastly, we did not measure plasma anthocyanins
due to cost constraints and instead chose to focus on flavonoids and phenolic acids, since the
assay allowed measurement of more polyphenolic compounds. Despite these limitations, this
was a comprehensive, highly controlled study examining the glycemic, insulinemic,
glucoregulatory hormone and ex-vivo oxidative stress responses following consumption of
polyphenol-fortified, high carbohydrate cereal bars.

**Conclusion**
Fortification with a high-dose of freeze-dried black raspberries blunted postprandial peak insulin and incretin hormone responses compared to the reference bar, and tended to increase postprandial glycemia compared to the low-dose raspberry bar. Together, these findings suggest that fortifying a high-carbohydrate bar with ~25 g raspberry powder, which contained ~1.2 g of total polyphenols, modulated postprandial glycemia and insulinemia in a dose-dependent manner, thus maintaining glucose availability in the postprandial period. Further research is warranted to determine whether this response has practical benefits in certain scenarios, e.g., when the food item is consumed prior to endurance exercise or to maximize cognitive performance during the later postprandial period. However, these glycemic effects did not translate into meaningful changes in appetite or ex-vivo oxidation of LDL within the immediate postprandial period at the dose consumed and/or with the method used to assess appetite.

Acknowledgements

The authors would like to thank the study participants and technical support personnel within USARIEM’s Military Nutrition Division, with whom this study would not have been possible.

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The authors’ contributions are as follows – TJS designed the study, collected data, conducted analyses and drafted the manuscript; JPK contributed to study design, collected data, conducted analyses, drafted portions of the manuscript related to satiety and revised the article for important intellectual content; MW and CW collected data and assisted in data analyses; CW also assisted in drafting the manuscript. AB and NFF conceived of the study, assisted in study design and revised the article for important intellectual content; C-YOC assessed LDL oxidation and plasma polyphenolic concentrations, and revised the article for important intellectual content; SM contributed to study design and revised the article for important intellectual content. All authors reviewed, edited, and approved the final manuscript.
The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or reflecting the views of the US Army or the Department of Defense. Any citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement of approval of the products or services of these organizations. Study funded by the US Army Medical Research and Materiel Command.

The authors have no financial or personal conflicts of interest to declare.

References

12. Schulze C, Bangert A, Kottra G et al. (2014) Inhibition of the intestinal sodium-coupled glucose transporter 1 (SGLT1) by extracts and polyphenols from apple reduces postprandial blood glucose levels in mice and humans. *Molecular nutrition & food research* 58, 1795-1808.


### Table 1. Pre-trial Energy and Macronutrient Intake

<table>
<thead>
<tr>
<th>Bar Type</th>
<th>Energy (kcal)</th>
<th>Fat (g)</th>
<th>CHO (g)</th>
<th>PRO (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>2280 ± 490</td>
<td>85 ± 27</td>
<td>261 ± 63</td>
<td>117 ± 30</td>
</tr>
<tr>
<td>Black Raspberry Low</td>
<td>2380 ± 740</td>
<td>92 ± 34</td>
<td>255 ± 87</td>
<td>131 ± 44</td>
</tr>
<tr>
<td>Black Raspberry High</td>
<td>2360 ± 710</td>
<td>88 ± 34</td>
<td>260 ± 94</td>
<td>134 ± 35</td>
</tr>
<tr>
<td>Cranberry Low</td>
<td>2400 ± 690</td>
<td>93 ± 31</td>
<td>263 ± 85</td>
<td>130 ± 39</td>
</tr>
<tr>
<td>Cranberry High</td>
<td>2300 ± 620</td>
<td>87 ± 32</td>
<td>252 ± 65</td>
<td>126 ± 32</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bar Type</th>
<th>Energy (kcal)</th>
<th>Fat (g)</th>
<th>CHO (g)</th>
<th>PRO (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>970 ± 120</td>
<td>29 ± 4</td>
<td>125 ± 16</td>
<td>54 ± 7</td>
</tr>
<tr>
<td>Black Raspberry Low</td>
<td>990 ± 170</td>
<td>30 ± 4</td>
<td>128 ± 17</td>
<td>54 ± 7</td>
</tr>
<tr>
<td>Black Raspberry High</td>
<td>990 ± 110</td>
<td>30 ± 4</td>
<td>130 ± 5</td>
<td>54 ± 7</td>
</tr>
<tr>
<td>Cranberry Low</td>
<td>980 ± 120</td>
<td>30 ± 5</td>
<td>127 ± 16</td>
<td>54 ± 7</td>
</tr>
<tr>
<td>Cranberry High</td>
<td>1000 ± 170</td>
<td>31 ± 7</td>
<td>130 ± 22</td>
<td>54 ± 7</td>
</tr>
</tbody>
</table>

All data is mean ± SD.

1 Indicates average daily intake in the two days prior to each trial.
**Table 2. Baseline body weight, glucose and insulin**

<table>
<thead>
<tr>
<th></th>
<th>Reference</th>
<th>Low&lt;sup&gt;1&lt;/sup&gt;</th>
<th>High&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Low&lt;sup&gt;3&lt;/sup&gt;</th>
<th>High&lt;sup&gt;4&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Weight (kg)</td>
<td>82.0 ± 13.9</td>
<td>81.9 ± 14.1</td>
<td>81.7 ± 13.8</td>
<td>82.1 ± 14.0</td>
<td>82.1 ± 13.9</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>90.6 ± 8.0</td>
<td>88.8 ± 7.9</td>
<td>88.6 ± 7.9</td>
<td>89.8 ± 7.3</td>
<td>90.6 ± 9.7</td>
</tr>
<tr>
<td>Insulin (uIU/mL)</td>
<td>6.8 ± 5.5</td>
<td>7.3 ± 6.5</td>
<td>6.3 ± 6.2</td>
<td>5.9 ± 4.7</td>
<td>6.2 ± 5.5</td>
</tr>
</tbody>
</table>

Values are mean ± SD; there were no significant difference between treatments at baseline (p > 0.3).

<sup>1</sup>Contains 10% freeze-dried black raspberry powder per total weight

<sup>2</sup>Contains 20% freeze-dried black raspberry powder per total weight

<sup>3</sup>Contains 0.5% cranberry extract per total weight

<sup>4</sup>Contains 1% cranberry extract per total weight
Table 3. Nutritional Composition and Acceptability of Cereal bars

<table>
<thead>
<tr>
<th></th>
<th>Reference</th>
<th>Low&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Low&lt;sup&gt;4&lt;/sup&gt;</th>
<th>High&lt;sup&gt;3&lt;/sup&gt;</th>
<th>High&lt;sup&gt;5&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Weight (g)</strong></td>
<td>124</td>
<td>122.9</td>
<td>124.9</td>
<td>121.6</td>
<td>125.5</td>
</tr>
<tr>
<td><strong>Energy (kcals)</strong></td>
<td>459</td>
<td>458.4</td>
<td>460.9</td>
<td>451.1</td>
<td>465.6</td>
</tr>
<tr>
<td><strong>Carbohydrates (g)</strong></td>
<td>100</td>
<td>100.7</td>
<td>99</td>
<td>101.7</td>
<td>102.2</td>
</tr>
<tr>
<td>Total Sugars (g)</td>
<td>45</td>
<td>45.5</td>
<td>44</td>
<td>45.3</td>
<td>45.4</td>
</tr>
<tr>
<td>Fructose (g)</td>
<td>3.9</td>
<td>5.8</td>
<td>4.1</td>
<td>4</td>
<td>4.3</td>
</tr>
<tr>
<td>Sucrose (g)</td>
<td>17.3</td>
<td>14.4</td>
<td>15.2</td>
<td>11.7</td>
<td>15.7</td>
</tr>
<tr>
<td>Glucose (g)</td>
<td>14.5</td>
<td>15.9</td>
<td>15.8</td>
<td>19.9</td>
<td>16.3</td>
</tr>
<tr>
<td>Maltose (g)</td>
<td>9.3</td>
<td>9.5</td>
<td>8.9</td>
<td>9.7</td>
<td>9.2</td>
</tr>
<tr>
<td>Total Fiber (g)</td>
<td>6.4</td>
<td>7.9</td>
<td>7.1</td>
<td>9.1</td>
<td>7.3</td>
</tr>
<tr>
<td>Soluble Fiber (g)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Insoluble Fiber (g)</td>
<td>6.4</td>
<td>7.9</td>
<td>7.1</td>
<td>9.1</td>
<td>7.3</td>
</tr>
<tr>
<td>Starch (g)&lt;sup&gt;6&lt;/sup&gt;</td>
<td>48.6</td>
<td>47.3</td>
<td>47.9</td>
<td>47.3</td>
<td>49.5</td>
</tr>
<tr>
<td>Carbohydrates (% total kcals)</td>
<td>87</td>
<td>88</td>
<td>88</td>
<td>88</td>
<td>88</td>
</tr>
<tr>
<td><strong>Fat (g)</strong></td>
<td>3.7</td>
<td>3.7</td>
<td>3.4</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Fat (% total kcals)</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td><strong>Protein (g)</strong></td>
<td>6.2</td>
<td>5.5</td>
<td>5.9</td>
<td>5.7</td>
<td>6.1</td>
</tr>
<tr>
<td>Protein (% total kcals)</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Overall acceptability</td>
<td>7.4 ± 1.2</td>
<td>6.9 ± 1.5</td>
<td>6.6 ± 1.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.2 ± 2.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.8 ± 1.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup>All bars were chemically analyzed for nutritional content (Covance Laboratories, Inc., Madison, WI).

<sup>2</sup>Contains 10% freeze-dried black raspberry powder per total weight

<sup>3</sup>Contains 20% freeze-dried black raspberry powder per total weight

<sup>4</sup>Contains 0.5% cranberry extract per total weight

<sup>5</sup>Contains 1% cranberry extract per total weight

<sup>6</sup>Starch content calculated as total carbohydrates minus total fiber and total sugar.

<sup>a</sup>Marginal model with Bonferroni corrections. Significantly different from reference P ≤ 0.01.
Table 4. Time to peak or nadir, and change from baseline to postprandial peak or nadir, blood concentrations of glucose, insulin, glucoregulatory and appetite-mediating hormones.

<table>
<thead>
<tr>
<th></th>
<th>Reference</th>
<th>Black raspberry</th>
<th>Cranberry</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Low&lt;sup&gt;4&lt;/sup&gt;</td>
<td>High&lt;sup&gt;5&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glucose ∆Max (mmol/L)</td>
<td>2.9 ± 1.2</td>
<td>2.8 ± 0.9</td>
<td>2.9 ± 0.9</td>
</tr>
<tr>
<td>Time to peak glucose (mins)</td>
<td>38 ± 11</td>
<td>38 ± 15</td>
<td>38 ± 17</td>
</tr>
<tr>
<td>Insulin ∆Max (uIU/mL)&lt;sup&gt;1&lt;/sup&gt;</td>
<td>90 ± 64</td>
<td>78 ± 55</td>
<td>75 ± 53&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Time to peak insulin (mins)</td>
<td>49 ± 25</td>
<td>50 ± 21</td>
<td>54 ± 32</td>
</tr>
<tr>
<td>GLP-1 ∆Max (pg/mL)&lt;sup&gt;2&lt;/sup&gt;</td>
<td>25 ± 15</td>
<td>21 ± 14</td>
<td>18 ± 13&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Time to peak GLP-1 (mins)</td>
<td>41 ± 40</td>
<td>33 ± 26</td>
<td>26 ± 14</td>
</tr>
<tr>
<td>GIP ∆Max (pg/mL)&lt;sup&gt;3&lt;/sup&gt;</td>
<td>201 ± 103</td>
<td>211 ± 82</td>
<td>162 ± 97&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Time to peak GIP (mins)</td>
<td>78 ± 31</td>
<td>95 ± 42</td>
<td>71 ± 36</td>
</tr>
<tr>
<td>C-peptide ∆Max (mg/dL)</td>
<td>8 ± 3</td>
<td>8 ± 3</td>
<td>8 ± 3</td>
</tr>
<tr>
<td>Time to peak C-peptide (mins)</td>
<td>49 ± 15</td>
<td>71 ± 31</td>
<td>62 ± 27</td>
</tr>
<tr>
<td>Ghrelin ∆Max (pg/mL)</td>
<td>-64 ± 51</td>
<td>-63 ± 50</td>
<td>-59 ± 58</td>
</tr>
<tr>
<td>Time to nadir glucose (mins)</td>
<td>58 ± 22</td>
<td>68 ± 31</td>
<td>67 ± 28</td>
</tr>
</tbody>
</table>

Values are mean ± SD. ∆Max, change from baseline to peak glucose, insulin, Glucagon-like peptide-1 (GLP-1), glucose-dependent insulino tropic polypeptide (GIP), c-peptide concentrations or nadir acylated ghrelin concentrations.

Data analyzed using marginal models with Bonferroni corrections. ∆Max analyses included fasting blood concentrations as a covariate.

<sup>1</sup>Significant main effect of bar within raspberry treatment (P = 0.02).<sup>a</sup>P = 0.03 versus reference.

<sup>2</sup>Trend for main effect of bar within raspberry treatment (P = 0.09).<sup>b</sup>P = 0.09 versus reference.

<sup>3</sup>Significant main effect of bar within raspberry treatment (P = 0.01).<sup>c</sup>P = 0.01 versus LO-Rasp.

<sup>4</sup>Contains 10% freeze-dried black raspberry powder per total weight.

<sup>5</sup>Contains 20% freeze-dried black raspberry powder per total weight.

<sup>6</sup>Contains 0.5% cranberry extract per total weight.

<sup>7</sup>Contains 1% cranberry extract per total weight.
Table 5. Appetite and ad libitum energy intake following consumption of fortified and reference cereal bars.

<table>
<thead>
<tr>
<th></th>
<th>Reference</th>
<th>Black raspberry</th>
<th>Cranberry</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Low(^3)</td>
<td>High(^4)</td>
</tr>
<tr>
<td><strong>AUC(_{0-180\text{ min}}) (cm*min)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hunger</td>
<td>-530 ± 372</td>
<td>-386 ± 418</td>
<td>-451 ± 448</td>
</tr>
<tr>
<td>Fullness(^1)</td>
<td>509 ± 459</td>
<td>423 ± 353</td>
<td>470 ± 359</td>
</tr>
<tr>
<td><strong>ΔMax (cm)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hunger</td>
<td>-4.8 ± 2.7</td>
<td>-4.7 ± 2.7</td>
<td>-4.6 ± 2.9</td>
</tr>
<tr>
<td>Fullness(^1)</td>
<td>4.7 ± 3.2</td>
<td>4.6 ± 2.3</td>
<td>4.6 ± 2.8</td>
</tr>
<tr>
<td><strong>Ad libitum energy intake (kcal)</strong></td>
<td>1177 ± 438</td>
<td>1093 ± 390</td>
<td>1169 ± 492</td>
</tr>
</tbody>
</table>

Values are mean ± SD. ΔMax, change from baseline to peak fullness or the hunger nadir. Data analyzed using marginal models with Bonferroni corrections. AUC and ΔMax analyses included fasting hunger or fullness as a covariate.

\(^1\)Trend for main effect of bar within cranberry treatment (P ≤ 0.08). \(^a\)P = 0.06 versus reference.

\(^2\)Trend for main effect of bar within raspberry treatment (P = 0.10). No significant post hoc differences.

\(^3\)Contains 10% freeze-dried black raspberry powder per total weight

\(^4\)Contains 20% freeze-dried black raspberry powder per total weight

\(^5\)Contains 0.5% cranberry extract per total weight

\(^6\)Contains 1% cranberry extract per total weight
Figure 1. Participant Disposition. ¹Potential volunteers attended the briefing after seeing informational flyers or as part of their voluntary assignment in the Natick Soldier, Research, Development and Engineering Center’s Human Volunteer Detachment (Natick, MA); ²Two individuals verbally indicated that they did not meet inclusion criteria; ³Not scheduled to participate due to scheduling conflicts; ⁴Withdrawn due to multiple failed catheter attempts.
Figure 2. Baseline and post-prandial glucose (A, B), insulin (C, D), and c-peptide (E, F) concentrations. Note: Error bars represent standard deviations. AUC, inset represents incremental area under the curve 0-180 mins post-prandial. Treatment effect on glucose AUC0-180min response (P=0.04): trend for higher glucose following consumption of black raspberry high versus black raspberry low (P=0.06).
Figure 3: Baseline and post-prandial GLP-1 (A, B), GIP (C, D) and acylated ghrelin (E, F) concentrations. Note: Error bars represent standard deviations. AUC inset represents incremental area under the curve 0-180 mins post prandial. *indicates significant difference between groups, p < 0.05. a-glucose-dependent insulinotropic polypeptide. b-glucagon-like peptide-1. Treatment effect on GIP AUC0-180min response (P=0.001): GIP lower following consumption of black raspberry high versus reference (P=0.014) and black raspberry low (P=0.003).
Figure 4: Ex-vivo LDL oxidation (lag time, seconds) of A) Reference vs black raspberry fortified bars and B) Reference vs cranberry fortified bars. Error bar represent standard deviations.
Figure 5: Baseline and Post-prandial concentrations of vanillic acid (A, B), caffeic acid (C, D) and coumaric acid (E, F). Note: Error bar represent standard deviations. AUCi inset represents incremental area under the curve 0-180 mins post prandial. *indicates significant difference between groups, p < 0.05.
Figure 6: Baseline and Post-prandial concentrations of ferulic acid (A, B), sinapic acid (C, D) and gentisic acid (E, F). Note: Error bar represent standard deviations. AUCi inset represents incremental area under the curve 0-180 mins post prandial. *indicates significant difference between groups, p < 0.05.