A dose–response evaluation of freeze-dried strawberries independent of fiber content on metabolic indices in abdominally obese individuals with insulin resistance in a randomized, single-blinded, diet-controlled crossover trial

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Scope: This study evaluated the dose–response relationship of strawberries, an anthocyanin-rich fruit, on postprandial glucose and insulin concentrations in individuals with insulin resistance (IR), including changes in plasma anthocyanins, markers of oxidative stress, and inflammation. Methods and Results: In a randomized controlled, four-arm, dose–response, crossover trial, 21 adults with IR consumed a high-carbohydrate, high-fat meal with one of four beverages containing 0 g freeze-dried whole strawberry powder (0g FDS, control), 10, 20, or 40 g FDS, controlled for fiber. Blood was collected at 0 min and at 30 min intervals postmeal until 2 h, then hourly until 6 h. Postmeal insulin concentrations (6 h) were significantly reduced after the 40-g FDS beverage compared to other beverages (p < 0.05). Postmeal 6 h glucose concentrations were not different, although mean insulin:glucose ratio was significantly different among beverages (p < 0.05). Pelargonidin-glucuronide was inversely associated with mean insulin concentrations after the 20 and 40 g FDS (p < 0.05). Oxidized low-density lipoprotein was reduced after 20 g FDS (p < 0.05) and IL-6 was not different among treatments. Strawberry intake reduced the insulin demand to manage postmeal glucose in obese individuals with IR, which was related to plasma anthocyanin/pelargonidin concentrations. Conclusion: The data support a role of strawberries in improving insulin sensitivity in people with IR.

1 Introduction

Insulin resistance (IR) is the hallmark feature of the metabolic syndrome and is a major risk factor for developing type 2 diabetes mellitus (T2DM) [1]. In addition to T2DM, IR contributes to cardiovascular diseases (CVD), stroke, macular degeneration, Alzheimer disease, and reduced longevity [1–5]. Obesity, elevated blood glucose, chronically high insulin and nonesterified fatty acid concentrations, and inflammation are important factors in the etiology of IR [6–9]. In westernized countries, 25% of the population has some degree of IR [10].

Fruit and vegetable consumption has been linked to reductions in chronic disease risk, and evidence highlights the health benefits of phytochemicals, such as polyphenols. However, guidance on how much, how often, and whether intake levels should differ among certain at risk groups is a critical gap in developing dietary recommendations for polyphenols and certain fruit and vegetable categories. Anthocyanins are a class of polyphenols that give plants their distinctive red, blue, and purple color. Berries are a rich source of anthocyanins [11]. Epidemiologic observations suggested that the risk of T2DM is inversely associated with the intake of anthocyanin rich foods, and specifically berries [12–15]. Improvements in insulin sensitivity and/or indices of...
oxidative stress and inflammation with berry or anthocyanin intake are possible reasons for the observed risk reductions in T2DM [14, 16].

Several factors play a role in glucose management; most notably, insulin sensitivity in peripheral tissues. Western dietary patterns, characterized by excessive energy intake and high-carbohydrate, high-fat meals, promote impaired insulin action and compensatory hyperinsulinemia, in part due to prolonged hyperglycemia, hyperlipemia, cellular redox imbalances (i.e., oxidative stress), and inflammation; all of which can be observed with a single meal [17–20]. Previous work on overweight men and women indicates that consuming a strawberry drink containing 10 g freeze-dried strawberry (FDS) powder (equivalent to ~110 g fresh fruit) with a high-carbohydrate, high-fat meal significantly attenuated unfavorable postprandial responses, including reducing the amount of insulin required to achieve glucose homeostasis [17, 18]. Whether these effects would extend to people with IR is uncertain and was the focus of the present study.

The present study tested the effect of four dietary intake levels of strawberry fruit on postprandial glucose and insulin kinetic variables in abdominally obese individuals with IR. The primary objective of the study was to characterize the “dose–response” relationship between strawberry intake and postprandial insulin and glucose concentrations to a standard high-carbohydrate, high-fat meal. Secondary objectives aimed to determine if meal-induced metabolic changes were related to plasma anthocyanin concentrations and acute changes in oxidative stress and inflammation.

2 Materials and methods

2.1 Study design and study participants

2.1.1 Design

A randomized, single-center, single-blinded, four-arm, placebo-controlled, 6-h postprandial, crossover study in obese individuals with IR was performed at the Clinical Nutrition Research Center (CNRC) at the Illinois Institute of Technology (Chicago, IL, USA). The study included four separate 6-h postprandial visits to the CNRC. The study was conducted according to the guidelines laid down in the Declaration of Helsinki and the International Conference on Harmonization-Good Clinical Practice, and all procedures involving human subjects were approved by the Institutional Review Board at the Illinois Institute of Technology. Written informed consent was obtained from all subjects before the initiation of the study. The study was conducted in Chicago, IL, USA (Clinical Trial.gov Registration number, NCT01199848).

2.1.2 Study participants

Adult men and women (≥ 18 years) who had a waist circumference of greater than 110 cm and any of the following: fasting blood glucose concentration between 5.5 and 6.9 mmol/L, fasting insulin concentration greater than 75th percentile cutoff of 13.13 μIU/mL, or homeostasis model assessment value for insulin resistance (HOMA-IR) ≥ 1.0 [21] were eligible to participate in the study provided that they did not meet any of the exclusion criteria. Exclusion criteria included potential participants who smoked or who had documented history of chronic disease, such as CVD, diabetes, and cancer, or who had fasting blood glucose ≥ 7.0 mmol/L on two occasions. Additionally, individuals taking medications or dietary supplements that could interfere with the study procedures or endpoints, such as antiinflammatory medications, lipid- or glucose-lowering medications, or antioxidant supplements, or who regularly consumed berry products (>2 cups per day) were not eligible for the study. Women who were pregnant or lactating or anyone reporting allergies or sensitivity to berry products was not eligible to participate in the study.

Twenty-nine subjects were enrolled and randomized and 25 subjects completed the study (Fig. 1). Four people dropped out due to personal reasons or time conflicts. Four additional subjects were excluded from the final analysis because they were found to not meet inclusion/exclusion criteria: one was found to be a current smoker, one was found to have consistently elevated glucose (>7.0 mmol/L) after study start, one was found to be insulin sensitive (IR < 1), and another was found to be taking nonsteroidal antiinflammatory medications. All were revealed at the end of the study.

2.2 Study meals and test beverages

Subjects received a standard breakfast meal accompanied with one of four beverages. The standard meal consisted of a bagel with cream cheese and margarine, a hard-boiled egg, cantaloupe, and whole milk and provided ~ 654 kcal. The beverages were milk based and contained 0, 10, 20, or 40 g FDS (California Strawberry Commission, Watsonville, CA) delivering ~320 kcal. Ten grams of FDS powder is equivalent to approximately 110 g fresh strawberries and each dose of FDS powder was equivalent to 0.7, 1.5, and ~3 cups of fresh strawberries, respectively. The nutrient composition of the meal and beverages is shown in Table 1. The amount of strawberry tested was based on the previous work using a similar beverage recipe and 10 g FDS [18, 22] and other groups that tested between 25 and 50 g FDS [15, 23]. All meals and beverages were prepared in the metabolic kitchen at the CNRC where strict food safety standards were maintained under the supervision of a registered dietitian.

2.3 Study procedures for postprandial visits

Eligible subjects after screening were enrolled and randomized to receive study beverages in one of four computer-generated sequences by the study statistician. On each postprandial visit day, subjects arrived to the CNRC in overnight
Fasted and well-hydrated condition. After the standard admission procedures were completed, a catheter/cannula was placed by a registered nurse in subject’s nondominant arm and a fasting/baseline blood sample was collected (0 min). Thereafter, subjects were provided the breakfast meal and were assigned strawberry beverage to consume in 20 min. Blood samples were collected at 30, 60, 90, 120, 180, 240, 300, and 360 min after the start of the breakfast meal. Postprandial visits were not less than 3 days apart and no more than 14 days apart. Subjects were asked to maintain their usual level of physical activity and consume their usual diet throughout the study with the exception of limiting all berry products during the study and limiting the consumption of polyphenolic-containing foods 3 days prior to each 6-h

Table 1. Nutrient composition of test beverages and standard breakfast meal for postprandial study days

<table>
<thead>
<tr>
<th></th>
<th>0 g FDS beverageb) + standard breakfastc)</th>
<th>10 g FDS beverage + standard breakfast</th>
<th>20 g FDS beverage + standard breakfast</th>
<th>40 g FDS beverage + standard breakfast</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calories (kcal)</td>
<td>973</td>
<td>972</td>
<td>975</td>
<td>979</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>36.9</td>
<td>36.9</td>
<td>36.9</td>
<td>37.8</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>146.2</td>
<td>148.1</td>
<td>149.8</td>
<td>151.4</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>25.4</td>
<td>25.1</td>
<td>25.3</td>
<td>25.8</td>
</tr>
<tr>
<td>Fiber (g)</td>
<td>12.3</td>
<td>12.3</td>
<td>12.3</td>
<td>12.3</td>
</tr>
<tr>
<td>Sugar (g)</td>
<td>89.9</td>
<td>89.0</td>
<td>88.6</td>
<td>85.9</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>49.7</td>
<td>58.0</td>
<td>69.7</td>
<td>88.0</td>
</tr>
<tr>
<td>Total anthocyaninsd) (mg/beverage)</td>
<td>0.04</td>
<td>42.2</td>
<td>87.9</td>
<td>154.5</td>
</tr>
</tbody>
</table>

a) FDS, freeze-dried whole strawberry powder (California Strawberry Commission, Watsonville, CA).
b) Beverages consisted of 0, 10, 20, or 40 g FDS, non-fat milk, evaporated milk, table sugar, strawberry-flavored Nestle Quick mix, Unifiber®, and water. All ingredients are commercially available. Beverages were prepared fresh before drinking.
c) Standard breakfast consisted of bagel with cream cheese and margarine, hard-boiled egg, whole milk, and cantaloupe; all foods were purchased from local grocery stores in Chicago, IL, USA and prepared fresh on each study day.
d) Values represent the mean of anthocyanin concentrations in each beverage randomly measured over the course of the study. Total anthocyanins include the mean of the sum of pelargonidin and cyanidin glycosides.
postprandial visit. Food diaries and subject interviews were used to monitor the subject compliance.

### 2.4 Metabolic markers and analytical chemistry analyses

Blood was collected from indwelling catheters/cannulas at specified time points into EDTA tubes and centrifuged at 12,857 x g for 15 min at 4°C for 15 min to obtain plasma. Aliquots of plasma were stored immediately at –80°C for subsequent analysis. Plasma triglycerides and glucose concentrations were measured using standardized enzyme-based assay kits (Cat. #TR210, intraassay % coefficient of variation (CV) 1.5–3.3 and interassay % CV 1.3–3.5, and Cat. #GL3815, intraassay % CV 2.0–4.5 and interassay % CV 3.5–5.9, respectively; Randox, Antrim, UK) on the Randox Daytona Auto Clinical Analyzer (Randox). Plasma insulin concentration was measured using the AlphaLISA method (AL204C, intraassay % CV 2.9–8.2 and interassay % CV 7.5–12.3, Perkin Elmer, Waltham, MA, USA). Plasma oxidized low-density lipoprotein (Ox-LDL) and IL-6 concentrations were measured using ELISA assay kits (Cat. #10-1143-01, intraassay % CV 5.5–6.2 and interassay % CV 4–6.2, Mercodia Inc., Winston Salem, NC, USA and Cat. #HS600B, intraassay % CV 6.9–7.8 and interassay % CV 6.5–9.6, R&D Systems, Minneapolis, MN, USA, respectively). All assay protocols were performed according to the manufacturers’ instructions and appropriate quality controls were used as applicable. The oxygen radical absorbance capacity (ORAC) assay was performed according to the method described by Prior et al. [24] with minor modifications for analyzing beverages and plasma [17, 25]. Anthocyanin analysis followed the procedures described previously [22, 25] and focused on measuring parent compounds and conjugated parent metabolites maintaining the base C6-C3-C6 structure, since these have been shown previously to be the main anthocyanin compounds increasing in plasma within the first few hours of consumption [17, 22], which is consistent with our study design. Agilent Technologies 1260 Infinity HPLC system with a 6460 Triple Quadrupole Mass Spectrometer (Agilent Technologies, Santa Clara, CA, USA) was used for the analysis. Briefly, samples (500 μL) were acidified to pH 2 with formic acid and 50 μL of delphinidin-3-O-glucoside (1 μg/mL) was added as an internal standard. Samples were extracted with 1.5 mL of acetonitrile, centrifuged at 12,857 x g for 15 min at 4°C and then reextracted with 1.5-mL methanol. Extracts were pooled, evaporated under nitrogen at 35°C, reconstituted in 250 μL of mobile phase, and centrifuged at 12,857 x g for 30 min at 4°C. Samples were transferred to amber HPLC vials and immediately analyzed by LC-MS/MS. Anthocyanin compounds were quantified using standards purchased from Chromadex Inc. (Santa Ana, CA, USA). Matrix-matched pelargonidin-3-O-glucoside (P3G) and cyanidin-3-O-glucoside (C3G) standard curves (range 0.1–1000 ng/mL) were used to relatively quantify all pelargonidin-based or cyanidin-based compounds, respectively.

### 2.4.1 Definitions and calculations

HOMA-IR was calculated using fasting insulin (μIU/mL) and fasting glucose (mg/dL) concentration divided by 405 [26]. Peak plasma concentrations (Cmax) are the highest concentration of analytes or anthocyanin metabolites measured during the postprandial period of 0–6 h. The peak incremental increase is baseline corrected magnitude of increased analyte concentration postmeal calculated by subtracting subjects’ respective fasting/baseline concentration from their highest postprandial concentration (Cmax – baseline/fasting). The time to maximum plasma concentrations (Tmax) was defined as the time in minutes at which Cmax was achieved. Incremental area under the 6-h concentration curves (AUC) was calculated using the linear trapezoidal method with baseline corrected values [27]. Relative bioavailability of anthocyanins was based on parent compounds and metabolites maintaining the parent C6-C3-C6 flavonoid structure. Relative bioavailability was calculated based on compound/metabolite AUC0-6 h corrected for individual plasma volume based on sex and body weight (kg) according to the methods described in the technical manual of American Association of Blood Banks where plasma volume is estimated to be 36 mL/kg for women and 40 mL/kg for men [28]. Percent bioavailability was calculated by dividing the plasma mass (nmol) by the mass of the anthocyanin compound per beverage (nmol) and multiplying by 100.

### 3 Statistical analysis

Subject characteristics were analyzed using descriptive statistics and tabulated. All efficacy variables were first examined for normality and data not conforming to normal distribution patterns were log transformed prior to analysis. Mixed-model analysis of repeated measures was performed on each quantitative efficacy variable to test the main effects of FDS treatment beverages (0, 10, 20, or 40 g FDS), time (min), and by time by treatment interaction using PROC MIXED via Window PC-SAS (version 9.3; SAS Institute Inc., Cary, NC, USA). The Kenward–Rogers correction and the method of restricted maximum likelihood were used in all mixed models. Anthocyanin pharmacokinetic parameters (Cmax, AUC, Tmax, and bioavailability) were analyzed using one-way analysis of variance (ANOVA) followed by a Bonferroni adjusted t-test for the pairwise multiple comparisons using SigmaPlot 11.0 (Systat Software Inc., San Jose, CA). Correlational analyses between endpoints of interest were conducted using Spearman rank correlation procedures at specific time points and general linear model (GLM) correlations derived from an analysis of variance to allow for repeated measures within subjects [29].

The results of the statistical analysis were presented as least square means ± SEMs unless indicated otherwise. Statistical significance was based on two-sided treatment comparison at the 5% significance level under a null hypothesis of no difference among treatments. Sample size estimates

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Table 2. Baseline characteristics of study participants

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total subjects (n = 21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>39.8 ± 13.8</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>40.2 ± 7.2</td>
</tr>
<tr>
<td>Midpoint waist circumference (cm)</td>
<td>117.5 ± 12.4</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>122.0 ± 13.7</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>71.8 ± 9.9</td>
</tr>
<tr>
<td>Capillary fasting glucose</td>
<td>5.0 ± 0.6</td>
</tr>
<tr>
<td>concentration (mmol/L)</td>
<td></td>
</tr>
<tr>
<td>Fasting plasma glucose concentration (mmol/L)</td>
<td>5.8 ± 0.5</td>
</tr>
<tr>
<td>Fasting plasma insulin concentration (µIU/mL)</td>
<td>14.9 ± 10.9</td>
</tr>
<tr>
<td>HOMA-IR (b)</td>
<td>3.9 ± 3.1</td>
</tr>
<tr>
<td>Race, n (%)</td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>9 (42.9)</td>
</tr>
<tr>
<td>African-American</td>
<td>12 (57.1)</td>
</tr>
<tr>
<td>Gender, n (%)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>5 (23.8)</td>
</tr>
<tr>
<td>Female</td>
<td>16 (76.2)</td>
</tr>
</tbody>
</table>

a) Values are the mean ± SD of variables at screening, except where noted otherwise.
b) Values represent the average of fasting values from each postprandial study day.

were based on the previous work with 10 g FDS beverage in overweight individuals assuming comparisons between FDS versus control beverage and mean difference in insulin integrated response of 8 µIU/mL, standard deviation of 11 [17]. A total sample size of 18 subjects would provide >80% power to detect significant treatment-related differences in insulin responses.

4 Results

4.1 Demographic and baseline characteristics

Twenty-nine participants were recruited. Four participants withdrew after the first visit for personal reasons and four participants were excluded from the analysis as described in section 2 and shown in Fig. 1. The evaluable data set included 21 individuals who collectively had a mean ± SD age of 39.8 ± 13.8 years and a mean BMI of 40.2 ± 7.2 kg/m² (Table 2). Subjects were generally hyperinsulinemic (mean fasting insulin ±SE 14.9 ± 10.9 µIU/mL) and had a mean fasting glucose of 5.8 ± 0.5 mmol/L. The IR of the group was 3.9 ± 3.1 according to the HOMA-IR calculation. Sixteen subjects met criteria for prediabetes based on the average of four fasting glucose concentrations (>5.6 mmol/L) from each postprandial visit. Body weights were maintained consistent throughout the study (data not shown). All beverages were well liked and tolerated. No adverse events related to treatments were reported during the study.

4.2 Composition of beverages consumed by study participants with the standard meal

The nutrient compositions of the study beverages are shown in Table 1 as consumed with the standard meal. Total energy consumed was ~975 kcal. The anthocyanin content of the beverages increased with increasing strawberry powder ranging from 0 to 155 mg (i.e., 0–369 µmol/ beverage). The ORAC values of the 0, 10, 20, and 40 g FDS beverages were 0 ± 0.0, 9.3 ± 0.7, 19.1 ± 0.6, and 33.6 ± 1.8 µmol Trolox equivalents (TE)/mL, respectively.

4.3 Effect of strawberry consumed with a standard Western-type meal on postprandial plasma glucose, insulin, and triglyceride responses in individuals with IR

Overall, the 40 g FDS beverage significantly reduced postmeal insulin concentrations over 6 h compared to all other beverages (~12% reduction), except the 20 g FDS beverage (p > 0.05), when consumed with the standard meal (main effect of treatment p = 0.04, log transformed, baseline adjusted, Table 3 and Fig. 2). Additionally, the 40 g FDS beverage resulted in the lowest absolute peak insulin concentration (p = 0.047, Fig. 2) and incremental increase from baseline (p = 0.03, Table 3 and Fig. 2) postprandially, which was marginally lower (p = 0.055) than the insulin peak after the 0 g FDS control beverage and not different from the 10 or 20 g FDS beverages (p > 0.05). No significant differences among treatments were observed for mean 6 h postprandial glucose concentrations or peak glucose concentrations (Table 3 and Fig. 3). However, the mean postprandial (6 h) insulin to glucose ratio was significantly different among treatments (p = 0.008), emphasizing the reduced insulin requirements after the 40-g FDS beverage to manage the postprandial glucose load (40 g FDS < 0 g FDS, p = 0.005; 40 g FDS < 10 g FDS, p = 0.003). The mean insulin to glucose ratio data suggested a trend for reduced insulin requirements with the 20 g FDS beverage compared to 0 g FDS (p = 0.11) and the 10 g FDS beverage (p = 0.08). The mean insulin to glucose ratio over the 6-h postprandial period was not different between the 20 and 40 g FDS beverages (p = 0.24). No significant differences in triglyceride concentrations were observed (Table 3). Peak concentrations of triglycerides from baseline were also not different among beverage treatments (data not shown).

4.4 Effect of strawberry consumed with a standard Western-type meal on postprandial plasma Ox-LDL and antioxidant capacity and IL-6

Oxidative stress was assessed using two markers: a specific marker of oxidative modification of LDL particles (Ox-LDL) and the nonspecific assessment of oxygen radical scavenging
capacity using the ORAC assay. Mean baseline concentrations of Ox-LDL were significantly different among subjects and treatments ($p < 0.05$). Normalizing responses to subjects own baseline revealed a significant reduction in Ox-LDL after the 20 g FDS beverage (–3.0 ± 0.8 U/L) compared to all other beverages; 0, 10, and 40 g FDS beverages (–0.1 ± 0.8, –0.3 ± 0.8, and –0.7 ± 0.8, respectively, $p < 0.05$, Fig. 4 and Table 3). The antioxidant capacity of plasma assessed by ORAC showed an ~10% increase in antioxidant capacity of plasma in the hydrophilic fraction after the 20 g FDS beverage compared to other beverages; however, this was not statistically significant ($p > 0.05$, data not shown).

A significant main effect of time ($p < 0.0001$) was observed for IL-6, but no difference was observed among treatments. In general, IL-6 responses gradually increased over the 6-h period with the highest values observed at the 6-h time point (0 versus 6 h, $p < 0.05$), but were not different among beverages (Table 3).

### 4.5 Anthocyanins in FDS beverages, relative bioavailability, and pharmacokinetic profile

The most abundant anthocyanins in the FDS beverages were P3G and C3G. Beverages containing 0, 10, 20, and 40 g FDS powder delivered 0.1 ± 0.0, 100.9 ± 2.7, 210.3 ± 5.7, and 368.8 ± 12.1 μmol (i.e., 0–155 mg) total anthocyanins, respectively ($p < 0.05$), among which P3G was 0, 81.3 ± 1.8, 166.8 ± 4.5, and 285.8 ± 8.9 μmol, respectively ($p < 0.05$). C3G concentrations were 0.1 ± 0.0, 3.7 ± 0.4, 9.7 ± 1.2, and 22.0 ± 1.3 μmol, respectively.

After drinking the 10, 20, and 40 g FDS beverages, the anthocyanin that appeared in plasma in greatest amount...
was the anthocyanin metabolite pelargonidin-O-glucuronide (PG). P3G and C3G were also detectable but at much lower concentrations. No anthocyanins were detected in plasma of subjects after drinking the 0 g FDS/control beverage. Pharmacokinetic analysis of the primary anthocyanin metabolites is presented in Table 4. Plasma concentration by time curve of the most abundant metabolite, PG, is illustrated in Fig. 5. In general, there was a significant dose-related increase in $C_{\text{max}}$ and AUC for all three plasma anthocyanins detected and their conjugated parent C6-C3-C6 metabolite; however, when expressed relative to dose, the percent bioavailability decreased as the dose/amount of anthocyanins increased (Table 4). Bioavailability of anthocyanins ranged from 0.1 to 2.1% differing between anthocyanins/metabolites and dependent on anthocyanin doses ($p < 0.05$). Relative bioavailability of the pelargonidin-based anthocyanins declined significantly between 10 and 20 g dose ($p > 0.05$), but not significantly between the 20 and 40 g FDS dose. Alternatively, bioavailability significantly declined between 10 and 20 g FDS ($p < 0.05$) and between 20 and 40 g FDS ($p < 0.05$) for C3G.

### 4.6 Correlational analysis between insulin, glucose, Ox-LDL and IL-6, and anthocyanin metabolites

#### 4.6.1 Insulin and glucose

Repeated measures correlational analysis revealed significant inverse associations within subjects between PG and insulin (with the 20 and 40 g FDS beverages, Spearman correlation

![Graph showing glucose concentration over time](https://via.placeholder.com/150)

**Figure 3.** Postprandial glucose concentrations in abdominally obese individuals with insulin resistance: dose–response effect of strawberry containing beverages (10, 20, and 40 g FDS) versus control beverage (0 g FDS) when consumed with a standard high-carbohydrate, high-fat meal, $n = 21$. FDS, freeze-dried whole strawberry powder.

![Graph showing changes in Ox-LDL](https://via.placeholder.com/150)

**Figure 4.** Changes in Ox-LDL in the plasma of abdominally obese individuals with insulin resistance after consuming a standard high-carbohydrate, high-fat meal with beverages containing different amounts of strawberry (10, 20, and 40 g FDS) compared to control beverage (0 g FDS), $n = 21$. *Significant differences among groups ($p < 0.05$). FDS, freeze-dried whole strawberry powder.
Table 4. Anthocyanin relative bioavailability and kinetic profile in plasma after consuming strawberry beverages with a standard meal in obese individuals with insulin resistance

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0 g FDS beverage</th>
<th>10 g FDS beverage</th>
<th>20 g FDS beverage</th>
<th>40 g FDS beverage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma bioavailability (^a)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pelargonidin-3-O-glucoside as pelargonidin-3-O-glucoside</td>
<td>0.00 ± 0.00</td>
<td>0.13 ± 0.02(^d)</td>
<td>0.11 ± 0.01(^b)</td>
<td>0.10 ± 0.01(^b)</td>
</tr>
<tr>
<td>Pelargonidin-based anthocyanins as pelargonidin glucuronide</td>
<td>0.00 ± 0.00</td>
<td>1.47 ± 0.10(^a)</td>
<td>1.16 ± 0.09(^b)</td>
<td>1.07 ± 0.08(^b)</td>
</tr>
<tr>
<td>Cyanidin-3-O-glucoside as cyanidin-3-O-glucoside</td>
<td>0.00 ± 0.00</td>
<td>2.11 ± 0.41(^a)</td>
<td>1.19 ± 0.14(^b)</td>
<td>0.85 ± 0.08(^b)</td>
</tr>
<tr>
<td>Pharmacokinetic variables (^b)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pelargonidin-3-O-glucoside</td>
<td>(C_{\text{max}}) (nmol/L)</td>
<td>0.00 ± 0.00(^a)</td>
<td>6.8 ± 0.6(^b)</td>
<td>10.7 ± 0.8(^c)</td>
</tr>
<tr>
<td>(T_{\text{max}}) (min)</td>
<td>0.00 ± 0.00(^a)</td>
<td>111.2 ± 6.2(^b)</td>
<td>121.8 ± 10.4(^b)</td>
<td>137.6 ± 11.8(^b)</td>
</tr>
<tr>
<td>(\text{AUC}_{0-6 \text{ h}}) (nmol·h/L)</td>
<td>0.00 ± 0.00(^a)</td>
<td>23.1 ± 2.8(^b)</td>
<td>38.8 ± 3.8(^c)</td>
<td>64.2 ± 4.3(^d)</td>
</tr>
<tr>
<td>Pelargonidin-glucuronide</td>
<td>(C_{\text{max}}) (nmol/L)</td>
<td>0.00 ± 0.00(^a)</td>
<td>72.7 ± 5.1(^b)</td>
<td>116.8 ± 10.1(^c)</td>
</tr>
<tr>
<td>(T_{\text{max}}) (min)</td>
<td>0.00 ± 0.00(^a)</td>
<td>162.4 ± 10.0(^b)</td>
<td>158.8 ± 8.8(^b)</td>
<td>172.9 ± 7.1(^b)</td>
</tr>
<tr>
<td>(\text{AUC}_{0-6 \text{ h}}) (nmol·h/L)</td>
<td>0.00 ± 0.00(^a)</td>
<td>317.7 ± 16.4(^b)</td>
<td>522.8 ± 8.0(^c)</td>
<td>829.0 ± 62.1(^d)</td>
</tr>
<tr>
<td>Cyanidin-3-O-glucoside</td>
<td>(C_{\text{max}}) (nmol/L)</td>
<td>0.00 ± 0.00(^a)</td>
<td>4.8 ± 0.5(^b)</td>
<td>6.9 ± 0.7(^c)</td>
</tr>
<tr>
<td>(T_{\text{max}}) (min)</td>
<td>0.00 ± 0.00(^a)</td>
<td>118.2 ± 9.8(^b)</td>
<td>130.6 ± 9.9(^b)</td>
<td>135.9 ± 7.3(^b)</td>
</tr>
<tr>
<td>(\text{AUC}_{0-6 \text{ h}}) (nmol·h/L)</td>
<td>0.00 ± 0.00(^a)</td>
<td>17.1 ± 2.9(^b)</td>
<td>26.0 ± 2.4(^c)</td>
<td>42.7 ± 3.8(^d)</td>
</tr>
</tbody>
</table>

\(a\) Values represent mean and standard error (SE) of calculated percent bioavailability of indicated strawberry anthocyanins/metabolites after drinking beverage treatments \((n = 17)\). Values with different letters are significantly different, \(p < 0.05\).

\(b\) Values represent mean and standard error (SE) of calculated pharmacokinetic variables of indicated strawberry anthocyanins/metabolites after drinking beverage treatments \((n = 17)\). Values with different letters are significantly different, \(p < 0.05\).

FDS, freeze-dried whole strawberry powder; AUC, area under the concentration curve; \(C_{\text{max}}\), concentration maximum = absolute peak concentration achieved during the 6 h period; \(T_{\text{max}}\), time of \(C_{\text{max}}\).

coefficients \((r)\), \(r = -0.31, p = 0.001\) and \(r = -0.20, p = 0.03\), respectively) and glucose (10, 20, and 40 g FDS beverages, \(r = -0.25, p = 0.01; r = -0.34, p = 0.0002;\) and \(r = -0.28, p = 0.003\), respectively). Correlational analysis by time point, reflecting correlation across subjects, corroborated these findings showing the strongest associations with PG and insulin in response to the 40 g FDS \((r = 0.53, p = 0.03)\) and 20 g \((r = -0.53, p = 0.04)\) beverages at 120 min. At 180, 240 and 300 min, representing clearence side of glucose curve, glucose and P3G were strongly and negatively correlated \((r = -0.70, p = 0.004; r = -0.80, p = 0.0002;\) and \(r = -0.54, p = 0.03\), respectively) after the 20 g FDS beverage. P3G was also

![Figure 5. Dose/amount–response profile of pelargonidin-glucuronide (PG) in the plasma of abdominally obese individuals with insulin resistance after consuming strawberry and control beverages with a standard meal, \(n = 17\). Different letters indicate differences in the area under the response curve (AUC), 0–6 h, \(p < 0.05\). FDS, freeze-dried whole strawberry powder.](www.mnf-journal.com)
inversely associated with insulin and glucose after the 10 g FDS beverage up to 90 min ($p < 0.05$).

### 4.6.2 Oxidative stress and inflammatory markers

Correlational analysis of anthocyanin metabolite data and changes in postprandial oxidative stress parameters revealed significant inverse association between changes in Ox-LDL and P3G, and PG and C3G after the 20 g FDS beverage ($r = -0.27, p = 0.03$; $r = -0.36, p = 0.003$; $r = -0.27, p = 0.03$, respectively); however, AUC analysis suggested that only C3G was negatively correlated with Ox-LDL ($r = -0.59, p = 0.02$).

A positive correlation between changes in IL-6 and all three metabolites after the 10 g and 20 g FDS beverages were evident ($p < 0.02$), however no associations were observed after the 40 g FDS beverage ($p > 0.05$).

### 5 Discussion

IR is a critical metabolic abnormality and possibly one of the most important common antecedents of CVD and T2DM; both major public health concerns in the United States and other parts of the world [30]. In this randomized controlled trial, we observed reduced postprandial insulin concentrations in obese individuals with IR when strawberries were included in a beverage equivalent to ~3 cups (40 g FDS, 440 g fresh weight) and drank with a high-carbohydrate, high-fat breakfast meal representative of Western eating patterns. Further, postprandial oxidative modification of LDL was reduced after the 20 g FDS beverage and inverse correlations with plasma anthocyanin metabolites and insulin, glucose, and Ox-LDL were observed. In the context of usual intake, the results of this study were observed in free-living subjects with limited intake of berries and anthocyanin-containing products, in general, and specifically leading into study days. The results agree with common observations that the effects of polyphenols appear to be most apparent in biology when the system is being challenged or is compromised, such as by smoking, consumption of energy dense, consumption of energy dense, density, high-fat Western type diets, obesity, IR [18, 31, 32], and that the presence of anthocyanin metabolites in plasma during certain challenges to the system may be an important factor in efficacy responses.

Strawberries contain appreciable amounts of (poly)phenols, including flavonols, flavanols, phenolic acids, and ellagitannins, but are best known for their anthocyanin content, which contribute >75% of total phenolic compounds [33]. The current study examined the effects of strawberry consumption at different doses in individuals already exhibiting IR. A growing area of interest is to develop a solid knowledge base for understanding the health value of fruits and vegetables in multiple populations, including those at higher risk for certain diseases. Anthocyanins and anthocyanin-rich foods have attracted particular attention owing to recent epidemiological and clinical reports describing reduced CVD and diabetes disease risk with increased intake

[15, 16, 34]. Strawberries are among the most commonly consumed anthocyanin-rich foods. Strawberries are available in fresh and frozen forms providing year round accessibility for inclusion in the diet regularly [35]. However, specific guidance on how much, how often and whether intake levels should differ among certain at risk groups is a critical gap in developing dietary recommendations. The results presented here begin to fill those gaps and suggest that consuming 1.5–3 cups (220–440 g fresh weight) of strawberries in the context of modern day dietary patterns can beneficially impact postprandial metabolic balance.

The impetus of this work was derived from our previous clinical work with strawberries using 10 g FDS and ~110 g fresh weight strawberries, which suggested improvements in postprandial insulin action in overweight men and women consuming a typical American high-carbohydrate, high-fat breakfast meal. In that study, there was a modest ~2.0 g difference in fiber content between the two dietary regimens [17], which may have contributed at least partially to the observed effects. Stull and colleagues showed improved insulin sensitivity using the euglycemic-insulinemic clamp method in obese individuals with IR after 6 weeks of consuming the equivalent of two cups of blueberries a day delivering ~7 g of fiber/day [14]. In a cross-sectional study, Jennings et al. reported lower fasting insulin and improved IR with increasing anthocyanin-rich food intake after controlling for multiple variables, including whole grains, but not for the broader variable of dietary fiber, which would also account for fruit and vegetable fibers [16]. The health benefits of dietary fiber are well recognized and the current study was designed to assess the effects of strawberries concentrating largely on the polyphenolic components and less on the fiber effects. Accordingly, fiber content was controlled across beverage groups and the bioavailability and pharmacokinetic profile of parent and conjugated strawberry anthocyanins were characterized and analyzed relative to metabolic, inflammatory, and oxidative stress outcome variables. As evidenced by this work, strawberries provide a benefit on postprandial insulin responses beyond the contribution of fiber. Further, we found inverse associations between insulin and glucose responses and P3G and PG starting early in the postmeal time period for the former that switched to PG with increasing strength during peak metabolite concentration time periods (~120–240 min) after the 20 g and 40 g FDS beverages. Correlations between anthocyanin metabolites and plasma Ox-LDL were observed after the 20 g FDS beverage suggesting some anti-oxidative protection or activity; however, this was not observed in the higher 40 g FDS condition where insulin activity appeared to be improved. Collectively, the data suggest that strawberry polyphenols including their anthocyanin content are acting on biological endpoints of interest for metabolic health in a dose-contingent manner. The beneficial effects of strawberries/polyphenol components may be observed (and maximized) at different intake and plasma concentration levels depending on the clinical endpoint and population under study. Further, the data suggest strawberry polyphenols are...
working beyond traditional antioxidant properties, and likely working through cellular mechanisms impacting insulin signaling. In skeletal muscle cells treated with strawberry extracts (prepared from the FDS of the present study) under varied metabolic stress conditions typical of diabetes, such as oxidative stress, hyperglycemia, and hyperlipidemia, we have shown favorable changes in phosphorylation patterns among serine (inhibitory, reduced) and tyrosine (stimulatory, increased) residues of the insulin receptor substrate-1, insulin receptor, and AKT/P13 pathways that are critical to effective insulin signaling and glucose uptake [36, 37]. These data are corroborated by other published preclinical mechanistic work on anthocyanins and insulin signaling [13, 38].

The study had the following limitations: (1) the study was single blinded—a placebo FDS powder was not available to make the study double blinded, (2) lack of plasma data on other non-anthocyanin strawberry polyphenols and their metabolites, such as flavonols or phenolic acids, may have yielded additional insight on the relationship between strawberry polyphenols and bioactivity, (3) additional inflammatory marker assessment that may have provided broader insight to the strawberry associated inflammatory-IR relationship, and (4) finally, the fiber controlled aspect of the study was a strength for identifying the insulin and insulin-glucose related effects of strawberries beyond the functional properties of their fiber content; however, this approach likely minimized observing a larger effect size of strawberry beverages from the control condition and a significant effect on glucose variables due to additive or synergistic effects of strawberry’s bioactive components, including their fiber content.

To the best of our knowledge, this is the first study to examine the dose/amount–response relationship between strawberry intake, an anthocyanin-rich food, and postprandial metabolism in people with IR. This is an important study because it is the type of data required for devising dietary recommendations for fruit intake and anthocyanin phytochemical intake targeting national health concerns, such as the growing population with metabolic disorders rooted by obesity and IR.

B.B.F. designed the research, reviewed data, and interpreted results, and she had primary responsibility for the final content of the manuscript; I.E. and E.P. conducted the research and acquired data; I.E., H.W., and L.V. performed laboratory analysis and interpretation; E.P. conducted statistical analyses; E.P. and B.B.F. wrote the manuscript. All authors read and approved the final manuscript.

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The authors have declared no conflict of interest. B. B.-F. has received honorarium for speaking by CSC. This trial was registered at clinicaltrials.gov as NCT01199848.

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