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The effect of dairy and non-dairy beverages consumed with high glycemic cereal on subjective appetite, food intake and post-prandial glycemia in young adults

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Abstract

Objective: To compare the effect of dairy and non-dairy beverages when consumed with carbohydrate at breakfast on subjective appetite, food intake (FI) and post-prandial glycemia (PPG) in healthy young adults.

Design: Twenty-six healthy males and females (13 males and 13 females; 23.0 ± 2.6 years; BMI: 22.3 ± 1.5 kg/m²) participated in a randomized crossover study. They consumed non-isocaloric amounts (250 mL) of almond beverage, soy beverage, 1% fat milk, yogurt beverage and water (control) with cereal and 120 min later, an ad libitum meal. Subjective appetite, PPG and insulin were measured at baseline and at intervals before and after the meal at which FI was measured.

Results: Post-treatment blood glucose was lowest following soy beverage compared to all treatments but was not different from milk ($p=0.0002$). There were no differences between any other treatments. However, over the first hour, PPG for all treatments was 27% lower compared to water ($p<0.0001$). Milk and yogurt beverage led to the highest insulin concentrations post-treatment ($p<0.0001$) but there were no differences between treatments post-meal. All treatments reduced appetite and led to lower FI at the meal compared to water but FI was lower after milk compared to all treatments except yogurt beverage ($p<0.0001$).

Conclusion: Both dairy and non-dairy beverages consumed with a high glycemic cereal at breakfast increased satiety and decreased FI compared to water with cereal. Despite higher carbohydrate content, all beverages led to similar or lower PPG than the water breakfast but dairy beverages increased insulin more than non-dairy beverages.

Key words: dairy, non-dairy beverages, appetite, food intake, glycemia, satiety

Introduction

Two-thirds of Canadian adults are overweight or obese (Kenneth and Eggleton 2016) and many have type 2 diabetes (T2D) (Public Health Agency of Canada 2011). Foods that increase satiety and decrease PPG have been proposed to be effective strategies against obesity and T2D. To encourage identification of these foods, Health Canada (HC) released two draft guidance documents for food health claims for “satiety” (Health Canada 2012) and “the reduction in post-prandial glycemic response” (Health Canada 2013). Both documents emphasize the testing of foods in usual serving sizes when tested alone or within a usual meal context.

Dairy beverages are often reported to promote satiety but effects on FI and PPG are less clear (Harper et al. 2007, Maersk et al. 2012). Milk proteins are known to contribute to the regulation of satiety, FI and PPG (Akhavan et al. 2014). However, milk as a whole has greater effects than that predicted from the additive effects of its components, highlighting the importance of consuming milk as a whole entity (Lorenzen et al. 2012, Panahi et al. 2014). Milk consumed with glycemic carbohydrate attenuates PPG (Panahi et al. 2013, Sun et al. 2015), but the effect of dairy beverages consumed in meals of typical serving sizes on satiety and FI has not been reported. Similarly the effects of consumption of non-dairy substitutes (Jacobsen 2013) that are often lower in energy and protein content than milk have not been reported.

Therefore, the primary objective of this study was to compare the effects of 1% milk, yogurt beverage, soy beverage, almond beverage and water consumed with a high glycemic breakfast cereal on PPG and satiety, as measured by subjective appetite, prior to and after an ad libitum meal 120 min later in healthy young men and women. The second objective was to determine

if a health claim could be made by applying HC's guidance document for comparative PPG claims. We hypothesized that dairy beverages consumed with breakfast cereal decrease PPG and later FI, and increase satiety more than dairy substitutes or the cereal alone.

Materials and Methods

Subjects

Men and women between the ages of 20-30 years with a body mass index (BMI) of 20.0-24.9 kg/m² participated. Participants were recruited through advertisements posted online and around the campus of the University of Toronto. Exclusion criteria included smokers, breakfast skippers, lactose intolerance and allergies to study foods, dislike of study foods, trying to lose or gain weight, elite athletes, use of protein or fibre supplements, prescription medication (excluding hormonal contraceptives), fasting blood glucose of ≥ 5.6 mmol/L, diabetes or any medical conditions that could interfere with study outcomes. Restrained eaters as identified by a score of ≥ 11 on the Eating Habits Questionnaire were also excluded (Herman and Polivy 1980). For women, regular monthly menstrual cycles were a requirement. Since reduced insulin sensitivity has been observed during the luteal phase of the menstrual cycle (Escalante Pulido and Alpizar Salazar 1999), females were scheduled only during their follicular phase. The sample size was based on previous short-term FI and glycemia studies with dairy (Panahi et al. 2013, El Khoury et al. 2014). Participants were financially compensated and the study protocol was approved by the Human Subject Review Committee at the University of Toronto Ethics Review Office.

Study Design

This experiment used a randomized, non-blinded, crossover design. Each treatment was provided once per week for men, but only during the first two weeks of their menstrual cycle

for women. The order of treatments was randomized using a randomization block design generated with a random generator script in SAS version 9.3 (SAS Institute Inc., Cary, NC, USA). Each of five treatments was consumed with cereal as part of a breakfast meal. An ad libitum meal was served 120 min following the breakfast meal. Subjective appetite, blood glucose and insulin were measured at baseline and at intervals before and after the ad libitum meal, during which FI was measured.

Treatments

Treatments were non-isocaloric serving sizes of: 1) almond beverage (Silk; Broomfield, USA), 2) soy beverage (Silk, Broomfield, USA), 3) 1% fat milk (Neilson Dairy, St-Laurent, Canada), 4) yogurt beverage (vanilla-flavoured) (Yop; General Mills, Mississauga, Canada) and 5) water (control). The serving size of 250 mL was chosen to represent one serving size as listed on the products' Nutrition Facts Table as required by HC's guidelines for a PPG health claim. Each treatment was part of a breakfast meal that also included 54 g of Cheerios cereal (General Mills, Mississauga, Canada). One serving size of Cheerios is listed as one cup or 27 g. Two cups were used in order to accentuate the glycemic responses to the beverage treatments. Plain Cheerios tested with 50 g available carbohydrates has a Glycemic Index (GI) of 74 compared to 50 g of glucose (GI = 100) (Foster-Powell et al. 2002). In addition to the beverage serving sizes, breakfast was chosen as the meal consistent with HC guidance that treatments be consumed at a suitable time of day and in a manner reflecting usual consumption. The nutritional composition of each beverage, the cereal and each breakfast is outlined in Table 1.

All beverages were served chilled in a cup. Participants were instructed to pour the treatment into the bowl of cereal and consume the breakfast with a spoon and finish any leftover liquid

in the bowl. An additional 100 mL of water was provided when they had finished eating to reduce the aftertaste of the breakfast meal.

Protocol

Each participant was scheduled to arrive at the same time (between 8:30 and 10 am) and day of the week to the Department of Nutritional Sciences at the University of Toronto for each of their five study sessions. Participants were required to observe a 12 h overnight fast, except for water, which was permitted until 1 h before the session. They were also instructed to refrain from vigorous exercise and alcohol consumption the day before their sessions and to eat similarly the evenings before.

Upon arrival, participants completed questionnaires to ensure no unusual deviations from their diet and lifestyle patterns from the previous day and current morning. If they were deemed unfit to participate (e.g. due to sickness), they were rescheduled. Participants then completed visual analogue scale (VAS) questionnaires to assess their “Food Intake and Activity Level”, “Sleep Habits”, “Stress Factors”, “Feelings of Fatigue”, “Physical Comfort” and “Motivation to Eat” (Panahi et al. 2013, El Khoury et al. 2014). The “Motivation to Eat” VAS consisted of four appetite questions assessing the participants’ “desire to eat” (DTE), “hunger”, “fullness” and “prospective food consumption” (PFC). An average appetite score was calculated from these four questions as previously described (Patel et al. 2013).

Participants provided a baseline finger prick capillary blood sample using a single-use lancet (Unistik; Oxfordshire, UK). Plasma blood glucose concentration was measured with a handheld glucometer (Accu-Chek Aviva; Roche Diagnostics Canada, Laval, Canada). Accuracy of each meter and batch of test strips were routinely checked using the control

solutions from the manufacturer as well as a standard glucose solution composed in the lab and measured using a glucose oxidase spectrophotometric method (GOPOD). Any meter or batch of test strips that did not measure within 3% of the control solutions were discarded. Additionally, each participant was assigned their own glucometer and same lot number of test strips for the duration of the study. A baseline measurement of ≥ 5.6 mmol/L suggested non-compliance with the fast or high fasting plasma glucose and the participant was rescheduled or excluded from the study.

Participants were instructed to consume the breakfast meal within 10 min while eating at a steady pace. After consumption, the palatability of the treatment was measured by VAS. Subjective appetite and blood glucose were measured at 15, 30, 45, 60, 75, 90 and 120 min (post-treatment period) from the time participants began eating the breakfast meal and insulin was measured at 0 (baseline), 30, 60, 90 and 120 min consistent with HC's guidance for measurement of PPG. Blood for insulin analysis was collected via finger prick with Microvette capillary tubes 300 Z (Sarstedt, Numbrecht, Germany). Blood was immediately centrifuged (20°C, 10000 rpm, 5 min) and the plasma were frozen at -80°C until measurements. Insulin levels were quantified with an insulin enzyme-linked immunoabsorbent (ELISA) kit (Alpco, Salem, USA). Participants were asked to remain seated throughout the session and were permitted to do quiet activities such as read or listen to music.

Satiety was measured over 120 min at which time participants were fed an ad libitum test meal. This duration of time, although does not adhere to HC's recommendation of 180 min, was chosen based on previous studies reporting sustained effects of dairy on satiety up to 120 min and no effect at 180 min (Dougkas et al. 2012, El Khoury et al. 2014). Participants sat in

individual booths separated by dividers and were not allowed any distractions (i.e. cellphones, books, etc.) during the length of the test meal. They were provided with three varieties of pizza (Deluxe, Pepperoni and Three Cheese) and were provided the same combinations at each session based on their preferences at the initial screening. Pizza was chosen as the test meal due to its ease to prepare, homogeneity of the food (no surrounding crust), palatability and accuracy of consumption measures and has been used in many previous studies (Akhavan et al. 2014, El Khoury et al. 2014, Panahi et al. 2014). Each pizza was cut into four slices containing approximately 50 kcal. Participants were allowed 20 min to eat and instructed to eat until they felt “comfortably full”. Three separate trays of pizza were provided at regular intervals regardless if the participant was finished eating the current tray or already full. Subjective appetite, blood glucose and insulin were measured at 140 and 170 min (post-meal period).

The nutritional composition of the pizzas is shown in Table 2 and method of cooking was reported previously (Akhavan and Anderson 2007). Test meal consumption was calculated by weighing the amount of pizza consumed and based on the nutritional information provided by the manufacturer. Ad libitum water intake at the test meal was measured by weight (g). Cumulative energy intake was calculated by adding the energy content of the fixed breakfast with the total energy consumed at the test meal. Caloric compensation, expressed as a percentage, was calculated using the following formula: $\text{Caloric compensation} = [(\text{kcal consumed at the test meal after the water control} - \text{kcal consumed at the meal after the treatment}) / (\text{kcal of the treatment (beverage)})] \times 100\%$. A caloric compensation of <100% indicates undercompensation while a score >100% indicates overcompensation (greater reduction) at the test meal for the energy consumed at breakfast (Panahi et al. 2013).

Statistical Analyses

Statistical analyses were conducted using SAS version 9.3 (SAS Institute Inc., Cary, NC). One-and two-factor repeated-measures analysis of variance (ANOVA; via PROC MIXED procedure) and two-factor repeated-measures analysis of covariance (ANCOVA; via PROC MIXED procedure) were performed to analyze the effects of treatment, time, sex and their interaction on dependent variables over the entire study period including changes from baseline for subjective appetite scores, blood glucose and insulin concentrations and means for FI, cumulative energy intake, water intake and caloric compensation. Absolute baseline values were used as covariates for subjective appetite, blood glucose and insulin. There were no significant interactions between treatment and sex for any of the variables therefore these results were pooled for both men and women. When there was a significant treatment-by-time interaction, one-factor ANOVA (PROC MIXED procedure) was followed by Tukey's post-hoc test to investigate the effect of treatment on changes from baseline for appetite, blood glucose and insulin at each time of measurement and means for FI, cumulative energy intake, water intake and caloric compensation. Post-treatment and cumulative changes from baseline were calculated from 15-120 min for subjective appetite and PPG and 30-120 min for insulin. Post-meal changes for all outcome measures were calculated from 140-170 min.

Total areas under the curve (tAUC) for subjective appetite and incremental areas under the curve (iAUC) for blood glucose and insulin were calculated separately for the post-treatment, post-meal and cumulative periods; post-treatment, post-meal and cumulative periods were 0-120 min, 120-170 min and 0-170 min, respectively, for all variables. To provide estimates of the efficacy of insulin action (Vuguin et al. 2001), glucose to insulin iAUC ratios and glucose to insulin mean changes from baseline ratios at 30 min were compared among treatments

during the post-treatment, post-meal and cumulative periods. Data are presented as mean $\pm$ standard error of the mean (SEM). Statistical significance was set at $p < 0.05$.

Data are reported as tAUC and iAUC for subjective appetite and blood glucose, respectively, as recommended by Health Canada guidance documents (Health Canada 2013) and common usage. However, expressing the data as mean changes from baseline is an important evaluation, as it provides a more precise evaluation of treatment effects. For example, a treatment that has a high initial peak and then falls and a treatment that has a sustained blood glucose response may result in the same iAUC. However, mean changes from baseline have the potential to provide a more detailed description of post-prandial glucose that may be physiologically relevant and missed by iAUC.

Results

Participant Characteristics

Twenty-six healthy males and females (sex: 13 males and 13 females; age: 23.0 ± 2.6 years; BMI: 22.3 ± 1.5 kg/m²) completed all five sessions. One female participant was excluded due to failure to schedule the required sessions and two males due to concurrent participation in another intervention study. Their results were not included in the analysis.

Palatability

Water with cereal was the least preferred compared to all other treatments with cereal, with no significant differences between other treatments ($p < 0.0001$). There were no differences in palatability of the test meal after any of the treatments (mean score = 69 mm, $p = 0.487$).

Food and Water Intake

Treatment affected FI ($p < 0.0001$), cumulative energy intake ($p < 0.0001$) and caloric compensation ($p = 0.0045$) but not water intake (Table 3). All treatments resulted in lower FI at the ad libitum meal than water. Energy intake was lower after milk than all treatments except yogurt beverage. There were no differences in FI between session number (i.e. participants ate similarly at their first session compared to their last) ($p = 0.6580$). Cumulative energy intake (breakfast + lunch) was also lowest after milk compared to all treatments except almond beverage. Similarly, compensation for energy in the beverages was greater for milk compared to soy ($p = 0.0348$) and yogurt beverages ($p = 0.0131$) but was not different from almond beverage ($p = 0.1679$). Almond beverage was not significantly different from yogurt beverage ($p = 0.0786$). Caloric compensation for milk and almond beverage calories averaged 190 and 170%, but for yogurt and soy beverage was $< 100\%$ at the ad libitum meal.

Subjective Appetite

Post-treatment (15-120 min) mean appetite changes from baseline were affected by time ($p < 0.0001$) and treatment ($p < 0.0001$) but not by a time-by-treatment interaction. Yogurt beverage, with the highest caloric content, suppressed appetite compared to all treatments ($p < 0.0001$; Table 4). All other beverages equally suppressed appetite more than water (Fig. 1). However, the tAUCs showed all treatments equally suppressed appetite compared to water with no differences between them ($p < 0.0001$; Table 5). Post-meal (140-170 min) mean appetite changes from baseline were affected by time ($p < 0.0001$) but not treatment (Table 4). Similarly, post-meal tAUCs were unaffected by treatments (Table 5).

An examination of the individual appetite scales (data not shown) showed that yogurt beverage reduced hunger and PFC compared to all other treatments in the post-treatment

($p < 0.0001$) and cumulative ($p < 0.0001$) periods. Post-meal, hunger was lower after water and soy beverage ($p = 0.0002$) and PFC ($p = 0.003$) compared to yogurt beverage, which is consistent with the higher FI following water and soy beverage at the test meal (Table 3). Yogurt beverage reduced DTE compared to all treatments except almond beverage in the post-treatment ($p < 0.0001$) and cumulative ($p < 0.0001$) periods. Post-meal, water reduced DTE compared to yogurt beverage ($p = 0.007$).

Blood Glucose Concentrations

Post-treatment (15-120 min) blood glucose mean changes from baseline were affected by treatment ($p < 0.0001$), time ($p < 0.0001$) and by a time-by-treatment interaction ($p < 0.0001$) (Table 4). Blood glucose peaks at 30 min were lowest following soy beverage compared to other treatments and did not return to baseline within the post-treatment period (Fig. 2). No differences between dairy and non-dairy beverages were observed for any other timepoints thereafter. However, by 90 and 120 min, only almond beverage led to higher blood glucose compared to water. Over the post-treatment period, blood glucose was lowest after soy beverage compared to all treatments but milk also led to lower blood glucose compared to water ($p < 0.0001$; Table 4). No differences were observed between almond and yogurt beverages compared to water ($p < 0.0001$; Table 4). However, an analysis of the first hour showed that blood glucose was 27% lower after the dairy and non-dairy beverages compared to water ($p < 0.0001$) (Table 4). In the post-treatment period, blood glucose iAUCs did not show a difference between soy beverage and milk (Table 5).

Post-meal (140-170 min) mean blood glucose changes from baseline were affected by time ($p < 0.0001$) but not treatment (Table 4). However, post-meal iAUCs showed yogurt and almond beverages equally reduced blood glucose compared to water (Table 5).

Insulin Concentrations

Post-treatment (30-120 min) mean insulin changes from baseline were affected by treatment ($p<0.0001$), time ($p<0.0001$) and by a time-by-treatment interaction ($p<0.0001$). Insulin peaks at 30 min were highest following yogurt beverage compared to all treatments and failed to return to baseline for all treatments within the post-treatment period (Fig. 3). Over the post-treatment period, insulin was highest after yogurt beverage but both dairy beverages led to higher insulin than non-dairy beverages and water ($p<0.0001$; Table 4). Results for insulin iAUCs were similar (Table 5). Post-meal (140-170 min) mean insulin changes from baseline were affected by treatment ($p=0.0052$), time ($p<0.0001$) but not by a time-by-treatment interaction ($p=0.06$). Milk led to lower insulin compared to water which is consistent with the lower FI following milk ($p=0.005$). There were no other differences between any other treatments. No differences were observed between treatments for insulin iAUCs (Table 5).

Blood Glucose to Insulin Ratios

In the post-treatment (0-120 min) and cumulative (0-170min) periods, yogurt led to the lowest glucose/insulin iAUC ratio but was not different from milk and soy beverage ($p<0.0001$; Table 6). The highest ratio was following almond beverage which was not different from water ($p<0.0001$). There were no differences between treatments in the post-meal period (120-170 min) (Table 6). The ratio for mean changes from baseline at 30 min was lowest following yogurt beverage and highest following water ($p=0.03$) with no differences between any other treatments (Table 6).

Discussion

In support of our hypothesis, both milk and yogurt beverage consumed with breakfast cereal reduced subjective appetite and FI compared to water with cereal but only milk led to full

energy compensation at a later meal and lower PPG. In contrast to expectations, almond and soy beverages also showed beneficial effects.

In the first hour following consumption of the treatments, PPG mean changes for dairy and non-dairy beverages averaged 27% lower than when water was consumed with cereal (Table 4), even though these beverages added 8-28 g to the carbohydrate load of cereal alone with water. Thus, it can be suggested that assessment of the PPG response based on carbohydrate alone, as ranked by the GI, may be misleading for meal guidance and support the recommendation of HC (Health Canada 2012; Health Canada 2013) that health claims for PPG should be measured in responses to serving sizes in meals as usually consumed.

The PPG responses of the breakfasts were not solely dependent on their carbohydrate contents. Although the yogurt beverage breakfast had 41% more carbohydrate than the water breakfast, and while the overall mean change in PPG response was similar in the post-treatment period (15-120 min), the peak response in the first hour (15-60 min) was 16% ($p < 0.0001$) lower than after the water and cereal breakfast (Table 4). These results are consistent with a recent study that reported lower blood glucose after chocolate milk with higher sugar content compared to orange juice (Panahi et al. 2013).

This response may be due in part to the protein content or the beverages as milk (Nilsson et al. 2004) and soy proteins (Kashima et al. 2016) stimulate insulin release. However, non-insulin dependent mechanisms also determine insulin and blood glucose responses. Increased efficacy of insulin following yogurt beverage is suggested by its low glucose to insulin ratio in the post-treatment period (Table 6) (Vuguin et al. 2001). The high carbohydrate content (Marciani et al. 2001), and not protein, is the likely cause of the higher insulin and fat content

(Maljaars et al. 2009) may have also contributed to slower gastric emptying. Fermentation is also a possible explanation since fermented milk beverages delay gastric emptying when compared to regular milks (Ostman et al. 2001, Sanggaard et al. 2004). Although only containing 1 g of protein, almond beverage did not differ in PPG from milk (9 g protein) and yogurt beverage (6 g protein). This can perhaps be explained by the addition of locust bean gum, sunflower lecithin and gellan gum in the brand of almond beverage used. Locust bean gum delays gastric emptying when added to rice puddings (Darwiche et al. 2003) and infant formula (Miyazawa et al. 2006) and gellan gum delayed gastric emptying in rats (Nishimukai et al. 2003).

Similarly, the soy beverage contained carrageenan which may provide an explanation for the lower PPG after the soy beverage compared to milk. Carrageenan (2.5g) added to 250 mL of 3.25% M.F. milk reduced blood glucose response compared to 3.25% M.F. milk alone at 30 min which is similar to results for soy beverage and milk in the present study. The authors attribute the results to delayed gastric emptying (Arshad et al. 2016).

Insulin responses corresponded with both the carbohydrate and protein contents of the treatments, however the differences in macronutrient content made it difficult to distinguish the degree of effect for each. Insulin after the yogurt beverage breakfast remained highest at all times. This sustained insulin response is likely due to its high carbohydrate content as, while the milk breakfast had more protein but less carbohydrate, post-prandial insulin was lower than after the yogurt beverage breakfast.

The observation that post-prandial insulin was higher after milk than after soy beverage appears to contrast with a recent study that compared a breakfast of white bread consumed

with 1% M.F. milk (15.1 g lactose, 8.6 g protein) or soy beverage (10.8 g sucrose, 8.6 g protein) and found higher post-prandial insulin following the soy breakfast (Sun et al. 2015). However, the present study allowed the carbohydrate content to represent a usual meal in order to define the functionality of the beverages in that context. In contrast, Sun et al. standardized the available carbohydrate content at 50 g to mimic a GI test by reducing the amount of bread. While it showed that both milk and soy consumed either prior to or with bread reduced PPG compared with bread alone, showing a similar result to the present study, it did not represent a meal as usually consumed.

Satiety in the post-treatment period was also not determined by protein content alone. All treatments reduced appetite compared to water as shown by tAUC, but yogurt beverage led to the lowest mean subjective appetite ratings, most likely due to its high energy content (Panahi et al. 2013). Additionally, sweetness has been shown to reduce appetite (Lavin et al. 2002) and the yogurt beverage contained 28 g of sugar from natural sources (lactose) and added sugar (sucrose) and was the sweetest tasting treatment. However, subjective appetite ratings were not predictive of FI which is consistent with reports of others (Tsuchiya et al. 2006, Harper et al. 2007).

Milk resulted in lower FI compared to non-dairy beverages. This is contrary to a previous study that compared soy beverage and milk when consumed alone (Panahi et al. 2013). In that study, there were no differences in FI measured at 120 min indicating perhaps a stronger effect on FI when milk is consumed with a meal. In addition, soy protein is considered a “fast” protein and milk is primarily made up of casein, a “slow” protein that coagulates in the stomach and delays gastric emptying (Bos et al. 2003). Therefore, lower FI may be attributed to the actions of casein. It is unlikely that energy content of the treatments played a role in

affecting FI since the difference between milk and soy beverage was only 10 calories and yogurt beverage resulted in the same FI as soy beverage and almond beverage despite much higher calories. Furthermore, lower FI after the milk breakfast translated to lower cumulative FI due to a decrease of energy intake at the test meal that reflected overcompensation for the calories added from milk at breakfast. This indicates that milk may be a good choice of beverage for the purpose of reducing energy intake at a later meal although longer-term effects need to be studied. Overcompensation (167%) for the energy added by almond beverage at the breakfast was likely due to its low energy content (60 kcal).

Undercompensation (72%) by yogurt beverage was surprising due to its high energy content (190 kcal). However, this energy content was contributed partly from the added sugars in the beverage which represents approximately 33% (63 kcal) of the total energy content. Protein is more satiating than fat or carbohydrate. Therefore, perhaps the relatively low protein to energy ratio and the high carbohydrate to energy ratio is an explanation for this undercompensation. In order to answer this hypothesis, this yogurt beverage would need to be compared with a higher protein or unsweetened product.

The lack of control for the macronutrient and energy content of treatments may be seen as a limitation of this study. However, the objective was to compare commercially-available products consumed in typical serving sizes at usual meals and therefore provide data with ecological validity. A limitation of this study design was the ad libitum nature of the test meal which does not allow for optimal measurement of a second-meal effect for glycemia and potentially causes blunting of treatment effects on insulin in the post-meal period. A fixed-meal based on calories per kilogram bodyweight such as one described in a previous study by (Akhavan et al. 2014) would have been more effective, however the measurement of FI

requires ad libitum feeding (Blundell et al. 2010). Furthermore, the measurement of gastrointestinal hormones as well as gastric emptying through methods like paracetamol administration would have contributed to better understanding of the mechanisms involved in appetite and glycemic regulation by dairy and non-dairy beverages. Finally, although the study included both healthy men and women, as recommended by HC for studies for health claims, the results cannot be extrapolated to unhealthy populations such as obese, pre-diabetic and diabetic individuals as well as the elderly which are all groups that would greatly benefit from dietary measures of appetite and glycemic control. Perhaps pre-meal treatment with the beverages would provide the greatest benefit (Sun et al. 2015).

The significance of this study is two-fold. First, both dairy and dairy substitutes markedly reduced blood glucose responses to a high glycemic cereal. Thus, it can be suggested that dietary advice based on the avoidance of carbohydrate foods based on the GI rating of carbohydrates measured alone may be misleading. In addition to a recent report showing its lack of reproducibility within and between individuals (Matthan et al. 2016), many studies show that responses to carbohydrate foods are greatly modified when consumed in a meal after protein (Akhavan et al. 2010) or within a meal with usual amounts of carbohydrate (Panahi et al. 2013, Sun et al. 2015). As with dietary guidance given on the basis of the GI of breakfast cereals, potatoes are not recommended as a starchy addition to a meal because of their high GI. Yet, when consumed with meat by children, FI and blood glucose were lower than when the meat was consumed with rice and pasta, providing evidence of the synergism of carbohydrate and protein functions within meals (Akilen et al. 2016). Clearly, a greater appreciation of the complementary benefits of food and food components as consumed in meals with usual serving sizes as recommended by HC is needed to set policy for dietary guidelines and labels on food.

Second, the results contribute to consideration of study designs that may be applied to PPG and satiety health claims as proposed by HC (Health Canada 2012, Health Canada 2013). In the present study design, most of the fundamental criteria such as using a healthy adult population, a crossover design, valid outcome measures and the recommended serving size for the treatments were fulfilled (Health Canada 2013). However, it was not a direct comparison of PPG response to the beverages alone but included a determination of their comparative effect consumed with carbohydrate. Thus, the present study did not give rise to any PPG comparative claim among the beverages. However, since the carbohydrate meal was the same across all breakfasts, the observed differences could be ascribed to the beverages. Satiety was measured but the design did not strictly meet satiety guidelines stating that the test food should never be of higher energy content than the reference food (Health Canada 2012). However, the reality is that serving sizes as defined by HC do not have similar caloric content. In addition, FI was measured at 120 min and not at 180 min as recommended by the satiety guidelines. By 120 min, the treatment effects were nearing baseline. Thus, it is unlikely that measurement at 180 min would have reflected differences due to treatment, the objective of making such comparisons. The study results also show that because the designs and criteria proposed in the guidance documents for post-prandial glycemia and satiety claims are different, it is difficult to derive both PPG and satiety claims from the same study.

In conclusion, both dairy and non-dairy beverages consumed with a high glycemic cereal at breakfast increased satiety and decreased FI compared to water with cereal. Despite adding carbohydrate to the breakfasts all led to similar or lower PPG than the water breakfast but dairy beverages increased insulin more than non-dairy beverages.

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Table 1. Nutritional information of treatments, breakfast cereal and breakfast

	Treatments				Cereal
	Almond beverage	Soy beverage	1% Milk	Yogurt beverage	
Nutrients ^A					
Energy (kcal)	60	100	110	190	200
Total Fat (g)	3	4	3	6	3
Saturated fat (g)	0.2	0.5	1.5	4	0.8
Trans fat (g)	0	0	0	0	0
Cholesterol (mg)	0	0	10	0	0
Sodium (mg)	160	110	120	70	340
Total Carbohydrate (g)	9	8	12	28	40
Fibre (g)	1	2	0	0	4
Sugars (g)	8	5	12	28	2
Protein (g)	1	7	9	6	6
% Energy					
Fat (%)	45	36	20	28	14
Carbohydrate (%)	53	24	44	59	72
Protein (%)	7	28	33	13	12
Breakfast Composition ^B					
Energy (kcal)	260	300	310	390	200
Fat (g)	6	7	6	9	3
Available Carbohydrate (g)	44	42	52	68	36
Protein (g)	7	13	15	12	6

^A Nutrient content of each product as provided on the labels by the manufacturer. Amounts given are per 250 mL of each treatment and 54 g of cereal.

^B Breakfast = treatment + cereal. Last column also represents nutritional composition for control (water) breakfast.

Table 2. Nutritional information of test meal

	Pizzas		
	Cheese	Pepperoni	Deluxe
Weight ^A	81 g	87	92
Nutrients ^B			
Energy (kcal)	180	180	170
Fat (total) (g)	5	5	5
Saturated fat (g)	3	2.5	2
Trans fat (g)	0.2	0.2	0.2
Cholesterol (mg)	20	20	15
Sodium (mg)	400	400	400
Total Carbohydrate (g)	23	23	24
Fibre (g)	1	1	1
Sugars (g)	2	3	2
Protein (g)	10	9	9

^AWeight of one pizza.^BNutrient content of each pizza as provided on the labels by the manufacturer.

Table 3. Energy intake, cumulative energy intake, water intake and caloric compensation^A

Treatment	Energy intake (kcal)		Water Intake (g)	Caloric Compensation (%) ^D
	Test meal ^B	Cumulative ^C		
Control ^E	835.8±66.8 ^a	1035.8±66.8 ^{ab}	322.9±30.5	—
Almond beverage	726.2±52.4 ^b	986.2±52.4 ^{bc}	311.6±30.2	166.9±59.9 ^{ab}
Soy beverage	743.6±57.5 ^b	1043.6±57.5 ^{ab}	336.5±29.8	85.3±29.8 ^b
1% Milk	624.3±41.3 ^c	934.3±41.3 ^c	328.4±27.8	190.2±31.8 ^a
Yogurt beverage	700.2±47.1 ^{bc}	1088.2±47.1 ^a	358.0±26.3	71.7±21.2 ^b
<i>p</i>	<0.0001	<0.0001	NS	0.0045

^A All values are means ± SEMs (*n* = 26). Values in the same column with different superscript letters are significantly different. *P* < 0.05 (treatment effect using one-way ANOVA via proc mixed procedure, Tukey's post-hoc).

^B Energy consumed in an ad libitum meal was measured at 120 min following treatment consumption.

^C Energy in breakfast meal (treatment + cereal) + energy from test meal.

^D Caloric compensation = [(kcal consumed at meal after the water control – kcal consumed at the meal after the beverage)/kcal in the beverage] x 100.

^E Water (250 mL).

Table 4. Effect of treatment on post-treatment, post-meal and cumulative mean changes from baseline for subjective appetite, blood glucose and insulin^A

		Control ^B	Almond beverage	Soy beverage	1% Milk	Yogurt beverage	<i>p</i>
Appetite (mm)	Post-treatment ^C	-23.6±1.8 ^a	-29.7±1.7 ^b	-26.2±1.8 ^b	-26.5±1.7 ^b	-35.4±1.8 ^c	<0.0001
	Post-meal ^D	-26.4±2.8	-25.3±2.5	-26.0±2.4	-24.0±2.3	-22.3±2.3	NS
	Cumulative ^E	-31.3±1.8 ^a	-35.8±1.7 ^{bc}	-32.7±1.8 ^b	-32.3±1.7 ^b	-40.6±1.7 ^c	<0.0001
Glucose (mmol/L)	Post-treatment	1.2±0.08 ^a	1.0±0.06 ^{ab}	0.7±0.06 ^c	0.9±0.07 ^b	1.0±0.07 ^a	<0.0001
	First hour ^F	1.5±0.1 ^a	1.1±0.08 ^b	0.8±0.08 ^c	1.2±0.1 ^b	1.3±0.1 ^b	<0.0001
	Post-meal	0.8±0.1	0.3±0.06	0.6±0.09	0.5±0.09	0.4±0.06	NS
	Cumulative	1.2±0.07 ^a	1.1±0.05 ^{ab}	0.9±0.05 ^c	1.0±0.06 ^{bc}	1.0±0.06 ^{ab}	<0.0001
Insulin (μU/ml)	Post-treatment	18.2±2.3 ^c	16.5±1.6 ^c	18.7±1.9 ^c	25.7±2.6 ^b	33.9±3.2 ^a	<0.0001
	Post-meal	34.4±4.1 ^a	28.0±3.0 ^{ab}	30.8±4.1 ^{ab}	24.1±2.9 ^b	26.9±3.4 ^{ab}	0.0052
	Cumulative	28.8±2.4 ^{bc}	26.0±1.9 ^c	29.1±2.4 ^{bc}	32.1±2.2 ^b	40.4±2.7 ^a	<0.0001

^AAll values are means ± SEMs (*n* = 26). Means within a row with different superscript letters are significantly different, *p* < 0.05 (treatment effect using two-way ANCOVA via proc mixed procedure, Tukey's post hoc).

^BWater (250 mL).

^CPost-treatment values are mean changes from baseline for all observations before the test meal: 15, 30, 45, 60, 75, 90 and 120 min for appetite and glucose and 30, 60, 90 and 120 min for insulin.

^DPost-meal values are mean changes from baseline of all observations after the test meal: 140 and 170 min.

^ECumulative values are mean changes from baseline for all observations for the entire session: 15-170 min.

^FFirst hour values are mean changes from baseline for all observations for the first hour post-treatment: 15-60 min.

Table 5. Effect of treatment on post-treatment, post-meal and cumulative tAUCs for subjective appetite and iAUCs for blood glucose and insulin^A

		Control ^B	Almond	Soy	Milk	Yogurt	<i>P</i>
Appetite tAUC (mm min)	Post-treatment ^C	5995.1±509.8 ^a	5014.5±355.5 ^b	5176.7±467.6 ^b	4903.4±414.6 ^b	4530.9±392.6 ^b	<0.0001
	Post-meal ^D	1059.0±92.2	959.0±72.3	957.5±83.7	1005.7±86.6	977.0±132.0	NS
	Cumulative ^E	7054.1±557.0 ^a	5997.7±392.3 ^b	6158.6±522.6 ^b	5923.2±468.0 ^b	5534.7±488.9 ^b	0.0002
Glucose iAUC (mmol min/L)	Post-treatment	145.8±13.0 ^a	131.8±11.0 ^a	97.4±9.7 ^b	120.4±10.0 ^{ab}	131.2±12.5 ^a	0.0002
	Post-meal	44.1±4.7 ^a	22.1±2.8 ^b	35.2±4.0 ^{ab}	30.4±5.9 ^{ab}	22.8±3.4 ^b	0.0006
	Cumulative	198.3±15.2 ^a	186.1±15.7 ^{ab}	153.0±13.1 ^b	171.6±12.5 ^{ab}	176.4±16.8 ^{ab}	0.0084
Insulin iAUC (µU min/mL)	Post-treatment	2702.9±240.6 ^{bc}	2252.7±225.5 ^c	2625.7±231.8 ^c	3450.3±322.9 ^b	4702.2±348.1 ^a	<.0001
	Post-meal	1959.8±194.0	1577.1±135.7	1737.3±197.9	1447.4±157.8	1587.6±189.0	0.04
	Cumulative	4754.6±318.8 ^{bc}	4222.4±306.0 ^c	4649.1±419.8 ^{bc}	5410.5±434.9 ^b	7101.0±473.6 ^a	<.0001

^AAll values are means ± SEMs (*n* = 26). Means within a row with different superscript letters are significantly different, *p* < 0.05 (treatment effect using one-way ANOVA via proc mixed procedure, Tukey's post hoc).

^BWater (250 mL).

^CPost-treatment values are AUCs of all observations before the test meal: 0, 15, 30, 45, 60, 75, 90 and 120 min for appetite and glucose and 0, 30, 60, 90 and 120 min for insulin.

^DPost-meal values are mean changes from baseline of all observations after the test meal: 120, 140 and 170 min.

^ECumulative values are AUCs for all observations for the entire session: 0-170 min.

Table 6. Effect of treatment on post-treatment, post-meal and cumulative ratios of blood glucose to insulin iAUC and on ratios of blood glucose to insulin changes from baseline at 30 min^A

		Control ^B	Almond beverage	Soy beverage	1% Milk	Yogurt beverage	<i>p</i>
Glucose/insulin iAUC ratio (mmol min L ⁻¹ /μU min mL ⁻¹)	Post-treatment ^C	0.06±0.007 ^{ab}	0.07±0.01 ^a	0.04±0.004 ^{bc}	0.04±0.006 ^{bc}	0.03±0.004 ^c	<.0001
	Post-meal ^D	0.03±0.004	0.02±0.002	0.02±0.003	0.02±0.003	0.02±0.004	NS
	Cumulative ^E	0.04±0.003 ^a	0.05±0.005 ^a	0.04±0.004 ^{ab}	0.04±0.005 ^{ab}	0.03±0.003 ^b	0.0006
Glucose/insulin changes from baseline ratio (mmol min L ⁻¹ /μU min mL ⁻¹)	30 min	0.20±0.04 ^a	0.18±0.02 ^{ab}	0.16±0.03 ^{ab}	0.14±0.03 ^{ab}	0.09±0.008 ^b	0.0346

^AAll values are means ± SEMs (*n* = 26). Means within a row with different superscript letters are significantly different, *p* < 0.05 (treatment effect using one-way ANOVA via proc mixed procedure, Tukey's post hoc).

^BWater (250 mL).

^CPost-treatment values are for all observations before the test meal: 0, 15, 30, 45, 60, 75, 90 and 120 min for appetite and glucose and 0, 30, 60, 90 and 120 min for insulin.

^DPost-meal values are for all observations after the test meal: 120, 140 and 170 min.

^ECumulative values are for all observations for the entire session: 0-170 min.

Figure Captions

Figure 1. Effect of treatments on average subjective appetite mean changes from baseline over time. Means with different superscripts are significantly different at each measured time (one-way ANOVA, Tukey-Kramer post-hoc test, $p < 0.05$). All values are mean $\pm$ SEM ($n = 26$).

Figure 2. Effect of treatments on blood glucose mean changes from baseline over time. Means with different superscripts are significantly different at each measured time (one-way ANOVA, Tukey-Kramer post-hoc test, $p < 0.05$). All values are mean $\pm$ SEM ($n = 26$).

Figure 3. Effect of treatments on insulin mean changes from baseline over time. Means with different superscripts are significantly different at each measured time (one-way ANOVA, Tukey-Kramer post-hoc test, $p < 0.05$). All values are mean $\pm$ SEM ($n = 26$).

Figure 1

Figure 2

Figure 3