**Effect of dairy and non-dairy snacks on postprandial blood glucose regulation in 9-14 year old children**

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Abstract

In adults, dairy consumption improves short-term blood glucose regulation. It is unknown whether these short-term benefits extend to children of different weight statuses. The objective of this study was to investigate the effect of a dairy and non-dairy snack in both normal weight (NW) and overweight/obese (OW/OB) children on blood glucose regulation and food intake (FI). In a repeated measures crossover design, 11 NW and 7 OW/OB children (age: 9-14y), consumed, in random order, a dairy (Greek yogurt, 198.9g, 171kcal, 0g fat, 17g protein) or non-dairy (mini sandwich type cookies, 37.5g, 175kcal, 7.5g fat, 1.3g protein) snack containing 25g of available carbohydrates. Ad libitum FI was measured 120 min after snack consumption. Blood glucose, insulin, C-peptide, and GLP-1 were measured at 0 min (before the snack), and 30, 60, 90, and 120 min after snack consumption. Insulin secretion was calculated from deconvolution of C-peptide. Hepatic insulin extraction was calculated as C-peptide divided by insulin. FI did not differ between snacks (P=0.55). Mean blood glucose was lower (P<0.001) and insulin higher (P<0.0001) in the 120 min after consuming the dairy snack. C-peptide concentrations (P=0.75) and insulin secretion (P=0.37) were not different between snacks. The increase in insulin was explained by reduced hepatic insulin extraction (P<0.01). Consumption of the dairy snack also increased mean GLP-1 concentrations (P<0.001). In conclusion, consumption of a dairy snack by NW and OW/OB children results in reduced postprandial blood glucose concentrations and elevated circulating insulin compared to a non-dairy snack possibly due to delayed hepatic insulin extraction.
Key words: Children, Blood Glucose, Insulin, C-peptide, Available Carbohydrate, Snacking, Obesity, Dairy, Cookies, GLP-1

Introduction

Almost one in three Canadian children (31.4%) are overweight or obese (Rao et al. 2016). Obesity is associated with an increased risk of chronic disease later in life including cardiovascular disease and Type 2 Diabetes Mellitus (T2DM) (Dixon 2010). Similar to obesity, the prevalence of T2DM in children and adolescents has increased (Vehik and Dabelea 2011) and 95% of Canadian children diagnosed with T2DM are obese at diagnosis (Amed et al. 2010).

The increase in childhood obesity and incidence of T2DM has paralleled an increase in snacking (Piernas and Popkin 2010) and children in this age group do not meet Eating Well with Canada’s Food Guide minimum recommended number of servings from each food group including milk and milk products (Wadsworth et al. 2012). Childhood snacking is characterized by foods with increased energy density and lower calcium density compared to non-snack eating occasions (Jahns et al. 2001). Therefore, the choice of snack products presents an opportunity to introduce nutrient-dense foods to children without detrimental effects on blood glucose and food intake (FI) control. Acute trials show that milk proteins attenuate the rise in postprandial blood glucose concentration when consumed with a high glycemic response food (Akhavan et al. 2011; Akhavan et al. 2010; Drummond et al. 2018,) and epidemiological studies demonstrate that yogurt consumption is inversely associated with the incidence of overweight and obesity (Martinez-Gonzalez et al. 2014). Furthermore, yogurt
specifically, has been associated with a decreased risk for T2DM in adults (Chen et al. 2014; O'Connor et al. 2014). Recent evidence in children has also identified that frequent yogurt consumption is associated with lower fasting insulin concentrations, lower insulin resistance, and higher insulin sensitivity (Zhu et al. 2014).

Our understanding of the short-term effect of snacking on blood glucose control in children remains incomplete. The increase in energy consumption associated with increased snacking occasions is often aligned with a high intake of available carbohydrates. Therefore, it is important to study how the various components of different snack foods, particularly macronutrients, affect blood glucose control and food intake (FI) in children. Dairy snacks are worthy of study because of their unique macronutrient composition containing high levels of bioactive proteins such as casein and whey protein. It is known that the ingestion of whey protein reduces glycaemia and increases circulating insulin in adults via a number of mechanisms (Luhovyy et al. 2007) including increased release of glucagon-like peptide-1 (GLP-1) and altered hepatic insulin extraction (HIE) (Akhavan et al. 2014; Lan-Pidhainy and Wolever 2010).

However, how these mechanisms function in children of different body weights in the context of a mixed macronutrient snack in its native food matrix is unknown. The study of these mechanisms in 9-14y old children is of particular interest as a number of hormonal changes affecting the insulin response are occurring and distinguish children of this age from adults (Moran et al. 2002). We hypothesized that a dairy snack with a high protein content would reduce postprandial glycaemia compared to a non-dairy snack with the same level of available carbohydrate. The objective of this study was to investigate the effects of a dairy and non-dairy snack on measures of blood glucose regulation (blood...
glucose, insulin, C-peptide, and GLP-1), *ad libitum* FI, and subjective appetite in the 120 min after snack consumption in children of different weight statuses.

**Materials and Methods**

**Participants**

Children between the ages of 9 and 14y were recruited to participate in this study from the Halifax Regional Municipality via word of mouth, posted flyers in locations frequented by children and their parents, and from our database of previous participants. At the time of recruitment children were either normal weight (NW) (5-85th percentile for age and gender) or overweight/obese (OW/OB) (>85th percentile for age and gender) according to the Canadian interpretation (2004) of the CDC growth charts (Ogden et al. 2002), which were the most current recommendations when the study was developed and conducted. All ethical components of this study were reviewed by the Mount Saint Vincent University Research Ethics Board and were found to be in compliance with the University’s Research Ethics Policy. Additionally, the study’s procedures were approved by IWK Health Centre Ethics Board and were conducted in accordance with the Declaration of Helsinki. The trial was registered at clinicaltrials.gov as NCT02484313.

Children were excluded from the study if they were unable to consume any of the foods used during the study, had recent, unusual fluctuations in body weight in the last 6 months, did not regularly consume breakfast, followed a special diet, had a medical condition requiring medication, had learning, emotional, or behavioural problems or were
not born at term (<37 and >42 weeks). Children provided written assent and their parents provided written consent prior to them participating in the study. During the in-person screening session the children’s age was collected as well as anthropometric measures including height (m), weight (kg), and body composition obtained using a Tanita Body Composition Analyzer (TBF-300A, Tanita, Arlington Heights, Illinois, USA) equipped with a stadiometer. Children also completed a Physical Activity Questionnaire to determine typical activity in metabolic equivalents (Ainsworth et al. 2000). A self-administered Tanner Staging (Tanner 1962) and puberty questionnaire was completed to determine the children’s stage of pubertal growth as puberty is associated with transient insulin resistance due to an increase in growth hormones, sex hormones, and insulin-like growth factors (Steinberger et al. 2009). Children were categorized as pre-early puberty (Tanner stages I-II) or mid-late puberty (Tanner stages III-V). A children’s version of the Dutch Eating Behaviour Questionnaire (DEBQ) was also completed to assess levels of dietary restraint, emotional, or external eating behaviours (van Strien and Oosterveld 2008).

Based on previously reported data (Mollard et al. 2014), it was determined that a sample size of seven children per weight status group would be sufficient to detect a 20% difference (1.6 mmol/L) in the blood glucose response at the peak (30 min) between the snacks (α-level of 0.05 and β-level of 0.80).

Session Protocol

A non-blinded, repeated measures crossover design was used to compare the effects of a dairy and non-dairy snack. Children arrived at the Canadian Centre for Vaccinology at the IWK Health Centre in Halifax, NS, twice, at the same time, on
separate days that were at least one week apart. Children would arrive at 9:30 AM or 10:00 AM (but consistent throughout), two hours after eating a standardized breakfast that contained skim milk (250 mL, 90 kcal; Scotsburn, Scotsburn, NS, Canada), Honey-Nut Cheerios® (26 g, 90 kcal; General Mills, Mississauga, ON, Canada) cereal and Tropicana Orange Juice® (236 mL, 110 kcal; Tropicana Products Inc, Bradenton, FL, USA). Prior to breakfast consumption the children had fasted for 12 hours with the exception of water. Immediately upon arrival, children completed 100 mm visual analogue scale (VAS) questionnaires assessing subjective appetite and physical comfort and had a baseline blood sample drawn by a registered nurse. In a randomized fashion, children consumed, at their regular pace, either a dairy (Strawberry low fat Greek yogurt; Liberté, St. Hubert, Canada) or non-dairy snack (Mini Oreo Mr. Christie’s Cookies; Kraft Canada Inc., Don Mills, Canada) (Table 1). The subjective sweetness and pleasantness of the snacks were assessed using 100 mm VAS questionnaires after consumption. Ad libitum FI was measured using a macaroni and cheese meal (Kraft Dinner Easy Mac, Kraft Canada, Don Mills, ON) 120 min after the snack was provided.

**Snacks**

The dairy and non-dairy snacks were matched for available carbohydrate (25 g), as the study’s primary outcome was blood glucose regulation. The portion size was selected to replicate the amount typically consumed as a snack. Snacks were prepared on study session days prior to the participants’ arrival. Children were asked to consume the entirety of the snacks at their normal pace, which occurred within 8 min. The order of snack was randomized for each participant using the Statistical Analysis Systems (SAS) version 9.2 (SAS Institute Inc., Carey, NC, USA) Proc Plan method.
Subjective Appetite

Similar to previous reports in children four dimensions of subjective appetite were measured using 100 mm VAS: desire to eat (DTE), hunger, fullness, and prospective food consumption (PFC) administered at 0 min (immediately before the snack), 15, 30, 45, 60, 90, and immediately before the test meal (120 min). Average appetite (AA) was calculated for each time point to obtain a composite measure of appetite, using the formula:

$$\text{AA score (mm)} = \frac{\text{DTE} + \text{hunger} + (100-\text{fullness}) + \text{PFC}}{4}$$ (Bellissimo et al. 2008a, Bellissimo et al. 2007, Bellissimo et al. 2008b, Patel et al. 2011).

Physical Comfort, Sweetness, and Palatability

As has been reported in children previously (Bellissimo et al. 2007; Bellissimo et al. 2008a; Bellissimo et al. 2008b) perceived sweetness and pleasantness of the snacks were evaluated using a 100 mm VAS questionnaire reading: “How sweet have you found the snack?” (“Not sweet at all” to “Extremely sweet”). The pleasantness of snacks was assessed using a VAS questionnaire reading: “How pleasant have you found the snack?” (“Not at all pleasant” to “Very pleasant”). Similarly, perceived physical comfort was measured at 0, 8, 30, 45, 60, 90, 120 min, and finally after the test meal (150 min) using a 100 mm VAS questionnaire reading: “How well do you feel?” (“Not well at all” to “Very well”).

Food Intake

Microwaveable macaroni and cheese (Kraft Dinner Easy Mac, Kraft Canada, Don Mills, ON) was used as the test food for the ad libitum test meal that occurred 120 min after snack consumption. The macaroni and cheese meal was familiar to children and had
a uniform macronutrient composition and energy density. One bowl was offered every 10 min for a total of three bowls. The weight (g) of the macaroni and cheese was collected before providing it to the children and the remaining weight (g) was collected after each 10 min interval. The weights were then converted to energy (kcal) values using the information provided by manufacturer. A bottle of water (Nestlé Waters Canada, Guelph, ON, Canada) was provided with the meal and the amount of water consumed (g) was recorded.

**Blood Collection Protocol (Glucose, Insulin and C-Peptide)**

Blood samples for blood glucose, insulin, and C-Peptide were drawn at 0, 30, 60, 90, and 120 min. According to the manufacturer’s instructions a shielded intravenous catheter (BD™ Insyte Autoguard™) connected to a Luer-Access split septum device (BD™ Q-Syte™) and Luer-Lok™ access device (BD Vacutainer®) was used. Once collected, samples were centrifuged at 1,300 g relative centrifugal force for 10 min at 4°C, aliquoted, and stored in a -80°C freezer.

**Blood Collection Protocol (GLP-1)**

The same system and sample preparation technique as discussed above was used to collect samples for GLP-1 however, samples were collected at 0, 30, 60, and 120 min in BD™ P800 tubes (Becton, Dickinson and Company, Mississauga, ON) which contain a proprietary cocktail of protease inhibitors including DPP-IV esterase.

**Blood Glucose, Insulin, C-peptide, and GLP-1 Analysis**

Blood glucose was determined using the YSI 2300 STAT Plus Glucose Analyzer (YSI Life Sciences Yellow Springs, OH, USA). Insulin (80-INSHU-E01.1, Diagnostics, Salem, NH, USA), C-peptide (80-CPTHU-E01.1, ALPCO Diagnostics, Salem, NH, USA).
USA), and GLP-1 (EZGLPHS-25K, EMD Millipore Corporation, St. Charles, MO, USA) were measured using a sandwich type enzyme-linked immunosorbent assay (ELISA).

Statistical Analysis

SAS version 9.2 was used for all statistical analyses with data reported as means ± SEM. Data was analyzed using a three-way analysis of variance (ANOVA) via the Proc Mixed model to determine the effect of snack, time, weight status, and their interactions on dependent variables including subjective appetite, blood glucose, serum insulin, C-peptide, and GLP-1. To determine the effect of snack and weight status on FI a two-way ANOVA was used. Paired t-tests were used to determine whether differences existed at baseline between snacks and unpaired t-tests to compare across weight statuses for dependent variables. When main effects and/or interaction terms were significant post hoc analyses were performed using Tukey-Kramer’s test, adjusted for multiple comparisons. Normality for biochemical measures was assessed using the Shapiro-Wilk test using the Univariate procedure in SAS 9.2 and non-normal data were log transformed prior to analysis. HIE was calculated by dividing mean C-peptide levels by mean serum insulin levels. The division of C-peptide by insulin levels is intended to represent HIE as a large proportion of insulin is removed by the liver during the first circulation and C-peptide is not (Eaton et al. 1983). The Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) was used to approximate insulin resistance for the purpose of characterizing the metabolic health of the children in each weight status group. To determine HOMA-IR the following calculation was used: (Baseline Glucose × Baseline Insulin) / 22.5 (Matthews et al. 1985). While it is recommended that fasted insulin and glucose values be used for determining HOMA-IR, the use of insulin and glucose values...
after a standardized meal have shown to be significantly correlated with fasted values (Hancox and Landhuis 2011). For each child HOMA-IR was calculated on both study session days and the mean of the two values was used for statistical analysis. Prehepatic insulin secretion (ISEC) was calculated by the deconvolution of plasma C-peptide concentrations using the ISEC software package developed and provided by Hovorka et al. (Hovorka et al. 1996). Total area under the curve (AUC) for all biochemical measures was calculated for 0-120 min using the trapezoid rule, as well as for AA. All AUC values were analyzed using a two-way ANOVA via the Proc Mixed procedure to evaluate the effect of snack, weight status, and their interaction. Correlations between dependent measures were conducted using Pearson correlation coefficients. Differences are reported as significant if $P < 0.05$. 

...
Results

Participants

In total 18 children completed this study, 11 NW children and 7 OW/OB children, their baseline characteristics appear in Table 2. Age ($P = 0.38$), weight ($P = 0.16$), height ($P = 0.87$), fat-free mass ($P = 0.82$), HOMA-IR ($P = 0.15$), and overall DEBQ scores ($P = 0.30$) did not differ between NW and OW/OB children. The OW/OB group had significantly greater BMI percentile scores ($P = 0.0001$), fat mass ($P < 0.001$), and lower levels of physical activity ($P = 0.01$).

Subjective Appetite

At baseline there was no difference between AA scores between snacks ($P = 0.44$) or between NW and OW/OB children ($P = 0.52$). Absolute AA scores rose over time ($P < 0.0001$) but were not affected by snack ($P = 0.62$) or weight status ($P = 0.46$) (Table
There was no difference between AA AUC scores between snacks ($P = 0.98$) or between children of different weight statuses ($P = 0.86$) (Table 3).

Subjective physical comfort and palatability of the snacks and the test meal

Physical comfort scores were not affected by weight status ($P = 0.18$), snack ($P = 0.09$), or time ($P = 0.45$) (Table 3). Perceived sweetness of the dairy ($P = 0.78$) and non-dairy ($P = 0.25$) snack did not differ between NW and OW/OB children. Perceived pleasantness of the dairy ($P = 0.22$) and non-dairy ($P = 0.42$) snack did not differ between NW and OW/OB children (Table 3). Overall, children did not perceive a difference in sweetness ($P = 0.11$) or pleasantness ($P = 0.09$) between the two snacks.

Food and water intake

FI did not differ between snacks ($P = 0.55$) or between children of different weight statuses ($P = 0.78$) (Table 3). Water intake did not differ between snacks ($P = 0.44$) or between NW and OW/OB children ($P = 0.40$) (Table 3).

Blood Glucose

There was no difference in blood glucose concentrations between snacks at baseline ($P = 0.58$) (Figure 1). Blood glucose concentrations were affected by snack ($P < 0.001$), time (increasing in the 120 min after snack consumption, $P < 0.0001$), and there was a snack × time interaction ($P = 0.03$) (Figure 1). Post hoc analysis revealed that blood glucose was lower after the dairy snack at 60 ($P < 0.001$) and 90 ($P < 0.001$) min (Figure 1). Blood glucose concentrations were not different between NW and OW/OB children ($P = 0.10$) in the 120 min after snack consumption (Table 4); however, at baseline NW (4.6 ± 0.1 mmol/L) children had lower BG than OW/OB (4.9 ± 0.1 mmol/L) children ($P = 0.04$). Blood glucose AUC was lower after the dairy snack ($P <
0.01) but was not different between weight statuses ($P = 0.15$) and there was no snack × weight status interaction ($P = 0.30$) (Table 4).

**Serum Insulin**

Insulin concentrations were not different between weight statuses ($P = 0.15$) or snacks ($P = 0.31$) at baseline however, in the 120 min after snack consumption they were affected by time ($P < 0.0001$), snack ($P < 0.0001$), and their interaction ($P < 0.0001$) (Figure 1). There was no effect of weight status on insulin concentrations ($P = 0.10$) (Table 4). Insulin concentrations were higher after the dairy snack at 30 ($P < 0.0001$) and 60 ($P = 0.0555$) min as determined by post hoc analysis (Figure 1). Insulin AUC was greater after the dairy compared to the non-dairy snack ($P < 0.0001$) but was not different between weight statuses ($P = 0.09$) and there was no interaction between the two ($P = 0.44$) (Table 4).

**Blood C-peptide**

No difference in C-peptide levels between NW and OW/OB children ($P = 0.69$) or between snacks ($P = 0.55$) was observed at baseline. Time ($P < 0.0001$), but not weight status ($P = 0.87$) or snack ($P = 0.75$) affected C-peptide concentrations in the 120 min after snack consumption (Figure 1). Neither snack ($P = 0.54$), weight status ($P = 0.31$), or their interaction ($P = 0.11$) affected AUC C-peptide concentrations (Table 4).

**Insulin Secretion**

At baseline ISEC was not different between NW and OW/OB children ($P = 0.39$) or between snacks ($P = 0.37$). ISEC was not affected by weight status ($P = 0.42$), time ($P = 0.23$), or snack ($P = 0.23$) in the 120 min after snack consumption (Figure 1).
was no effect of weight status \((P = 0.61)\), snack \((P = 0.97)\), or their interaction \((P = 0.57)\) on AUC ISEC (Table 4).

**Hepatic Insulin Extraction**

At baseline, there was no effect of snack \((P = 0.16)\) or weight status \((P = 0.26)\) on HIE values. In the 120 min after snack consumption, HIE was affected by snack \((P < 0.01)\), time \((P < 0.0001)\), and their interaction \((P = 0.0001)\) but, there was no effect of weight status \((P = 0.34)\) (Figure 1). HIE was significantly reduced after the dairy snack at 30 min \((P < 0.001)\) (Figure 1).

**Plasma GLP-1**

GLP-1 concentrations were not different at baseline between snacks \((P = 0.18)\) or between NW and OW/OB children \((P = 0.08)\). GLP-1 concentrations in the 120 min following snack consumption were affected by snack \((P < 0.001)\), weight status \((P = 0.03)\) and time \((P < 0.01)\) (Figure 1). Post hoc analysis revealed that GLP-1 concentrations increased between 0 and 30 min \((P = 0.0047)\). GLP-1 AUC concentrations were not affected by weight status \((P = 0.73)\), snack \((P = 0.25)\), or their interaction \((P = 0.59)\) (Table 4).

**Associations of Dependent Measures**

Mean blood glucose concentrations in the 120 min after snack consumption \((r = 0.72, P = 0.001)\) and glucose AUC values \((r = 0.72, P = 0.001)\) were positively correlated with body fat mass. Mean insulin values \((r = 0.47, P = 0.05)\) were positively correlated with mean GLP-1 values. Mean C-peptide values were negatively correlated with BMI percentile \((r = -0.52, P = 0.03)\) and mean GLP-1 values \((r = -0.60, P < 0.01)\). Mean ISEC was positively correlated with mean GLP-1 values \((r = 0.80, P < 0.0001)\). HIE was
positively correlated with weight \( (r = 0.47, P = 0.0467) \) and fat free mass \( (r = 0.64, P < 0.01) \). HIE was not correlated with mean GLP-1 \( (r = -0.11, P = 0.53) \) or GLP-1 AUC \( (r = -0.11, P = 0.51) \).

Discussion

This study is one of the first to examine the acute effect of commercially available foods, rather than isolated food components such as whey protein, on postprandial blood glucose control and short-term FI in children. In both NW and OW/OB children consumption of a dairy snack caused a reduction in blood glucose concentrations and an increase in circulating insulin levels compared with a non-dairy snack. The increase of circulating insulin levels in response to both snacks was transient and lasted only up to 90 min after the dairy and 60 min after the non-dairy snack. Neither C-peptide nor ISEC were different after the two snacks however, HIE was reduced after the dairy snack suggesting the higher levels of circulating insulin may be explained by a reduction in the
postprandial removal of insulin by the liver during the first pass rather than increased
insulin secretion by the β-cells (Lan-Pidhainy and Wolever 2010).

In agreement with previous reports in adults, the dairy snack caused an increase in
insulinaemia beyond what would be predicted from the concurrent glycaemia alone
(Liljeberg, Elmstahl and Bjorck 2001; Ostman et al. 2001). It is not fully understood what
components of the dairy food matrix produce large increases in circulating insulin
however, the effects do not appear to be abated by fermentation (Ostman et al. 2001).
Others have observed the sharp rise in insulin after dairy product consumption is
correlated with the appearance of leucine, valine, lysine, and isoleucine in the
bloodstream (Nilsson et al. 2004). A possible mechanism for the dairy induced increase
in insulin is incretin release in humans (Akhavan et al. 2014; Lan-Pidhainy and Wolever
2010), as was supported by the absolute values in the present study (~44% increase in
GLP-1 after the dairy snack compared to baseline). It has also been shown that dairy
protein reduces dipeptidyl peptidase-4 (DPP-IV) activity, the enzyme responsible for
GLP-1 degradation, in mice (Gunnarsson et al. 2006) although these results were not
replicated in humans with T2DM after consuming whey protein (Jakubowicz et al. 2014).
It is unknown if healthy humans would respond similarly to T2DM patients and recent in
vitro evidence supports the ability of various milk protein fractions to inhibit DPP-IV
(Nongonierma and FitzGerald 2014). In the present study GLP-1 secretion did not beget
insulin secretion and it is not known if more GLP-1 was secreted or simply allowed to
survive longer due to potential DPP-IV inhibition by the high protein, dairy snack
provided.
C-peptide concentrations and ISEC did not differ between snacks indicating the observed increase in insulin was not due to a greater production of insulin. In apparent contrast, a similar study in adults by Akhavan et al. found an increase in C-peptide and ISEC after whey protein compared to a calorically void preload but this increase was significantly less than glucose alone (Akhavan et al. 2014). This study detected a significant difference in the first 30 min after consumption, a period during which they had sampled blood at 20 and 30 min whereas the present study did not obtain a blood sample at 20 min thus, the timing of sampling may explain the discord.

The disparity between insulin and ISEC levels may be explained by increased HIE. Blood C-peptide divided by insulin provides a measure of HIE after the first pass through the liver as proinsulin is enzymatically converted to equimolar concentrations of insulin and C-peptide however, while insulin can be removed by the liver through the first pass C-peptide cannot. It is estimated 50% of insulin is removed by this mechanism before entering circulation in a fasted state (Eaton et al. 1983) and can be reduced to 20% after feeding (Caumo et al. 2007). The present results are in agreement with previous work demonstrating that in healthy adults protein in combination with carbohydrate results in a greater increase in circulating insulin concentrations than carbohydrate alone, independent of C-peptide and ISEC (Lan-Pidhainy and Wolever 2010).

The cause of reduced HIE after consumption of the dairy snack is unclear but it may be due to the intrinsic properties of the dairy food matrix. It has been observed that HIE is reduced by glucose ingestion but unchanged by glucose infusion suggesting that nutrient interaction with the small intestine plays a key role in reducing HIE (Meier et al. 2007). GLP-1 is integral to insulin regulation and is released from the L-cells in the distal
small intestine in response to nutrient sensing in the proximal small intestine (Schirra et al. 1996). HIE has been shown to be reduced only by exogenous, not endogenous, production of incretins in adults (Meier et al. 2007) which is supported by our present finding of no association between GLP-1 and HIE. The present observation in conjunction with previous observations by Lan-Pidhainy and Wolever (2010) suggest that whether the dairy protein is primarily derived from casein (Greek yogurt) or whey similar effects on circulating insulin levels and HIE are observed, indicating a similarity that the two proteins share is responsible for the effect. Both proteins contain appreciable amounts of the amino acids isoleucine, leucine, and lysine (Sindayikengera and Xia 2006) all three of which have been shown to be correlated with insulin concentrations after dairy product consumption (Nilsson et al. 2004). Future research directly evaluating whether increases in circulating isoleucine, leucine, and lysine are casually responsible for increases in circulating insulin is warranted.

At the test meal FI did not differ between the dairy and non-dairy snack. A lack of effect could have been due to the small caloric dose of the snack (170.5-175 kcal). Previous research however, has shown that NW and OW/OB boys were able to compensate for a similar caloric load of glucose given 30 min before an ad libitum test meal (Bellissimo et al. 2008a) suggesting that the longer duration (120 min) to the test meal may have been a more influential factor. The effect of duration to the test meal is supported by a previous study comparing dairy beverages to other commercially available beverages that noted differences in FI at a test meal 60 min but not 120 min later (Panahi et al. 2013). Furthermore, no difference in ad libitum FI was observed after 20 adult men consumed varying dairy snacks or orange juice with caloric content ranging
from 141 to 167 kcal 120 min later despite the different types of foods provided and their protein to carbohydrate ratios (El Khoury et al. 2014). Similarly, in adult women no differences in meal initiation or subsequent FI was observed when 160 kcal yogurt snacks were given in the afternoon (Ortinau et al. 2013). While higher levels of circulating insulin and reduced levels of glucose have been associated with reduced FI (Air et al. 2002), in this study insulin and glucose levels were not correlated with FI. This lack of correlation can be explained by the fact that differences in glucose and insulin levels were no longer present after 90 min and any anorexigenic action of insulin may have exerted would have dissipated by 120 min.

One limitation of the study is that the power calculations that guided participant recruitment were based on changes in blood glucose levels and not secondary measures such as subjective appetite. Therefore, the results of secondary measures should be interpreted with caution. An additional limitation of this study was that it was powered to detect differences between snacks within weight statuses potentially confounding any inferences made about differences in response to snacks between weight statuses. Finally, the ISEC software is based on C-peptide data derived from an adult population and while a similar program using data from a pediatric population would have been preferred, to our knowledge, no such data exists.

In conclusion, a dairy snack reduced postprandial blood glucose levels in the 120 min following consumption compared to a non-dairy snack likely due to an increase in circulating insulin levels possibly caused by a reduction in HIE in both NW and OW/OB children 9-14y old children however, no effect on subjective appetite or FI was observed. Additionally, the present study confirms that similar to adults, dairy consumption
increases GLP-1 in children. Therefore, combining protein and carbohydrate in a dairy food matrix and provided to children as a snack may hold benefit with regards to metabolic regulation, particularly blood glucose regulation.

Acknowledgements

GHA conceived the hypothesis; BL and GHA designed the study; BJG, MG and AL conducted the experiments; BJG performed statistical analysis and wrote the paper; BL supervised graduate students (BJG, MG and AL) and participated in writing the manuscript, JH and NB contributed to the study design and assisted in writing the manuscript, YA, NTG and FN assisted in data analysis and writing the manuscript, BL had primary responsibility for the final content. All authors read and approved the final manuscript.

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Conflict of interest. The authors declare no conflict of interest.
**References**


10.1016/j.numecd.2014.05.015


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Tanner, J.M. Growth at adolescence; with a general consideration of the effects of hereditary and environmental factors upon growth and maturation from birth to maturity: Springfield, Ill; 1962.


Table 1. Characteristics of the Snacks

<table>
<thead>
<tr>
<th></th>
<th>Dairy (Greek Yogurt)</th>
<th>Non-Dairy (Sandwich Type Cookies)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (g)</td>
<td>199</td>
<td>37.5</td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>170.5</td>
<td>175</td>
</tr>
<tr>
<td>Total fat (g)</td>
<td>0</td>
<td>7.5</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>17</td>
<td>1.3</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>26.1</td>
<td>26.3</td>
</tr>
<tr>
<td>Sugars (g)</td>
<td>23.9</td>
<td>15.0</td>
</tr>
<tr>
<td>Fibre (g)</td>
<td>1.1</td>
<td>1.3</td>
</tr>
<tr>
<td>Available Carbohydrate (g)</td>
<td>25.0</td>
<td>25.0</td>
</tr>
</tbody>
</table>
## Table 2. Baseline Characteristics of Children

<table>
<thead>
<tr>
<th></th>
<th>All Children (n=18)</th>
<th>Normal Weight Children (n=11)</th>
<th>Over Weight/Obese Children (n=7)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>11.57 ± 0.41</td>
<td>11.87 ± 0.58</td>
<td>11.10 ± 0.55</td>
<td>0.38</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>48.39 ± 3.58</td>
<td>44.35 ± 4.32</td>
<td>54.76 ± 5.80</td>
<td>0.16</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.53 ± 0.04</td>
<td>1.53 ± 0.05</td>
<td>1.52 ± 0.06</td>
<td>0.87</td>
</tr>
<tr>
<td>BMI percentile(^1)</td>
<td>69.89 ± 6.10</td>
<td>54.73 ± 6.61(^a)</td>
<td>93.71 ± 1.43(^b)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Fat mass (kg)(^2)</td>
<td>11.46 ± 1.53</td>
<td>7.69 ± 1.02(^a)</td>
<td>16.86 ± 2.17(^b)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fat-free mass (kg)(^2)</td>
<td>39.19 ± 2.75</td>
<td>38.64 ± 3.90</td>
<td>39.97 ± 4.03</td>
<td>0.82</td>
</tr>
<tr>
<td>Tanner Stage I-II(^3)</td>
<td>14</td>
<td>8</td>
<td>6</td>
<td>-</td>
</tr>
<tr>
<td>Tanner Stage III-V(^3)</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1.47 ± 0.08</td>
<td>1.40 ± 0.10</td>
<td>1.59 ± 0.15</td>
<td>0.30</td>
</tr>
<tr>
<td>-------------------</td>
<td>--------------</td>
<td>--------------</td>
<td>--------------</td>
<td>------</td>
</tr>
<tr>
<td>Physical Activity</td>
<td>53.11 ± 6.50</td>
<td>63.55 ± 7.93</td>
<td>33.97 ± 6.29</td>
<td>0.01</td>
</tr>
<tr>
<td>(MetS/week)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Food Intake</td>
<td>450.15 ± 25.40</td>
<td>436.78 ± 31.46</td>
<td>471.16 ± 43.58</td>
<td>0.78</td>
</tr>
<tr>
<td>(kcal)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.13 ± 0.26</td>
<td>1.80 ± 0.29</td>
<td>2.63 ± 0.45</td>
<td>0.15</td>
</tr>
</tbody>
</table>

Data are presented as means ± SEM.

Values with different subscript in each horizontal line are different from each other (unpaired t-test, P < 0.05).

1 Based on the Center for Disease Control growth charts (Ogden et al. 2002).

2 Estimated using a Tanita Body Composition Analyzer (Model TBF-300A, Tanita, Arlington Heights, Illinois, USA).

3 Estimated using self-administered Tanner staging questionnaires (Tanner 1962).

4 Determined using a child specific version of the Dutch Eating Behaviour Questionnaire (van Strien and Oosterveld 2008).

5 Assessed using a Physical Activity Questionnaire (Ainsworth et al. 2000).

Abbreviations: BMI, body mass index; DEBQ, Dutch Eating Behaviour Questionnaire; HOMA-IR, homeostasis model assessment of insulin resistance; MetS, Metabolic Equivalents
Table 3. Food and Water Intake at 120 min, Average Appetite and Physical Comfort in the 120 min after snack consumption, and Sensory Characteristics of the Snacks

<table>
<thead>
<tr>
<th></th>
<th>All Children</th>
<th>Normal Weight Children</th>
<th>Over Weight/Obese Children</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dairy (Greek Yogurt) (n=18)</td>
<td>Non-Dairy (Sandwich Type Cookies) (n=18)</td>
<td>Snack (P)</td>
</tr>
<tr>
<td>Food Intake (kcal)</td>
<td>440.1 ± 39.6</td>
<td>458.8 ± 30.2</td>
<td>0.55</td>
</tr>
<tr>
<td>Water Intake (g)</td>
<td>280.1 ± 61.8</td>
<td>248.1 ± 41.2</td>
<td>0.44</td>
</tr>
<tr>
<td>Perceived Sweetness (mm VAS)</td>
<td>59.7 ± 6.2</td>
<td>69.0 ± 5.1</td>
<td>0.11</td>
</tr>
<tr>
<td>Perceived Pleasantness (mm)</td>
<td>63.6 ± 7.9</td>
<td>81.1 ± 5.5</td>
<td>0.09</td>
</tr>
<tr>
<td>Physical Comfort (mm VAS)</td>
<td>74.3 ± 2.0</td>
<td>78.5 ± 1.9</td>
<td>0.09</td>
</tr>
<tr>
<td>Average Appetite (mm VAS)</td>
<td>53.7 ± 5.3</td>
<td>53.2 ± 5.4</td>
<td>0.62</td>
</tr>
<tr>
<td>Average Appetite (AUC)</td>
<td>5984.7 ± 629.6</td>
<td>5958.2 ± 655.0</td>
<td>0.98</td>
</tr>
</tbody>
</table>
Data are presented as means ± SEM.

No measures were affected by snack, weight status, or their interaction.

Abbreviations: AUC, area under the curve; VAS, visual analogue scale.
Table 4. Effect of Snack and Weight Status on Blood Glucose, Serum Insulin, C-Peptide, ISEC, HIE, and GLP-1

<table>
<thead>
<tr>
<th></th>
<th>Normal Weight (n = 11)</th>
<th>Over Weight/Obese (n = 7)</th>
<th></th>
<th>Greek Yogurt</th>
<th>Cookies</th>
<th>P</th>
<th>Weight Status X Snack Interaction (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Blood Glucose (mmol/L)</td>
<td>4.6 ± 0.1</td>
<td>4.9 ± 0.1</td>
<td>0.10</td>
<td>4.5 ± 0.1</td>
<td>4.9 ± 0.1</td>
<td>0.0006</td>
<td>0.03</td>
</tr>
<tr>
<td>Blood Glucose AUC (mmol/L • min/mL)</td>
<td>550.7 ± 14.7</td>
<td>588.9 ± 14.4</td>
<td>0.15</td>
<td>541.4 ± 13.1</td>
<td>589.7 ± 15.9</td>
<td>0.0058</td>
<td>0.30</td>
</tr>
<tr>
<td>Mean Serum Insulin (pmol/mL)</td>
<td>106.79 ± 7.83</td>
<td>139.83 ± 12.80</td>
<td>0.10</td>
<td>141.93 ± 11.73</td>
<td>97.35 ± 6.90</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Serum Insulin AUC (pmol • min/L)</td>
<td>14082.19 ± 1236.69</td>
<td>18344.05 ± 1783.72</td>
<td>0.09</td>
<td>18989.52 ± 1395.73</td>
<td>12489.64 ± 1232.33</td>
<td>&lt; 0.0001</td>
<td>0.44</td>
</tr>
<tr>
<td>Mean C-Peptide (pmol/L)</td>
<td>1017.82 ± 57.17</td>
<td>1057.12 ± 76.39</td>
<td>0.87</td>
<td>1050.36 ± 63.38</td>
<td>1015.64 ± 66.72</td>
<td>0.75</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>C-Peptide AUC (pmol • min/L)</td>
<td>28178.17 ± 6563.90</td>
<td>43250.99 ± 10682.78</td>
<td>0.31</td>
<td>32939.12 ± 5459.52</td>
<td>35140.53 ± 10462.14</td>
<td>0.54</td>
<td>0.11</td>
</tr>
<tr>
<td>ISEC (pmol/kg)</td>
<td>5.27 ± 0.40</td>
<td>4.17 ± 0.29</td>
<td>0.42</td>
<td>4.66 ± 0.35</td>
<td>5.02 ± 0.42</td>
<td>0.23</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>ISEC AUC (pmol/kg)</td>
<td>HIE</td>
<td>Mean GLP-1 (pmol/L)</td>
<td>GLP-1 AUC (pmol • min/L)</td>
<td></td>
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<td>------------------------</td>
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<tr>
<td></td>
<td>449.41 ± 60.11</td>
<td>17.65 ± 2.16</td>
<td>3.34 ± 0.25</td>
<td>105.68 ± 18.50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>391.71 ± 51.31</td>
<td>11.52 ± 1.81</td>
<td>5.95 ± 0.64</td>
<td>91.03 ± 32.57</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>0.61</td>
<td>0.34</td>
<td>0.03</td>
<td>0.73</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>431.92 ± 51.61</td>
<td>12.51 ± 1.32</td>
<td>5.14 ± 0.52</td>
<td>123.38 ± 24.69</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>422.03 ± 66.69</td>
<td>18.05 ± 2.48</td>
<td>3.57 ± 0.31</td>
<td>74.87 ± 21.99</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.97</td>
<td>&lt;0.01</td>
<td>&lt;0.001</td>
<td>0.25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.57</td>
<td>0.0001</td>
<td>0.53</td>
<td>0.59</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as means ± SEM (n = 18 except where specified).

*P* values represent comparisons of the two columns to the left via a two-way ANOVA.

Abbreviations: AUC, area under the curve; GLP-1, glucagon-like peptide; HIE, hepatic insulin extraction; ISEC, insulin secretion.
Figure 1. The effect of a dairy and non-dairy snack on blood glucose, insulin, GLP-1, C-Peptide, ISEC, and Hepatic Insulin Extraction

Data are presented as means ± SEM (n = 18).

Abbreviations: ISEC, insulin secretion.

Blood glucose concentrations were affected by snack ($P < 0.001$), time ($P < 0.0001$), and their interaction ($P = 0.03$, Three-way ANOVA).

Serum insulin concentrations were affected by time ($P < 0.0001$), snack ($P < 0.0001$), and their interaction ($P < 0.0001$, Three-way ANOVA).

C-peptide concentrations were affected by time ($P < 0.0001$), but not snack ($P = 0.75$) and there was a snack by treatment interaction ($P = 0.03$, Three-way ANOVA)

HIE was affected by snack ($P < 0.01$), time ($P < 0.0001$), and their interaction ($P = 0.0001$, Three-way ANOVA).

GLP-1 concentrations were affected by snack ($P < 0.001$), time ($P < 0.001$) but not their interaction ($P = 0.76$, Three-way ANOVA).

ISEC was not affected by snack ($P = 0.23$), time ($P = 0.23$), or their interaction ($P = 0.16$, Three-way ANOVA).

*indicates significance ($P < 0.05$; Tukey’s post hoc test).