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Mechanism of action of whole milk and its components on glycemic control in healthy young men☆,☆☆,★

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Abstract

Milk reduces post-meal glycemia when consumed either before or within an *ad libitum* meal. The objective of this study was to compare the effect of each of the macronutrient components and their combination with whole milk on postprandial glycemia, glucoregulatory and gastrointestinal hormones and gastric emptying in healthy young men. In a randomized, crossover study, 12 males consumed beverages (500ml) of whole milk (3.25% M.F.) (control), a simulated milk beverage based on milk macronutrients, complete milk protein (16g), lactose (24g) or milk fat (16g). Whole and simulated milk was similar in lowering postprandial glycemia and slowing gastric emptying while increasing insulin, C-peptide, peptide tyrosine tyrosine (PYY) and cholecystokinin (CCK), but simulated milk resulted in higher (41%) glucagon-like peptide-1 (GLP-1) and lower (43%) ghrelin areas under the curve (AUC) than whole milk (P=.01 and P=.04, respectively). Whole and simulated milk lowered glucose (P=.0005) more than predicted by the sum of AUCs for their components. Adjusted for energy content, milks produced lower glucose and hormone responses than predicted from the sum of their components. The effect of protein/kcal on the AUCs was higher than fat/kcal for insulin, C-peptide, insulin secretion rate, GLP-1, CCK and paracetamol (P<.0001), but similar to lactose except for CCK and paracetamol, which were lower. The response in PYY and ghrelin was similar per unit of energy for each macronutrient. In conclusion, milk lowers postprandial glycemia by both insulin and insulin-independent mechanisms arising from interactions among its macronutrient components and energy content.

Keywords: Whole milk; Milk macronutrients; Glycemic control; Gastric emptying; Glucoregulatory and gastrointestinal hormones

1. Introduction

Epidemiological studies have linked frequent dairy consumption with healthier body weights [1] and lower risk of type 2 diabetes (T2D) [2]. This possible link between milk consumption and obesity and T2D is of growing interest because milk and its components contribute to metabolic control, including postprandial glycemia [3,4].

Fluid milk products reduce post-meal glycemia when consumed either before or within an *ad libitum* meal by healthy young adults [3,4] or with carbohydrate foods [5]. In a comparison of familiar beverages consumed before a meal, including isovolumetric (500ml) servings of 2% milk, 1% chocolate milk, orange juice, soy beverage, infant formula and water, postprandial glycemia following a pizza meal was lowest after milk [4]. Similarly, 1% milk, in contrast to other caloric and noncaloric beverages, when consumed at a pizza meal, reduced postprandial glycemia and produced the lowest post-meal appetite [3]. Milk consumed with carbohydrate foods, such as bread and pasta, also reduces postprandial glycemia than observed after the carbohydrate food alone [5].

The effect of milk on postprandial glycemia has been attributed to its stimulatory effect on insulin [6] because milk proteins, when consumed in beverage form or with carbohydrate, reduce glycemia [7], consistent with a rise in blood insulin concentrations [6,8]. Although milk proteins stimulate insulin, attributed to the rapid digestion and absorption of their branched-chain amino acids [9], post-meal reduction of glycemia after milk consumption may not be only due to its protein content and insulin release [7]. Milk proteins release gut hormones including glucagon-like peptide-1 (GLP-1), peptide tyrosine tyrosine (PYY) and cholecystokinin (CCK) which also

Abbreviations: T2D, type 2 diabetes; M.F, milk fat; GLP-1, glucagon-like peptide-1; CCK, cholecystokinin; PYY, peptide tyrosine tyrosine; BMI, body mass index; ANOVA, analysis of variance; ISEC, Insulin SECretion; DPP-IV, dipeptidyl peptidase IV.

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affect blood glucose, delay stomach emptying [7,10,11] and suppress food intake [10,12].

In addition, milk is a complex mixture of proteins (whey protein and casein in the ratio of 20:80), fats (saturated, monounsaturated and polyunsaturated and trans-fatty acids), carbohydrate (lactose), and micronutrients with a wide range of bioactivities [13]. Milk fat is an important regulator of metabolic responses via the ileal brake, slowing stomach emptying and stimulating the release of gastrointestinal peptides such as CCK and PYY [14]; however, its relevance to glycemic control by milk has not been reported. Furthermore, lactose contributes to lower glycemia than other sugars or starch [5], but also simulated glucoregulatory and gastrointestinal hormones [15]. Thus, each of the macronutrients of milk may contribute to reducing glycemia and food intake [15–17]; however, there are no reports of the individual and collective contribution of milk's macronutrient components to the effect of whole milk.

Therefore, we hypothesized that regulation of postprandial glycemia after milk consumption occurs through both insulin and insulin-independent actions due to interactions among its macronutrient components and energy content. The objective was to compare the effects of isovolumetric (500ml) beverages of whole milk (3.25% M.F.), each of its macronutrient components (protein, lactose and fat) and their combination (a simulated milk beverage) on postprandial glycemia, glucoregulatory and gastrointestinal hormones and gastric emptying in healthy young men.

2. Methods and materials

2.1. Participants

Participants were recruited through advertisements posted at the University of Toronto campus. Healthy men between 20 and 30 years of age with a body mass index (BMI) of 20–24.9kg/m^2 were eligible to participate. Exclusion criteria included smoking, dieting, skipping breakfast, lactose intolerance or allergies to milk, taking medications that may affect glucose metabolism, diabetes (fasting blood glucose ≥7.0mmol/L) or other metabolic diseases that could interfere with study outcomes. Based on previous clinical studies on gastrointestinal hormones with the sample size required for blood glucose response, 12 participants were recruited and completed the sessions [18]. Participants were financially compensated for completing the study. The procedures of the study were approved by the Human Subject Review Committee, Ethics Review Office at the University of Toronto.

2.2. Beverages

Beverages included isovolumetric amounts (500ml) of (1) whole milk (3.25% M.F.; Neilson Dairy, Toronto, ON, Canada) (control); (2) complete milk protein (16g, whey protein/casein ratio of 20:80; American Casein Company, Burlington, NJ, USA); (3) lactose (24g; Davisco Foods International Inc., Eden Prairie, MN, USA); (4) milk fat (16g, from unsalted butter, 80% M.F.; Lactantia, Parmalat Canada Inc., Toronto, ON, Canada); and (5) a simulated milk beverage consisting of complete milk protein (16g), lactose (24g) and milk fat (16g). Each of the macronutrients and that in the simulated milk beverage were formulated at the same concentration as in whole milk.

Whole milk (3.25% M.F.), which contains the highest fat content compared to other commercially available types of milk, was used as the control for the following reason. In our previous study [4], despite infant formula and chocolate milk having similar carbohydrate contents, infant formula, containing a higher fat content, resulted in lower pre-meal blood glucose concentrations compared to chocolate milk. Thus, we hypothesized that the fat component may also contribute to glycemic control. A simulated milk beverage was provided to assess the effects of the combination of macronutrients without the possible interference of other components of whole milk on glycemic control.

Complete milk protein and lactose beverages were prepared at the Department of Nutritional Sciences at the University of Toronto by adding each of the powders to 500ml of water and stirred at room temperature for 20min until mixed. Milk fat beverages were prepared by the Department of Food Science at the University of Guelph from butter (80% M.F.; Lactantia, Parmalat Canada Inc.). Butter was added to water (at 4.35%), heated to 75°C and mixed using an industrial mixer. During the mixing process, a saturated monodiglyceride (0.2%, Danisco, Toronto, ON, Canada) was added as an emulsifying agent. The fat mixture was run through a two-stage homogenizer (Model 31MR [APV Gaulin Inc., Wimington, MA, USA], at 17.5/3.5MPa) to reduce the size and size distribution of milk fat globules. The milk fat beverage was pasteurized at 75°C for 15min, then heated to 90°C and poured into 500-ml sterilized bottles. Simulated milk beverages were prepared at the Department of Nutritional Sciences at the University of Toronto by adding lactose (24g) and protein (16g) (both in powder form) to 460ml of the ready-made milk fat beverages to achieve a volume of 500ml with 16g of fat and stirred at room temperature for 20min.

Paracetamol (1.5g, Panadol; GlaxoSmithKline) was dissolved in each of the five beverages so the rate of its appearance in the blood can be used as a proxy to measure rate of gastric emptying [19]. Vanilla extract (1.2ml; Flavorganics, Newark, NJ, USA) and sucralose (0.02g; McNeil Specialty Products Company, New Brunswick, NJ, USA) were added to all beverages to equalize palatability and sweetness and blind the participants to the beverages. All beverages were isovolumetric (500ml) based on the commercially available serving size of milk beverages and were served chilled. The nutritional composition of the beverages is provided in Table 1.

2.3. Protocol

This study was a randomized, crossover, single-blind design consisting of five sessions separated by a 1-week washout period to minimize any carryover effects. Individuals who fulfilled eligibility requirements were invited to participate in the study. Participants attended the Department of Nutritional Sciences at the University of Toronto following a 12-h overnight fast, except for water, which was permitted until 1h before each session. To minimize within subject variability, all participants were scheduled to arrive at the same time and on the same day of the week for each treatment, instructed to refrain from alcohol consumption and to maintain the same dietary and exercise patterns the evening before each test. To ensure that these instructions were followed, participants completed a questionnaire detailing pre-session information about their diet and lifestyle patterns. The order of beverages was randomized using a randomization block design, which was generated with a random generator script in SAS version 9.2 (SAS Institute Inc, Cary, NC, USA).

On arrival, participants completed visual analog scale questionnaires assessing their "sleep habits," "stress factors," "food intake and activity level," "feelings of fatigue" and "motivation to eat" [20,21]. Before the beginning of each test, each subject provided a baseline finger-prick capillary blood sample using a Monoejector Lancet device (Sherwood Medical, St. Louis, MO, USA) to ensure compliance with fasting instructions. Plasma concentration of glucose was measured with a glucose meter (Accu-Chek Compact; Roche Diagnostics Canada, Laval, Quebec, Canada). A baseline measurement of>5.5mmol/L indicated noncompliance with the fasting instructions, and participants were rescheduled accordingly.

Following the finger-prick blood glucose measurement, an indwelling intravenous catheter was inserted in the antecubital vein by a registered nurse and a baseline blood sample was obtained. Immediately thereafter, each person was instructed to consume one of the five beverages within 5min at a constant pace. Blood samples were collected at 0min (baseline) and at 30, 45, 60, 90, 120, 150 and 180min. Participants were asked to remain seated for the duration of the experimental session and were permitted to read, do homework or listen to music.

2.4. Blood parameters

Blood was collected in 8.5ml BD P800 tubes (BD Diagnostics, Franklin Lakes, NJ, USA) containing spray-dried K_2EDTA anticoagulant and a proprietary cocktail of additives which includes DPP-IV, esterase and other protease inhibitors to prevent the proteolytic breakdown of hormones. The tubes were centrifuged at 1300 RCF for 20min at 4°C. Collected plasma samples were aliquoted in Eppendorf tubes and stored at −70°C for analyses. Plasma concentrations of glucose, insulin, C-peptide, GLP-1, PYY, CCK, ghrelin and paracetamol were measured.

Plasma glucose was measured using the enzymatic hexokinase method (intra-CV (coefficient of variation): <5%; inter-CV: <8%; Roche Diagnostic). Insulin was assessed with an electrochemiluminescence assay "ECLIA" (intra-CV: <3%; inter-CV: <7%; Roche Diagnostic). These analyses were performed by the Pathology and Laboratory Medicine

Table 1
Nutritional composition of beverages

Composition[a]	Beverages[b]				
	Whole milk (3.25% M.F.)	Simulated milk beverage	Protein	Lactose	Fat
Energy (kcal)	300	300	64	96	144
Fat (total) (g)	16	16	0	0	16
Carbohydrate (g)					
Lactose (g)	24	24	0	24	0
Protein (g)	16	16	16	0	0
Whey (g)	3.2	3.2	3.2	0	0
Casein (g)	12.8	12.8	12.8	0	0

[a] Composition of each beverage as provided by the manufacturer.
[b] Amounts given are per 500ml serving. Paracetamol (1.5g), vanilla extract (1.2ml) and sucralose (0.02g) were added to all beverages.