MILK AND ITS COMPONENTS IN THE REGULATION OF SHORT-TERM APPETITE, FOOD INTAKE AND GLYCEMIA IN YOUNG ADULTS

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ABSTRACT

The hypothesis that milk consumption decreases short-term appetite and food intake and improves glycemic control compared with other caloric beverages in healthy young adults was explored in four experiments. The first two experiments compared isovolumetric amounts (500 ml) of milk (2% M.F.), chocolate milk (1% M.F.), a soy beverage, infant formula, orange juice and water on satiety and food intake and blood glucose before and after a meal provided at 30 min (Experiment 1) and 120 min (Experiment 2). Pre-meal ingestion of chocolate milk and infant formula (highest in calories) reduced food intake at 30 min, but not 2 h. Only milk reduced post-meal blood glucose in both experiments suggesting that its macronutrient composition is a factor in blood glucose control. Experiment 3 compared the effects of ad libitum consumption of milk (1% M.F.), regular cola, diet cola, orange juice and water at a pizza meal on fluid and ad libitum food intake and post-meal appetite and glycemia. Fluid volume consumed was similar, but all caloric beverages added to total meal-time energy intake. However, milk lowered post-meal blood glucose and appetite score. In Experiment 4, the effect of isovolumetric (500 ml) beverages of whole milk (3.25% M.F.) and each of its macronutrient components, protein (16 g), lactose (24 g), and fat (16 g) on glycemic control and gastrointestinal hormonal responses were examined. The reduction in post-prandial glycemia was mediated by interactions between its macronutrient components and associated with hormonal responses that slow stomach emptying and increase glucose disposal. Thus, the results of this research do not support the hypothesis that milk consumption decreases short-term appetite and food intake compared with other beverages; however, milk improves glycemic control by insulin-dependent and independent mechanisms.

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You taught me to persevere
You gave me the courage to believe in myself
You instilled in me the desire to learn
You pushed me to succeed and achieve my goals

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I learned that knowledge makes us humble
I learned that diligence and hard work make us successful

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
AUC	Area Under the Curve
BMI	Body Mass Index
BCAA	Branched-Chain Amino Acids
CHO	Carbohydrate
CCK	Cholecystokinin
CNS	Central Nervous System
CLA	Conjugated Linoleic Acid
CMP	Caseinomacropeptide
DPP-IV	Dipeptidyl Peptidase IV
FI	Food Intake
FFA	Free Fatty Acids
H	Hour
GI	Glycemic Index
GIP	Glucose-Dependent Insulinotropic Peptide
GLP-1	Glucagon-Like Peptide-1
GLP-1R	GLP-1 Receptors
GMP	Glycomacropeptide
II	Insulin Index
Kcal	Kilocalories
Min	Minutes
NS	Not Statistically Significant
PYY	Peptide Tyrosine Tyrosine
SEM	Standard Error of the Mean
SSB	Sugar-sweetened Beverages
T2D	Type 2 Diabetes
VAS	Visual Analogue Scale

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CHAPTER 1

INTRODUCTION

CHAPTER 1. INTRODUCTION

Over the last 30 years, overweight and obesity have become characteristic of the majority of Canadians. Approximately 60% of adults and 30% of children in Canada are overweight or obese [1]. The Canadian Health Measures Survey, conducted during 2007-2009, estimated the prevalence of metabolic syndrome at 19.1% in the adult population [2, 3], as defined by a combination of three or more of the following: a) abdominal circumference > 102 cm for men and > 88 cm for women; b) hypertension; c) hyperglycemia; and d) dyslipidemia [4]. Obesity is a major risk factor for other co-morbidities such as type 2 diabetes, marked by elevated blood glucose at fasting and postprandial, in the presence of insulin resistance [5]. According to the 2011 Canadian Diabetes Association report, 285 million people in Canada are affected directly and indirectly by diabetes and an estimated 9 million people have been diagnosed with diabetes or pre-diabetes [6]. This can lead to a variety of complications including cardiovascular, kidney, and vision damage.

Although consumer interest is increasing for foods that may help prevent or treat obesity and diabetes, effective dietary countermeasures against these diseases and related metabolic complications have not yet been established. There are two general approaches for their prevention and treatment, including pharmacological and nutritional. Since pharmacological approaches have many disadvantages such as adverse events and large costs, dietary approaches remain the safest way to improve metabolic functions. Thus, there is an urgency to find effective dietary approaches to reduce obesity and to identify functional properties of foods for the prevention and management of obesity and metabolic diseases.

In Canada and the United States, water, orange juice, cow's milk, and soy beverages have been suggested rather than sugar-sweetened beverages (SSB) for both adults and children. To provide guidance on beverage consumption, the Beverage Guidance Panel constructed a beverage hierarchy with water as a primary option followed by calorie-free tea or coffee, then low-fat milk and soy beverages, non-caloric sweetened beverages, caloric beverages with some nutrients and lastly, calorically sweetened beverages with no nutrients [7]. However, this was not based on comparisons of these commonly consumed beverages on satiety, food intake and metabolic regulation. These beverages differ considerably in their energy content and composition.

Milk is inexpensive, readily available and a good source of high quality nutrients and a recommended component of the diets of Canadians. Despite the known benefits of milk and milk products, the majority of Canadians do not consume two to three servings per day as recommended in Canada's Food Guide [8]. According to data from the Canadian Dairy Information Centre, the per capita consumption of milk in Ontario was 17% less in 2006 than it was in 1986 in Ontario [9]. The most popular type of milk in the last 20 years has been 2% milk, which has experienced a decline in consumption from 74 L in 1986 to 40 L per capita [9]. The same trend is observed for whole milk, for which consumption was reduced by 50% from 1986 to 2006 [9]. There have been many reasons for this, including increased consumption of SSB and dietary recommendations to avoid saturated fat.

Nevertheless, the decrease in milk consumption has coincided with an increase in the prevalence of metabolic diseases including obesity, type 2 diabetes, and metabolic syndrome. Epidemiologic studies have often linked frequent milk consumption with healthier body weights and lower risk of type 2 diabetes and metabolic syndrome [10-13], but many have not [14-16]. A small number of randomized control trials failed to show consistent benefits of increased consumption of dairy products in ad libitum diets on body weight, waist circumference, cardiovascular risk markers [17], indicators of chronic inflammation and endothelial function [18] or characteristics of metabolic syndrome; however, reduced waist circumference and sagittal abdominal diameter were found in those who started with low baseline calcium intake [19]. In contrast, other randomized control trials have shown that increased dairy consumption prevents or slows an increase in body weight [20-22]. These failures of observational and intervention studies to support findings of the epidemiologic studies emphasize a need for better understanding of the metabolic effects of consuming dairy products. Composition, amount, timing and frequency of consumption are primary determinants of the impact of a food on metabolic regulation, especially glucose utilization and disposal [23, 24].

In contrast to the inconsistencies in the epidemiologic data and outcomes of randomized control trials, results of many short-term studies of the physiological properties of milk components and their effect on short-term satiety, food intake and blood glucose add physiological plausibility to benefits of dairy consumption found in epidemiologic studies. The role of individual milk components [20, 25, 26], especially milk proteins and calcium, on metabolic regulatory mechanisms have received considerable attention. Whey protein in beverage form increases satiety, reduces food intake and post-prandial glycemia by insulin-

dependent and independent mechanisms [26, 27]. However, milk is a complex fluid matrix consisting of many components including proteins, carbohydrates, fats, vitamins and minerals. Yet, the effect of milk as a natural complex fluid on the mechanisms regulating satiety, food intake, and blood glucose has not been elucidated nor has the role of the individual macronutrients in contributing to these effects of milk been described. Therefore, the focus of this thesis is on the effect of milk and its components on short-term satiety, food intake and post-prandial glycemia, and mechanisms of action in healthy young adults.

CHAPTER 2

LITERATURE REVIEW

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2.1. Introduction

As background for this thesis research, the following literature review is composed of eight main sections. After a brief introduction on beverage consumption, the relationship between consumption of milk and obesity, type 2 diabetes and metabolic syndrome and the role of milk components in these relationships is examined. This is followed by a review of experimental studies assessing the effect of milk and its components on satiety, food intake and glycemic control. A later section provides a brief orientation to the physiological mechanisms of satiety, food intake and glycemic control and is followed by an examination of what is known about the role of milk and its mechanisms of action on these parameters.

2.2. Beverage Consumption

Beverages are a central part of the diet. Fluids (water and other beverages) deliver more than 80% of the total daily water intake [28]. Beverages can also be a major source of calories and nutrients. The Beverage Guidance Panel was created to offer guidance on the relative health and nutritional advantages and possible health risks of a variety of beverage types through a systematic review of the relevant scientific literature and to understand the effects of beverages on overall energy intake [7]. In the United States, more than 21% of calories are consumed from beverages which is over double than it was several decades ago [7]. Between 1977 and 2001, the percentage of energy obtained from calorically sweetened soft drinks and fruit drinks, which are separate from fruit juices, has risen from 3.9% to 7.1% of total daily calories (from 70 to 189 kcal per day) for the average American aged two years and older. Based on data from the Canadian Community Health Survey, soft drinks and other sweetened beverages such as fruit drinks account for only 4% of calories in the Canadian diet [29].

In Canada and the United States, water, orange juice, cow's milk, and soy beverages have been suggested rather than sugar-sweetened beverages (SSB) for both adults and children. To provide guidance on beverage consumption, the Beverage Guidance Panel constructed a beverage hierarchy with water as a primary option followed by calorie-free tea or coffee, then low-fat milk and soy beverages, non-caloric sweetened beverages, caloric beverages with some nutrients and lastly, calorically sweetened beverages with no nutrients [7]. However, this was

not based on comparisons of these commonly consumed beverages on satiety, food intake and metabolic regulation. These beverages differ considerably in their energy content and composition.

Beverages are suggested to have weak satiety properties and produce poor dietary compensation. Studies examining appetite (e.g. hunger, fullness, desire to eat and prospective consumption) support the notion that fluids are less satiating than solid foods [30, 31]. Furthermore, caloric compensation, which is the adjustment in energy intake made by individuals in later meals in response to earlier food intake, has been examined with solid and semi-solid foods and fluids. It has been hypothesized that liquid calories bypass satiety compared with solid calories and therefore, promote positive energy intake and body weight [32, 33]. However, evidence that liquids have less impact on satiety than solids remains inconclusive for two reasons. First, only one report has directly tested the hypothesis that liquid calories are less satiating than solid calories by examining the effect of pure macronutrients (e.g. sugars or proteins) with similar tastes, ingredients, volumes and matrices on satiety and food intake [34]. Furthermore, the majority of clinical studies have compared the effect of solid and liquid forms of fruit [35], soup [36-38] or yogurt with fruits [39] on satiety and food intake. Second, this hypothesis has not been supported by some experimental studies [40-42]. For example, iso-caloric (300 kcal) beverages of regular cola (24 oz) and fat-free raspberry cookies (3 oz) resulted in similar food intakes both 20 min and 2 h later [42], suggesting that energy content of liquid or solid foods consumed prior to a meal may be the primary factor affecting short-term food intake [42] and not the food form.

The effect of liquid compared with solid sources of calories has been the focus of much debate, primarily because it has been used to explain that the concurrent rise in obesity and metabolic diseases is associated with higher consumption of SSB including fruit and soft drinks [43, 44]. Furthermore, the increase in SSB consumption [45] has been concurrent with a decrease in milk consumption [46-48]. However, short-term studies comparing the effect of milk and SSB on satiety and food intake have failed to shed light on the association [49-51].

2.3. Relationship Between Milk Consumption, Obesity, Type 2 Diabetes and Metabolic Syndrome

Milk and dairy products are of interest for many reasons. Several epidemiological studies suggest a strong inverse association between higher dairy intake and reduced risk of obesity [52], type 2 diabetes [12] and metabolic syndrome [12, 53]. Furthermore, milk and dairy products are moderately inexpensive, readily available and a good source of high quality nutrients including protein, calcium, magnesium and phosphorus. The possible link between dairy food consumption and symptoms of the metabolic syndrome is of growing interest and has the potential to exert favorable effects on determinants of energy balance and the regulation of metabolism. Although the beneficial effects of dairy have been attributed primarily to its protein content [54-56], other components including lactose [57], calcium [21, 58, 59], medium-chain triglycerides [60], and conjugated linoleic acids (CLA) [61, 62] may also play a role in body weight and metabolic control.

Many cross-sectional epidemiological studies have linked frequent dairy consumption with healthier body weights and lower risk of type 2 diabetes [11, 63]. In many of these studies [16, 64, 65], the inverse relationship between milk and dairy consumption and the incidence of obesity and type 2 diabetes have been attributed to dietary patterns including low-fat [66], but not high-fat dairy products. However, observational studies have not provided consistent results. This may be due to diverse study populations [67, 68], ethnicities [69], methods of intake measurement [15] that do not measure habitual intake [70], the wide range of composition in milk and dairy products [14, 16], and that associations between diet and health markers are detected most easily for frequently consumed items and thus, fail to detect associations with those less frequently consumed. Furthermore, dairy intake alone is not necessarily a marker of a healthier dietary pattern, but is concordant with an overall healthier diet. Dietary patterns characterized by high intakes of milk and dairy products, particularly low-fat dairy products, combined with higher intakes of whole grains, fruits and vegetables, are also inversely associated with the risk of weight gain and obesity [12, 44, 52] and the incidence of type 2 diabetes [71, 72]. The number of long-term randomized clinical trials conducted is still modest, and show inconsistent outcomes. Some failed to show consistent benefits of increased consumption of dairy products in ad libitum diets on body weight, waist circumference, cardiovascular risk markers [17], indicators of chronic inflammation and endothelial function [18] or characteristics

of metabolic syndrome; however, reduced waist circumference and sagittal abdominal diameter were found in those who started with low baseline calcium intake [19]. In contrast, others showed a positive benefit of dairy consumption on body weight [20-22]. Again, these discrepancies can be attributed to variations in study design and duration, sample size, quantity of dairy and habitual dairy/calcium intakes prior to the study.

Epidemiological studies are primarily based on subjective food recalls and in intervention studies, beverages or ingredients are typically part of the diet. As a result, the role of dairy and its potential physiologic mechanisms underlying the observed effects cannot be isolated, but the epidemiologic data are sufficiently encouraging to test hypotheses on the physiological actions of dairy. Composition, amount, timing and frequency of consumption are primary determinants of the impact of a food on satiety, food intake and metabolic regulation, especially glucose utilization and disposal [23, 24].

2.4. Milk

Milk is a white liquid produced by the mammary glands of mammals. It is described as an oil-in-water emulsion or colloid of butterfat globules within a solution of lactose, soluble proteins, minerals, vitamins and other components [73]. Upon standing for 12 to 24 hours, fresh milk has a tendency to separate into a high-fat cream layer on top of a larger, low-fat milk layer. Processing of milk, available for consumption, has led to many differences in the fat composition, ranging from whole to skim milk. Milk lipids are combined into milk fat globules in raw milk, which are reduced in size during homogenization; a mechanical treatment of the fat globules that prevents a cream layer from separating out of the milk. This is achieved by passing milk under high pressure through narrow tubes, which results in a decrease in the average diameter and an increase in number and surface area of the fat globules. Homogenization has negligible effects on the composition of milk and the nutritional properties of the milk lipids are not affected [74, 75]; however, it may affect milk structure and its bioactivities. Casein proteins, which are in the form of micelles, are attracted to the newly exposed fat surfaces. The exposed fat globules are vulnerable to certain enzymes present in milk, which could break down the fats and produce rancid flavors. To prevent this, the enzymes are inactivated by pasteurizing the milk immediately before or during homogenization.

2.4.1. Milk Components

Several components of milk may contribute to its effects on satiety, food intake, body weight, blood pressure, lipid levels and insulin sensitivity. The components of milk possess both physical and physiological properties that may contribute to its beneficial effects on satiety, food intake and glycemic control. Human milk and milk from other mammals is a complex mixture of proteins (whey protein and casein), fats (saturated, mono- and poly-unsaturated fatty acids), and carbohydrates (lactose), which, in addition to providing high quality nutrients, possess a wide range of bioactivities. In addition to the macronutrient content, milk also contains numerous micronutrients including vitamins and minerals and also biologically active substances, such as immunoglobulins, enzymes, cytokines, hormones and growth factors. Thus, the health benefits of milk may be due, in part, to all of these components individually and function together as part of the matrix in milk.

2.4.2. Milk Proteins

Milk proteins, including whey protein and casein, have functional properties for the control of food intake and glycemia. These properties may partly explain the association found in some studies between higher dairy consumption and lower body weights, and decreased risk of developing hyperglycemia and diabetes [55]. Both of the major protein fractions in milk, whey protein and casein, suppress short-term food intake and contribute to metabolic regulation through the stimulation of many hormones regulating food intake and glucose utilization [55, 56].

The total protein content and ratio between protein fractions in milk show interspecies differences. For instance, cow's milk contains a total protein content of 3.4%, while human milk contains 1%. Casein and whey proteins are high quality proteins with a ratio of 80:20 in cow's milk and 40:60 in human milk, respectively [76]. Furthermore, milk proteins are considered a significant source of bioactive peptides for which a growing number have been found in milk protein hydrolysates and fermented dairy products. The primary structure of milk proteins are encrypted with at least 26 bioactive peptides and many of them have been isolated from dairy products, including sour milk, cheeses and yogurts [77].

2.4.2.1. Physical Properties

Casein and whey proteins make up 80% and 20% of cow milk proteins, respectively, and each are made up of complex proteins of different characteristics [78]. Bovine casein consists of αs_1-, αs_2-, β-, and κ-casein [78]. Most of the casein proteins exist in a colloidal particle known as the casein micelle that range from 50 to 250 nm in diameter [79]**.** Its biological function is to carry large amounts of highly insoluble calcium phosphate in liquid form and to form a clot in the stomach for more efficient nutrition. Besides casein protein, calcium and phosphate, the micelle also contains citrate, minor ions, lipase and enzymes [79]. The distinguishing property of all caseins is their low solubility at pH 4.6. Caseins, when broken down by proteolytic enzymes, produce bioactive peptides that affect cardiovascular, nervous, immune and digestive systems [80]. For example, caseinomacropeptide (CMP) and glycomacropeptide (GMP), which is the glycosylated form of CMP, is found in κ-casein. After hydrolysis into para-κ-casein by chymosin during cheese-making, they become whey components which are removed with the whey liquid.

Whey proteins are a group of milk proteins including α-lactalbumin, β-lactoglobulin, serum albumin, immunoglobulins, lactoferrin and secretory components that remain soluble in milk serum or whey after precipitation of caseins at pH 4.6 [54, 78]. Whey proteins are precursors of angiotensin converting enzyme (ACE)-inhibitory peptides called lactokinins [81], which have antihypertensive properties. Similar to casein and soy protein hydrolysates, the peptides, α- and β-lactorphin [82], have an effect on adipocyte lipogenesis because of their ACE-inhibitory activities and may also suppress food intake through peripheral opioid receptors [83]. Peptides released during digestion of whey protein may contribute to its health-promoting properties. They possess many physiological functions such as the modulation of blood pressure, hyperglycemia, inflammatory processes and food intake regulatory systems. However, these actions are not limited only to whey proteins and peptides, but are also due to synergy between whey proteins and other whey components such as calcium. Some research has suggested that consuming milk containing calcium attenuates fat and weight gain and decreases blood pressure significantly more than that of calcium supplementation alone [21].

2.4.2.2. Physiological Properties

The differences in the physical properties of whey protein and casein contribute to their functionality in food systems and in their physiological effects when ingested. Whey protein is considered a “fast protein” and is rapidly digested, whereas, casein is a “slow protein” and is more slowly digested. The categorization of whey protein and casein as “fast” and “slow” are based on their rate of absorption as indicate by plasma amino acid concentrations and contributions to protein synthesis after their consumption [10]. In humans, the intake of whey protein results in a rapid rise in plasma amino acids that peak 40 min to 2 h after its consumption and returns to baseline concentrations after 3 to 4 h. In contrast, casein results in lower plasma amino acid concentrations that increase more slowly, but maintains an extended plateau that lasts at least 7 h after its ingestion [10]. Whey protein is a soluble protein, whereas, casein clots in the stomach, which delays its gastric emptying and thus, results in a slower release of amino acids [84, 85].

Although the reasons for the observed benefits of increased dairy consumption and lower prevalence of obesity and chronic diseases remain unclear, the physiologic actions of their proteins, beyond providing essential amino acids for protein synthesis, has been offered as an explanation [25, 86]. The high content of the branched-chain amino acids (BCAA) and bioactive peptides in milk protein play a variety of metabolic roles in regulating thermogenesis [87], blood pressure [88], satiety [89-91], short-term food intake [56, 92] and blood glucose [93-95]. Healthy subjects ingesting a mixture of lysine, valine, threonine, leucine, and isoleucine had similar glycemic and insulinemic responses to those after consumption of intact whey protein [96], suggesting that the BCAA content in whey protein reduces glycemia by stimulating insulin. However, unlike whey protein, a free amino acid mixture failed to stimulate glucose-dependent insulinotropic peptide (GIP), which along with glucagon-like peptide-1 (GLP-1), account for 50-70% of insulin secretion. In addition, the intact whey resulted in lowered post-prandial glucose through both insulin and insulin-independent pathways [27]. Bioactive peptides derived from intact whey protein [96] stimulate gastrointestinal hormones such as GLP-1 and GIP, and other hormones that slow stomach emptying [27].

Many studies show that milk proteins increase satiety, but not necessarily predict later food intake, but the results can be partially explained by the protein source, quantity and time of measurements [92]. In a randomized, single-blind study, a breakfast, with 25% of energy from

casein, was more satiating than a breakfast with 10% of energy from casein to lunch three to four hours later in healthy young adults [97]. However, there were no differences in energy intake at lunch. Similarly, a decrease in subjective appetite ratings was found after consumption of whey protein (15 g), providing 10% of the energy content of breakfast, compared with casein or soy protein for up to four hours [98]; however, no differences in energy intake were found. On the other hand many studies of shorter intervals between treatment and a meal show that milk proteins not only increase satiety, but decrease food intake [26, 92].

Of the milk proteins, preloads (45-50 g) of whey protein have been shown to decrease food intake more than casein at meals consumed 30-90 min later [92], while casein reduced food intake more than whey protein at 180 min [99]. However, these earlier studies used doses well above that which may be obtained from usual serving sizes and provided little evidence that milk consumed in usual amounts contributes to food intake regulation. More recent studies indicate that protein intake from milk in the range of usual serving sizes, defined as 250 ml (9 g protein), but often consumed in larger amounts of 360 to 500 ml (18 g protein), is of functional significance for food intake control through the combination of the effects of whey in early, and casein in later, satiety. For example, milk (600 ml, 21 g protein) consumed with a toast and jam breakfast reduced food intake and measures of hunger and increased fullness over four hours to lunch time compared with a fruit based beverage (600 ml, 0 g protein) [100].

Much less attention has been given to the potential role of milk consumption in controlling satiety, food intake and glycemia. There are many reports of the effect of whey protein, consumed alone, in beverage form or when consumed with carbohydrate on glycemic control [95, 101-103]. Addition of whey protein (18 g) to a carbohydrate-containing meal resulted in a 21% lower post-prandial blood glucose response and 57% higher plasma insulin concentration over 120 min in individuals with type 2 diabetes [102]. In another study, whey protein was suggested to be the primary insulinotropic factor because a 50% greater increase in insulin response was found after preloads of carbohydrate (25 g) with whey protein (18.2 g) than with milk or cheese [101]. However, the greater insulinotropic effect of whey protein alone [25, 54, 86] most likely arises from its rapid digestion and high content of BCAAs [101], and the lesser effect of milk or cheese may also be attributed to its food form, casein and fat content.

The importance of non-insulinogenic mechanisms of dairy in reducing post-prandial glycemia is shown by two recent studies comparing doses (10-40 g) of whey protein, consumed 30 min prior to a meal of fixed size [95] and glucose and whey protein [27]. In the first, lower

post-meal (30-170 min) blood glucose was found in the presence of a lower, not higher, post-meal insulin AUC with increasing doses of whey protein. Although the mechanism by which pre-meal whey protein resulted in improved post-meal glucose control with a lower requirement for insulin was unclear, it was proposed that non-insulinogenic pathways of glucose control where involved [95]. Slower stomach emptying is expected after whey protein because of the release of hormones such as CCK, GLP-1, GIP and PYY from the intestinal enteroendocrine cells [25] and this was shown in a study comparing whey protein with glucose which led to similar reductions in post-meal plasma glucose by both insulin-dependent and independent-mechanisms [27]. Gastric emptying is also reduced by protein ingestion consumed either alone or with carbohydrate or when consumed before a meal [104].

2.4.3. Milk Fats

While milk protein is positively associated with health benefits, milk fat has been considered a risk factor for many cardiovascular diseases (CVD) due to its saturated fat content. The association between consumption of milk fat and increased risk for CVD has led to the emergence of reduced-fat and fat-free milk and dairy products. However, the overall health effects of milk fat may be more favorable than considered by present views [105]. Research relating dairy consumption with higher serum lipid levels and CVD mortality [106] have limitations [107]. While the saturated fat content of milk has been reported to elevate blood cholesterol concentrations, recent studies have found that a diet supplemented with milk reduced blood lipids [108, 109], suggesting that milk may contain components that counterbalance the effect of the saturated fatty acids (FA) in milk [110]. Furthermore, the FA in milk have several important metabolic functions and may play a role in the regulation of satiety, food intake and glycemia through their short-term effects on hormonal responses.

2.4.3.1. Physical Properties

Milk fat contains numerous components that possess functional properties and serve as an important delivery medium for nutrients, including the fat-soluble vitamins A, D, E, and K [111]. Furthermore, the amount, type and quality of fat in the diet plays a role in the development of metabolic diseases. The majority of raw cows' milk contains about 4% total fat, but the concentrations vary extensively. Factors such as breed, season, type of feed, nutritional status,

stage of lactation, age and health of the cow, intervals between milking and the point during milking when the sample is taken play a role in the fat content of milk [112]. Milk contains, on average, a total fat content of 33 g/L, where triglycerides account for approximately 95% of this lipid portion. Other milk lipids include diglycerides (~2% of the lipid fraction), cholesterol (< 0.5%), phospholipids (~1%), and free FA (< 0.5%). Fatty acids are classified as short-chained (C4:0), medium-chained (C6:0-C10:0) and long-chained (> C12:0) [113]. Milk fat is comprised of 65% saturated, 30% mono-unsaturated and 3% poly-unsaturated FA. The FA in cow's milk are the most variable constituents of milk [114]. Furthermore, milk fats can vary in their effects on satiety, food intake and glycemia due to differences in chain length and degree of unsaturation of the FA and variability in the composition [115-118].

2.4.3.2. Physiological Properties

Milk fat is a key regulator of metabolic responses through the ileal brake. This inhibitory distal to proximal feedback mechanism controls the transit and processing of food through the gastrointestinal tract to enhance the digestion and absorption of nutrients [117]. Reduced gastric emptying, increased small intestinal transit time, and decreased gastric and pancreatic secretions occur as a result [119]. Duodenal fat stimulates the release of CCK, PYY and other gastrointestinal hormones involved in the regulation of satiety and food intake [119]. Fat also increases satiety and reduces food intake when infused into the ileum. Both animal and human studies suggest that the satiating effect of fat from the ileum is even greater than that from the duodenum [117]. This may be due to its physicochemical properties such as FA chain length and unsaturation [117]. Milk fat, has 32.2% (% weight) of C18:1 plus 2.4 % of C18:2 of total lipids in whole milk [120] and contains the highest amount of short-chained fatty acids of any food which may contribute to the satiety value of milk. In a randomized, crossover study comparing shea oil [(high in stearic acid (C18:0)], canola oil [high in linoleic acid (C18:1)] and safflower oil [high in linloneic acid (C18:2)] FA infused into the ileum, canola and safflower oil were found to increase fullness, reduce hunger and increase CCK concentrations [117] compared to shea oil in healthy individuals. Another study showed that emulsions enriched with linoleic acid (C18:2) reduced food intake more than did oleic (C18:1) or stearic (C18:0) acids [121]. Only one study has investigated the effect of CLA on food intake and measures of subjective appetite [116]. After undergoing a three-week energy restricted diet, participants were provided

either 1.8 g or 3.6 g of CLA or a placebo for 13 weeks. CLA (3.6 g) reduced subjective appetite more than CLA (1.8 g) after an overnight fast, but failed to suppress food intake at breakfast [103]. These findings suggest that the dairy products, where these FA naturally occur, may contribute to the ileal brake phenomenon while fat-free dairy products do not; however, no intervention studies have compared full-fat versus fat-free milk products with respect to satiety, food intake and body weight gain. Furthermore, the effect of milk fat on post-prandial glycemia control has not yet been reported; although the primary fatty acid in milk, palmitate, has been shown to increase hepatic glucose production at low to normal blood glucose concentrations [122].

2.4.4. Milk Carbohydrates

The main source of milk carbohydrate is lactose and is dissolved in whey, the serum phase of fluid milk. In addition to lactose, fresh milk contains other carbohydrates in small amounts, including glucose, galactose, and oligosaccharides.

2.4.4.1. Physical Properties

Lactose is a disaccharide comprising of glucose and galactose. Because of the anomeric carbon on the right side, lactose can exist as two isomers, alpha or beta, in which the hydroxyl on the anomeric carbon would point upward on the ring structure. Regular pasteurization of milk has no major effect on lactose; however, spray-drying or ultra-high temperature pasteurization, used to prolong the shelf-life of milk, can cause isomerization of lactose to lactulose and prevent physical discomfort in individuals with lactose intolerance.

2.4.4.2. Physiological Properties

Lactose is hydrolyzed by the lactase enzyme to glucose and galactose which are absorbed in the small intestine. Inadequate activity of this enzyme results in lactose intolerance. While many lactose-intolerant individuals tend to avoid dairy products, it has been shown that a minimum of one cup of milk can be consumed without suffering symptoms, and that tolerance can be further improved by including milk as part of a meal or choosing yogurt or hard cheeses as alternatives [123].

Lactose produces a lower glycemic response than sucrose or glucose, and therefore, contributes to the low glycemic effect of milk. This may be either because lactose is less well absorbed than sucrose or because galactose elicits a lower glycemic response than fructose [124]. In healthy subjects, consumption of 50 g of galactose did not elicit a rise in blood glucose [125]. When consumed by obese participants, lactose (56 g) led to lower glycemic and insulin responses and lower food intake at 180 min compared to glucose (56 g) [126]. Another study comparing the glycemic indices for 25 g carbohydrate in white bread, lactose and whole milk found that the glycemic index (GI) of whole milk was significantly lower than either white bread or lactose, but the insulin index (II) was significantly greater for whole milk compared to lactose [127] indicating lactose and protein had additive effects.

2.4.5. Milk Vitamins and Minerals

Milk is a significant source of many micronutrients with recognized health benefits. In dietary patterns, milk and dairy products provide more than 10% of many nutrient requirements, especially vitamins and minerals, including vitamins A and B12, thiamin, calcium, magnesium, phosphorus, potassium and zinc [128]. The bioavailability of some of these minerals and trace elements such as magnesium, calcium, selenium, iron and zinc is improved by dairy proteins [129]. Furthermore, the role of many of these minerals in the etiology and treatment of metabolic disorders has been a subject of interest [130]. For example, inadequate calcium and or dairy intake has been suggested to increase the risk of a positive energy balance and developing obesity and metabolic syndrome [86]. Milk is a significant source of calcium which not only plays an important role in body weight regulation [131], but may also improve lipid profiles [132, 133]. Epidemiological studies have also consistently reported a negative relationship between calcium intake and blood pressure [134, 135]. Many Canadians do not meet the recommended intakes for many nutrients provided by milk including vitamin D, calcium, potassium and magnesium [136]. Thus, it would be challenging for individuals to achieve the nutritional requirements for these nutrients in the absence of milk consumption or when other nondairy calcium sources are consumed [137].

2.4.6. Milk-based Infant Formulas and Milk Substitutes

The composition of cow's milk is often compared with cow's milk-based infant formula which has been associated with increased obesity in children [138], although it was recently pointed out that there is little evidence for this [139]. The development of infant formulas as breast milk substitutes to meet the amino acid requirements of infants has been guided primarily by the protein content and composition of human milk including its fat content. Most commercial formulas will resemble human milk in the whey:casein ratio [138]. Furthermore, infant formulas use one or more vegetable oils, such as soybean, corn or safflower oil, in addition to coconut oil, in order to produce a fatty acid profile similar to human milk [138]. The primary source of carbohydrate in infant formula is lactose. However, there are no reports in the literature on the effects of infant formula compared with human or cow milk on intake regulation or glucose control in either infants or older humans.

In addition, because of the perceived negative effects of cow's milk, milk substitutes based on soy protein (e.g. soy beverages) have been developed, but there has been concern of the estrogenic effects of its isoflavone content [140]. Soy protein contains a single homogeneous protein fraction and is classified as a "fast" protein, similar to whey [92]. Thus, it is digested rapidly compared with casein or cow's milk which may lead to it having physiological effects different from milk-based formula [138]. Surprisingly, there are a lack of studies investigating the effect of milk beverages or their substitutes on short- and long-term food intake, appetite and metabolic control.

2.5. Physiology of Food Intake Regulation

The physiological and neurological mechanisms that regulate both short- and long-term food intake are extremely complex. The central nervous system (CNS) is involved in the regulation of food intake which receives feedback from sensory properties of food, mechanical and chemical receptors in the gut, circulating metabolites and hormones. The hypothalamus in the brain integrates signals arising from the gastrointestinal tract, liver and pancreas.

The physiological mechanisms that govern when, what and how much we eat are modulated by pre- and post-absorptive signals in response to food consumption. Pre-absorptive signals arise from gastric distension, gastric emptying and through secretion of various gastrointestinal hormones in response to ingested food. Post-absorptive signals are generated

after nutrients have been digested and have entered circulation where they stimulate satiety centers in the brain. The following sections will provide a brief overview of the current understanding of the mechanisms behind short-term food intake regulation in humans as background for the research study on mechanisms by which milk regulated satiety, food intake and post-prandial glucose.

2.5.1. Hormonal Regulation of Satiety and Food Intake

Many of the signals that result in a decrease in food intake in the short-term are activated by gastrointestinal hormonal responses to food ingestion. These hormones are predominantly synthesized and secreted from three main organs including the pancreas, stomach and intestine. The gut recognizes the composition of the food consumed and sends signals to various organs in anticipation of the metabolic requirements for the handling of nutrients derived from the digestion and absorption of that specific food. Macronutrient ingestion stimulates the release of pancreatic hormones such as insulin and many gastrointestinal peptides involved in satiety including GLP-1, PYY, CCK and ghrelin.

2.5.1.1. Pancreatic Hormones

Insulin is a key endocrine and metabolic hormone secreted by the β-cells of the pancreas in response to nutrient ingestion. Insulin is an important central satiety signal [141] and modulates food intake more than any of the gastrointestinal satiety hormones [142, 143]. The strongest predictor of insulin secretion is plasma glucose and it affects short-term food intake and satiety in several ways [144-146]. Insulin acts to increase the satiating properties of blood glucose and vice versa. Insulin-sensitive glucose transporters, GLUT-4 and GLUT-8, have been detected in appetite-regulating areas of the brain, including the hypothalamus [147]. These glucose transporters are translocated to the plasma membrane of glucose-sensing hypothalamic neurons, increasing their sensitivity to blood glucose concentrations and therefore suppressing appetite [147]. At the same time, glucose metabolism in pancreatic β-cells enhances secretion of insulin [148]. The products of glycolysis activate membrane depolarization in β-cells and subsequent release of insulin [148]. This positive feedback loop leads to higher glucose and insulin concentrations following a meal and as a result increases post-prandial satiety.

2.5.1.2. Gastric Hormones

In between meals, satiety decreases and once again gives rise to hunger. Ghrelin, primarily produced by the A-cells of the stomach, is the only orexigenic (appetite stimulating) hormone that exerts its major effects within the CNS to enhance appetite and food intake and increase gastric motility and emptying [149]. A rise in plasma ghrelin stimulates appetite and food intake by binding to its receptors (GRLN-R) in the arcuate nucleus of the hypothalamus; an area not protected by the blood brain barrier, and therefore, able to respond to peripheral ghrelin concentrations [150]. Ghrelin is implicated in short-term meal-time hunger and meal initiation partly because circulating ghrelin concentrations rise before a meal and decrease after food ingestion in proportion to caloric load [151]. In addition, ghrelin enhances food intake by increasing the number of meals initiated, without varying their size, and stimulates several appetitive feeding behaviours [152].

Two major forms of ghrelin are found in plasma: acyl and des-acyl ghrelin [153]. Plasma concentrations of acyl ghrelin together with that of total ghrelin (acyl and des-acyl) decline significantly after ingestion of nutrients [153]. These findings indicate that the regulation of acyl ghrelin in the short-term was almost the same as that of total ghrelin. After a meal, plasma ghrelin concentrations decrease, giving way to appetite suppressing hormones [150].

2.5.1.3. Gastrointestinal Hormones

Although the pancreas, liver, muscle, adipose tissue and CNS all play a role in the regulation of food intake, the gastrointestinal tract is the first tissue affected by nutrient ingestion. There are more than 20 different regulatory peptides released by the gastrointestinal tract during nutrient ingestion through a widespread neural network that exert anorexigenic (appetite suppressing) effects in the brain, particularly in the hypothalamus. Although many of these peptides are also synthesized in the brain, the focus of this section is on the gastrointestinal tract as a major source of these hormones in the peripheral circulation.

These gastrointestinal hormones also regulate satiety and food intake by their effect on insulin secretion [154, 155], and gastric emptying [156]. Thus, the role of insulin compared with gut hormones on the regulation of food intake is not clear. GLP-1, CCK and PYY exert their satiety and metabolic effects through their actions on inhibiting gastric emptying. CCK, released by endocrine I-cells in the duodenum and jejunum of the small intestine [157, 158], and GLP-1

[159] and PYY [160], secreted from L-cells in the distal parts of the GI tract, also cross the blood-brain barrier (BBB) to directly transmit signals that inhibit appetite and gastric emptying.

Nutrients stimulate the release of these hormones differently. Dietary protein, fat and carbohydrates all stimulate the release of PYY, but to different degrees and time-courses [161]. CCK [162-164] is primarily released after fat and protein ingestion, but not carbohydrate. Although GLP-1 concentrations are increased in response to all three macronutrients, several studies reported a higher GLP-1 response after protein compared to fat and carbohydrate [126, 165, 166].

2.5.2. Gastric Distension and Emptying Rate

Pre-absorptive satiety signaling begins when food enters the stomach, causing an increase in gastric volume, which triggers appetite-suppressing signals directly to the CNS [167]. Independent of nutrient content, stretch receptors in the gastric wall signal gastric distension, mostly through the afferent fibres of the vagus nerve [168], to the brain, which has a negative feedback on hunger [169]. The effect of small or moderate volumes of food on gastric distension is short-lived and does not produce a sensation of satiety *per se* [170]. Increasing the volume of the preload affects satiety and short-term food intake due to an increase in gastric distension and post-gastric mechanisms. For example, 600-ml isocaloric (499 kcal) milk-based preloads suppressed satiety and food intake at 30 min more than the 300-ml preload in normal-weight men (n= 20) [171]. Furthermore, gastric distension-induced satiety can also be modulated by gut hormones such as CCK [172] and GLP-1 [173]. In another study, women consumed 13% less food 30 min after a 400-ml isocaloric preload (611 kcal) compared with a 200-ml preload infused intragastrically, bypassing cognitive and sensory cues of food [174].

The rate at which food passes through the stomach into the duodenum (gastric emptying rate) is also a major pre-absorptive determinant of satiety. Gastric emptying rate contributes to short-term satiation and satiety in two ways. First, it reduces or prolongs the time the stomach is distended. Second, it controls the rate at which the proximal small intestine is exposed to nutrients [175]. Among several factors affecting gastric emptying, the physical state, caloric and nutrient contents of food play major roles. With a mixed meal in the stomach, liquids are emptied at a faster rate than solids [176, 177], which is why it has been proposed that liquid compared to solid foods fail to increase satiety and suppress short-term food intake [32, 178,

179]. Furthermore, meals with a higher caloric content empty from the stomach at a slower rate than meals with a lower caloric content. The effect of nutrients on gastric emptying is regulated by feedback mechanisms from the small intestine. Of the macronutrients, fat and protein (e.g. milk proteins) are more potent inhibitors of gastric emptying than carbohydrate [57] [180], due in part, to the release of gastrointestinal hormones such as CCK, PYY and GLP-1.

2.5.3. Post-absorptive Satiety Signals

Post-absorptive signals are produced after nutrients have been digested and absorbed by the gut where they stimulate satiety centers in the brain via endocrine and metabolic actions. The primary post-absorptive satiety signals are derived from increased concentrations of glucose, amino acids and fatty acids in the blood and the brain as encompassed in the glucostatic, aminostatic and lipostatic theories of intake regulation.

The glucostatic theory of appetite regulation [181] postulates that fluctuations in blood glucose concentrations, as detected by glucoreceptors in the hypothalamus, trigger appropriate changes in appetite and consequently food intake [182]. Since glucose is the main energy substrate of brain cells, increased availability of glucose produces more ATP reflecting greater utilization of glucose in neurons, thereby, increasing satiety [183, 184]. According to this theory, a rise in blood glucose concentrations leads to increased feelings of satiety and terminates food intake; whereas, a drop in blood glucose concentrations stimulates appetite and triggers the onset of feeding [182]. This means that, glucose uptake and utilization may play a key role in the control of satiety and energy intake regulation [182]. Since peripheral glucose utilization is induced by insulin, increased insulin concentrations in response to glucose and certain amino acids has also been shown to promote satiety and suppress short-term food intake [142, 185]. Insulin crosses the BBB, by a saturable transport system, to act within the brain to help control appetite [186].

Similar to the glucostatic theory, the aminostatic hypothesis suggests that post-absorptive elevations of certain amino acids in the blood and brain may be accompanied by suppression of appetite and food intake [187]. While the post-absorptive mechanisms regulating satiety and food intake by proteins and amino acids is unclear, a number of mechanisms have been proposed.

Another metabolic theory that has been suggested to contribute to the perception of post-prandial satiety is the lipostatic hypothesis [188]. Transport mechanisms and enzymes for both fat oxidation and synthesis are present in the brain and inhibitors of fatty acid oxidation increase food intake. Although this could be a peripheral effect, it is clear that the hypothalamus senses a nutrient surfeit in fatty acid metabolism arising from circulating lipids from either dietary sources or adipose tissue [188].

2.6. Regulation of Glycemia

Post-prandial glucose is crucial to overall glycemic control. High post-prandial glucose has been independently related with adverse metabolic consequences including increased oxidative stress and endothelial inflammation, abnormal vascular reactivity, glycation, and hypercoagulability [189].

Glycemic control is regulated by complex interactions among neural, metabolic and hormonal signals. Plasma glucose concentrations originate from three sources including endogenous glucose from glycogenolysis and gluconeogenesis and exogenous glucose from post-meal intestinal absorption [190]. During the fasting state, hepatic glucose is produced from glycogenolysis, the breakdown of glycogen to produce glucose, and gluconeogenesis, the formation of glucose primarily from lactate and amino acids [190]. During the fed state, post-prandial glucose concentrations depend on several factors including carbohydrate absorption, secretion of insulin, glucagon and other gastrointestinal hormones, their coordinated effects on glucose metabolism and gastric emptying rate [190] [191]. Furthermore, the magnitude and time of the peak glucose concentration depends on timing, quantity and composition of the meal [191].

2.6.1. Hormonal Regulation of Glycemia

2.6.1.1. Pancreatic Hormones

Glycemic control is achieved through many regulatory pathways; however, one of the key pancreatic hormones secreted in response to increased blood glucose following ingestion of a meal is insulin. During the fed state, the main action of insulin is to stimulate glucose uptake by binding to its receptors in adipose, liver and muscle cells. The rate of glucose uptake in the peripheral tissues and endogenous glucose production in the liver is constant and prevents blood

glucose elevation [192]. Insulin helps regulate post-prandial glucose in three ways. First, insulin acts on the cells of insulin-sensitive peripheral tissues, mainly in skeletal muscle, by stimulating GLUT4 receptors to increase glucose uptake [193]. Second, insulin acts on the liver to promote glycogenesis. Third, insulin sends signals via the portal vein to the liver to suppress production and release of glucose through glycogenolysis and gluconeogenesis [194].

Glycogenolysis and gluconeogenesis are primarily regulated by the hormone glucagon which is secreted from the α-cells of the pancreas. Glucagon is the major counterpart to insulin and maintains basal plasma glucose concentrations within a normal range during the fasting state and is suppressed following meal ingestion. These two hormones are secreted in a coordinated, pulsatile manner in a reciprocal fashion at approximately 5-min intervals [195]. Carbohydrate is the most potent stimulator of insulin. Unlike carbohydrate, protein ingestion has been found to stimulate the release of both insulin and glucagon [104, 196]. Although not fully understood, protein-induced glucagon secretion contributes to the prevention of insulin-induced hypoglycemia. Therefore, proteins may contribute to long-term glycemic control by reducing and preventing hypoglycemic episodes. In contrast, ingested fat does not independently stimulate insulin secretion; however, when consumed with carbohydrate, it may have a significant effect on plasma glucose and/or insulin responses in healthy humans [197].

2.6.1.2. Gastric Hormones

Recent research supports an important role for ghrelin in the regulation of glucose homeostasis [198]. Ghrelin has an inverse secretory pattern to insulin [152, 199, 200] and glucose [201], suggesting an inhibitory feedback between ghrelin and insulin [198]. Insulin has been shown to suppress circulating ghrelin concentrations, independent from changes in glucose concentrations [201]. The post-prandial ghrelin response is affected by the macronutrient composition of the meal. Among the different macronutrients, carbohydrates may be the most effective in reducing post-prandial ghrelin concentrations [202] although decreased ghrelin levels have consistently been found after milk-based proteins [203]. After consumption of a high-fat meal, ghrelin concentrations have been shown to decrease [202] or to increase [204]. If decreased, the decline has been characterized by a slower return to baseline than after a high-carbohydrate meal [205]. For example, after protein (55 g) and lactose (56 g) preloads, ghrelin concentrations were suppressed at 60 min and remained stable for 1 h before returning to pre-

prandial concentrations; however, after glucose (56 g), ghrelin returned to baseline levels within one hour of the nadir and exceeded this level in the next hour [126]. In another study, whey protein (10-20 g) compared to glucose (10-20 g) did not suppress ghrelin concentrations compared with a water control [206]. This may be explained by the small amounts of whey protein or glucose used in the study. Previous studies reporting a suppression of ghrelin examined the effect of nutrients either as part of a meal [180, 207] or used higher amounts of the nutrients [208].

2.6.1.3. Gastrointestinal Hormones

The effect of GLP-1 on glycemic control occurs primarily through its action on pancreatic hormones (i.e. insulin secretion and glucagon suppression), thereby inhibiting hepatic glucose production and lowering blood glucose concentrations [154, 155]. GLP-1 potently stimulates insulin secretion in a glucose-dependent manner. However, due to the rapid cleavage of these incretins by the dipeptidyl peptidase IV (DPP- IV) enzyme, which deactivates the hormones, only a small percentage of the total GLP-1 secreted reaches pancreatic β-cells to stimulate insulin secretion [209]. Furthermore, GLP-1 contributes to the maintenance of blood glucose beyond its effect on insulin secretion [210, 211]. The peripheral activation of GLP-1 receptors (GLP-1R) enhances hepatic insulin action to replenish hepatic glycogen stores [212] as well as to increase disposal of meal-derived glucose by activation of neurons with the hypothalamus [213]. The GLP-1R expressed in the gastrointestinal tract exert inhibitory actions on gastric emptying [209].

Furthermore, PYY in the portal vein stimulates hepatic and pancreatic vagal afferents that enhance glucose disposal, thereby, augmenting portal-mediated glucose clearance [214, 215]. CCK has also been suggested to exert its insulinotropic action through different mechanisms other than directly stimulating secretion. This includes potentiating the release of other gut hormones during meal ingestion that enhance insulin secretion [216] and inhibiting gastric emptying [217].

2.6.2. Gastric Emptying

During the fed state, gastric emptying, which is the time it takes for the food to empty from the stomach and enter the small intestine, is a major determinant of post-prandial glycemia.

Delayed gastric emptying is associated with lower glycemia [218-221]. In the post-prandial state, the regulation of gastric emptying by the gastrointestinal hormones such as GLP-1[156], PYY [160, 222] and CCK [223] has a major influence on glucose homeostasis [83, 172, 220].

2.7. Milk in the Regulation of Satiety, Food Intake and Glycemia

Increased dairy consumption has been reported to result in reduced [21, 44, 52], unchanged [224, 225], or increased [226] body weight. However, clinical studies that used either body weight change or food intake as outcomes failed to provide conclusive evidence on the impact of a diet rich in milk on body weight and adiposity [39, 51, 100, 227]. High protein diets such as those that contain whey proteins [22], may suppress hunger and food intake, thereby reducing fat deposition and improving insulin sensitivity, but do not prove that dairy consumption will do so. Milk components, such as proteins, have been explored in their effect on short-term appetite and food intake, but there has been little investigation on whole milk as a complete entity on satiety and food intake, and glycemic control.

2.7.1. Satiety and Food Intake

Satiety and food intake are often measured in short-term studies using a preload study design. In comparisons of milk and beverages with caloric sweeteners, there is evidence that the composition of the calories is a factor in the response, although calories are a primary factor in determining short-term effects [50, 51]. In one of the few studies of milk, chocolate milk compared with regular cola, decreased subjective ratings of hunger and prospective consumption and increased satiety and fullness as measured by visual analogue scales (VAS) [51]. The study compared isovolumetric (500 mL) and isocaloric (900 kJ) beverages of regular cola (0 g protein, 53 g CHO) and chocolate milk (12.6 g protein, 36 g CHO) provided in randomized order to healthy young men (n = 22) 30 min before an ad libitum lunch [51]. Food intake after chocolate milk was 4% less than after regular cola, but the difference was not significant. The enhanced satiety after chocolate milk may be explained by its protein content; however, the lack of effect on food intake may be due to the low energy content of both beverages that were consumed shortly before the meal [51]. In another study, isovolumetric (600 mL) and isocaloric beverages (1062 kJ) of skim milk were compared with a fruit drink at breakfast on post-meal satiety and food intake measured at an ad libitum lunch 240 min later [100]. Overweight men and women (n

= 34) consumed skim milk (25 g protein, 36 g lactose, <1 g fat) or a fruit drink (<1 g protein, 63 g sugar, <1 g fat) drink with a fixed-energy breakfast (1923 kJ). Satiety ratings before the meal were higher after consumption of skim milk than the fruit drink [100] and food intake was suppressed by 8.5% after skim milk compared to the fruit drink. More recently, a crossover study compared isovolumetric (500 ml) amounts of semi-skimmed milk (950 kJ), regular cola (900 kJ), diet cola (7.5 kJ) or water in 24 obese men and women on subjective appetite, ghrelin, GLP-1, GIP and ad libitum energy intake 4 h after intake of the test beverages. Although energy intake was not different among the beverages, milk increased satiety, GLP-1 and GIP responses compared with regular cola; however, ghrelin, consistent with its role as an energy sensor [142], was reduced by 20% after both milk and regular cola compared to water.

Therefore, it is hypothesized that the effect of milk on satiety and food intake is mainly mediated through the effect of milk proteins on the release of satiety hormones; however, there are a lack of studies investigating how consumption of milk as a complete entity influences short-term food intake and appetite.

2.7.2. Glycemic Control

Epidemiologic studies have found inverse relationships between milk consumption and type 2 diabetes [11, 59, 64]; however, few clinical studies have investigated the effects of milk consumed alone or in meals on glycemic control before and after meals.

Short-term experimental studies on milk proteins support the hypothesis that milk consumption before or as part of a meal offers benefits to glycemic control and not only because milk proteins stimulate insulin. It has been suggested that milk consumed with carbohydrate foods modifies the glycemic response to their ingestion. For example, high milk-containing breakfasts resulted in lower glycemic responses in healthy individuals and persons with type 2 diabetes compared with high fiber or high fat-containing breakfasts [228]. Furthermore, adding milk to low GI mixed meals elicited an insulinotropic effect and a lower glycemic response [229]. The increase in insulin after both whole and skim milk when consumed alone indicates that fat plays little role, but is related to its lactose and protein content [127]. The insulinotropic effect after milk consumption has been attributed to its protein content, particularly BCAA, because of the association between post-prandial insulin and rise in the plasma amino acids valine, leucine, and isoleucine [101], but the effect of proteins relative to its lactose content has

not been reported. Furthermore, release of hormones such as CCK, GLP-1 and PYY after milk proteins, suggest non-insulinogenic mechanisms of milk in reducing post-prandial glycemia [27]. Therefore, it remains to be determined whether milk consumed prior to or with meals improves post-prandial glycemia without increasing the insulin requirement in healthy adults.

2.8. Summary and Research Rationale

The primary reason for promoting milk consumption has been its nutrient content, but its functional benefits for the maintenance of a healthy body weight and metabolic control may be equally important. Data from neither epidemiological nor randomized control trials have confirmed that dairy consumption is negatively associated with the prevalence of obesity, type 2 diabetes, and metabolic syndrome. Yet, experimental studies in both animal models and humans point to milk proteins having many functional properties essential to metabolic regulation that go beyond the provision of essential amino acids and support the hypothesis that there are similar metabolic benefits to be derived by consuming dairy products. Milk, although one of the most frequently consumed beverages, and when consumed as a complete entity on the mechanisms regulating satiety, food intake, and blood glucose has received little attention.

Therefore the objective of this thesis is to describe the effect and mechanism of action of milk and its components consumed before or within a meal on satiety, food intake and pre- and post-meal blood glucose. It is anticipated that the results will add descriptive, causal and mechanistic support for the inverse associations found between milk consumption, obesity, type 2 diabetes and metabolic syndrome and encourage milk consumption for more reasons that the provision of essential nutrients.

CHAPTER 3

HYPOTHESES AND OBJECTIVES

CHAPTER 3. HYPOTHESES AND OBJECTIVES

3.1. General Hypothesis and Objective

Hypothesis

- Milk consumption suppresses short-term appetite and food intake and reduces post-prandial glycemia through insulin-dependent and independent mechanisms.

Objective

- To identify the effects of milk on satiety, food intake, and post-prandial glycemia compared to other beverages and describe the mechanism of action of milk and its components.

3.2. Specific Hypotheses and Objectives

Chapter 4: ENERGY AND MACRONUTRIENT CONTENT OF FAMILIAR BEVERAGES INTERACT WITH PRE-MEAL INTERVALS TO DETERMINE LATER FOOD INTAKE, APPETITE AND GLYCEMIC RESPONSE IN YOUNG ADULTS

Hypothesis:

- Both energy and the macronutrient composition of recommended beverages when consumed before and between meals is a factor in the regulation of appetite, food intake, and post-meal glycemic response.

Objective:

- To investigate the effects of isovolumetric amounts, representing two serving sizes, of 2% milk, 1% chocolate milk, a soy beverage, infant formula (based on cow’s milk but with 1% vs 3% protein and with an increased whey to casein ratio), orange juice and water on food intake either 30 or 120 minutes later as well as on subjective appetite and glycemic response pre- and post-meal in healthy young men and women.

Chapter 5: CALORIC BEVERAGES CONSUMED FREELY AT MEAL-TIME ADD CALORIES TO AN AD LIBITUM MEAL

Hypothesis:

- Consuming caloric beverages ad libitum and reflecting thirst, as part of a meal, reduces meal-time food intake, post-meal subjective appetite and glycemic response in healthy young adults

Objective:

- To compare the effects of ad libitum consumption of water, 1% milk, regular cola, diet cola, and orange juice at a pizza meal on ad libitum energy and fluid intake, and post-meal subjective appetite and glycemic response in healthy young men and women.

Chapter 6: MECHANISM OF ACTION OF WHOLE MILK AND ITS COMPONENTS ON SATIETY AND GLYCEMIC CONTROL IN HEALTHY YOUNG MEN

Hypothesis:

- Milk improves glycemic control through the interaction between its macronutrient components by both insulin-dependent and independent mechanisms.

Objective:

- To describe and compare the effects of isovolumetric beverages (500 ml) of whole milk (3.25% M.F.), each of its macronutrient components (protein, lactose, and fat) and their combination on gastric emptying rate, glycemic and appetite hormone response in healthy young men.

CHAPTER 4

ENERGY AND MACRONUTRIENT CONTENT OF FAMILIAR BEVERAGES INTERACT WITH PRE-MEAL INTERVALS TO DETERMINE LATER FOOD INTAKE, APPETITE AND GLYCEMIC RESPONSE IN YOUNG ADULTS

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CHAPTER 4

Energy and macronutrient content of familiar beverages interact with pre-meal intervals to determine later food intake, appetite and glycemic response in young adults

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4.1. Abstract

The objective was to compare the effects of pre-meal consumption of familiar beverages on appetite, food intake, and glycemic response in healthy young adults. Two short-term experiments compared the effect of consumption at 30 (Experiment 1) or 120 min (Experiment 2) before a pizza meal of isovolumetric amounts (500 mL) of water (0 kcal), soy beverage (200 kcal), 2% milk (260 kcal), 1% chocolate milk (340 kcal), orange juice (229 kcal) and cow's milk-based infant formula (368 kcal) on food intake and subjective appetite and blood glucose before and after a meal. Pre-meal ingestion of chocolate milk and infant formula reduced food intake compared to water at 30 min, however, beverage type did not affect food intake at 2 h. Pre-meal blood glucose was higher after chocolate milk than other caloric beverages from 0-30 min (Experiment 1), and after chocolate milk and orange juice from 0-120 min (Experiment 2). Only milk reduced post-meal blood glucose in both experiments, suggesting that its effects were independent of meal-time energy intake. Combined pre- and post-meal blood glucose was lower after milk compared to chocolate milk and orange juice, but did not differ from other beverages. Thus, beverage calorie content and inter-meal intervals are primary determinants of food intake in the short-term, but macronutrient composition, especially protein content and composition, may play the greater role in glycemic control.

Keywords: beverages, milk, glycemic response, food intake, appetite, macronutrient composition, calories, protein

4.2. Introduction

The prevalence of obesity and overweight in the Canadian population has doubled over the past 25 years, while per capita milk consumption has decreased [230]. Similarly, in the United States milk consumption has decreased [231] and energy-containing sweetened beverages have increased [232, 233]. Many population studies of recent years have associated higher milk and dairy intake with healthier body weights and reduced risk of developing characteristics of the metabolic syndrome including hyperglycemia [12, 52]. Based on the composition of milk [56, 234], this may be causally based.

In Canada and the United States, cow's milk, soy beverages, orange juice and water have been recommended over sugar-sweetened beverages for both adults and children. The Beverage Guidance Panel created a beverage hierarchy to guide beverage consumption, with water as a primary choice followed by calorie-free tea or coffee, then low-fat milk and soy beverages, non-caloric sweetened beverages, caloric beverages with some nutrients and lastly, sugar or high fructose corn syrup sweetened beverages [7]. However, this was not based on comparisons of these frequently consumed beverages on food intake and metabolic regulation. Furthermore, in spite of the high sugar content, fruit juices such as orange juice are often considered healthy beverages.

Sugar composition and protein content are major determinants of the blood glucose and insulin responses and subjective appetite and food intake [56, 235, 236]. Glucose alone is more glycemic than sucrose or lactose and protein, when consumed with carbohydrate, reduces subsequent glycemic response [101-103, 237]. Yet, there have been no reports on usually consumed beverages differing in composition on later food intake [49, 51, 100]. Isocaloric (248 kcal) servings of orange juice, regular cola, and 1% milk when compared with sparkling water given 2 h 15 min before a meal showed no differences in ad libitum food intake [49], but this study did not consider the nutritional merits of these beverages. Milk products provide not only more essential nutrients than juices or other beverages, but also proteins (whey protein and casein) that contribute to glycemic control [56, 164, 218] and satiety and reduce food intake [56, 92]. However, in contrast to data on the second-meal effect of solid food consumption on reducing post-meal glycemic response [238, 239], there are no reports of the effects of pre-meal consumption of beverages on post-meal glycemia as a marker of metabolic control and post-meal

satiety. Post-meal glycemia is important for achieving overall glycemic control and has been independently associated with adverse metabolic outcomes [189].

Therefore, we hypothesized that both energy and the macronutrient composition of recommended beverages when consumed before and between meals is a factor in the regulation of appetite, food intake, and post-meal glycemic response. The objective of the present study was to investigate the effects of isovolumetric amounts, representing two serving sizes, of 2% milk, 1% chocolate milk, a soy beverage, infant formula (based on cow's milk but with 1% vs 3% protein and with an increased whey to casein ratio), orange juice and water on food intake either 30 or 120 minutes later as well as on subjective appetite and glycemic response pre- and post-meal in healthy young men and women.

4.3. Subjects and Methods

4.3.1. Subjects

Participants were recruited through advertisements posted on the University of Toronto campus. Men and women between 20 and 30 years of age with a body mass index (BMI) of 20 to 24.9 kg/m^2 were eligible to participate. Exclusion criteria included smoking, dieting, skipping breakfast, lactose intolerance, allergies to milk or soy, diabetes (fasting blood glucose $\geq$ 7.0 mmol/L) or other metabolic diseases that could interfere with study outcomes. Restrained eaters, identified by a score of $\geq$ 11 on the Eating Habits Questionnaire [240] and those taking medications were also excluded. The sample size required was based on previous short-term food intake studies on milk protein [56, 92]. Subjects 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.

4.3.2. Beverages

Beverages included isovolumetric amounts (500 mL) of the following: 1) water (control); 2) milk (2% M.F) (Neilson Dairy; ON, Canada) because it is the most commonly consumed type of milk amongst Canadians; 3) chocolate milk (1% M.F.) (Neilson Dairy; ON, Canada) because it is the most commonly consumed flavoured milk; 4) soy beverage (Silk Soy Beverage: Original; Broomfield, CO) because it is an alternative for individuals who are vegetarian or lactose-intolerant; and 5) cow's milk-based infant formula (Enfamil; Mead Johnson

Nutrition & Company, ON, Canada) because it mimics breast milk in protein content and in the ratio of whey to casein. In experiment 2, orange juice (Tropicana Pure Premium, no pulp; Tropicana Products Inc., Bradenton, Florida, United States) was also provided for three reasons. First, it is the most commonly consumed fruit juice and is a recommended beverage. Second, sugar sweetened beverages have been hypothesized to by-pass food intake regulatory systems [184], but no comparison of non-carbonated sweetened beverages with beverages of more complex compositions have been reported. Third, the objective was to compare the effects of chocolate milk with orange juice, which has similar sugar content but is absent of protein, in order to examine whether chocolate milk confers glycemic benefits despite its sugar content. The nutritional composition of each beverage is outlined in **Table 4.1**. All beverages were isovolumetric (500 mL) representing two servings (cups). Beverages were served chilled.

4.3.3. Protocol

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 1 h 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 and 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, subjects completed a questionnaire detailing pre-session information about their diet and lifestyle patterns. Because impaired insulin sensitivity has been observed after an oral glucose tolerance test in the luteal phase of the menstrual cycle in healthy women [241], women were scheduled for the sessions during their follicular phase. The beverages were provided in random order once per week for men and for women, in random order once per week during the first two weeks of their menstrual cycles.

On arrival, participants completed visual analog scale (VAS) questionnaires assessing their “Sleep Habits”, “Stress Factors”, “Food Intake and Activity Level”, “Feelings of Fatigue”, and “Motivation to Eat”. A composite score of the 4 appetite questions in the “Motivation to Eat” VAS was calculated to obtain the average appetite score [142, 242] for statistical analysis.

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). Plasma concentration of glucose was measured with a glucose meter (Accu-Chek Compact; Roche

Diagnostics Canada, Laval, Canada). A baseline measurement of > 5.5 mmol/L suggested non-compliance with the fasting instructions and subjects were rescheduled accordingly.

Following the completion of baseline measurements, each person was instructed to consume the beverage within 5 min at a constant pace. In experiment 1, subjective appetite and blood glucose were measured at 10, 20 and 30 min from the time subjects began drinking the beverages (pre-meal) and immediately following the meal at 50 min and 65, 80, 95, 110, 140 and 170 min (post-meal) after consumption of the beverages. In experiment 2, the same outcomes were measured at 10, 20, 30, 45, 60, 90 and 120 min (pre-meal) as well as 140, 170, 230, and 260 min (post-meal) after consumption of the beverages. Subjects were asked to remain seated for the duration of the experimental session and were permitted to read, do homework or listen to music.

Food intake was measured at an ad libitum pizza meal (McCain Foods Ltd, Florenceville, NB) 30 min (experiment 1) or 120 min (experiment 2) after consumption of the beverages. Subjects were provided 3 varieties of pizza (Deluxe, Pepperoni, and Three Cheese) based on their preferences and were asked to eat until they were "comfortably full". They were allowed 20 min to eat. Detailed information of the nutrient content of the pizza and method of cooking was reported previously [142, 242]. Test meal consumption was calculated from the weight of the consumed pizza based on the compositional information provided by the manufacturer. Water intake was measured by weight (g).

Cumulative energy intake was calculated by adding the energy consumed from the pre-meal beverage to the energy consumed at the pizza test meal [142]. Caloric compensation, expressed as a percentage, was calculated by subtracting the calories consumed following the beverage from those following the water control divided by the calories in the beverage and multiplied by 100. A caloric compensation of < 100% indicated that the subject had low compensation for the beverage energy at the test meal, while a score > 100% indicated overcompensation for the beverage energy at the test meal.

4.3.4. Statistical Analyses

Statistical analyses were conducted using SAS version 9.2 (SAS Institute Inc, Cary, NC). The effect of beverage on food intake, cumulative energy intake and water intake was not dependent on sex, as indicated by no sex by beverage interaction (two-factor ANOVA),

therefore, these results were pooled for both men and women. The effect of beverage on food intake, cumulative energy intake, and water intake were tested by one-factor ANOVA followed by Tukey's post hoc test.

Three-factor repeated-measures analysis of variance (ANOVA; via PROC MIXED procedure) were performed to analyze the effects of sex, time, beverage, and their interaction on dependent variables including pre- and post-meal blood glucose response and average appetite scores. There was no significant interaction, indicating that the effect of beverages on blood glucose and average appetite over time is not affected by sex. Thus, the reported results were pooled for both men and women as well. Furthermore, two-factor ANOVA was performed on pooled blood glucose and average appetite to analyze the effects of time, beverage and time by beverage interaction. When an interaction was statistically significant, one-factor ANOVA was followed by Tukey's post hoc test to investigate the effect of beverage on absolute blood glucose and appetite at each time of measurement.

Pearson's correlation coefficients were used to detect associations between dependent measures. All results are presented as mean ± standard error of the mean (SEM). Statistical significance was concluded with a *p*-value less than 0.05.

4.4 Results

4.4.1. Subject characteristics

In experiment 1, 29 healthy subjects (sex: 16 males and 13 females; age: 22.4 ± 0.4 years; BMI: 21.9 ± 0.3 kg/m^2) completed the study. In experiment 2, 31 healthy subjects (sex: 16 males and 15 females; age: 23.5 ± 0.5 years; BMI: 22.3 ± 0.3 kg/m^2) completed the study. There was one dropout in experiment 1 and three in experiment 2 due to participant time constraints.

4.4.2. Food and water intake

In experiment 1, beverage was the main factor affecting food intake at the test meal (p = 0.007), cumulative energy intake ($p < 0.0001$) and water intake (p = 0.03, **Table 4.2**). Chocolate milk and infant formula suppressed food intake more at 30 min than water ($p < 0.05$). Energy intake after milk, soy beverage and water were not different. Cumulative energy intakes (beverage plus pizza) were not different among the caloric beverages, but were higher than the control ($p < 0.0001$). More water was consumed at the pizza meal following chocolate milk and

2% milk than after the water control, but there was no difference between the soy beverage and infant formula. Compensation for energy consumed in the drinks was low for all beverages at the meal, averaging only 32% ($p > 0.05$, Table 4.2).

In experiment 2, beverage was the main factor affecting cumulative energy intake ($p < 0.0001$), but did not affect either food or water intake at the test meal ($p > 0.05$, Table 4.2). Cumulative energy intake from the beverage and pizza was significantly greater following infant formula compared to either soy beverage or water, however, there were no significant differences amongst the other beverages ($p < 0.0001$). Water intake at the test meal was similar after all beverage treatments ($p > 0.05$). Compensation for energy consumed in the caloric beverages at the test meal was low, averaging 13% for all caloric beverages, with no difference among them ($p > 0.05$, Table 4.2).

4.4.3. Blood glucose concentrations

In experiment 1, pre-meal blood glucose concentrations (0-30 min) were affected by time ($p < 0.0001$), beverage ($p < 0.0001$) and time and beverage interaction ($p < 0.0001$), but not sex or time, beverage and sex interaction. Pre-meal blood glucose concentrations at 10, 20 and 30 min were higher after all beverages compared to water and higher after chocolate milk than after all other beverages at 10, 20 and 30 min (**Fig. 4.1A**). Post-meal blood glucose (50-170 min) was affected by time ($p = 0.03$), beverage ($p < 0.0001$) and time and beverage interaction ($p < 0.0001$), but not sex or time, beverage and sex interaction. Post-meal blood glucose concentrations immediately after the pizza meal at 50 min were lower after all of the beverages than after water. Chocolate milk resulted in the lowest post-meal blood glucose concentration at 80 min while both chocolate milk and milk led to a reduction in blood glucose at 95 min compared to all other beverages (Fig. 4.1A).

Over the entire pre-meal period (0-30 min), blood glucose concentrations were significantly higher following all caloric beverages compared to the water control ($p < 0.0001$, **Table 4.3**). In addition, chocolate milk resulted in a higher blood glucose compared to milk, soy beverage and infant formula ($p < 0.0001$). Over the entire post-meal period (50-170 min) blood glucose concentrations were reduced after milk and chocolate milk compared to water, soy beverage and infant formula ($p < 0.0001$). Cumulative blood glucose concentrations (0-170 min)

were lower after milk compared to chocolate milk consumption, but neither differed from the other beverages ($p = 0.005$, Table 4.3).

In experiment 2, pre-meal blood glucose concentrations (0-120 min) were affected by time ($p < 0.0001$), beverage ($p < 0.0001$) and time and beverage interaction ($p < 0.0001$), but not sex or time, beverage and sex interaction. Pre-meal blood glucose concentrations at 10, 20 and 30 min were higher for all beverages compared to water, however, chocolate milk and orange juice resulted in the highest concentrations at 30 min (**Fig. 4.1B**). Post-meal blood glucose (140-260 min) was affected by time ($p < 0.0001$), beverage ($p < 0.0001$) and time and beverage interaction ($p = 0.0002$), but not sex or time, beverage and sex interaction. Milk resulted in the lowest blood glucose response at 170 min compared to all beverages (Fig. 4.1B).

Over the entire pre-meal period (0-120 min), blood glucose concentrations were significantly higher following all beverages compared to water, however, chocolate milk and orange juice resulted in a significantly higher blood glucose than milk, soy beverage and infant formula ($p < 0.0001$, Table 4.3). Over the post-meal period (140-260 min), blood glucose was reduced by chocolate milk, milk, infant formula and orange juice compared to water, with milk resulting in the lowest concentrations ($p < 0.0001$). Cumulative blood glucose (0-260 min) was lower after all beverages compared to chocolate milk and orange juice consumption, but with orange juice resulting in higher overall blood glucose concentrations ($p < 0.0001$, Table 4.3).

4.4.4. Average subjective appetite scores

In experiment 1, pre-meal average appetite (0-30 min) was affected by time ($p < 0.0001$), beverage ($p < 0.0001$) and sex ($p < 0.0001$), but not time, beverage and sex interaction. Pre-meal appetite was suppressed more at 10 and 20 min following the caloric beverages than at 30 min. After consumption of the beverages at 10 and 20 min, pre-meal appetite was reduced from baseline (74.4 mm) by an average of 16.9 mm and 15.4 mm, respectively, for all beverages (**Fig. 4.2A**). Post-meal average appetite (50-170 min) was affected by time ($p < 0.0001$) and beverage ($p = 0.002$), but not sex or time, beverage and sex interaction. After the meal (50 min), there was an average reduction in appetite scores of 51.1 mm from just before the subjects consumed the meal (**Table 4.4**).

In experiment 2, pre-meal average appetite (0-120 min) was affected by time ($p < 0.0001$), beverage ($p < 0.0001$) and sex ($p = 0.02$), but not time, beverage and sex interaction.

Pre-meal subjective appetite was significantly reduced at 10, 20, 30, 45, 60, 90 and 120 min after consumption of all beverages compared to the water control (**Fig. 4.2B**). Pre-meal average subjective appetite was significantly reduced following all beverages compared to the water control ($p < 0.0001$, Table 4.4). Post-meal average appetite (140-260 min) was affected by time ($p < 0.0001$) and beverage ($p = 0.006$), but not sex or time, beverage and sex interaction. There were no significant differences in post-meal average appetite scores amongst beverages (Table 4.4).

4.4.5. Relations between dependent measures

In experiment 1, food intake was not associated with pre- or post-meal blood glucose concentrations, but was associated with pre- and post-meal average subjective appetite ($r = 0.54$, $p < 0.0001$). Pre-meal blood glucose was inversely associated with pre-meal subjective appetite ($r = -0.09$, $p = 0.03$).

In experiment 2, food intake was inversely associated with pre- and post-meal blood glucose concentrations ($r = -0.34$, $p = 0.002$) and post-meal subjective appetite ($r = -0.38$, $p < 0.0001$). Pre-meal average subjective appetite was associated with pre-meal blood glucose concentrations ($r = -0.11$, $p < 0.0001$) and food intake ($r = 0.39$, $p = 0.0004$).

4.5. Discussion

The results of this study support the hypothesis that energy content of recommended beverages is a primary factor in the control of short-term satiety and food intake, but show that macronutrient composition is a factor only in decreasing the glycemic response to a later meal. The caloric content of the beverages was the primary determinant of food intake suppression at 30 min, but neither macronutrient nor caloric composition were factors at 2 h. However, 2% milk, but not infant formula, reduced post-meal blood glucose possibly due to its higher concentration and ratio of casein to whey proteins.

Energy content of the beverages was a major factor contributing to food intake suppression at 30 min, but macronutrient composition was not. The beverages differed considerably in their protein, fat and carbohydrate content, however, these characteristics had little effect on food intake. Only the two beverages with the highest energy content, 1% chocolate milk (340 kcal) and infant formula (368 kcal) led to the suppression of food intake at

30 min. Based on previous studies and findings from the current study, beverages of caloric contents in the range provided in these studies reduce subsequent food intake when the time between beverage and test meal is short (e.g. 60 min or less) [243], but not at later times [49, 50]. Compensation occurs at the test meal for the calories in beverage of >150 kcal, but not after smaller energy beverage preloads and time intervals greater than 60 min. The importance of energy and not composition in the short-term is also supported by previous research. Energy intakes were similar at a test meal provided 30 min following energy-matched beverages (900kJ/215 kcal) of chocolate milk or cola [51] or at 50 min following an energy-matched beverage (1.5MJ/358 kcal) of milk or drinks sweetened with sucrose or high-fructose corn syrup [244]. Furthermore, no differences in energy intake were found after consumption of isovolumetric amounts of semi-skimmed milk, regular cola, diet cola and water 4 h prior to an ad libitum meal [245].

Increasing the time interval between a caloric beverage and an ad libitum meal reduces the effect of the beverage on meal-time food intake [246]. In experiment 2, food intake was measured at 2 h because it is a common inter-meal period and our objective was to determine if the effects of realistic, albeit large, servings of chocolate milk, infant formula and milk on food intake and blood glucose would be sustained over this period of time. Although the calorie content of milk, milk substitutes and orange juice reduced subjective satiety over the 2 h and immediately prior to the meal, there was no effect of these beverages on food intake. However, at that time, the differences from control in subjective appetite were small. Conversely, subjective appetite was not different among beverages at 30 min in experiment 1, yet food intake differences were found. Thus, as often observed, subjective appetite is not a reliable predictor of food intake [49].

Differences in the carbohydrate composition between the beverages provide an explanation for their effect on pre- and post-meal blood glucose concentrations. The chocolate milk and orange juice were highest in sugar content and resulted in higher pre-meal and total blood glucose concentrations. Compared to all of the beverages, 2% milk resulted in the lowest post-meal blood glucose in both experiments even though it had a higher sugar content than the soy beverage, perhaps due to its protein content. Proteins are shown to stimulate insulin release in both healthy and diabetic individuals when ingested with or without carbohydrate [101, 247]. In this study, the soy beverage contained 14 g of protein compared to 18 g in the milk, but did not attenuate blood glucose suggesting that both the amount and composition of protein plays an

important role in post-meal glucose control [92, 248]. However, it is also well known that food proteins differ in their effects on insulin and glucose metabolism and milk protein may be more insulinotropic [98]. Although the reduction in post-meal blood glucose following chocolate milk may have been due to the lesser food intake, there were no differences in food intake among the beverages at 2 h. These data lead to the suggestion that the reduction in post-meal blood glucose following 2% milk in both experiments was not driven by the energy content, but by its macronutrient composition, in particular its protein content. Furthermore, post-meal blood glucose reductions may be due to an initial insulin response, but other mechanisms including stimulation of gastrointestinal peptides and slower stomach emptying may explain the reductions in blood glucose [206], however, insulin was not measured pointing to a limitation in the study and interpretation of results.

The importance of milk proteins to glycemic control is indicated in experiment 2. Chocolate milk, which contains 3% protein, had a similar sugar content (56 g) to orange juice, but resulted in lower overall blood glucose. Milk compared with infant formula also sustained a lower post-meal blood glucose. This suggests that both concentration and ratio of proteins in milk are a factor. The total protein content in milk is 3% and the casein to whey ratio is 80:20 while infant formula contains a protein concentration of only 1% and has a casein to whey ratio of 40:60 to simulate human milk composition [138].

In both experiments, incomplete meal-time compensation occurred for all pre-meal caloric beverages, resulting in higher cumulative intakes compared with water, which suggests that water may be the best choice as a between meal beverage for the purpose of maintaining energy balance by reducing total energy intake [50, 232]. However, for between meal consumption, it cannot be concluded that liquid calories lead to only increased cumulative intakes. Milk provides many essential nutrients that may be inadequate in diets and should therefore be recommended for between meal consumption over many other energy-containing beverages or snacks. However, the contribution of energy-containing beverages including milk to energy balance in the long-term requires further study.

4.6. Conclusion

In conclusion, beverage calorie content and inter-meal intervals are primary determinants of food intake in the short-term, but macronutrient composition, especially protein content and composition, may play the greater role in glycemic control.

4.7 Acknowledgements

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Table 4.1. Nutritional composition of beverages

Nutrients*	Beverages				
	Chocolate milk (1% M.F.)	Milk (2% M.F.)	Soy beverage	Infant formula**	Orange juice
Energy (kcal)	340	260	200	368.2	229
Fat (total) (g)	5	10	8	19.1	0
Saturated fat (g)	3	6	1	8.1	0
Trans fat (g)	0	0.2	0	0	0
Cholesterol (mg)	20	40	0	12.0	0
Sodium (mg)	240	240	240	98.4	0
Carbohydrate (g)	56	24	16	39.6	54
Fiber (total) (g)	0	0	2	0	0
Sugars (total) (g)	56	24	12	39.6	46
Protein (g)	18	18	14	7.6	4

*Nutrient content of each beverage as provided by the manufacturer. Amounts given are per 500 mL serving.

**Powdered infant formula prepared according to manufacturer label instructions.

Table 4.2. Energy intake, cumulative energy intake, water intake and caloric compensation[*]

	Beverage	Energy Intake		Water Intake	Caloric Compensation[‡]
		Test Meal[**]	Cumulative[†]		
		kcal		*g*	*%*
Experiment 1	Control[§]	1022 ± 75[a]	1022 ± 75[a]	253 ± 32[a]	
	Chocolate milk (1% M.F.)	880 ± 72[b]	1220 ± 72[b]	302 ± 29[b]	42 ± 11
	Milk (2% M.F.)	954 ± 76[ab]	1214 ± 76[b]	301 ± 31[b]	26 ± 16
	Soy beverage	967 ± 83[ab]	1167 ± 83[b]	279 ± 27[ab]	28 ± 19
	Infant formula	905 ± 79[b]	1273 ± 79[b]	288 ± 31[ab]	32 ± 12
	p	0.007	<0.0001	0.03	NS
Experiment 2	Control[§]	1144 ± 89	1144 ± 89[c]	313 ± 27	
	Chocolate milk (1% M.F.)	1093 ± 83	1433 ± 83[a]	341 ± 27	15 ± 14
	Milk (2% M.F.)	1165 ± 91	1424 ± 91[ab]	352 ± 29	8 ± 14
	Soy beverage	1119 ± 88	1319 ± 88[b]	359 ± 27	13 ± 21
	Infant formula	1114 ± 90	1482 ± 90[a]	322 ± 26	8 ± 11
	Orange juice	1189 ± 86	1409 ± 86[ab]	348 ± 31	20 ± 12
	p	NS	<0.0001	NS	NS

[*]All values are means ± SEMs (n=29 in experiment 1 and n=31 in experiment 2). Values in the same column with different superscript letters are significantly different, $P < 0.05$ (treatment effect using proc mixed, Tukey's post hoc).
[**]Energy consumed in an ad libitum meal was measured at 30 min in experiment 1 and 120 min in experiment 2 following beverage consumption.
[†]Energy in beverages + energy from the meal
[‡]Caloric compensation = [(kcal consumed at the meal after the water control – kcal consumed at the meal after the beverage)/kcal in the beverage] x 100.
[§]Water (500 mL)

Table 4.3. Overall mean blood glucose concentrations for the pre- and post-meal periods in experiments 1 and 2[*]

	Beverage	Pre-meal (mmol/L)[**]	Post-meal (mmol/L)[†]	Total (mmol/L)[‡]
Experiment 1	Control[§]	4.6 ± 0.05^{c}	5.9 ± 0.12^{a}	5.4 ± 0.09^{ab}
	Chocolate milk (1% M.F.)	5.7 ± 0.07^{a}	5.5 ± 0.09^{b}	5.6 ± 0.07^{a}
	Milk (2% M.F.)	5.1 ± 0.06^{b}	5.5 ± 0.07^{b}	5.3 ± 0.06^{b}
	Soy beverage	5.1 ± 0.06^{b}	5.7 ± 0.11^{ab}	5.5 ± 0.08^{ab}
	Infant formula	5.0 ± 0.07^{b}	5.7 ± 0.11^{ab}	5.4 ± 0.08^{ab}
	p	<0.0001	0.02	0.003
Experiment 2	Control[§]	4.7 ± 0.1^{d}	6.4 ± 0.1^{a}	5.3 ± 0.1^{c}
	Chocolate milk (1% M.F.)	5.7 ± 0.1^{a}	6.0 ± 0.1^{bc}	5.8 ± 0.1^{a}
	Milk (2% M.F.)	5.0 ± 0.1^{c}	5.9 ± 0.1^{c}	5.4 ± 0.1^{c}
	Soy beverage	5.0 ± 0.1^{c}	6.2 ± 0.1^{ab}	5.4 ± 0.1^{c}
	Infant formula	5.1 ± 0.1^{c}	6.0 ± 0.1^{bc}	5.4 ± 0.1^{c}
	Orange juice	6.0 ± 0.1^{b}	6.0 ± 0.1^{bc}	6.0 ± 0.1^{b}
	p	<0.0001	<0.0001	<0.0001

[*]All values are means ± SEMs (n = 29 in experiment 1 and n = 31 in experiment 2). Means within a column with different superscript letters are significantly different, $P < 0.05$ (treatment effect using proc mixed, Tukey's post hoc).

[**]Pre-meal values are means of all observations before the test meal: 0, 10, 20 and 30 min in experiment 1 and 0, 10, 20, 30, 45, 60, 90 and 120 min in experiment 2.

[†]Post-meal values are means of all observations after the test meal: 50, 65, 80, 95, 110, 140 and 170 min in experiment 1 and 140, 170, 200, 230 and 260 min in experiment 2.

[‡]Total values are means of all observations: 0-170 min in experiment 1 and 0-260 min in experiment 2.

[§]Water (500 mL)

Table 4.4. Overall mean average appetite scores for the pre- and post-meal periods in experiments 1 and 2[*]

	Beverage	Pre-meal (mm)[**]	Post-meal (mm)[†]
Experiment 1	Control[‡]	69 ± 3.3[a]	19 ± 1.6
	Chocolate milk (1% M.F.)	60 ± 3.4[b]	20 ± 1.8
	Milk (2% M.F.)	60 ± 3.5[b]	21 ± 1.9
	Soy beverage	64 ± 3.4[b]	20 ± 1.6
	Infant formula	62 ± 3.9[b]	18 ± 1.8
	p	<0.0001	NS
Experiment 2	Control[‡]	72 ± 3.0[a]	23 ± 1.8
	Chocolate milk (1% M.F.)	60 ± 3.4[b]	20 ± 2.1
	Milk (2% M.F.)	60 ± 3.4[b]	21 ± 2.2
	Soy beverage	59 ± 3.8[b]	21 ± 2.3
	Infant formula	60 ± 3.3[b]	24 ± 2.1
	Orange juice	60 ± 3.5[b]	23 ± 2.1
	p	< 0.0001	NS

[*]All values are means ± SEMs (n = 29 in experiment 1 and n = 31 in experiment 2). Means within a column with different superscript letters are significantly different, $P < 0.05$ (treatment effect using proc mixed, Tukey's post hoc).
[**]Pre-meal values are means of all observations before the test meal: 0, 10, 20 and 30 min in experiment 1 and 0, 10, 20, 30, 45, 60, 90 and 120 min in experiment 2. [†]Post-meal values are means of all observations after the test meal: 50, 65, 80, 95, 110, 140 and 170 min in experiment 1 and 140, 170, 200, 230 and 260 min in experiment 2.
[‡]Water (500 mL)

Fig. 4.1A

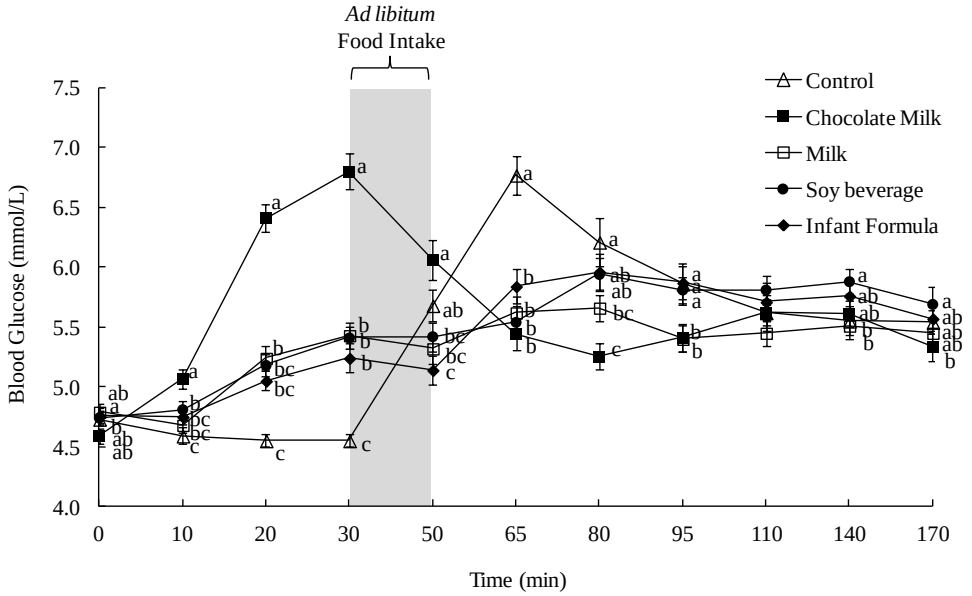

Fig. 4.1B

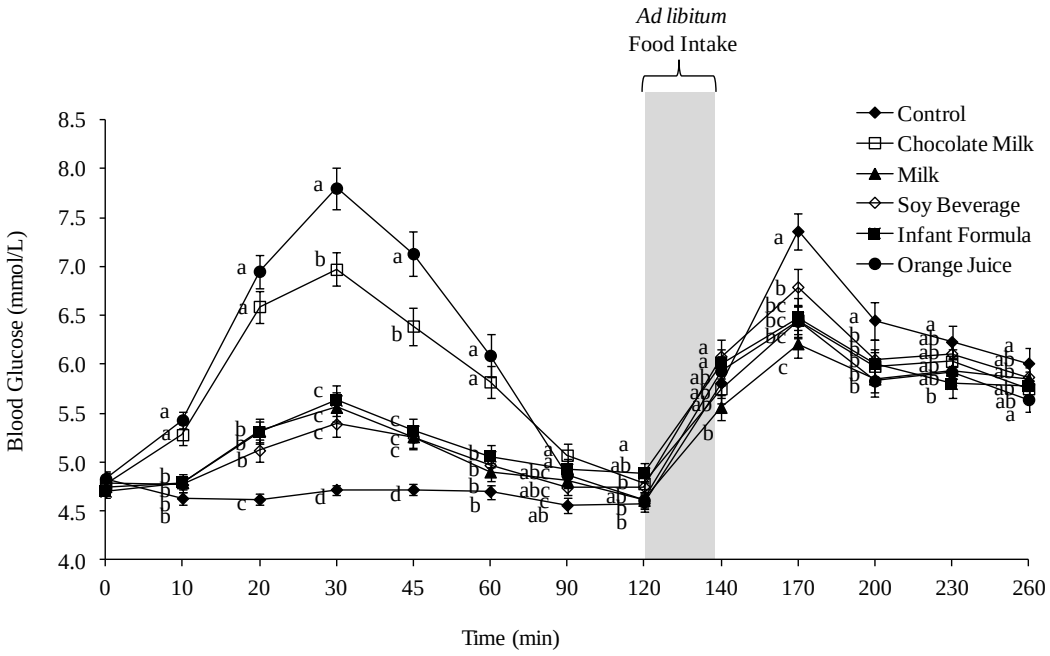

Fig. 4.1. Effect of beverages on blood glucose concentrations over time. **A**) Experiment 1. **B**) Experiment 2. Means with different superscripts are significantly different at each measured time (one-way ANOVA, Tukey-Kramer post-hoc test, $P < 0.05$). All values are mean ± SEM (n = 29 in experiment 1; n = 31 in experiment 2).

Fig. 4.2A

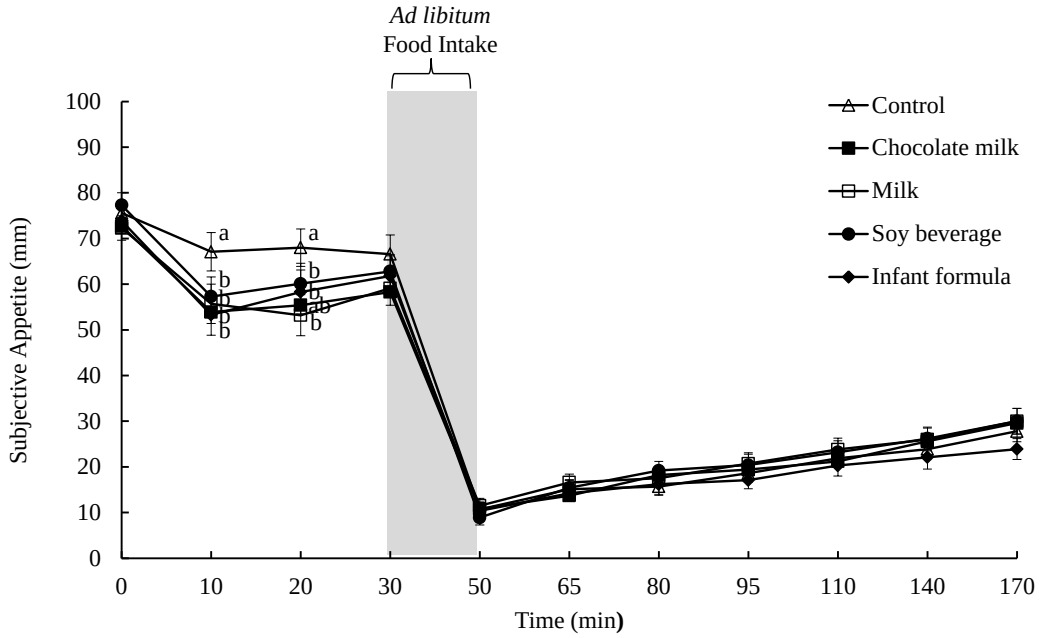

Fig. 4.2B

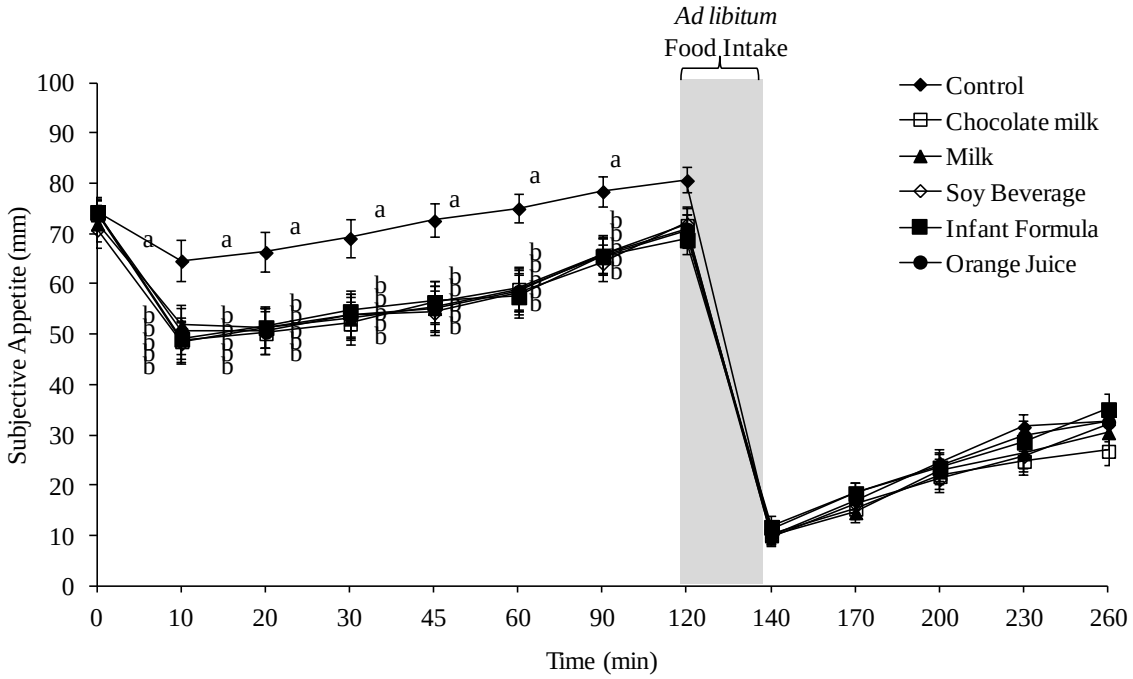

Fig. 4.2. Effect of beverages on average subjective appetite over time. **A**) Experiment 1. **B**) Experiment 2. Means with different superscripts are significantly different at each measured time (one-way ANOVA, Tukey-Kramer post-hoc test, $P < 0.05$). All values are mean ± SEM (n = 29 in experiment 1; n = 31 in experiment 2).

CHAPTER 5

CALORIC BEVERAGES CONSUMED FREELY AT MEAL-TIME ADD CALORIES TO AN AD LIBITUM MEAL

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CHAPTER 5

Caloric Beverages Consumed Freely at Meal-time Add Calories to an Ad libitum Meal

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5.1. Abstract

The objective was to compare the effects of ad libitum consumption of commonly consumed meal-time beverages on energy and fluid intakes and post-meal average subjective appetite and blood glucose in healthy adults. In a randomized controlled design, 29 males and females consumed to satiation an ad libitum pizza meal with one of five beverages in unlimited amount including water (0 kcal), 1% milk (44 kcal/100 ml), regular cola (44 kcal/100 ml), orange juice (44 kcal/100 ml) and diet cola (0 kcal). Food and fluid intakes were measured at the meal. Average subjective appetite and blood glucose were measured before and for 2 h after the meal. Although energy intake from pizza was similar amongst all beverage treatments, the amount of fluid consumed (g) varied among the beverages with intake of orange juice higher than regular and diet cola, but not different from water or milk. Meal-time ingestion of caloric beverages, milk, orange juice and regular cola, led to higher total meal-time energy intakes compared to either water or diet cola. Post-meal blood glucose area under the curve (AUC) was lower after milk than after meals with water, orange juice and regular cola and post-meal average subjective appetite AUC was lower after milk than after meals with water. Meal intakes of nutrients including protein, calcium, phosphorus, zinc, vitamins B12, A and D were higher at the meal with milk compared to the other beverages. Thus, caloric beverages consumed ad libitum during a meal add to total meal-time energy intake, but 1% milk favours a lower post-meal blood glucose and average subjective appetite score and adds to nutrient intake.

Keywords: meal-time beverages, milk, glycemic response, food intake, fluid intake, appetite, macronutrient composition, calories

5.2. Introduction

Several studies in adults and children have suggested that consumption of energy-yielding beverages is associated with positive energy balance, obesity and type 2 diabetes [249, 250]. Sugar-sweetened beverages have come under particular examination as their consumption now exceeds that of milk and fruit and vegetable juices in the United States [251, 252]. Caloric beverages provide approximately 81% of total daily water intake and contribute significantly to overall dietary and calorie intake [253, 254]. Based on survey data of dietary records, American adults ages 19 years and older consume an average of about 400 kcal per day from energy-containing beverages including regular soda, energy and sports drinks, milk, 100% fruit juice and fruit drinks [255, 256].

The calorie content of beverages varies widely and some including regular sodas, fruit drinks and alcoholic drinks contain calories, but provide little or no essential nutrients. Others, however, such as fat-free or low-fat milk and 100% fruit juice provide nutrients and have a similar energy density (energy per unit weight) to regular soda. To limit excess calories and maintain a healthy weight, the 2010 Dietary Guidelines for Americans advise individuals to drink water and other beverages with few or no calories, in addition to recommended amounts of low-fat or fat-free milk and 100% fruit juices [255]. Similarly, the Beverage Guidance Panel created a beverage hierarchy to guide beverage consumption, recommending water as the primary choice followed by calorie-free tea or coffee, then low-fat milk and soy beverages, non-caloric sweetened beverages, caloric beverages with some nutrients and lastly, sugar or high fructose corn syrup sweetened beverages [7].

Energy-yielding beverages are commonly used as snacks, meal accompaniments or meal replacements. Although energy from beverages has been claimed to be less satiating than that from solid foods in some studies [257] and hypothesized to bypass intake regulatory systems [184], there is still no agreement on the impact of liquid calories on energy intake [258]. The majority of studies examining the effect of beverages on appetite and food intake have been conducted on beverages alone prior to a meal [51, 259-261]. One report has shown that caloric beverages consumed at meal-time do not affect the amount of food eaten at an ad libitum meal, however, in this report the amount was controlled to isovolumetric amounts (360 ml) of water, diet cola, regular cola, fruit juice, and 1% milk [50]. Energy intakes from the lunch-time foods were not different among the beverages, but total energy intakes with caloric beverages were

higher than non-caloric beverages [50]. A limitation of these prior studies is that they have not allowed thirst to be a factor in beverage intake during a meal. The effect of ad libitum consumption of beverages at meal-time on energy intake has not been reported.

Although the past focus has been primarily on the energy contribution of beverages to excess energy intake, it is also shown that carbohydrate containing beverages raise blood glucose, which is of concern due to the high prevalence of insulin resistance [249]. However, the effect of their macronutrient composition on blood glucose has received little attention. We previously showed that isovolumetric amounts (500 ml) of 2% milk given prior to a meal reduced post-meal blood glucose in healthy men and women compared to other beverages including chocolate milk, soy beverage, orange juice, infant formula and water [260], suggesting a benefit to milk beyond its calories and nutrient contribution to a meal. This may be attributed to its protein content as milk proteins, when consumed alone in beverage form or with carbohydrate, reduce glycemic response [56, 237, 262]. The effect of beverages consumed ad libitum as part of a meal on post-prandial satiety and blood glucose concentrations has not been reported.

We hypothesized that consuming caloric beverages ad libitum and reflecting thirst, as part of a meal, reduces meal-time food intake, post-meal subjective appetite and glycemic response in healthy young adults. Therefore, the objective of the present study was to compare the effects of ad libitum consumption of water, 1% milk, regular cola, diet cola, and orange juice at a pizza meal on ad libitum energy and fluid intake, and post-meal subjective appetite and glycemic response in healthy young men and women.

5.3. Subjects and Methods

5.3.1. Subjects

Participants were recruited through advertisements posted from the University of Toronto campus. Men and women between 20 and 30 years of age with a body mass index (BMI) of 20 to 24.9 kg/m^2 were eligible to participate. Exclusion criteria included smoking, dieting, skipping breakfast, lactose intolerance or allergies to milk, diabetes (fasting blood glucose $\geq$ 7.0 mmol/L) or other metabolic diseases that could interfere with study outcomes. Restrained eaters, identified by a score of $\geq$ 11 on the Eating Habits Questionnaire [240], and those taking medications were also excluded. The sample size required was based on previous short-term

food intake studies on milk protein [56, 92] to detect a 150 kcal difference in food intake with a power of 0.90 and an alpha of < 0.05. 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.

5.3.2. Beverages

Beverages included: 1) water (control); 2) milk (1% M.F.) (Neilson Dairy; ON, Canada); 3) regular cola (Coca Cola Canada Ltd; ON, Canada); 4) diet cola (Coca Cola Canada Ltd; ON, Canada); and 5) orange juice (Tropicana Pure Premium, no pulp; Tropicana Products Inc., Bradenton, Florida, United States). The nutritional composition of each beverage is outlined in **Table 5.1**. All beverages were served ad libitum in 500 ml containers. Beverages were served chilled.

5.3.3. Protocol

A standard breakfast (300 kcal) consisted of a single serving of a ready-to-eat breakfast cereal (Honey Nut Cheerios; General Mills, Mississauga, Canada), a 250 mL box of 2% milk (Sealtest Skim Milk, Markham, Canada) and a 250 mL box of orange juice (Tropicana Pure Premium, no pulp; Tropicana Products Inc., Bradenton, Florida, United States). Breakfasts were given to participants to be consumed at their preferred time in the morning (0600-0900) after a 12 h overnight fast and were asked not to consume any food between the breakfast and the study session 4 h later (1000-1300), but were permitted to drink water until 1 h before the session. Participants attended the Department of Nutritional Sciences at the University of Toronto for the sessions. 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 and 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. Because impaired insulin sensitivity has been observed after an oral glucose tolerance test in the luteal phase of the menstrual cycle in healthy women [241], women were scheduled for the sessions during their follicular phase. The beverages were provided in random order once per week for

men and for women, in random order once per week during the first two weeks of their menstrual cycles.

On arrival, participants completed visual analog scale (VAS) questionnaires assessing their "Sleep Habits", "Stress Factors", and "Food Intake and Activity Level" over the previous 24 h before the study session as well as their activity since waking up, "Feelings of Fatigue", "Thirst" and "Motivation to Eat". The Motivation to Eat questionnaire tool, used to assess subjective appetite, was composed of four individual VAS which measured: (1) desire to eat ("very weak" to "very strong"); (2) hunger ("not hungry at all" to "as hungry as I've ever felt"); (3) fullness ("not full at all" to "very full"); (4) prospective consumption ("nothing at all" to "a large amount"). Each VAS consisted of a 100-mm line, which subjects marked with an "X" to indicate their feelings at the given moment (Hamedani et al., 2009). Scores were determined by measuring the distance (in mm) from the left starting point of the line to the intersection of the "X". A composite score of the 4 appetite questions in the "Motivation to Eat" VAS was calculated at each time of measurement to obtain an average subjective appetite score by the formula: [desire to eat + hunger + (100 – fullness) + prospective food consumption]/4 [142, 242] for statistical analysis.

Before the beginning of each test, each participant provided a baseline finger prick capillary blood sample using a Monoejector Lancet device (Sherwood Medical, St. Louis, MO). Plasma concentration of glucose was measured with a glucose meter (Accu-Chek Compact; Roche Diagnostics Canada, Laval, Canada). A baseline measurement of > 5.5 mmol/L suggested non-compliance with the fasting instructions and participants were rescheduled accordingly.

Following the completion of baseline measurements, each person was instructed to consume, up to satiation, one of the five beverages provided in random order along with a pizza meal over 20 min. Both beverages and the pizza meal were served ad libitum to enable the participant to reach satiation. Post-meal average subjective appetite and blood glucose were measured at 20, 30, 45, 60, 75, 90, 105 and 120 min from the time participants began drinking and eating. Participants were asked to remain seated for the duration of the experimental session and were permitted to read, do homework or listen to music.

Food intake was measured at an ad libitum pizza test meal (McCain Foods Ltd, Florenceville, NB) and fluid intake was assessed based on the measurement of the ad libitum consumption of the beverage served with the pizza meal. Participants were instructed to eat and

drink until they were “comfortably full” and were provided 3 varieties of pizza (Deluxe, Pepperoni, and Three Cheese) based on their preferences. They were allowed 20 min to eat. Detailed information of the nutrient content of the pizza and method of cooking was reported previously [142, 242]. Food intake was calculated from the weight of the consumed pizza based on the compositional information provided by the manufacturer. Beverage intake was measured by weight (g). Energy intake from the beverage was calculated based on the amount ingested and the caloric content as indicated by the manufacturer. Total caloric intake was calculated by adding the energy consumed from the pizza meal and beverage.

Nutrient intakes for the beverages were calculated using information from the manufacturer’s label for the 13 core nutrients required on food labels. Nutrients not indicated on the label were taken from information provided by United States Department of Agriculture database and Canadian Nutrient File. Total nutrient intakes were calculated by adding the nutrient intakes consumed from the pizza meal and beverage.

5.3.4. Statistical analyses

Statistical analyses were conducted using SAS version 9.2 (SAS Institute Inc, Cary, NC). The effect of beverage on total calories consumed, food intake, and beverage intake was not dependent on sex, as indicated by no sex by beverage interaction (two-factor ANOVA), therefore, these results were pooled for both men and women. The effect of beverage on total calories consumed, food intake, and beverage intake were tested by one-factor ANOVA followed by Tukey’s post hoc test.

Three-factor repeated-measures analysis of variance (ANOVA; via PROC MIXED procedure) were performed to analyze the effects of sex, time, beverage, and their interaction on dependent variables including post-meal blood glucose response and average subjective appetite scores. There was no significant interaction, indicating that the effect of beverages on blood glucose and average subjective appetite over time was not affected by sex. Thus, reported results were pooled for both men and women as well. Furthermore, two-factor ANOVA was performed on pooled blood glucose, blood glucose area under the curve (AUC) and average subjective appetite to analyze the effects of time, beverage and their interaction. When an interaction was statistically significant, one-factor ANOVA was followed by Tukey’s post hoc test to compare

the effect of beverages on absolute blood glucose and average subjective appetite scores at each time of measurement.

Pearson's correlation coefficients were used to detect associations between dependent measures. All results are presented as mean ± standard error of the mean (SEM). Statistical significance was concluded with a *P*-value less than 0.05.

5.4. Results

5.4.1. Participant characteristics

Twenty-nine healthy participants (sex: 15 males and 14 females; age: 22.1 ± 0.4 years; BMI: 22.3 ± 0.3 kg/m^2) completed the study. There were three dropouts due to participant time constraints.

5.4.2. Food, beverage and total caloric intake

Beverage composition was the main factor affecting calories from beverage intake ($P < 0.0001$) and total meal-time calories ($P < 0.0001$), but had no effect on energy intake from pizza ($P = 0.25$) (**Table 5.2**). Fluid volume from all beverages was not different from the control although intake of orange juice was higher than regular and diet cola ($P < 0.0001$). Calories from the beverages reflected the caloric content and were similar for the caloric beverages, milk, orange juice and regular cola. Total calories from the ad libitum intakes of beverage with pizza was higher following the caloric beverages, milk, orange juice and regular cola compared to either water or diet cola ($P < 0.0001$).

5.4.3. Nutrient Intakes

Beverage composition affected nutrient intakes from the test meal ($P < 0.05$) (**Table 5.3**). Intakes of nutrients including protein, total fat, saturated fat, cholesterol, calcium, phosphorus, zinc, vitamins B12, A and D were higher after the consumption of 1% milk compared to the other beverages ($P < 0.0001$), however, intakes of carbohydrates and sugars were highest after orange juice and regular cola consumption ($P < 0.0001$).

5.4.4. Subjective appetite scores

Post-meal average subjective appetite (20-120 min) was affected by time ($P < 0.0001$) and beverage ($P < 0.0001$), but not sex, time and beverage interaction or time, beverage and sex interaction. Post-meal subjective appetite was lower at 45, 60, 75 and 90 min after consumption of all beverages compared to water ($P = 0.0003$) (**Fig. 5.1A**).

Subjective appetite AUC (0-120 min) was suppressed after milk compared with water (P = 0.02), but was not different from other beverages (**Fig. 5.1B**).

5.4.5. Blood glucose concentrations

Post-meal blood glucose (20-120 min) was affected by time ($P < 0.0001$), beverage ($P < 0.0001$), sex ($P = 0.03$) and time and beverage interaction ($P < 0.0001$). The interaction is explained by higher blood glucose after the meals with orange juice and regular cola than milk, diet cola and water at 20 and 30 min (**Fig. 5.2A**). Milk with the meal resulted in lower post-meal blood glucose at 45, 60, 75 and 105 min than all other beverages (Fig. 5.2A). Blood glucose AUC (0-120 min) was lower after milk compared to the water, orange juice and regular cola consumption ($P < 0.0001$) (**Fig. 5.2B**).

5.4.6. Relations between dependent measures

Blood glucose AUC was not associated with food and beverage intake, total calories or subjective appetite AUC, but was positively associated with the carbohydrate content of the meal (pizza and beverage) ($r = 0.21$, $P = 0.01$) and beverage ($r = 0.17$, $P = 0.03$), but not pizza alone. Furthermore, the reduction in subjective appetite AUC was negatively correlated with food intake ($r = -0.32$, $P < 0.0001$) and carbohydrate content of the meal ($r = -0.22$, $P = 0.008$) and pizza ($r = -0.28$, $P = 0.0005$), but not with fluid or calorie intake from total meal-time calories consumed or carbohydrate content of the beverages alone. Baseline thirst was positively correlated with fluid intake ($r = 0.28$, $P = 0.0007$), pizza intake ($r = 0.22$, $P = 0.009$) and total calories ($r = 0.17$, $P = 0.047$).

5.5. Discussion

In contrast to our hypothesis, meal-time energy intake from food was not affected by ad libitum consumption of caloric beverages. Fluid intake at the meals was similar to water with an overall average of 470 ml for all beverages. Caloric consumption (energy from pizza and beverage) averaged 199 kcal more following caloric beverages compared to water and diet cola. Although all caloric beverages added to meal-time energy intake, milk provided more nutrients, reduced post-meal glycemia and subjective appetite. The results lead to the suggestion that water or diet beverages should be recommended at meal-time. However, 1% milk favors lower post-meal blood glucose and adds to nutrient intake, and thus should be recommended over other caloric beverages.

These novel data arise as a result of both beverage and food being served ad libitum. Although drinks are frequently consumed ad libitum with meals, previous studies on meal-time effects of beverages have provided fixed amounts [50] and as a result thirst was not a factor in determining the amount of calories consumed. Therefore, the present study assumed thirst to be the driver of fluid intake at an ad libitum meal. In support, baseline thirst was positively correlated with fluid intake and total meal-time calories consumed and the volume ingested was similar among beverages indicating that satisfaction of thirst was a priority while eating. In our study, meal-time beverages suppressed post-meal thirst similarly; therefore, providing fluid intake to thirst was not a factor in determining meal-time intake.

The present results, showing failure to compensate at the meal for energy content of the beverages, are consistent with these in which the meal-time beverages have been served in fixed quantities [261]. For example, isovolumetric (360 mL) amounts of orange juice, regular cola, diet cola and 1% milk given with a meal showed no differences in ad libitum food intake when compared with water, however, energy-containing beverages increased cumulative energy intake compared to non-caloric beverages while reducing hunger ratings [50]. In the current study, the complete lack of compensation for beverage calories consumed within a meal is surprising as caloric beverages consumed 30 min before a meal result in lower meal-time food intake [263]. Beverages of caloric contents in the range consumed in this study reduce subsequent food intake when the time between beverage and test meal is short (e.g. 60 min or less) [243], but not at later times [49, 245]. Compensation, although it is often incomplete, occurs at the test meal for the calories in pre-meal beverages of >150 kcal, but not if beverage energy content is lower or if the

time interval is greater than 60 min [243]. For example, pre-meal ingestion of 1% chocolate milk (340 kcal) and infant formula (368 kcal) reduced energy intake compared to isovolumetric amounts (500 ml) of water (0 kcal), 2% milk (260 kcal), and soy beverage (220 kcal) 30 min later while subjective appetite decreased after all caloric beverages [263]. However, incomplete meal-time compensation occurred for all pre-meal caloric beverages, resulting in higher cumulative intakes compared with water and diet cola.

The reason why energy content of the beverages within the meal did not affect food intake is unclear. One reason may be that simultaneous ingestion of solid foods and beverages and their interaction affect the rate of gastric emptying [264]. Factors such as meal phase (liquid versus solid), composition, volume and caloric content have been reported to affect the rate of gastric emptying [265]. For example, incorporation of dextrose into the liquid phase of a mixed solid and liquid meal resulted in a delay of both solid and liquid emptying and gastric emptying of a solid meal only commenced when approximately 80% of a simultaneously ingested liquid meal had emptied from the stomach [266, 267]. Furthermore, solids may exert their impact on satiety primarily through energy content rather than volume [268], whereas beverages show a stronger effect of volume and a weaker effect of energy [261].

Total energy intake at meal-time was the determinant of post-prandial appetite. While all beverages had similar effects on meal-time food intake, the addition of caloric beverages suppressed post-meal appetite for a longer period of time than water reflecting the higher calorie intake. However, the complexity of the interaction between meal-time beverage composition and post-meal appetite is illustrated by the effect on post-meal appetite. Surprisingly, although absent of calories, diet cola also suppressed post-meal appetite more than water and similar to the caloric beverages at 85 and 100 min after the meal. The role of carbonation may be an explanation as it has been suggested to decrease appetite by increased gastric distension resulting from the liberation of carbon dioxide. But this influence is shown to be relatively transient lasting between 40-60 min and depends on factors such as eructation [269-271]. Less likely is a direct effect of the sweeteners, aspartame and acesulfame-potassium, in diet cola, [272], which have not been shown to affect appetite when provided in small amounts [273]. Furthermore, although fluid intake at the meal was similar for all beverages, 1% milk was the only one to reduce subjective appetite AUC compared to water suggesting that this may be due to protein content. Milk proteins have been shown to suppress appetite and food intake [92].

Another novel aspect of this study was the measure of post-prandial glucose response to meals containing beverages which showed that macronutrient composition of beverages influence post-meal glucose response. Post-meal glycemia is important for achieving overall glycemic control and has been independently associated with adverse metabolic outcomes [189]. In this study, orange juice and regular cola were higher in carbohydrate content and resulted, as expected, in the highest blood glucose concentrations immediately after the meal and at peak (30 min), whereas water, diet cola and 1% milk resulted in similar, but lower peak values. Although similar in energy content to caloric beverages, ad libitum intake of 1% milk resulted in lower overall AUC post-meal blood glucose concentrations compared to water, orange juice and regular cola, but similar to diet cola. This result is consistent with a previous study showing that pre-meal consumption of 2% milk when compared with water, 1% chocolate milk, soy beverage, infant formula and orange juice reduced post-meal glycemic response to a meal either 30 min or 2 h later [260]. The attenuated glucose response following meal ingestion is unlikely related to a lower meal-time carbohydrate intake since the total carbohydrate content of the meal was higher with milk (169 g) compared to water (121 g) and diet cola (117 g). Instead, this may be due to the effect of milk proteins in stimulating insulin release [247], and gut hormones that slow stomach emptying such as glucagon-like peptide (GLP-1) and peptide tyrosine tyrosine (PYY) [206].

Surprisingly, blood glucose AUC following consumption of diet cola was reduced and not different from that of milk. It is generally assumed that glucose metabolism is not altered by aspartame and acesulfame-potassium because diet sodas contain no calories from carbohydrate [274]. However, recent data from animal studies indicates that artificial sweeteners play an active metabolic role within the gut. Artificial sweeteners in the absence of carbohydrate do not stimulate secretion of insulin or incretin hormones such as GLP-1 in humans [275] or animals [276]. However, it has been suggested that artificial sweeteners in the presence of carbohydrate, through stimulating gut taste receptors and synergizing with glucose-mediated stimulation of GLP-1 [277] which enhances insulin release, lower blood glucose. Unfortunately, because insulin was not measured, it remains to be determined if this is the mechanism of action.

In addition to its benefit to post-meal glycemic control and lower appetite, the contribution of 1% milk to meal-time nutrient intake recommends it as a preferred beverage at meals if water is not consumed. Higher intakes of protein, calcium, magnesium, phosphorus, zinc, vitamins B12, A and D were found after consumption of milk than with other beverages. It

is difficult for individuals to meet the nutritional requirements for many of these nutrients in the absence of milk consumption.

There are some limitations in this study. First, food and beverage choice was not free and was limited to pizza and one of the five beverages, suggesting that results may not be applied to other meal-time foods and beverages. Second, these findings cannot be extrapolated to obese populations since normal weight and obese individuals were reported to differ in their eating and drinking patterns [278]. Finally, hormonal regulators of hunger, thirst and glycemic control were not measured. The assessment of the physiological mediators of energy balance and glucose homeostasis would provide additional mechanistic insight into the impact of beverage composition at a meal on food intake and glycemic control and remains a topic for further study.

5.6. Conclusion

In conclusion, caloric beverages consumed ad libitum during a meal add to total meal-time energy intake, but 1% milk favours a lower post-meal blood glucose and subjective appetite score and adds to nutrient intake.

5.7. Acknowledgements

This study was supported by the National Science and Engineering Research Council of Canada (NSERC-CRDJP 385597-09), Dairy Farmers of Ontario, and Kraft Canada Inc. We would also like to thank Nothando Swan, Undergraduate Research Opportunity Student (UROP), and Shari Berengut, research assistant, for their assistance during the study. None of the authors declared a conflict of interest.

Table 5.1. Nutritional composition of beverages

	Beverages			
Nutrients[1]	Milk (1% M.F.)	Regular Cola	Diet Cola	Orange Juice
Energy (kcal)	110	110	0	110
Fat (total) (g)	2.5	0	0	0
Saturated fat (g)	1.5	0	0	0
Trans fat (g)	0	0	0	0
Sodium (mg)	120	30	35	0
Carbohydrate (g)	12	30	0	27.5
Sugars (total) (g)	12	30	0	23
Protein (g)	9	0	0.1	2.1
Calcium (mg)	330	0	0	16

[1]Nutrient content of each beverage as provided by the manufacturer. Amounts given are per 250 ml serving.

Table 5.2. Effect of various beverages at meal-time on food intake, beverage intake and total caloric intake[1]

Beverage	Energy Intake		Beverage Intake[4]	Beverage Intake
	Total Calories[2]	Food Intake[3]		
	kcal		*g*	*kcal*
Water	962 ± 52[a]	962 ± 52	456 ± 40[abc]	0 ± 0[a]
Milk (1% M.F.)	1127 ± 57[b]	905 ± 53	506 ± 50[ab]	223 ± 22[b]
Orange Juice	1192 ± 55[b]	952 ± 50	544 ± 39[a]	239 ± 17[b]
Regular Cola	1110 ± 57[b]	915 ± 51	443 ± 31[bc]	195 ± 14[b]
Diet Cola	926 ± 63[a]	926 ± 63	373 ± 34[c]	0 ± 0[a]
P	<0.0001	NS	<0.0001	<0.0001

[1]All values are means ± SEMs; n=29. Values in the same column with different superscript letters are significantly different, $P < 0.05$ (beverage effect using proc mixed, Tukey's post hoc).
[2]Energy from beverages + energy from the pizza meal.
[3]Energy consumed in an ad libitum pizza meal.
[4]Energy consumed in an ad libitum beverage.

Table 5.3. Nutrient intakes (beverage and pizza meal)

Nutrient	Water	1% Milk	Orange Juice	Regular Cola	Diet Cola	*P*-value
Protein (g)	46.3 ± 2.5^{bc}	61.3 ± 3.0^{a}	50.2 ± 2.5^{b}	44.1 ± 2.5^{c}	44.8 ± 3.0^{c}	< 0.0001
Total Fat (g)	32.1 ± 1.7^{b}	35.1 ± 1.8^{a}	31.7 ± 1.7^{b}	30.5 ± 1.7^{b}	30.9 ± 2.1^{b}	0.0003
Saturated fat (g)	12.3 ± 0.7^{b}	14.7 ± 0.7^{a}	12.3 ± 0.6^{b}	11.9 ± 0.7^{b}	12.0 ± 0.8^{b}	< 0.0001
Trans fat (g)	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.1	NS
Cholesterol (mg)	80.2 ± 4.3^{b}	95.0 ± 4.8^{a}	79.4 ± 4.2^{b}	76.3 ± 4.5^{b}	77.2 ± 5.2^{b}	< 0.0001
Carbohydrate (g)	121.2 ± 6.5^{c}	137.5 ± 7.0^{b}	178.9 ± 8.1^{a}	166.8 ± 8.2^{a}	116.6 ± 7.9^{c}	< 0.0001
Fibre (g)	10.7 ± 0.6	10.1 ± 0.6	10.6 ± 0.6	10.2 ± 0.6	10.3 ± 0.7	NS
Sugars (g)	21.4 ± 1.2^{c}	43.6 ± 2.5^{b}	71.4 ± 3.9^{a}	71.9 ± 4.0^{a}	20.6 ± 1.4^{c}	< 0.0001
Calcium (mg)	652.1 ± 34.6^{b}	1255.6 ± 71.4^{a}	688.1 ± 34.2^{b}	615.2 ± 34.5^{b}	634.03 ± 42.5^{b}	< 0.0001
Iron (mg)	8.7 ± 0.5	8.2 ± 0.5	8.6 ± 0.5	8.3 ± 0.5	8.8 ± 0.6	NS
Magnesium (mg)	110.4 ± 5.7^{b}	153.6 ± 7.7^{a}	162.8 ± 7.4^{a}	99.8 ± 5.6^{b}	105.3 ± 7.0^{b}	< 0.0001
Phosphorus (mg)	865.3 ± 46.4^{b}	1294.6 ± 65.6^{a}	948.3 ± 46.3^{b}	867.9 ± 47.1^{b}	866.2 ± 57.5^{b}	< 0.0001
Potassium (mg)	807.9 ± 43.3^{c}	1544.7 ± 87.1^{b}	1826.1 ± 90.2^{a}	777.3 ± 43.3^{c}	808.1 ± 53.6^{c}	< 0.0001
Sodium (mg)	2036.1 ± 108.9	2165.3 ± 113.9	2010.2 ± 105.2	1983.5 ± 43.3	2005.4 ± 133.8	NS
Zinc (mg)	6.1 ± 0.3^{b}	7.9 ± 0.4^{a}	6.4 ± 0.3^{b}	5.9 ± 0.3^{b}	5.9 ± 0.4^{b}	< 0.0001
Vitamin C (mg)	6.4 ± 0.3^{b}	10.7 ± 0.6^{a}	163.6 ± 11.4^{b}	6.1 ± 0.3^{b}	6.2 ± 0.4^{b}	< 0.0001
Folate (mg)	380.2 ± 20.3^{b}	383.2 ± 21.1^{b}	496.5 ± 22.7^{a}	361.7 ± 20.3^{b}	366.0 ± 24.7^{b}	< 0.0001
Vitamin B-12 (ug)	2.4 ± 0.1^{b}	4.6 ±0.3^{a}	2.3 ± 0.1^{b}	2.2 ± 0.1^{b}	2.3 ± 0.2^{b}	< 0.0001
Vitamin A (ug, RAE)	213.8 ± 11.4^{b}	397.2 ± 22.1^{a}	211.6 ± 11.1^{b}	203.4 ± 11.4^{b}	205.8 ± 13.9^{b}	< 0.0001
Vitamin D (ug)	0.4 ± 0.0^{b}	4.8 ± 0.4^{a}	0.4 ± 0.0^{b}	0.3 ± 0.0^{b}	0.4 ± 0.0^{b}	< 0.0001
Caffeine (ug)	0 ± 0^{c}	0 ± 0^{c}	0 ± 0^{c}	36.1 ± 2.5^{b}	44.8 ± 4.1^{a}	< 0.0001

[1]All values are means ± SEMs; n = 29. Means within a row with different superscript letters are significantly different, $P < 0.05$ (beverage effect using proc mixed, Tukey's post hoc).

Fig. 5.1A

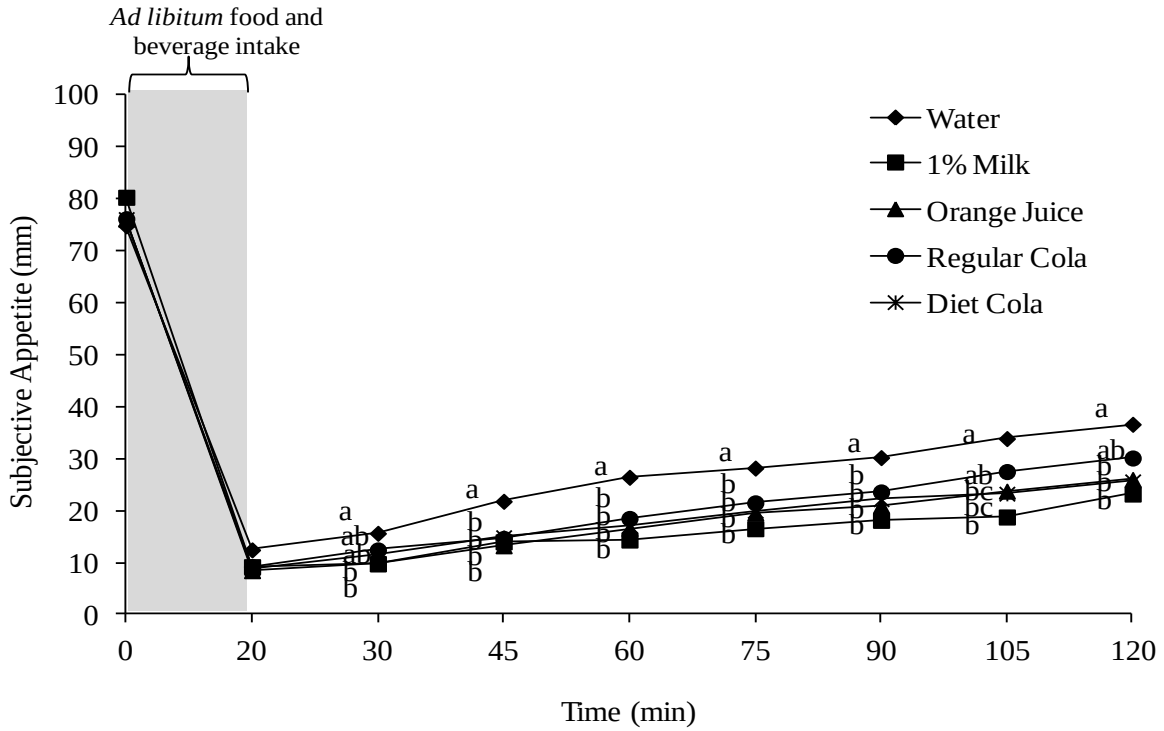

Fig. 5.1B

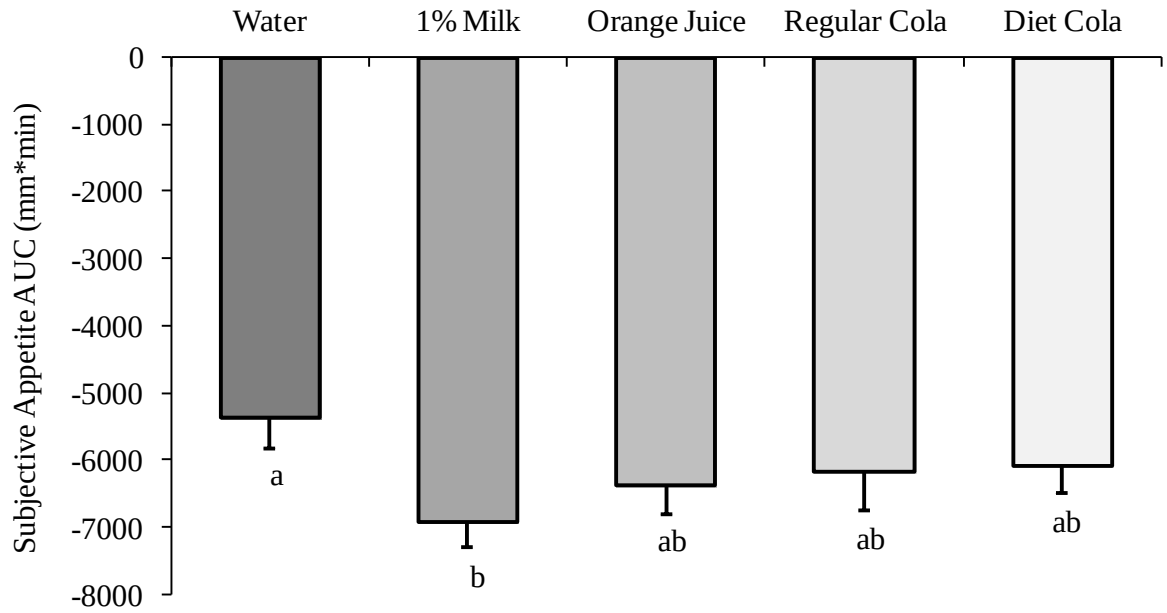

Fig. 5.1. Effect of ad libitum meal-time food and beverage intake on **A**) absolute subjective appetite scores over time; and **B**) subjective appetite AUC (mm*min). Means with different letters are significantly different at each measured time (one-way ANOVA, Tukey-Kramer post-hoc test, $P < 0.05$). All values are means ± SEMs; n = 29.

Fig. 5.2A.

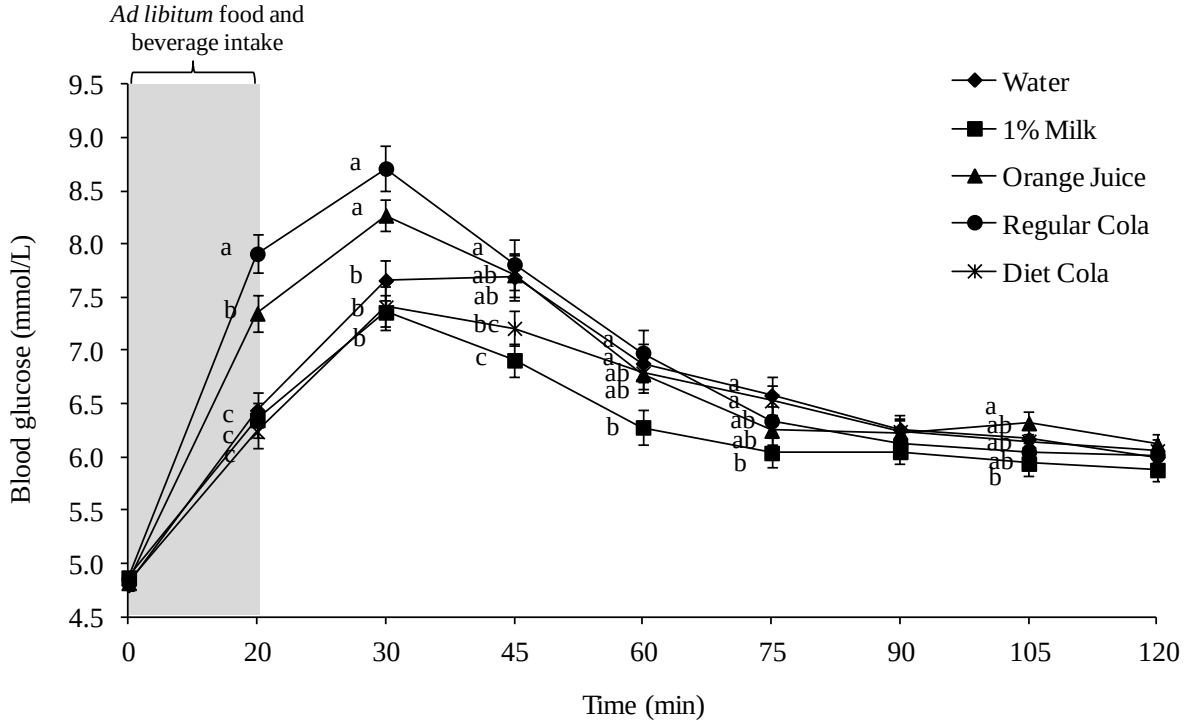

Fig. 5.2B

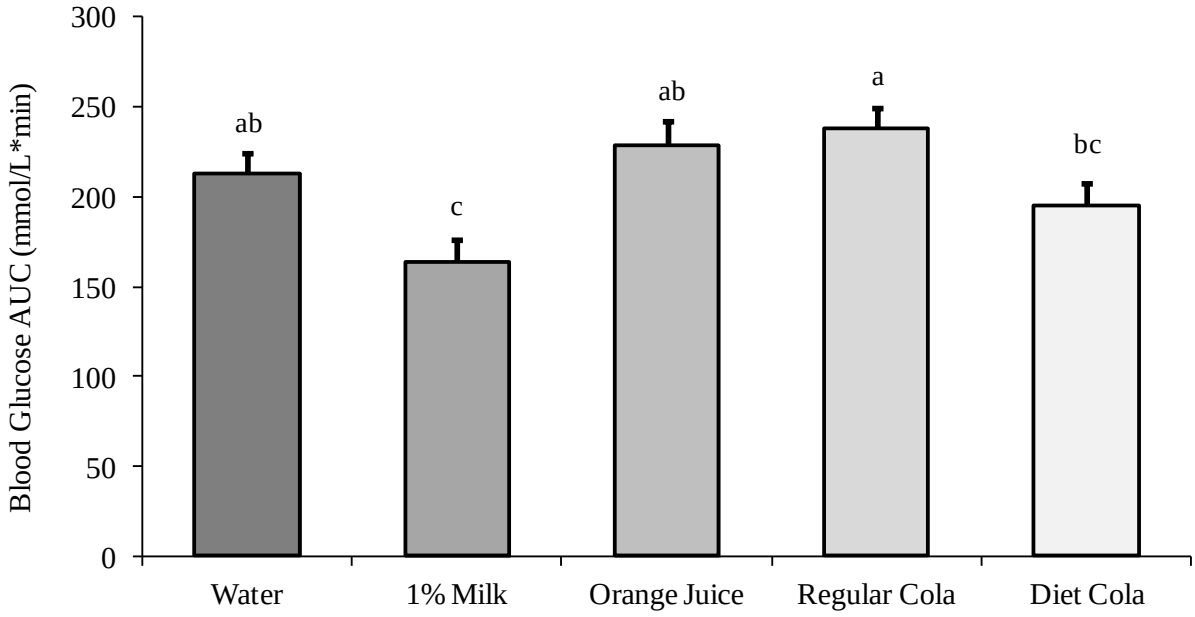

Fig. 5.2. Effect of ad libitum meal-time food and beverage intake on **A**) blood glucose concentrations over time; and **B**) blood glucose AUC (mmol/L*min). Means with different letters are significantly different at each measured time (one-way ANOVA, Tukey-Kramer post-hoc test, $P < 0.05$). All values are means ± SEMs; n = 29.

CHAPTER 6

MECHANISM OF ACTION OF WHOLE MILK AND ITS COMPONENTS ON GLYCEMIC CONTROL IN HEALTHY YOUNG MEN

The following chapter is a reproduction of a manuscript that will has been invited for resubmission to the Journal of Nutrition (MS ID#: NUTRITION/2013/180786).

CHAPTER 6

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

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6.1. Abstract

Milk reduces post-meal glycemic excursions when consumed either before or within an ad libitum meal by healthy adults; however, the role of the macronutrient components and mechanisms underlying this response have not been defined. Therefore, the objective of this study was to compare the effect of each of the macronutrient components and their combination with whole milk on post-prandial glycemia and gastrointestinal hormones in healthy young men. In a randomized, crossover study, 12 males consumed beverages (500 mL) of whole milk (3.25% M.F.) (control), a simulated milk beverage based on milk macronutrients, complete milk protein (16 g), lactose (24 g), or milk fat (16 g). Paracetamol (1.5 g) was added to all beverages to measure gastric emptying. Whole and simulated milk were similar in lowering post-prandial glycemia and stimulating insulin, PYY and CCK, but whole milk resulted in lower (41%) GLP-1 and higher (43%) ghrelin areas under the curve (AUC) than simulated milk (P = 0.01 and P = 0.04, respectively). When compared to the sum of AUCs for the component responses both whole and simulated milk resulted in lower glucose AUCs (P = 0.0005). Whole milk also produced a lower CCK AUC. When AUCs were adjusted for the energy content of the treatments, the milks produced lower glucose and hormone responses than predicted from the sum of component effects. The impact of protein energy was higher on insulin, GLP-1, CCK, and paracetamol, whereas for lactose energy, the impact was higher on glucose and glucose/insulin ratio. Fat energy had little effect. Thus, the lower post-prandial glycemia of whole milk than predicted from the sum of its components is mediated by interaction among its macronutrient components.

6.2. Introduction

Epidemiological studies have linked frequent dairy consumption with healthier body weights [12] and lower risk of type 2 diabetes (T2D) [11], where milk is a major contributor to dairy intake. This possible link between milk consumption and obesity and T2D is of growing interest because milk contributes to the regulation of glucose metabolism [279, 280].

Fluid milk products reduce post-meal glycemic excursions when consumed either before or within an ad libitum meal by healthy young adults [279, 280]. In a comparison of familiar beverages consumed before a meal, including isovolumetric (500 ml) servings of 2% milk, 1% chocolate milk, orange juice, soy beverage, infant formula and water, milk was the only beverage leading to a decrease in post-prandial glycemic response after a later meal [280]. Similarly 1% milk, in contrast to other caloric and non-caloric beverages, when consumed with a meal, reduced post-prandial glycemia [279]. Other studies show that consuming milk with bread and pasta lowers post-prandial glycemia [127].

Short-term studies show that milk proteins, when consumed alone in beverage form or with carbohydrate, reduce glycemia [26] due to the stimulation of insulin [101, 281]. Although milk proteins stimulate insulin, attributed to the rapid digestion and absorption of their branched-chain amino acids [103], post-meal reduction of glycemia after milk consumption may not be simply related to its insulin action. Release of gut hormones including glucagon-like peptide-1 (GLP-1), peptide tyrosine tyrosine (PYY), and cholecystokinin (CCK) delay stomach emptying [26, 54, 85]. These responses are also associated with suppression of food intake after consumption of milk proteins [54, 248], which also contributes to lower post-prandial glycemia.

Although the beneficial effects of milk have been attributed primarily to its protein content [26, 54, 99], milk is a complex mixture of proteins (whey protein and casein in the ratio of 20:80), fats (saturated, mono- and poly-unsaturated and trans fatty acids), and carbohydrate (lactose), and in addition to providing high quality nutrients, possesses a wide range of bioactivities [234]. Milk fat is an important regulator of metabolic responses via the ileal brake, which leads to slower stomach emptying and stimulates the release of gastrointestinal peptides such as CCK and PYY [117]; however, its relevance to glycemic control by milk has not been reported. Furthermore, lactose contributes to lower glycemia than other sugars or starch [127]. Each of the macronutrients of milk may contribute to reducing food intake and glycemia [92,

197, 282]; however, there are no reports comparing the individual and collective contribution of milk's macronutrient components to the effect of whole milk.

Therefore, we hypothesized that the physiological effects of milk on post-prandial glycemia are mediated by synergism interactions among its macronutrients and not predicted by studies of the individual components in isolation. The objective of this study was to compare the effects of isovolumetric (500 ml) 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 post-prandial glycemic and gastrointestinal hormone response and gastric emptying rate in healthy young men.

6.3. Methods

6.3.1. Participants

Participants were recruited through advertisements posted on the University of Toronto campus. Healthy men between 20-30 years of age with a body mass index (BMI) of 20-24.9 kg/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 or appetite, diabetes (fasting blood glucose $\geq$ 7.0 mmol/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 [27]. 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.

Beverages

6.3.2. Beverages

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

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.) was used as the control for two reasons. In our previous study [280], despite infant formula and chocolate milk having similar carbohydrate contents, infant formula, containing a higher fat content, resulted in a lower pre-meal blood glucose concentrations compared to chocolate milk. Furthermore, whole milk contains the highest fat content compared to other commercially available types of 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 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 500 ml of water and stirred at room temperature for 20 min 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, Toronto, ON, Canada). 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 17.5/3.5 MPa) to reduce the size and size distribution of milk fat globules. The milk fat beverage was pasteurized at 75°C for 15 min, 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 (24 g) and protein (16 g) (both in powder form) to 460 ml of the ready-made milk fat beverages to achieve a volume of 500 ml with 16 g of fat and stirred at room temperature for 20 min.

Paracetamol (1.5 g, Panadol; GlaxoSmithKline) was dissolved in each of the five beverages so the appearance in the blood can be used as a proxy to measure rate of gastric emptying [283]. Vanilla extract (1.2 ml, Flavorganics, Newark, NJ, USA), and sucralose (0.02 g, 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 (500 ml) based on the commercially available serving size of milk beverages and were served chilled. The nutritional composition of the beverages is provided in **Table 6.1**.

6.3.3. Protocol

This study was a crossover, randomized, single-blind design consisting of five sessions separated by a one-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 1 h 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 (VAS) questionnaires assessing their "Sleep Habits", "Stress Factors", "Food Intake and Activity Level", "Feelings of Fatigue", and "Motivation to Eat" [34, 242]. 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, CA). A baseline measurement of > 5.5 mmol/L indicated non-compliance 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 5 min at a constant pace. Blood samples were collected at 0 min (baseline) and at 30, 45, 60, 90, 120, 150 and 180 min. Participants were asked to remain seated for the duration of the experimental session and were permitted to read, do homework or listen to music.

6.3.4. Blood Parameters

Blood was collected in 8.5 mL 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 [284]. The tubes were centrifuged at 1300 RCF for 20 min at 4°C. Collected plasma samples were aliquoted in Eppendorf tubes and stored at -70 C for analyses. Plasma concentrations of glucose, insulin, GLP-1, PYY, CCK, ghrelin and paracetamol were measured.

Plasma glucose was measured using the enzymatic hexokinase method (intra-CV: <5%; inter-CV: <8%; Roche Diagnostic, Laval, QC, Canada). Insulin was assessed with an electrochemiluminescence assay "ECLIA" (intra-CV: <3%; inter-CV: <7%; Roche Diagnostic, Laval, QC, Canada). These analyses were performed by the Pathology and Laboratory Medicine Department at Mount Sinai Hospital (Toronto, ON, Canada). The remaining biomarkers were measured at the Department of Nutritional Sciences, University of Toronto.

To assess gastric emptying rate, free paracetamol (acetaminophen) was measured with a commercially-available paracetamol enzymatic assay **(**intra-CV: <2.4%; inter-CV: <2.6%; Cambridge Life Sciences, Ely, Cambridge, UK). Human active GLP-1 (intra-CV: <8%; inter-CV: <5%; #EGLP-35K), total ghrelin (intra-CV: <2%; inter-CV: <8%; #EZGRT-89K), and total PYY (intra-CV: <6%; inter-CV: <8%; #EZHPYYT66K) were measured with ELISA kits (Millipore, Billerica, MA, USA). Human CCK-8 [CCK-(26-33)] was measured with enzyme immunoassay kit (intra-CV: <10%; inter-CV: <15%; #EK-069-04, Phoenix Pharmaceuticals, Inc, Burlingame, CA, USA).

Plasma concentrations of glucose, insulin, and ghrelin were measured at all sampling times. However, due to the high cost of the kits and measurements, plasma concentrations of paracetamol, GLP-1 and PYY were measured at 0 (baseline), 30, 45, 60, 90, 150 and 180 min and CCK measured at 0 (baseline), 30, 45, 60, 90 and 150 min.

6.3.5. Statistical Analyses

Statistical analyses were conducted using SAS version 9.2 (SAS Institute Inc, Cary, NC, USA). Two-factor repeated-measures analysis of variance (ANOVA; via PROC MIXED procedure) were performed to analyze the effects of time, beverage, and their interaction on dependent variables including plasma glucose, insulin, paracetamol, and gastrointestinal hormones. When an interaction was found, one-factor ANOVA was performed followed by Tukey's post hoc test to compare the effect of beverages at each time of measurement. For

analysis of all parameters, the baseline value was subtracted from post-prandial responses to normalize between-subject differences.

Incremental areas under the curve (AUC) using the trapezoidal rule [285] were calculated for all measures. AUCs were calculated for each of the treatments both before and after adjusting for the variances in energy content of the treatments by calculating AUCs per 50 kcal for each treatment. For each parameter, AUC values represented the areas enclosed between the pre-ingestion baseline and the post-ingestion response curve until the return to baseline. Incremental AUC for whole milk and the simulated milk beverage were compared to the sum of the components by adding for each individual their AUCs for protein, lactose and fat (AUC_{sum}) for glucose, insulin, and gastrointestinal hormones.

Glucose to insulin ratios were compared among treatments by calculating both the ratios of blood glucose to insulin AUC, and the ratios of changes from baseline of blood glucose to insulin at 30 min. Glucose to insulin ratio is an accepted approach to evaluate the efficacy of insulin action over the post-prandial period [286]. The lower the ratio, the higher the efficacy of insulin action [287, 288].

Pearson's correlation coefficients were used to detect associations between dependent measures. All results are presented as mean ± standard error of the mean (SEM). Statistical significance was concluded with a *P*-value less than 0.05.

6.4. Results

6.4.1. Subject characteristics

Twelve healthy males (age: 22.4 ± 0.4 years; BMI: 21.9 ± 0.3 kg/m^2) completed the study. Three participants withdrew from the study after the first session (n = 2 personal time constraints; n = 1 physical discomfort after the lactose beverage).

6.4.2. Post-prandial responses unadjusted for energy content

6.4.2.1. Plasma glucose and insulin concentrations

Plasma glucose concentrations were affected by time ($P < 0.0001$), beverage ($P = 0.002$) and an interaction between time and beverage ($P < 0.0001$). Plasma glucose concentrations were higher after lactose, whole milk and simulated milk beverage compared to protein and fat

beverages at 30 min ($P < 0.0001$) and 45 min ($P = 0.03$) (**Fig 6.1A**). Plasma glucose AUCs for treatments was highest after lactose compared to fat and protein beverages, but not different from whole milk or the simulated milk beverage ($P = 0.005$) (**Table 6.2**). Whole milk and the simulated milk beverage resulted in a 56% lower AUC than the sum of protein, lactose and fat over 180 min ($P = 0.005$).

Plasma insulin concentrations were affected by time ($P < 0.0001$), beverage ($P < 0.0001$) and an interaction between time and beverage ($P < 0.0001$). Plasma insulin concentrations were higher after whole milk and simulated milk beverage than after fat and protein beverages at the peak time of 30 min ($P < 0.0001$) and after lactose, protein and fat beverages at 45 ($P < 0.0001$), 60 ($P < 0.0001$), and 120 min ($P < 0.0001$) (**Fig 6.1B**). Plasma insulin AUCs were higher after whole milk and the simulated milk beverage than after protein, lactose and fat ($P < 0.0001$) (Table 6.2). Whole milk and the simulated milk beverage resulted in a similar insulin AUC to each other and to the sum of protein, lactose and fat.

6.4.2.2. Glucose to insulin ratios

Glucose to insulin ratios of AUC was not affected by time, beverage or an interaction between time and beverage. At 30 min, the time point at which glucose and insulin peaked, the ratios were not different among beverages (Table 6.2). The ratios of glucose to insulin AUCs were lower after whole milk, the simulated milk beverage and protein compared to lactose, but were not different from fat ($P = 0.007$) (Table 6.2).

6.4.2.3. Plasma GLP-1 concentrations

Plasma GLP-1 concentrations were affected by time ($P < 0.0001$), beverage ($P < 0.0001$) and an interaction between time and beverage ($P < 0.0001$). The interaction is explained by the higher plasma GLP-1 concentrations after whole milk compared to protein, lactose and fat at 30 min ($P = 0.007$) and 45 min ($P < 0.0001$) and the higher GLP-1 concentrations at 60 and 90 min ($P < 0.0001$) after the simulated milk beverage compared to whole milk and its components (Fig 6.2A). Plasma GLP-1 AUC was higher after whole milk compared to lactose and protein, lower than simulated milk, but not different from fat ($P < 0.0001$) (Table 6.2). The simulated milk beverage resulted in 41% higher AUC than whole milk, but was not different from the sum of protein, lactose and fat.

6.4.2.4 Plasma PYY concentrations

Plasma PYY concentrations were affected by time ($P = 0.02$) and beverage ($P < 0.0001$) but not by a time and beverage interaction. Whole milk and the simulated milk beverage resulted in the highest PYY concentrations at 45 min ($P = 0.0004$), 60 min ($P = 0.0007$), 90 min ($P < 0.0001$), 150 min ($P = 0.004$) and 180 min ($P = 0.003$) compared to its components (**Fig 6.2B**). Plasma PYY AUC was higher after whole milk and the simulated milk beverage compared to each of the components ($P < 0.0001$) (Table 6.2), but similar to that predicted from the sum of the components.

6.4.2.5 Plasma CCK concentrations

Plasma CCK concentrations were affected by time ($P < 0.0001$) and beverage ($P = 0.002$), but not by a time and beverage interaction. Plasma CCK concentrations were increased after whole milk compared to the fat beverage at 30 min ($P = 0.02$) (**Fig 6.2C**), but were not different from the other beverages. The simulated milk beverage resulted in the highest plasma CCK concentrations at 45 min ($P = 0.005$) and 60 min ($P = 0.02$). Furthermore, CCK AUC was higher after the simulated milk beverage compared to protein, lactose and fat ($P < 0.0001$), but not different from whole milk (Table 6.2). Whole milk and the simulated milk beverage resulted in 200% and 60% lower AUCs than the sum of protein, lactose and fat ($P = 0.0005$).

6.4.2.6 Plasma ghrelin concentrations

Plasma ghrelin concentrations were affected by time ($P = 0.004$) and beverage ($P < 0.0001$), but not time and beverage interaction. All beverages reduced plasma ghrelin concentrations, however, the simulated milk beverage resulted in the lowest ghrelin concentrations at 30 min ($P = 0.0005$), 45 min ($P = 0.0008$), 90 ($P = 0.007$), 150 ($P = 0.0004$) and 180 min ($P = 0.02$) compared to whole milk and its separate components (**Fig 6.2D**) and remained below baseline for the duration of the study. Plasma ghrelin concentrations after protein and lactose beverages returned to above baseline levels after 120 min (Fig 6.2D). Ghrelin AUC below the line was larger after the simulated milk beverage compared to protein, lactose and fat ($P = 0.0003$), but not different from whole milk (Table 6.2). In addition, the

simulated milk beverage resulted in a lower (43% AUC) ghrelin ($P < 0.04$) than whole milk although post hoc analyses did not indicate differences.

6.4.2.7 Gastric emptying rate (plasma paracetamol concentrations)

Plasma concentrations of paracetamol were affected by time ($P < 0.0001$), beverage ($P < 0.0001$) and an interaction between time and beverage ($P < 0.0001$). Plasma paracetamol concentrations were lower after whole milk and protein beverages at 45 min ($P < 0.0001$) and after whole milk, the simulated milk beverage and protein at 60 min compared to lactose and fat beverages ($P < 0.0001$) (**Fig 6.3**). Whole milk and protein reduced plasma paracetamol AUC compared to fat ($P < 0.0001$), but was not different from the simulated milk beverage (Table 6.2).

6.4.3. Post-prandial responses adjusted for energy content

The AUCs for glucose and all hormones adjusted for energy content show that the responses were similar for whole milk and the simulated milk beverage (**Table 6.3**). All AUCs after the whole and simulated milk beverages were lower than predicted by summing their component effects. The effect of protein per kcal on the AUCs was highest compared to all treatments for insulin, GLP-1, PYY, CCK and paracetamol ($P < 0.0001$). Lactose per kcal had the greater effect on glucose and insulin, although was less than for protein and resulted in the highest glucose/insulin ratio.

6.4.4. Relations between dependent measures

Blood glucose AUC was associated with AUC for insulin ($r = 0.30$, $P = 0.02$), but not GLP-1, PYY, CCK or paracetamol. Insulin AUC was positively associated with AUC for PYY ($r = 0.31$, $P = 0.02$), but not GLP-1 or CCK and negatively associated with ghrelin ($r = -0.38$, $P = 0.003$) and paracetamol ($r = -0.53$, $P < 0.0001$). GLP-1 AUC was negatively associated with paracetamol ($r = -0.30$, $P = 0.02$), but was not associated with PYY, CCK or ghrelin. PYY AUC was negatively correlated with AUC for ghrelin ($r = -0.31$, $P = 0.02$) and paracetamol ($r = -0.52$, $P < 0.0001$). CCK and ghrelin AUCs were positively correlated with paracetamol ($r = 0.29$, $P = 0.02$), ($r = 0.31$, $P = 0.02$), respectively. Caloric content of beverages were positively correlated

with insulin (r = 0.56, P < 0.0001) and PYY (r = 0.35, P = 0.005), but not GLP-1 or CCK and negatively associated with ghrelin (r = -0.29, P = 0.03) and paracetamol (r = -0.28, P = 0.03).

6.5. Discussion

The hypothesis that low post-prandial glycemia of milk is mediated by interactions among its macronutrients and not predicted by glycemic responses to the individual components in isolation is supported by this study. Although post-prandial glycemia after whole and simulated milks was much lower than predicted from the sum of the glycemic effects of protein, lactose and fat, insulin and other hormones reflected the sums of the components, with the exception of a lower CCK after whole milk. A novel finding was that the simulated milk beverage resulted in a higher AUC for GLP-1 and lower AUC (greater suppression) for ghrelin compared to whole milk over the duration of the study. In contrast, the AUCs expressed per kcal show that the source of energy differs in impact per kcal on glucose and hormone responses. The stimulatory effects of whole and simulated milks per kcal were markedly attenuated compared with that predicted by the sum of the AUCs for protein, lactose and fat.

The much lower post-prandial glycemia for whole and simulated milk than that predicted from its component effects, but with no increase in insulin, suggest that alternate mechanisms may account for this observation. The glycemic response, calculated as the sum of the AUCs for protein, lactose and fat, was blunted by 56% in the milks (Table 6.2). These results are consistent with those showing that fat and protein reduce post-prandial glycemia of carbohydrate [102, 197, 218, 289]. Recent studies showed that whey protein ingestion reduced post-meal glycemia in a dose-dependent manner without increased insulin concentrations [26] and when compared with glucose led to similar reductions in post-meal plasma glucose by both insulin-dependent and independent mechanisms including those hormones that affect glucose utilization and stomach emptying rate [27].

Plasma insulin, GLP-1 and PYY AUCs were similar after whole milk and the simulated milk beverage compared to the sum of the individual responses to protein, lactose or fat showing an additive effect (Table 6.2). Because the ratio of glucose/insulin was lowest among the milk beverages, we suggest that the efficiency of insulin was enhanced or the lower glucose was due to actions of other hormones and delayed stomach emptying rate [27]. Enhanced insulin function was suggested in a recent study in mice that took a similar approach to evaluating a diet

and its macronutrient components [290]. The authors concluded that, compared to isocaloric glucose, there is a marked early insulin response to mixed meal ingestion, which emanates from a synergistic, rather than an additive, effect of the individual macronutrients in the mixed meal [290]. Similarly, the interactions between beverage and time for insulin (Fig 6.1A) and GLP-1 (Fig 6.2B) in the present study show a more rapid and greater peak rise at 30 min, perhaps due to a stronger first phase response, but an early measure would be required to support this suggestion [291]. In addition, both GLP-1 [292] and PYY [215] augment portal-mediated glucose clearance.

The most probable explanation for the insulin-independent actions of whole milk on blood glucose control resides in the effect of milk proteins on gastric emptying [218]. The role of GLP-1, PYY, and CCK in regulating gastric emptying rate is supported in our study by the negative correlations observed between these hormones and paracetamol concentrations. GLP -1 [21], PYY [22] and CCK [157] rapidly cross the blood-brain barrier to directly transmit signals that inhibit gut motility and delay gastric emptying. Slowing gastric emptying rates contribute to lower post-prandial glycemia without concurrent increases in insulin [27].

Paracetamol AUCs were similar after whole milk, the simulated milk beverage and protein, and lower after protein than lactose and fat (Table 6.2). Thus, these results are consistent with other studies reporting reduced gastric emptying rate after protein consumed either alone [27, 164] or with carbohydrate [218]. Macronutrients, particularly protein and fat, stimulate the release of CCK from the I-cells of the small intestine and delay stomach emptying [293], but in the present study lactose was equal to fat.

Overall ghrelin responses to whole milk demonstrated an additive effect of its macronutrient components (Table 6.2). These results are consistent with previous studies demonstrating that post-prandial inhibition of ghrelin is influenced by a meal's macronutrient content [294, 295] including carbohydrates [202] and milk-proteins [296]. Plasma ghrelin concentrations remained suppressed after whole milk, the simulated milk beverage and fat for the duration of the experiment (3 hours) while there was a rebound after protein and lactose at 2 hours (Fig 6.2D). Expressed per kcal, the effects of the macronutrients on ghrelin concentrations were similar among treatments, indicating that energy, irrespective of source is the primary determinant of ghrelin release (Table 6.3). However, the AUC of the sum of the components was greater than after whole milk suggesting a blunting of the response to energy in the whole

milk. This is perhaps due to components other than the macronutrients because the blunting was not apparent for the simulated milk.

A surprising result was that the simulated milk beverage resulted in a much higher AUC for GLP-1 (Fig 6.2A) and greater suppression of ghrelin as indicated by a larger AUC below baseline for ghrelin (Fig 6.2D) compared to whole milk. The ingestion of fats, carbohydrates and protein have been reported to stimulate a rise in GLP-1 [296, 297], which enhances glucose-stimulated insulin secretion [297]. However, the enhanced effect of the simulated milk beverage on GLP-1 suggests that other factors influence the efficacy of milk in stimulating GLP-1 secretion or delaying its catabolism. The milk protein concentrate used in this study contained micellar casein in a powder form after spray drying which results in a large particle size that is difficult to dissolve and while similar in chemical composition to milk, it was not in structure as assessed at the University of Guelph (data not shown). In addition, homogenization of whole milk rearranges physical properties of casein micelles such that they adsorb to fat which affects milk structure and bioactivities [298] further contributing to differences in effects of the two milks. A better understanding of the cause of these differences may have been achieved if the milk macronutrients were obtained from the same whole milk.

The primary objective of this study was to examine to what degree the individual macronutrients in whole milk contributed to glycemic control and gut hormones and whether the sum of the individual effects of the macronutrients, in their respective caloric concentrations, was equal to their effect when present as whole milk. Thus, the beverages used in the present study differed in caloric content, ranging from 64 to 304 kcal. Caloric content of beverages was positively correlated with insulin and PYY, but not GLP-1 or CCK, and negatively associated with ghrelin and paracetamol suggesting only some of these hormones are expressed in proportion to energy content [299]. Therefore, the responses were adjusted to a common basis of energy (Table 6.3). However, expressing the AUC responses per unit of energy shows that adding the effect of the protein, lactose and fat energy leads to a total response that predicts a large overestimate of their effect on post-prandial glycemia (Table 6.3). When responses were expressed per kcal for each treatment, whole milk and the simulated milk beverage reduced glycemic excursions more than the sum of their components. Protein per calorie had the strongest stimulatory effect on insulin, GLP-1 and CCK. These results are consistent with the known response of GLP-1 to proteins [296] and do not support the view that carbohydrate and fat are more potent stimulators [297]. In contrast, lactose per kcal had the largest effect on

glucose response and a somewhat smaller effect than protein on insulin response. Nutrients given in isolation are all individually capable of direct stimulation of post-prandial hormonal responses, but will respond differently when provided in a whole food matrix pointing to macronutrient interactions that may impact hormonal responses. However, the mechanism of synergism between macronutrients remains to be resolved. The approach of adjusting to expression per kcal is supported by observations in a recent study comparing whey protein and glucose at equicaloric doses. Pre-meal consumption of whey protein (10 and 20 g) compared with glucose (10 and 20 g) led to similar post-prandial insulin concentrations, but lower plasma glucose, higher GLP-1 and PYY concentrations and reduced gastric emptying rate [27].

The approach taken herein to assess the contribution of macronutrient components in foods with intrinsic health properties is rarely used and shows that the whole is more than a sum of the parts. However, there are some limitations in this study. First, the paracetamol absorption test is an indirect measure of gastric emptying rate of liquids in humans [164]. Although it provides a reasonably accurate and inexpensive estimate [300, 301], it does not have the precision of scintigraphy (gold standard) and other non-invasive methods such as the C_{13}-acetate breath test, magnetic resonance imaging and ultrasound, which are technically challenging and require specialized equipment [301]. While the quantitative rates of stomach emptying may not be as accurate, the primary purpose of the measure was to compare the response to beverages, and differences were found. Second, total ghrelin accounting for both acyl and des-acyl ghrelin was measured in this study. Acylated ghrelin is now recognized as the preferred measure as it relates more clearly to functionality [302]; however, total ghrelin is reflected by acyl ghrelin as previously reported [303]. Finally, because this study assessed the short-term effects of whole milk on glycemia and hormonal responses in healthy young men, its effects in women and on long-term glycemic control are unclear. Nonetheless, the results of our study add to the accumulating evidence that milk has potential in the dietary management T2D and provide encouragement to conduct both short and longer term studies in this population.

6.6. Conclusion

In conclusion, the lower post-prandial glycemia of whole milk than predicted from the sum of its components is mediated by an interaction among its macronutrient components.

6.7. Acknowledgements

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TABLE 6.1. Nutritional composition of beverages.

Composition[1]	Beverages[2]				
	Whole Milk (3.25% M.F.)	Simulated Milk Beverage	Protein	Lactose	Fat
Energy (kcal)	300	304	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

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

TABLE 6.2. Incremental areas under the curve (AUC) for glucose, insulin, gastrointestinal hormones, gastric emptying rate (plasma paracetamol), and glucose to insulin ratios after consumption of 500 ml of beverages.[1]

Biomarkers[2,3]	Whole Milk	Simulated Milk Beverage	Protein	Lactose	Fat	*P*-value[5]
Glucose (mmol*min*L^{-1})						
AUC	18.3 ± 3.1^{ab}	18.3 ± 3.3^{ab}	10.4 ± 3.2^{b}	23.9 ± 5.1^{a}	7.4 ± 1.4^{b}	0.005
$AUC_{(sum)}$	18.3 ± 3.1^{b}	18.3 ± 3.3^{b}		∑ 41.7 ± 6.8^{a}		0.0005
Insulin (pmol*min*L^{-1})						
AUC	4745 ± 912^{a}	5248 ± 820^{a}	1930 ± 340^{b}	1979 ± 618^{b}	684 ± 209^{b}	<0.0001
$AUC_{(sum)}$	4745 ± 912^{a}	5248 ± 820^{a}		∑ 4592 ± 564		NS
GLP-1 (pg*min*L^{-1})						
AUC	91 ± 18^{b}	153 ± 18^{a}	42 ± 7^{c}	35 ± 9^{c}	50 ± 10^{bc}	<0.0001
$AUC_{(sum)}$	91 ± 18^{b}	153 ± 18^{a}		∑ 128 ± 17^{ab}		0.01
PYY (pg*min*L^{-1})						
AUC	1768 ± 310^{a}	2056 ± 357^{a}	470 ± 67^{b}	697 ± 138^{b}	701 ± 169^{b}	<0.0001
$AUC_{(sum)}$	1768 ± 310	2056 ± 357		∑ 1868 ± 265		NS
CCK (ng*min*L^{-1})						
AUC	1.0 ± 0.1^{ab}	1.6 ± 0.2^{a}	0.9 ± 0.2^{b}	0.7 ± 0.1^{b}	0.8 ± 0.1^{b}	0.004
$AUC_{(sum)}$	1.0 ± 0.1^{b}	1.6 ± 0.2^{ab}		∑ 2.4 ± 0.3^{a}		0.0005
Ghrelin (pg*min*L^{-1})						
AUC	-1440 ± 313^{ab}	-2515 ± 533^{a}	-925 ± 329^{b}	-896 ± 240^{b}	-828 ± 180^{b}	0.002
$AUC_{(sum)}$	-1440 ± 313	-2515 ± 533		∑ -2649 ± 449		0.04
Paracetamol (mmol*min*L^{-1})						
AUC	7.0 ± 0.6^{bc}	7.7 ± 0.5^{abc}	6.8 ± 0.4^{c}	8.6 ± 0.5^{ab}	9.1 ± 0.6^{a}	<0.0001
Ratio glucose/insulin[2]						
AUC	0.007 ± 0.002^{b}	0.005 ± 0.001^{b}	0.008 ± 0.003^{b}	0.035 ± 0.015^{a}	0.023 ± 0.008^{ab}	0.007
At 30 min[4]	0.004 ± 0.001	0.004 ± 0.001	0.002 ± 0.001	0.007 ± 0.005	0.002 ± 0.004	NS

[1]All values are ± SEM. $n = 12$. Values in the same row with different superscript letters are significantly different, $P < 0.05$ (beverage effect using one-factor ANOVA with proc mixed procedure, Tukey's post-hoc).

[2]AUC are calculated as change from baseline from 0-150 min for CCK, and 0-180 min for glucose, insulin, GLP-1, PYY, ghrelin, paracetamol, and glucose to insulin ratios.

[3]AUC for whole milk and the simulated milk beverage were compared to the sum (∑) of the components by adding AUCs for protein, lactose and fat (AUC_{sum}) for glucose, insulin, GLP-1, PYY, CCK and ghrelin.

[4]Values are ratios of glucose to insulin changes from baseline at 30 min.

[5]NS = Non Significant.

TABLE 6.3. Energy-adjusted incremental areas under the curve (AUC) per 50 kcal for glucose, insulin, gastrointestinal hormones and gastric emptying rate (plasma paracetamol) after consumption of 500 ml of beverages.[1]

Biomarkers[2,3]	Whole Milk	Simulated Milk Beverage	Protein	Lactose	Fat	*P*-value[5]
Glucose (mmol*min*L^{-1})						
AUC	3 ± 0.5^{b}	3 ± 0.5^{b}	8 ± 2.5^{ab}	12 ± 2.5^{a}	2.5 ± 0.5^{b}	0.0005
$AUC_{(sum)}$	3 ± 0.5^{b}	3 ± 0.5^{b}		∑ 22.5 ± 4^{a}		<0.0001
Insulin (pmol*min*L^{-1})						
AUC	790 ± 150^{bc}	865 ± 150^{bc}	1500 ± 250^{a}	1050 ± 300^{ab}	250 ± 150^{c}	<0.0001
$AUC_{(sum)}$	790 ± 150^{b}	865 ± 150^{b}		∑ 2850 ± 600^{a}		<0.0001
GLP-1 (pg*min*L^{-1})						
AUC	16.5 ± 3^{b}	28 ± 3.5^{ab}	33 ± 5.5^{a}	18.5 ± 4.5^{ab}	17.5 ± 3.5^{b}	0.01
$AUC_{(sum)}$	16.5 ± 3^{b}	28 ± 3.5^{b}		∑ 69 ± 9^{a}		<0.0001
PYY (pg*min*L^{-1})						
AUC	295 ± 50	340 ± 60	365 ± 55	365 ± 70	245 ± 60	NS
$AUC_{(sum)}$	295 ± 50^{b}	340 ± 60^{b}		∑ 975 ± 110^{a}		<0.0001
CCK (pg*min*L^{-1})						
AUC	160 ± 25^{b}	260 ± 40^{b}	555 ± 125^{a}	205 ± 40^{b}	200 ± 35^{b}	0.0006
$AUC_{(sum)}$	160 ± 25^{b}	260 ± 40^{b}		∑ 960 ± 135^{a}		<0.0001
Ghrelin (pg*min*L^{-1})						
AUC	-240 ± 50	-415 ± 90	-725 ± 255	-465 ± 125	-315 ± 55	NS
$AUC_{(sum)}$	-240 ± 50^{b}	-415 ± 90^{b}		∑ -1505 ± 285^{a}		<0.0001
Paracetamol (mmol*min*L^{-1})						
AUC	1.5 ± 0.1^{d}	1.5 ± 0.1^{d}	6 ± 0.5^{a}	4.5 ± 0.5^{b}	3.5 ± 0.5^{c}	<0.0001
Ratio glucose/insulin[2]						
AUC	0.1 ± 0.04^{b}	0.1 ± 0.03^{b}	0.1 ± 0.03^{b}	0.2 ± 0.05^{a}	0.1 ± 0.02^{b}	0.006
At 30 min[4]	0.2 ± 0.05	0.2 ± 0.05	0.1 ± 0.05	0.4 ± 0.3	0.1 ± 0.2	NS

[1]All values are ± SEM. $n = 12$. Values in the same row with different superscript letters are significantly different, $P < 0.05$ (beverage effect using proc mixed procedure, Tukey's post-hoc).
[2]AUC are calculated as change from baseline from 0-150 min for CCK, and 0-180 min for glucose, insulin, GLP-1, PYY, ghrelin, paracetamol, and glucose to insulin ratios.
[3]AUC for whole milk and the simulated milk beverage were compared to the sum (∑) of the components by adding AUCs for protein, lactose and fat (AUC_{sum}) for glucose, insulin, GLP-1, PYY, and ghrelin.
[4]Values are ratios of glucose to insulin changes from baseline at 30 min.
[5]NS = Non Significant.

Fig 6.1A.

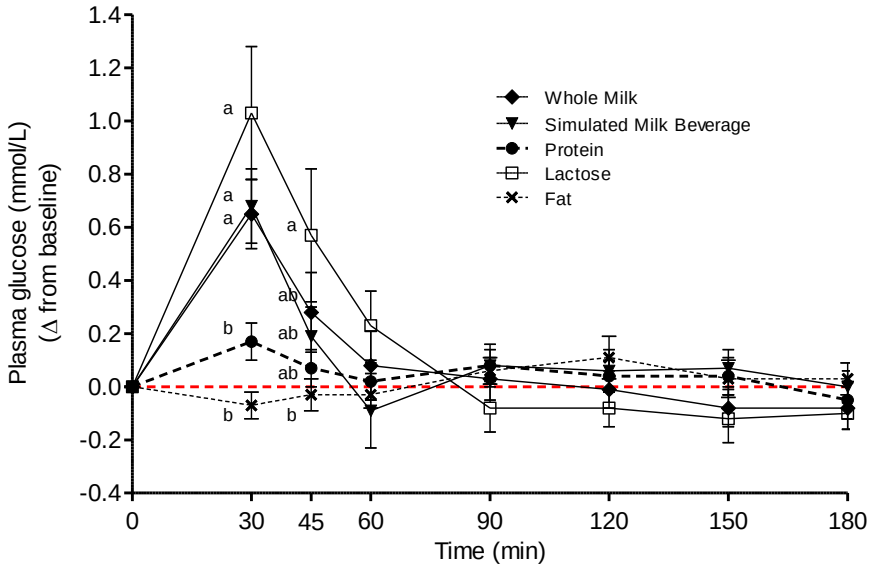


Fig 6.1B.

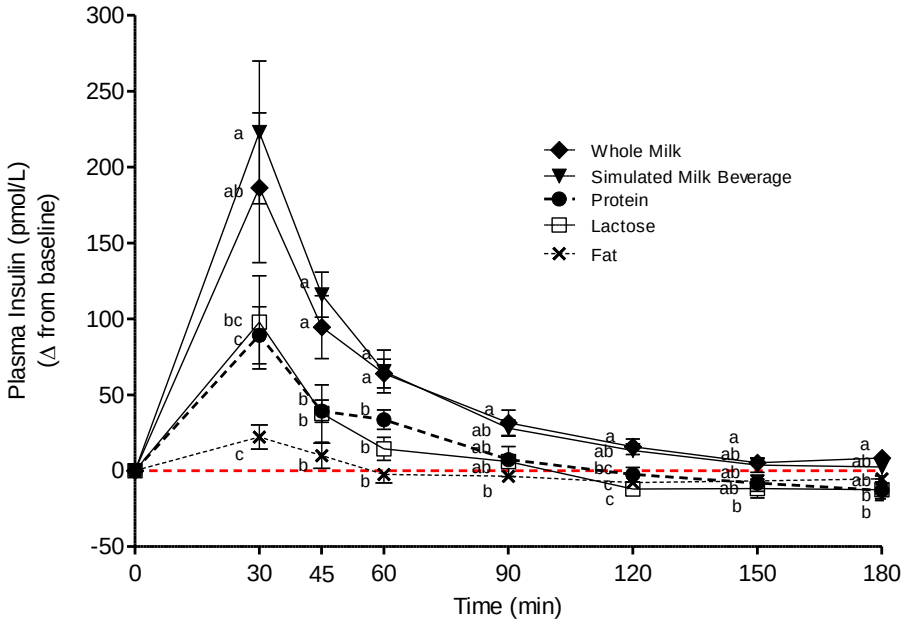


Figure 6.1. Effect of whole milk and its separate components on **A)** plasma glucose and **B)** insulin concentrations. Results are shown as change from baseline. Means with different superscripts are significantly different at each measured time [two-way ANOVA, time ($P < 0.0001$), treatment ($P < 0.0001$), time-by-beverage interaction ($P < 0.0001$), followed by one-way ANOVA, Tukey-Kramer post-hoc test, ($P < 0.05$)]. All values are means ± SEMs; n = 12.

Fig 6.2A.

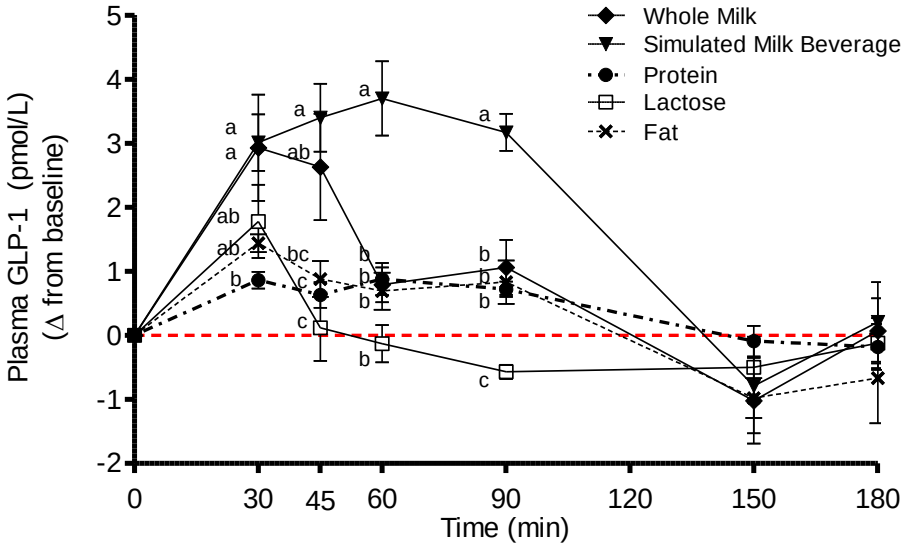
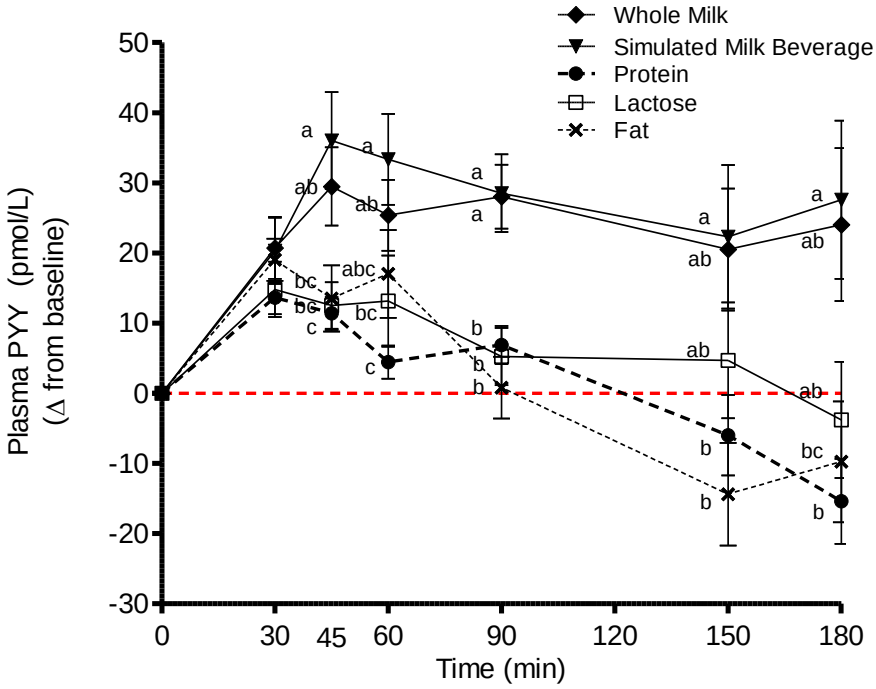


Fig 6.2B.



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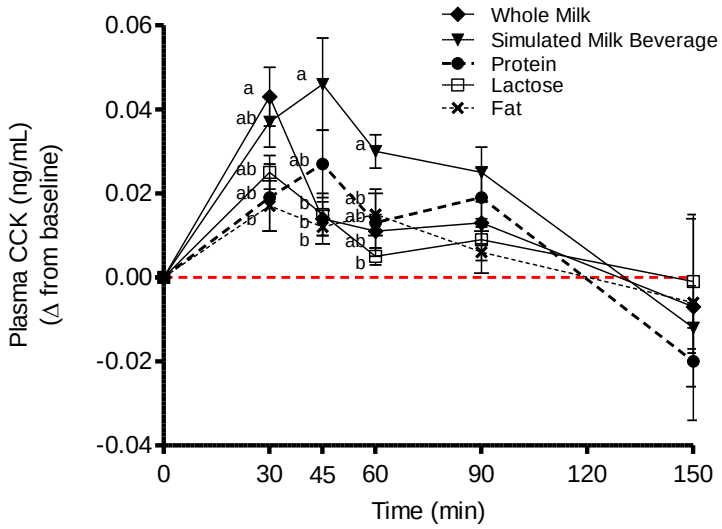

Fig 6.2D.

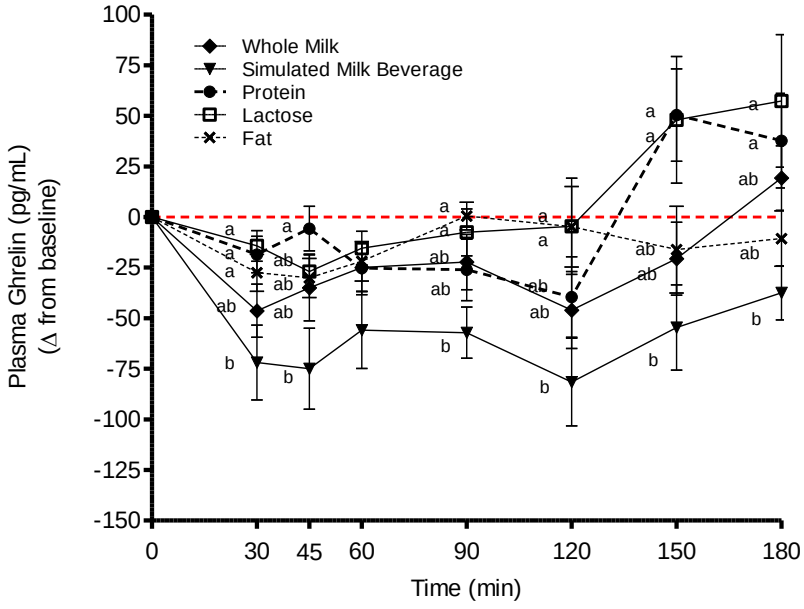

Figure 6.2. Effect of whole milk and its separate components on gastrointestinal hormones **A)** GLP-1; **B)** PYY; **C)** CCK; and **D)** ghrelin. Results are shown as change from baseline. Means with different superscripts are significantly different at each measured time [two-way ANOVA, time ($P < 0.05$), treatment ($P < 0.05$), time-by-beverage interaction for GLP-1 ($P < 0.0001$) and for PYY, CCK and ghrelin (P = NS), followed by one-way ANOVA, Tukey-Kramer post-hoc test, ($P < 0.05$)]. All values are means ± SEMs; n = 12.

Fig 6.3.

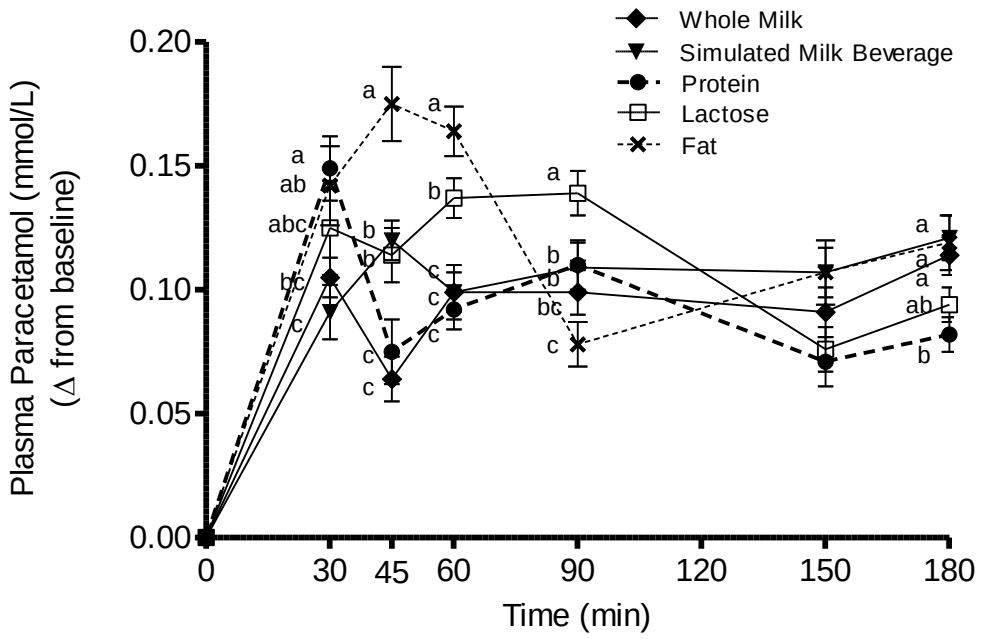

Figure 6.3. Effect of whole milk and its separate components on gastric emptying rate (plasma paracetamol concentrations). Results are shown as change from baseline. Means with different superscripts are significantly different at each measured time [two-way ANOVA, time ($P < 0.0001$), treatment ($P < 0.0001$), time-by-beverage interaction ($P < 0.0001$), followed by one-way ANOVA, Tukey-Kramer post-hoc test, ($P < 0.05$)]. All values are means ± SEMs; n = 12.

CHAPTER 7

GENERAL DISCUSSION

CHAPTER 7. GENERAL DISCUSSION

These studies further the understanding of the role of milk consumption on the regulation of satiety, food intake and glycemia. However, the hypothesis that milk decreases short-term appetite and food intake compared with other beverages was not supported. Suppression of appetite and food intake was determined primarily by energy content of the beverages. Composition of milk was important however, as milk reduced post-prandial glycemia by both insulin-dependent and independent mechanisms. The reproducibility of the lower post-meal glycemic responses after milk consumption compared to other beverages was confirmed by not only the consistent results between the first three studies (Chapters 4 and 5), but also by corresponding physiological responses from the study of satiety and glucoregulatory hormones (Chapter 6). Thus, this research provides evidence supporting a recommendation of milk consumption before and within meals for glycemic control and for achieving a more favorable nutrient intake.

The Beverage Guidance Panel ranked beverages from the lowest to the highest value based on caloric and nutrient contents and related health benefits and risks [7]. It recommended that SSB be replaced over time by other beverages with higher nutritional value and fewer calories, but also made the implicit assumption that the effect of calories was also determined by macronutrient content. However, these studies are the first to compare the effects of frequently consumed beverages on satiety, food intake and post-prandial glucose response. Consistent with many studies [49-51] the primary role of energy content of beverages and not composition as determinants of short-term satiety and food intake was supported. The quantity and time of beverage energy intake affected the magnitude and duration of the satiety response food intake at the pizza meals (Chapters 4 and 5). Energy contents ranging from 200-368 kcal, but not macronutrient composition, of familiar beverages (2% milk, 1% chocolate milk, orange juice, soy beverage, infant formula and water) consumed before meals was the primary factor reducing meal-time food intake 30 min later, but the effect on food intake was not noted at 120 min in healthy young adults (Chapter 4). Subjective satiety was reduced by the caloric beverages compared with water, but this response did not reflect the energy content. Again, these results are consistent with many studies showing that measures of subjective appetite do not provide an accurate prediction of later food intake nor distinguish among relatively small differences in energy content of food ingested [34, 49, 97]. In this study the volume (500 ml) in addition to the

energy content of the beverages may have been a factor, but as shown by the water control, volume alone was not a factor in the satiety response.

Two additional novel observations were made in Chapter 5. First, all caloric beverages, including milk, consumed freely during an ad libitum meal did not reduce meal time pizza intake and simply added calories to the meal. Second, the volume consumed was similar among beverages at an average of 470 ml, and independent of content, showing that thirst is a primary factor driving beverage consumption at a meal and supports the hypothesis that caloric beverages by-pass energy intake control mechanisms [32, 33]. Taken together with the results of the pre-meal consumption studies (Chapter 4), these results add emphasis to the conclusion that time of consumption of caloric beverages is a significant factor determining their effect on energy intake and ultimately energy balance.

To the time of this research, no studies have been reported of comparisons of the effects of milk and commonly consumed beverages on post-prandial glycemia. Both the pre-meal (Chapter 4) and within meal (Chapter 5) studies showed that milk was unique among the beverages for reducing post-prandial glycemia and led to the detailed investigation of its mechanisms reported in Chapter 6. Although previous research has shown that whey reduces post-meal glycemia by both insulin and non-insulin dependent mechanisms [26, 27], this was the first study to investigate mechanisms by which milk and its components contribute to regulation of post-prandial glycemia. The pre-meal studies of Chapter 4 showed that 2% milk resulted in the lowest mean post-meal glucose concentrations and this effect was enhanced by duration of time between beverage consumption and the pizza meal. That is, consuming milk 2 h before the meal showed a clearer distinction between other beverages than when it was consumed at 30 min. The within meal study (Chapter 5) clearly shows a strong effect of consuming milk, even if it adds calories, on reducing post-prandial glycemia compared with orange juice and regular cola of similar energy content. Thus, while the energy, but not macronutrient content of the beverages was the primary factor affecting food intake, composition was important in determining post-prandial glycemia. These observations led to the study of mechanisms in Chapter 6.

As discussed in Chapter 6, post-prandial glycemia of whole milk was lower than predicted from the sum of its components, protein, lactose and fat. In contrast, insulin and other hormones including GLP-1, PYY, and ghrelin reflected the sums of the components, with the exception of lower CCK after whole milk (Chapter 6). The lower post-prandial glycemia for

whole and simulated milk than that predicted from its component effects indicated that lower glycemic response occurred through insulin-independent mechanisms as well as insulin-dependent mechanisms as shown in our previous studies on the mechanism of action of whey protein [27]. Whole milk and the simulated milk beverage led to a lower post-prandial glycemic response than lactose alone, but with no increase in insulin, above that accounted for by the sum of its separate components. This may have been due to enhanced efficacy of insulin as the post-prandial reduction of blood glucose after whey consumption occurs without increased insulin synthesis as measured by C-peptide [27]. As noted, the most probable explanation for the insulin-independent actions of whole milk on blood glucose control resides in the effect of milk proteins on gastric emptying [218]. Gut hormones released by milk include several that function to delay gastric emptying, including GLP-1, PYY and CCK. Even though the use of paracetamol as a marker of stomach emptying is only a crude estimate, its effect of milk and its components on gut hormones combined with the estimated delay in stomach emptying show that slower stomach emptying is a plausible explanation for the reduced post-prandial glycemia after milk consumption, as observed in Chapters 4 and 5. In addition, whole milk led to higher plasma concentrations of GLP-1 and PYY that are known to be involved in the neural regulation of energy and glucose homeostasis. GLP-1 [292] and PYY [214, 215] in the portal vein stimulate hepatic and pancreatic vagal afferents that enhance glucose disposal, thereby, augmenting portal-mediated glucose clearance.

Another novel finding of this research was the outcome of effects of the simulated milk beverage indicating that function of milk macronutrients may be compromised by processing. The enhanced response of GLP-1 and lower ghrelin concentrations after the simulated milk beverage compared to whole milk resulted from mixing isolated milk proteins, lactose, and milk fat (Chapter 6). The milk protein concentrate used in this study contained micellar casein as in milk. However, the spray drying process results in a larger particle size that is more insoluble and not of the same structure as in milk. On the other hand, an explanation may reside in the effect of homogenization of whole milk, which was not done to the simulated milk. Homogenization has little effect on the composition, but has important effects on milk structure and bioactivities [75]. However, it is also possible that raw milk loses some of its metabolic functionality when processed for commercial use [298]. The traditional objectives of milk processing are to ensure a long shelf-life, produce satisfactory sensory properties, and eliminate pathogens [304]. More recently processing strategies have been developed to separate milk

components into discrete molecule classes within each of carbohydrate, protein and fat to be used as ingredients in a broader range of food applications. Whether physiologic functionality is lost in the pursuit of food functionality is unknown. Furthermore, in addition to the macronutrient content, the micronutrient content including vitamins and minerals that are not present in milk may have also played a role. Although the component(s) of the mixture that accounts for these differences between homogenized milk and simulated milk remain to be investigated, the explanation may lead to innovation of existing dairy products and of milk components to prolong the benefits of dairy in the control of post-prandial glycemia and other physiologic functions.

Although the current research does not fully elucidate the mechanism of action of milk on glycemic control, it indicates that improved post-meal blood glucose after milk consumption may be beneficial for glucose control in not only healthy individuals, but also those with insulin resistance. Importantly, the present research, along with our previous studies of the mechanism of action of whey protein [26, 27], show that dairy protein and milk functions in controlling post-prandial glycemia by both insulin-dependent and independent mechanisms. Milk and milk proteins may be unique in this effect compared to other proteins as indicated by the greater reduction in post-prandial glycemia after milk compared with a soy beverage milk substitute (Chapter 4).

Overall, the results of this research lead to the hypothesis that milk consumption may provide a strategy for management of blood glucose by reducing post-meal glycemia (Chapter 4-6). More recently, there is increased appreciation of their functional contributions in the maintenance of a healthy body weight and metabolic regulation. Several studies [20, 305, 306] suggest that milk and dairy sources of calcium and vitamin D exert stronger effects on weight loss than non-dairy sources of these nutrients [131]. Dairy vitamins and minerals are also factors modulating lipid metabolism and energy expenditure through the reduction of body fat mass [307] and increased thermogenesis [308], fecal fat excretion [309] and fat oxidation [224, 310, 311]. Dairy medium-chain triglycerides [60] and CLA [312] increase energy expenditure and fatty acid beta-oxidation, contributing to decreased adiposity and increased lean body mass. Thus, current research provides encouragement to pursue longer term randomized control trials on post-prandial glycemia as well as to evaluate the role of milk consumption on body weight and lipid metabolism. For the present, it also supports encouraging consumption of the

recommended amounts of dairy as indicated in food guides and replacing milk calories for other caloric beverages.

7.1. Study Design: Strengths and Limitations

Strengths

A major strength of the designs was their ecological validity for providing dietary guidance. The first three studies compared usually consumed beverages differing in composition and in amounts typically consumed by individuals between or at meals. Furthermore, these studies in which pre-meal, post-meal, and within meal effects of milk and other beverages on blood glucose was measured in a total of three studies that complimented each other, and combined, give a convincing picture of a role for milk in post-prandial glucose control. What could have been unremarkable studies arising from the satiety and food intake data became very important by providing short-term evidence supporting plausibility for the inverse associations of milk and type 2 diabetes reported in epidemiologic studies. The follow-up study of the mechanisms added strength to the overall interpretation and provides confidence in the conclusion that milk can play a role as a beverage between and with meals for control of post-prandial glycemia. In addition, the adjustment of the effect of milk and its components for energy content emphasized that the metabolic effects of the macronutrients differs and is not directly attributed to energy content. Finally, all studies were adequately powered.

Limitations

There are some limitations in these studies. First, there was a lack of double-blinding, but this is not an unusual weakness of food and beverage studies. Inherent differences in taste, smell, and preparation of the various beverages is conspicuous and thus, not possible to double-blind. Second, food choice was not free and was limited to pizza, suggesting that results may not be applied to other meal-time foods. Third, insulin was not measured in Chapters 4 and 5 pointing to some limitations in the interpretation of the reduced post-prandial glycemia results. Fourth, while the use of paracetamol [164, 301, 313] in the beverages in the final study is accepted as a reasonably accurate estimate for gastric emptying rate, at least for comparative purposes, it is less accurate for quantifying stomach emptying [314]. The gold standard method for measurement of gastric emptying is scintigraphy, which is a challenging method and requires

expensive equipment and special licensing for radioactive substances [315]. Fifth, total ghrelin accounting for both acyl and des-acyl ghrelin was measured in this study. Acylated ghrelin is now recognized as the preferred measure as it relates more clearly to functionality [302]; however, total ghrelin is reflected by acyl ghrelin as previously reported [303]. Sixth, although the short-term effects of milk on satiety, food intake, glycemia and hormonal responses were assessed; its effects on long-term satiety, food intake regulation and glycemic control remain uncertain. Finally, due to the strict inclusion criteria in the current studies, the study population is not representative of the population as a whole. Because age, BMI, sex and the presence of metabolic disorders can affect the interaction between dietary components and satiety, food intake and glycemia, further studies are required to determine if the benefits of milk will extend to these populations.

7.2. Significance and Implications

The decrease in milk consumption and simultaneous rise in obesity, type 2 diabetes, and metabolic syndrome require effective countermeasures. By describing the action of fluid milks on physiologic mechanisms regulating satiety, food intake, and glycemic control we will be able to communicate that milk has health benefits that go beyond its essential nutrient role and could be healthy choice to replace other caloric beverages. Furthermore, these novel experiments shed new insight into an understanding of insulin-independent mechanisms of satiety and glycemic control originating in the gastrointestinal tract from consumption of milk.

This research may also help to increase the consumption of milk. It will bring an understanding to Canadians about the importance of milk in their daily diet and may lead to a decrease in chronic disease rates, reduction in Federal and Provincial health care costs and increase in a healthier population. These short-term studies of milk and other beverages will provide the foundation for meaningful intervention studies. Furthermore, the results of this research may lead to increased consumption of milk by healthy individuals of various age groups in general, and by overweight and obese individuals in particular. Consumption may also increase to comply with Canada's Food Guide and help clinicians and dietitians recognize the value of dairy for metabolic control, reduce dependency on pharmaceutical approaches, and reduce the burden of metabolic diseases on the health care system. This research adds to the accumulating evidence that milk has potential in the dietary management of obesity, type 2

diabetes and metabolic syndrome and provides encouragement to conduct both short and longer term studies in these populations.

CHAPTER 8

GENERAL SUMMARY AND CONCLUSIONS

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In conclusion, the results of this research do not support the hypothesis that milk consumption decreases short-term appetite and food intake compared with other beverages; however, milk improves glycemic control by insulin-dependent and independent mechanisms.

Thus, the short-term effects on satiety and food intake of milk consumed prior to or with meals do not provide support for the inverse associations found between milk consumption and body weight. However, the improvement in post-prandial glycemia and increased nutrient intakes contributed by milk may be an explanation for long-term health benefits and reduced risk of obesity, type 2 diabetes and metabolic syndrome.

CHAPTER 9

FUTURE DIRECTIONS

CHAPTER 9. FUTURE DIRECTIONS

This research provided physiological explanations for the role of milk consumption on satiety, suppression of short-term food intake and improved glycemic control in healthy young adult men and women. Thus, the results add some biological plausibility to the inverse associations found between milk consumption and type 2 diabetes; however, longer term studies examining body weight, hyperglycemia, hypertension and hyperlipidemia which are other risk factors for metabolic syndrome are needed to elucidate the effects of milk. Several long-term intervention studies have failed to show consistent benefits of increased dairy consumption in ad libitum diets on body weight, waist circumference and characteristics of the metabolic syndrome [17, 18]. Furthermore, few studies have examined the effects of reduced-fat milk (0% to 2% M.F.) compared with whole milk on weight gain or other health outcomes [316]. Therefore, future studies should consider diet composition, type and amount of dairy, timing and frequency of consumption and target populations with a high risk for metabolic diseases.

Energy intake was not measured in the metabolic study (Chapter 6) and should be a priority in future research examining hormonal regulation of food intake and the post-meal effects of milk and its components. A follow-up study assessing the reproducibility and validity of our glycemic and gastrointestinal hormone responses after milk consumption should also be conducted. In addition, to address the interesting effects of the simulated milk beverage and whole milk in Chapter 6, in vitro studies are needed to elucidate the structure of milk components affecting physiologic functions. Furthermore, because there is strong evidence that milk proteins play a central role in the effects of milk consumption on control of glycemic excursions and satiety, in vitro studies focusing on the digestion kinetics of milk proteins can be correlated with the results of our in vivo metabolic responses. This will help to increase the understanding of the physiological effects of milk and aid in the development of innovative dairy formulations which vary in macronutrient components (i.e. total protein, casein, whey protein and lactose) while holding all others constant.

The rise in obesity and its co-morbidities provides a rationale for developing the Beverage Guidance System. Because some beverages provide primarily calories and can contribute significantly to a positive energy balance, reducing their consumption is an important

component of a broader strategy to reduce energy intake. The Panel has also noted that evidence indicates that SSB have replaced milk in the diet, which has resulted in a reduction in the intake of important essential nutrients. Although this Beverage Guidance System provides a sense of the relative energy and nutrient densities and health benefits and risks linked with each category of beverages (and also the relative importance of each beverage), it is not possible to provide clear recommendations regarding specific quantities. Future research should examine different amounts of these beverages over the longer term in order to elucidate their effects on body weight and other metabolic parameters.

CHAPTER 10

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CHAPTER 11

APPENDICES

APPENDIX 1. Sample Size Calculation

Sample size calculation when testing for the mean of a normal distribution (two-sided alternative), for within subject designs, is:

$$n = [(z_{1-\alpha/2} + z_{1-\beta}) \cdot \sigma/\Delta]^2$$

$\alpha = 0.05$, probability of Type 1 error
$\beta = 0.20$, probability of type II error
Z 0.975 = 1.96
Z 0.80 = 0.84
$\sigma = 186.2$ kcal
$\Delta = 157.0$ kcal
n = 26

Values were taken from a previous study [142]. σ represents standard deviation, Δ represents the minimal difference in food intake between the sugar beverage and water control. n is the number of subjects required.

APPENDIX 2. Subject Characteristics

Chapter 4											
Experiment 1						Experiment 2					
Subject	Sex	Age	Weight	Height	BMI	Subject	Sex	Age	Weight	Height	BMI
No.		(y)	(kg)	(m)	(kg/m^2)	No.		(y)	(kg)	(m)	(kg/m^2)
1	M	22	76.3	1.78	24.1	1	M	29	65.1	1.72	22.0
2	M	26	68.6	1.76	22.1	2	M	22	68.3	1.73	22.8
3	M	26	79.8	1.84	23.6	3	M	21	81.6	1.86	23.6
4	M	27	68.9	1.83	20.6	4	M	26	72.0	1.87	20.6
5	M	25	68.1	1.73	22.8	5	M	20	86.5	1.89	24.2
6	M	20	75.3	1.84	22.2	6	M	20	66.3	1.76	21.4
7	M	26	59.6	1.70	20.6	7	M	20	70.9	1.81	21.6
8	M	20	68.9	1.75	22.5	8	M	28	71.0	1.77	22.7
9	M	23	79.3	1.84	23.4	9	M	21	86.0	1.86	24.9
10	F	22	65.4	1.71	19.2	10	M	23	79.3	1.86	22.9
11	F	22	57.6	1.63	21.7	11	M	30	82.7	1.82	25.0
12	F	21	59.5	1.65	23.5	12	M	26	79.2	1.86	22.9
13	F	24	63.9	1.69	20.1	13	M	21	80.1	1.81	24.4
14	F	25	51.8	1.62	19.7	14	M	21	71.9	1.81	21.9
15	M	23	56.0	1.59	22.2	15	M	22	66.6	1.75	21.7
16	M	20	57.6	1.65	21.2	16	F	23	58.4	1.70	20.2
17	M	21	63.9	1.75	20.9	17	F	21	62.6	1.63	23.6
19	F	23	57.4	1.66	20.8	19	F	21	61.5	1.77	20.1
20	F	20	51.7	1.61	19.9	20	F	25	59.8	1.60	23.4
21	F	20	64.3	1.64	23.9	21	F	30	54.6	1.62	20.9
22	F	23	75.1	1.77	24.0	22	M	24	70.4	1.76	22.7
23	F	20	50.0	1.46	23.5	23	F	24	75.9	1.80	23.4
24	M	21	83.8	1.88	23.7	24	F	21	48.5	1.54	20.5
25	F	21	57.2	1.67	20.5	25	F	25	53.2	1.56	21.9
26	M	20	60.7	1.68	21.5	26	F	26	50.9	1.58	20.5
27	F	20	59.4	1.63	22.4	27	F	24	54.6	1.65	20.1
28	M	25	78.0	1.85	22.8	30	F	20	56.4	1.68	22.6
29	F	23	66.5	1.75	21.7	31	F	22	50.1	1.47	23.2
30	M	21	64.2	1.74	21.2	33	F	23	57.9	1.63	21.8
						34	F	24	66.3	1.75	22.0
						36	F	24	54.8	1.54	23.1
	MEAN	22.4	65.1	1.71	21.9		**MEAN**	23.5	66.6	1.72	22.3
	SEM	0.42	1.7	0.02	0.3		**SEM**	0.53	2.0	0.02	0.3

Chapter 5

Subject No.	Sex	Age (y)	Weight (kg)	Height (m)	BMI (kg/m^2)
1	M	22	73.7	1.74	24.3
2	M	21	68.1	1.75	22.2
3	M	23	73.4	1.75	24.0
4	M	24	80.7	1.81	24.6
5	M	24	67.6	1.74	22.3
6	M	21	57.3	1.63	21.6
7	M	21	58.1	1.61	22.4
8	M	26	71.1	1.86	20.6
9	M	21	64.8	1.72	21.9
10	M	25	57.2	1.69	20.0
11	M	21	64.8	1.62	24.7
12	M	20	67.9	1.72	23.0
13	M	20	64.8	1.79	20.2
14	M	22	66.2	1.73	22.1
16	M	21	69.7	1.71	23.8
17	F	20	64.9	1.69	22.7
18	F	20	53.0	1.60	20.7
19	F	24	70.3	1.77	22.4
20	F	20	62.1	1.65	22.8
21	F	26	54.9	1.56	22.6
23	F	20	47.4	1.53	20.2
24	F	20	53.6	1.55	22.3
25	F	23	69.4	1.73	23.2
26	F	25	57.9	1.57	23.5
27	F	21	59.4	1.67	21.3
28	F	21	62.2	1.73	20.8
30	F	22	48.8	1.50	21.7
31	F	24	63.6	1.66	23.1
32	F	24	71.1	1.88	20.1
	MEAN	22	63.6	1.69	22.2
	SEM	0.4	1.5	0.02	0.3

Chapter 6

Subject No.	Age (y)	Weight (kg)	Height (m)	BMI (kg/m^2)
1	21	62.3	1.62	23.7
3	23	71.8	1.78	22.4
4	25	58.1	1.70	20.1
6	30	78.5	1.78	24.5
7	21	85.4	1.86	24.7
8	22	67.1	1.72	22.7
9	25	58.3	1.67	20.9
10	22	67.7	1.68	24.0
12	22	69.8	1.70	24.1
13	22	67.7	1.72	22.9
14	23	74.2	1.78	23.4
15	22	74.0	1.74	24.4
MEAN	23	69.6	1.73	23.2
SEM	0.7	2.3	0.02	0.4

APPENDIX 3. Pizza Meal Composition

Nutritional Information Per 100g	Pepperoni	Deluxe	Three Cheese
Protein (g)	11	9.1	13
Total Fat (g)	7.7	6.2	8.4
Carbohydrate (g)	28	27	29
Energy (kcal)	219	195	237

McCain Foods: Deep and Delicious, 5” Pizza

APPENDIX 4. Consent Forms

Chapter 4: Energy and macronutrient content of familiar beverages interact with pre-meal intervals to determine later food intake, appetite and glycemic response in young adults (Experiment 1).

Department of Nutritional Sciences
FitzGerald Building, 150 College Street, 3rd Floor
Toronto, ON M5S 3E2
CANADA

The Effect of Isovolumetric Preloads of Various Types of Milk on Food Intake, Subjective Appetite and Glycemic Response in Healthy Young Men and Women

Information Sheet and Consent Form

Investigators: Dr. G. Harvey Anderson, Professor
Department of Nutritional Sciences, University of Toronto
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Funding Source:
Funding for this project is provided by Dairy Farmers of Ontario.

Background and Purpose of Research:

In 2004, almost 60% of adult Canadians were overweight or obese. This is a serious health problem because obesity and being overweight are related to many risk factors of disease, including increased blood sugar. Overweight and obesity can be treated by changing what we eat. It is important to find food-based ways to prevent and treat overweight and obesity.

Milk and milk substitutes are types of food components that can be used to decrease hunger and control blood glucose. This study will investigate whether different types of milk can be used for these purposes. The information obtained from this study will be used to better understand the effects of different types of food components on the health of young men and women and may lead to future studies in other groups, including overweight individuals and children.

The purpose of this research project is to determine the effects of drinking milk compared with chocolate milk, a soy beverage, infant formula and water on blood sugar and appetite in young men and women.

This study will have 52 participants.

Invitation to Participate:

You are being invited to take part in this study. If you choose to take part, you will be asked to drink milk or a milk substitute five times (five sessions) one week apart. At each session, your appetite, blood sugar and salivary cortisol (reflecting stress) will be measured after eating the treatment and a pizza lunch. Each session will take up to 3 ½ hours of your time.

Eligibility:

To participate in this study you must be a healthy male or female and between 20-30 years old. You must be a nonsmoker and you cannot be taking any medications. The study will take place in the Department of Nutritional Sciences, Rm 305, 334, 331 and 331A, FitzGerald Building, 150 College Street, Toronto, ON.

Procedure:

To find out if you can take part in this study, you will be asked to fill out questionnaires, which ask questions about your age, if you smoke, exercise, your health, if you are on any medications and your eating habits. Your height and weight will be measured.

If you can take part, you will be asked to fill out questionnaires about the foods you like. You will be scheduled to meet with us for five sessions over five weeks. You will be asked to fast for 12 hours prior to your session (no eating for 12 hours before coming in except for water).

You will be asked to arrive at the FitzGerald Building between 07:50 a.m. and 10:00 a.m. You will be asked to stick to your normal routine, including exercise and to eat a similar meal the night before each session. You can drink water up to one hour before meeting with us.

At each session, you will be asked to drink a milk beverage (500 ml), give blood samples and to complete questionnaires at the times outlined in the table below. Eleven times during each session, for a total of 55 times over the whole study, you will be asked to provide blood by finger-prick to measure blood sugar. Blood will be sampled before drinking the treatment and at 10, 20, 30, 50, 65, 80, 95, 110, 140 and 170 minutes after drinking the treatment. You will be asked to fill out visual analog scale (VAS) questionnaires measuring your appetite, physical comfort, energy/fatigue and stress as well as the palatability (pleasantness) of the treatment and pizza throughout the study sessions. You will be served a pizza meal 30 minutes after you drink the treatment. In addition, salivary cortisol is a useful measure of stress and will be measured every 30 minutes. Each session will last up to 3 ½ hours.

Example of Time and Activity Schedule for Each Session

Time	**Activity**
07:50	Arrive at the laboratory (fasted for 12 hours)
07:50	Fill in Sleep, Stress, and VAS questionnaires and take first blood sample
08:00-08:05	Drink the treatment (0 minutes)
08:00-08:35	Blood sampling and VAS questionnaires at 10, 20 and 30 minutes
08:35-08:55	Pizza served and eaten (30-50 minutes)
08:55-10:55	Blood sampling and VAS questionnaires at 50, 65, 80, 95, 110, 140 and 170 minutes

VAS: Visual Analogue Scale

Voluntary Participation and Early Withdrawal:

It is hoped that you will finish all five sessions. However, you may choose to stop being in the study at anytime without any problems.

Early Termination:

Not applicable.

Risks:

All of the foods and beverages that you will be asked to consume are prepared hygienically in the kitchen and present minimal risk. You may feel dizzy following the overnight fast, but this is rare. If this happens, you will likely feel fine once you drink the treatment provided to you.

The risks and discomfort will come from the blood sampling procedure. Great care will be taken when taking your finger-prick blood samples. The investigator will help you. To make sure that you are not exposed to another person's needle, we will ask you to sit away from other study participants. We will put a needle into the finger-prick gun before taking each blood sample and then put it into the safety container. There is very little risk of infection. We will clean your finger with a new alcohol swab before and after each finger-prick and will use a new sterile needle each time. You will be provided with your own finger-prick gun for the entire study.

Some discomfort will be felt as a result of a sharp momentary pain caused as the needle enters the skin. However, because the lancet needle is very small the pain felt is usually less than you might feel from skin puncture during vaccination or if a blood sample is taken by a needle inserted in a vein. There might be slight bruising under the skin, but this will be minimized by applying pressure after the finger is pricked and blood sugar is measured. A total of 11 finger-pricks will be conducted per session and may result in some discomfort.

You may experience flatulence (passing gas) and feelings of gastrointestinal discomfort (bloating) from the treatments. This is rare and there is no health risk linked with these effects.

In addition, there are no anticipated risks from measuring salivary cortisol. Saliva collection is hygienic, carried out by chewing new cotton wool swabs at the indicated time points to obtain fluid samples. Sampling is also painless and can be repeated without difficulty.

There is always a possibility that you will become ill following consumption of food, but that is very unlikely in this study. All treatments as well as pizza are freshly prepared at the time of your session. The pizzas are stored frozen and cooked accordingly to the manufacturer's instructions immediately before you are served.

Benefits:

You will not benefit directly from taking part in this study. You will be shown your blood sugar results and, if they are not normal, you will be told and advised to talk to your doctor. The foods and beverages will be provided for free.

Confidentiality and Privacy:

Confidentiality will be respected and no information that shows your identity will be released or published without your permission unless required by law. Your name, personal information and signed consent form will be kept in a locked filing cabinet in the investigator's office. Your results will not be kept in the same place as your name. Your results will be recorded on data sheets and in computer records that have an ID number for identification, but will not include your name. Your results, identified only by an ID number, will be made available to the study sponsor if requested. Only study investigators will have access to your individual results.

Publication of Results:

The results of the study may be presented at scientific meetings and published in a scientific journal. If the results are published, only average and not individual values will be reported.

Possible Commercialization of Findings:

This study is preliminary. Once these products are tested more widely in future studies, results may lead to commercialization of a product, new product formulation, changes in the labeling of a product and/or changes in the marketing of a product. You will not share in any way from the possible gains or money made by commercial application of findings.

Alternative Treatment/ Therapy:

Not applicable.

New Findings:

If anything is found during the course of this research which may change your decision to continue, you will be told about it.

Compensation:

You will be paid $36 per session ($180 in total). Reimbursement for travel expenses ($6 per session) is included in each session total. If you withdraw from the study before finishing or asked to withdraw, you will be paid for the sessions you have already finished.

Injury Statement:

If you begin to feel sick following participation in the study, please seek medical advice as soon as possible. We will provide your medical specialist with information about the food you have consumed during the session, so take our phone number with you.

Rights of Subjects:

Before agreeing to take part in this research study, it is important that you read and understand your role as described here in this study information sheet and consent form. You waive no legal rights by taking part in this study. If you have any questions or concerns about your rights as a participant you can contact the Ethics Review Office at ethics.review@utoronto.ca or call 416-946-3273.

If you have any questions after you read through this information please do not hesitate to ask the investigators for further clarification.

Dissemination of findings:

A summary of results will be made available for you to pick up after the study is done.

Copy of informed consent for participant:

You are given a copy of this informed consent to keep for your own records.

Consent:

I acknowledge that the research study described above has been explained to me and that any questions that I have asked have been answered to my satisfaction. I have been informed of the alternatives to participation in this study, including the right not to participate and the right to withdraw. As well, the potential risks, harms and discomforts have been explained to me. I understand that I will receive compensation for my time spent participating in the study.

As part of my participation in this study, I understand that I may come in contact with other study participants because our session times overlap. I agree to keep anything I learn about other participants confidential and know that other participants have agreed to do the same for me.

I hereby agree and give my authorized consent to participate in the study and to treat confidential information in a restrictive manner as described above. I have been given a copy of the consent form to keep for my own records.

_____	_____	_____
Participant Name	Signature	Date
_____	_____	_____
Witness Name	Signature	Date
_____	_____	_____
Investigator Name	Signature	Date

Chapter 4: Energy and macronutrient content of familiar beverages interact with pre-meal intervals to determine later food intake, appetite and glycemic response in young adults (Experiment 2).

Department of Nutritional Sciences
FitzGerald Building, 150 College Street, 3rd Floor
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CANADA

The Effect of Isovolumetric Preloads of Various Types of Milk on Food Intake, Subjective Appetite and Glycemic Response in Healthy Young Men and Women

Information Sheet and Consent Form

Investigators: Dr. G. Harvey Anderson, Professor
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Funding Source:

Funding for this project is provided by Dairy Farmers of Ontario.

Background and Purpose of Research:

In 2004, almost 60% of adult Canadians were overweight or obese. This is a serious health problem because obesity and being overweight are related to many risk factors of disease, including increased blood sugar. Overweight and obesity can be treated by changing what we eat. It is important to find food-based ways to prevent and treat overweight and obesity.

Milk and milk substitutes are types of food components that can be used to decrease hunger and control blood glucose. This study will investigate whether different types of milk can be used for these purposes. The information obtained from this study will be used to better understand the effects of different types of food components on the health of young men and women and may lead to future studies in other groups, including overweight individuals and children.

The purpose of this research project is to determine the effects of drinking milk compared with chocolate milk, a soy beverage, infant formula and water on blood sugar and appetite in young men and women.

This study will have 52 participants.

Invitation to Participate:

You are being invited to take part in this study. If you choose to take part, you will be asked to drink milk or a milk substitute five times (**six** sessions) one week apart. At each session, your appetite, blood sugar and salivary cortisol (reflecting stress) will be measured after eating the treatment and a pizza lunch. Each session will take up to 4 ½ hours of your time.

Eligibility:

To participate in this study you must be a healthy male or female and between 20-30 years old. You must be a nonsmoker and you cannot be taking any medications. The study will take place in the Department of Nutritional Sciences, Rm 305, 334, 331 and 331A, FitzGerald Building, 150 College Street, Toronto, ON.

Procedure:

To find out if you can take part in this study, you will be asked to fill out questionnaires, which ask questions about your age, if you smoke, exercise, your health, if you are on any medications and your eating habits. Your height and weight will be measured.

If you can take part, you will be asked to fill out questionnaires about the foods you like. You will be scheduled to meet with us for five sessions over five weeks. You will be asked to fast for 12 hours prior to your session (no eating for 12 hours before coming in except for water).

You will be asked to arrive at the FitzGerald Building between 07:50 a.m. and 10:00 a.m. You will be asked to stick to your normal routine, including exercise and to eat a similar meal the night before each session. You can drink water up to one hour before meeting with us.

At each session, you will be asked to drink a beverage (500 ml), give blood samples and to complete questionnaires at the times outlined in the table below. Fifteen times during each session, for a total of **78** times over the whole study, you will be asked to provide blood by finger-prick to measure blood sugar. Blood will be sampled before drinking the treatment and at **10, 20, 30, 45, 60, 90, 120 140, 170, 200, 230 and 260** minutes after drinking the treatment. You will be asked to fill out visual analog scale (VAS) questionnaires measuring your appetite, physical comfort, energy/fatigue and stress as well as the palatability (pleasantness) of the treatment and pizza throughout the study sessions. You will be served a pizza meal 120 minutes (2 hours) after you drink the treatment. In addition, salivary cortisol is a useful measure of stress and will be measured every hour. Each session will last up to 4 ½ hours.

Example of Time and Activity Schedule for Each Session

Time	**Activity**
07:50	Arrive at the laboratory (fasted for 12 hours)
07:50	Fill in Sleep, Stress, and VAS questionnaires and take first blood sample
08:00-08:05	Drink the treatment (0 minutes)
08:00-10:05	Blood sampling and VAS questionnaires at **10, 20, 30, 45, 60, 90, 120** minutes
10:05-10:25	Pizza served and eaten (120-140 minutes)
10:25-12:25	Blood sampling and VAS questionnaires at 140, 170, 200, 230, and 260 minutes

VAS: Visual Analogue Scale

Voluntary Participation and Early Withdrawal:

It is hoped that you will finish all **six** sessions. However, you may choose to stop being in the study at anytime without any problems.

Early Termination:

Not applicable.

Risks:

All of the foods and beverages that you will be asked to consume are prepared hygienically in the kitchen and present minimal risk. You may feel dizzy following the overnight fast, but this is rare. If this happens, you will likely feel fine once you drink the treatment provided to you.

The risks and discomfort will come from the blood sampling procedure. Great care will be taken when taking your finger-prick blood samples. The investigator will help you. To make sure that you are not exposed to another person's needle, we will ask you to sit away from other study participants. We will put a needle into the finger-prick gun before taking each blood sample and then put it into the safety container. There is very little risk of infection. We will clean your finger with a new alcohol swab before and after each finger-prick and will use a new sterile needle each time. You will be provided with your own finger-prick gun for the entire study.

Some discomfort will be felt as a result of a sharp momentary pain caused as the needle enters the skin. However, because the lancet needle is very small the pain felt is usually less than you might feel from skin puncture during vaccination or if a blood sample is taken by a needle inserted in a vein. There might be slight bruising under the skin, but this will be minimized by applying pressure after the finger is pricked and blood sugar is measured. A total of **13** finger-pricks will be conducted per session and may result in some discomfort.

You may experience flatulence (passing gas) and feelings of gastrointestinal discomfort (bloating) from the treatments. This is rare and there is no health risk linked with these effects.

In addition, there are no anticipated risks from measuring salivary cortisol. Saliva collection is hygienic, carried out by chewing new cotton wool swabs at the indicated time points to obtain fluid samples. Sampling is also painless and can be repeated without difficulty.

There is always a possibility that you will become ill following consumption of food, but that is very unlikely in this study. All treatments as well as pizza are freshly prepared at the time of your session. The pizzas are stored frozen and cooked accordingly to the manufacturer's instructions immediately before you are served.

Benefits:

You will not benefit directly from taking part in this study. You will be shown your blood sugar results and, if they are not normal, you will be told and advised to talk to your doctor. The foods and beverages will be provided for free.

Confidentiality and Privacy:

Confidentiality will be respected and no information that shows your identity will be released or published without your permission unless required by law. Your name, personal information and signed consent form will be kept in a locked filing cabinet in the investigator's office. Your results will not be kept in the same place as your name. Your results will be recorded on data sheets and in computer records that have an ID number for identification, but will not include your name. Your results, identified only by an ID number, will be made available to the study sponsor if requested. Only study investigators will have access to your individual results.

Publication of Results:

The results of the study may be presented at scientific meetings and published in a scientific journal. If the results are published, only average and not individual values will be reported.

Possible Commercialization of Findings:

This study is preliminary. Once these products are tested more widely in future studies, results may lead to commercialization of a product, new product formulation, changes in the labeling of a product and/or changes in the marketing of a product. You will not share in any way from the possible gains or money made by commercial application of findings.

Alternative Treatment/ Therapy:

Not applicable.

New Findings:

If anything is found during the course of this research which may change your decision to continue, you will be told about it.

Compensation:

You will be paid $36 per session (**$216** in total). Reimbursement for travel expenses ($6 per session) is included in each session total. If you withdraw from the study before finishing or asked to withdraw, you will be paid for the sessions you have already finished.

Injury Statement:

If you begin to feel sick following participation in the study, please seek medical advice as soon as possible. We will provide your medical specialist with information about the food you have consumed during the session, so take our phone number with you.

Rights of Subjects:

Before agreeing to take part in this research study, it is important that you read and understand your role as described here in this study information sheet and consent form. You waive no legal rights by taking part in this study. If you have any questions or concerns about your rights as a participant you can contact the Ethics Review Office at ethics.review@utoronto.ca or call 416-946-3273.

If you have any questions after you read through this information please do not hesitate to ask the investigators for further clarification.

Dissemination of findings:

A summary of results will be made available for you to pick up after the study is done.

Copy of informed consent for participant:

You are given a copy of this informed consent to keep for your own records.

Consent:

I acknowledge that the research study described above has been explained to me and that any questions that I have asked have been answered to my satisfaction. I have been informed of the alternatives to participation in this study, including the right not to participate and the right to withdraw. As well, the potential risks, harms and discomforts have been explained to me. I understand that I will receive compensation for my time spent participating in the study.

As part of my participation in this study, I understand that I may come in contact with other study participants because our session times overlap. I agree to keep anything I learn about other participants confidential and know that other participants have agreed to do the same for me.

I hereby agree and give my authorized consent to participate in the study and to treat confidential information in a restrictive manner as described above. I have been given a copy of the consent form to keep for my own records.

_____	_____	_____
Participant Name	Signature	Date
_____	_____	_____
Witness Name	Signature	Date
_____	_____	_____
Investigator Name	Signature	Date

Chapter 5: Caloric beverages consumed freely at meal-time add calories to an ad libitum meal

Department of Nutritional Sciences
FitzGerald Building, 150 College Street, 3rd Floor
Toronto, ON M5S 3E2
CANADA

The effects of composition of beverage consumed with a mixed meal on meal-time food intake and post-meal appetite and glycemia in healthy young men and women

Information Sheet and Consent Form

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Funding Source:

Funding for this project is provided by Collaborative Research and Development program funded by Natural Sciences and Engineering Research Council of Canada together with Dairy Farmers of Ontario and Kraft Canada Inc.

Background and Purpose of Research:

In 2004, almost 60% of adult Canadians were overweight or obese. This is a serious health problem because obesity and being overweight are related to many risk factors of disease, including increased blood sugar. Overweight and obesity can be treated by changing what we eat. It is important to find food-based ways to prevent and treat overweight and obesity.

The consumption of milk and dairy products was reported to protect against weight gain and obesity, opposite to soft drinks and fruit drinks. This study will test whether incorporating milk into a pizza mixed meal, instead of fruit juice and soft drinks, can control the feeling of hunger and blood sugar. The information obtained from this study will be used to better understand the importance of adding milk into meals, rather than other beverages, on the health of young men and women.

The purpose of this study is to find out the effects of drinking milk as part of a carbohydrate-rich meal, in comparison to soft drinks and fruit juice, on blood sugar and appetite in young men and women.

This study will have 30 participants.

Invitation to Participate:

You are being invited to take part in this study. If you choose to take part, you will be asked to drink various beverages five times (five sessions), one week apart. At each session, your appetite (feelings of hunger) and blood sugar will be measured after drinking the test beverages with a pizza lunch. Each session will take up to 2 ½ hours of your time.

Eligibility:

To participate in this study, you must be a healthy male or female and between 20-30 years old. You must be a nonsmoker and you cannot be taking any medications. The study will take place in the Department of Nutritional Sciences, Rooms 305, 329A, 334, 331 and 331A, FitzGerald Building, 150 College Street, Toronto, ON.

Procedure:

To find out if you can take part in this study, you will be asked to fill out questionnaires, which will ask questions about your age, if you smoke, exercise, your health, if you are on any medications and your eating habits. Your height and weight will be measured.

If you can take part, you will be asked to fill out questionnaires about the foods you like. You will be scheduled to meet with us five times over five weeks. You will be asked to fast for 12 hours prior to your session (no eating for 12 hours before coming in except for water).

Following the 12-hour overnight fast, you will be instructed to consume a standardized breakfast (6-9 am), of fixed composition and caloric content, and to arrive at the FitzGerald Building 4 hours later (10 am-1 pm) to start the session. You will be asked to stick to your normal routine,

including exercise, and to eat a similar meal the night before each session. You can drink water up to one hour before meeting with us.

At each session, you will be asked to drink one of the following five beverages, 1% milk, fruit juice, soft drink, diet soft drink and water, with a pizza mixed meal up to satiation, to give blood samples and to complete questionnaires at the times outlined in the table below. Nine times during each session, for a total of 45 times over the whole study, you will be asked to provide blood by finger-prick to measure blood sugar. Blood will be sampled before consuming the test beverages with the pizza lunch and at 20, 30, 45, 60, 75, 90, 105 and 120 minutes after their consumption. You will be asked to fill out visual analog scale (VAS) questionnaires measuring your appetite (hunger), physical comfort, energy/fatigue and stress as well as the palatability (pleasantness) of the beverage and the pizza meal throughout the study sessions. Each session will last up to 2 ½ hours.

Example of Time and Activity Schedule for Each Session

Time	**Activity**
09:50am	Arrive at the laboratory
09:50am	Fill in Sleep, Stress, and VAS questionnaires and take first blood sample
10:00am-10:20am	Consume the test beverage with the pizza meal (0 minutes)
10:20am-12:00pm	Blood sampling and VAS questionnaires at 20, 30, 45, 60, 75, 90, 105 and 120 minutes

VAS: Visual Analogue Scale

Voluntary Participation and Early Withdrawal:

It is hoped that you will finish all five sessions. However, you may choose to stop being in the study at any time without any problems.

Early Termination:

Not applicable.

Risks:

The served breakfast meal and the test beverages are commercially available and safe for human consumption. In addition, the pizza that you will be also asked to consume are prepared hygienically in the kitchen at the time of the session and present minimal risk. You may feel dizzy following the overnight fast, but this is rare. If this happens, you will likely feel fine once you consume the breakfast meal provided to you.
The risks and discomfort will come from the blood sampling procedure. Great care will be taken when taking your finger-prick blood samples. The investigator will be there to help you. To make sure that you are not exposed to another person's needle, we will ask you to sit away from other study participants. We will put a needle into the finger-prick gun before taking each blood sample and then put it into the safety container. There is very little risk of infection. We will clean your finger with a new alcohol swab before and after each finger-prick and will use a new

sterile needle each time. You will be provided with your own finger-prick gun for the entire study.

Some discomfort will be felt as a result of a sharp momentary pain caused as the needle enters the skin. However, because the lancet needle is very small, the pain felt is usually less than you might feel from skin puncture during vaccination or if a blood sample is taken by a needle inserted in a vein. There might be slight bruising under the skin, but this will be minimized by applying pressure after the finger is pricked and blood sugar is measured. A total of 9 finger-pricks will be conducted per one session and 45 throughout the whole study (five sessions).

You may experience flatulence (passing gas) and feelings of gastrointestinal discomfort (bloating) from the drinks. This is rare and there is no health risk linked with these effects.

There is always a possibility that you will become ill following consumption of food, but that is very unlikely. All foods and drinks are safe to consume and the pizzas are freshly prepared at the time of your session. The pizzas are stored frozen and cooked accordingly to the manufacturer's instructions immediately before you are served.

Benefits:

You will not benefit directly from taking part in this study. You will be shown your blood sugar results and, if they are not normal, you will be told and advised to talk to your doctor. The foods and drinks will be provided for free.

Confidentiality and Privacy:

Confidentiality will be respected and no information that shows your identity will be released or published without your permission unless required by law. Your name, personal information and signed consent form will be kept in a locked filing cabinet in the investigator's office. Your results will not be kept in the same place as your name. Your results will be recorded on data sheets and in computer records that have an ID number for identification, but will not include your name. Your results, identified only by an ID number, will be made available to the study sponsor if requested. Only study investigators will have access to your individual results.

Publication of Results:

The results of the study may be presented at scientific meetings and published in a scientific journal. If the results are published, only average and not individual values will be reported.

Possible Commercialization of Findings:

This study will just provide additional scientific evidence about the importance of incorporating milk, instead of other caloric beverages, into a meal. There is no potential commercialization of findings.

Alternative Treatment/ Therapy:

Not applicable.

New Findings:

If anything is found during the course of this research which may change your decision to continue, you will be told about it.

Compensation:

You will be paid $46 per session ($230 in total). Reimbursement for travel expenses ($6 per session) is included in each session total. If you withdraw from the study before finishing or asked to withdraw, you will be paid for the sessions you have already finished.

Injury Statement:

If you begin to feel sick following participation in the study, please seek medical advice as soon as possible. We will provide your medical specialist with information about the food you have consumed during the session, so take our phone number with you.

Rights of Subjects:

Before agreeing to take part in this research study, it is important that you read and understand your role as described here in this study information sheet and consent form. You waive no legal rights by taking part in this study. If you have any questions or concerns about your rights as a participant you can contact the Ethics Review Office at ethics.review@utoronto.ca or call 416-946-3273.

If you have any questions after you read through this information, please do not hesitate to ask the investigators for further clarification.

Dissemination of findings:

A summary of results will be made available for you to pick up after the study is done.

Copy of informed consent for participant:

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As part of my participation in this study, I understand that I may come in contact with other study participants because our session times overlap. I agree to keep anything I learn about other participants confidential and know that other participants have agreed to do the same for me.

I hereby agree and give my authorized consent to participate in the study and to treat confidential information in a restrictive manner as described above. I have been given a copy of the consent form to keep for my own records.

_____	_____	_____
Participant Name	Signature	Date
_____	_____	_____
Witness Name	Signature	Date
_____	_____	_____
Investigator Name	Signature	Date

Chapter 6: Mechanism of action of whole milk and its components on satiety and glycemic control in healthy young men

Department of Nutritional Sciences
FitzGerald Building, 150 College Street, 3rd Floor
Toronto, ON M5S 3E2
CANADA

The effect of pre-meal consumption of milk components on subjective appetite, glycemic control and gastrointestinal hormone response in healthy young men

Information Sheet and Consent Form

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Funding Source:

Funding for this project is provided by Collaborative Research and Development program funded by Natural Sciences and Engineering Research Council of Canada together with Dairy Farmers of Ontario and Kraft Canada Inc.

Background and Purpose of Research:

In 2004, almost 60% of adult Canadians were overweight or obese. This is a serious health problem because obesity and being overweight are related to many risk factors of disease, including increased blood sugar. Overweight and obesity can be treated by changing what we eat. It is important to find food-based ways to prevent and treat overweight and obesity.

Milk and its components such as sugar (eg. lactose), protein and fat can possibly be used to decrease hunger and control blood glucose, which is the purpose of this study. The information obtained from this study will be used to better understand the effects of different types of food components on the health of young men and may lead to future studies in other groups, including women, overweight individuals and children.

The purpose of this research project is to determine the effects of drinking milk compared with lactose, protein and fat drinks on appetite, blood glucose, insulin and hormonal responses after their consumption.

This study will have 16 participants.

Invitation to Participate:

You are being invited to take part in this study. If you choose to take part, you will be asked to drink five different drinks. These include milk, lactose drink, protein drink, fat drink and a drink containing lactose, protein and fat on five separate sessions over 5 weeks. Your appetite will be assessed by filling out Visual Analogue Scale questionnaires and your blood will be sampled by a registered nurse to be measured for blood glucose, insulin and hormonal responses after consumption of the drinks. Each session will take up to 3 ½ hours of your time.

Eligibility:

To participate in this study you must be a healthy male and between 20-30 years old. You must be a nonsmoker and you cannot be taking any medications. The study will take place in the Department of Nutritional Sciences, Rm 305, 334, 331 and 331A, FitzGerald Building, 150 College Street, Toronto, ON.

Procedure:

To find out if you can take part in this study, you will be asked to fill out questionnaires, which ask questions about your age, if you smoke, exercise, your health, if you are on any medications and your eating habits. Your height and weight will be measured.

If you can take part, you will be asked to fill out questionnaires about the foods you like. You will be scheduled to meet with us for five sessions. You will be asked to fast for 12 hours the night before the session (no eating for 12 hours except for water) and arrive at the FitzGerald Building between 7:50 a.m. and 10:00 a.m on the day of the session. Please do not eat before meeting with us in the morning. You will be asked to stick to your normal routine, including

exercise and to eat a similar meal the night before each session. You can drink water up to one hour before meeting with us.

At each session, an indwelling intravenous catheter will be inserted in your vein by a registered nurse to collect blood samples. The catheter will remain in your arm over the study session and be used to sample blood in small amounts during the test. Blood will be used to measure blood sugar and appetite-controlling (hunger) hormones. Nine blood samples will be taken during each experimental session, an amount of 90 ml (under 3 ounces). A total of 450 ml of blood will be collected during the entire study. To obtain blood samples, a nurse will insert a catheter (a needle attached to a plastic tube) into a vein in your arm. After the nurse collects the first sample at baseline (0 minutes), you will consume one of the beverages within five minutes. Each beverage will contain 1.5 g of paracetamol, which is an over-the-counter analgesic that is found in numerous cold medications and is used to measure gastric emptying. After you finish the drink, we will collect blood samples at 10, 20, 30, 45, 60, 80, 90, 120 and 180 minutes after baseline. You will be asked to fill out visual analog scale (VAS) questionnaires measuring your appetite, physical comfort, energy/fatigue and stress as well as the palatability (pleasantness) of the treatment and pizza throughout the study sessions. Each session will last up to 3 ½ hours.

Example of Time and Activity Schedule for Each Session

Time	**Activity**
07:50	Arrive at the laboratory (fasted for 12 hours)
07:50	Fill in Sleep, Stress, and VAS questionnaires and take first blood sample
08:00-08:05	Drink the treatment (0 minutes)
08:05-11:00	Blood sampling and VAS questionnaires at 10, 20, 30, 45, 60, 90, 120, and 180 minutes

VAS: Visual Analogue Scale

Voluntary Participation and Early Withdrawal:

It is hoped that you will finish all five sessions. However, you may choose to stop being in the study at any time without any problems.

Early Termination:

Not applicable.

Risks:

All of the foods and beverages that you will be asked to consume are prepared hygienically in the kitchen and present minimal risk. You may feel dizzy following the overnight fast, but this is rare. If this happens, you will feel fine once you drink the treatment provided to you.

The risks and discomfort will come from the blood sampling procedure. Some discomfort will be felt as a result of a sharp momentary pain caused when the venous catheter or syringe needle is put into your arm by a nurse. The pain felt is usually similar to that you might experience from

skin puncture during vaccination or if a blood sample is taken by a needle at your doctor's office. There is very little risk of infection. Before the catheter or needle is inserted, the area is cleaned with antiseptic (alcohol) by the nurse. There might be bruising under the skin, but this will be minimized by applying pressure after the catheter or needle is removed.

The total amount of blood taken during the study (over five weeks) is 450 ml, which amounts to a single blood donation. This may be too much if other amounts of blood are lost. Thus, we recommend that you do not donate blood during or within one month of the end of the study.

A total of 9 blood samples will be taken per session and may result in some discomfort.

The paracetamol absorption test for the determination of gastric emptying is a safe test. The amount used in this study is below the recommended daily limit for adults of 4 g and no studies have reported adverse reactions to weekly administration of this dose. However, acute overdoses of paracetamol can lead to toxic liver damage and renal impairment. Hence, you will be asked to refrain from using paracetamol or any analgesic drugs containing it during the study.

You may experience flatulence (passing gas) and feelings of gastrointestinal discomfort (bloating) from the treatments. This is rare and there is no health risk linked with these effects.

Benefits:

You will not benefit directly from taking part in this study. You will be shown your blood sugar results and, if they are not normal, you will be told and advised to talk to your doctor. The beverages will be provided for free.

Confidentiality and Privacy:

Confidentiality will be respected and no information that shows your identity will be released or published without your permission unless required by law. Your name, personal information and signed consent form will be kept in a locked filing cabinet in the investigator's office. Your results will not be kept in the same place as your name. Your results will be recorded on data sheets and in computer records that have an ID number for identification, but will not include your name. Your results, identified only by an ID number, will be made available to the study sponsor if requested. Only study investigators will have access to your individual results.

Publication of Results:

The results of the study may be presented at scientific meetings and published in a scientific journal. If the results are published, only average and not individual values will be reported.

Possible Commercialization of Findings:

This study is preliminary. Once these products are tested more widely in future studies, results may lead to commercialization of a product, new product formulation, changes in the labeling of a product and/or changes in the marketing of a product. You will not share in any way from the possible gains or money made by commercial application of findings.

Alternative Treatment/ Therapy:

Not applicable.

New Findings:

If anything is found during the course of this research which may change your decision to continue, you will be told about it.

Compensation:

You will be paid $45 per session ($225 in total). Reimbursement for travel expenses ($6 per session) is included in each session total. If you withdraw from the study before finishing or asked to withdraw, you will be paid for the sessions you have already finished.

Injury Statement:

If you begin to feel sick following participation in the study, please seek medical advice as soon as possible. We will provide your medical specialist with information about the food you have consumed during the session, so take our phone number with you.

Rights of Subjects:

Before agreeing to take part in this research study, it is important that you read and understand your role as described here in this study information sheet and consent form. You waive no legal rights by taking part in this study. If you have any questions or concerns about your rights as a participant you can contact the Ethics Review Office at ethics.review@utoronto.ca or call 416-946-3273.

If you have any questions after you read through this information please do not hesitate to ask the investigators for further clarification.

Dissemination of findings:

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Copy of informed consent for participant:

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I hereby agree and give my authorized consent to participate in the study and to treat confidential information in a restrictive manner as described above. I have been given a copy of the consent form to keep for my own records.

_____	_____	_____
Participant Name	Signature	Date
_____	_____	_____
Witness Name	Signature	Date
_____	_____	_____
Investigator Name	Signature	Date

APPENDIX 5. Screening Questionnaires

Phone Screening Questionnaire

Recruitment Screening Questionnaire

Sleep Habit Questionnaire

Eating Habit Questionnaire

Food Acceptability Questionnaire

5.1. Recruitment Screening Questionnaire

NOTE: After you are recruited for the study, you will be assigned an ID# which will be used on your forms and data throughtout the study.

NAME: _____ AGE: _____

ADDRESS: _____

PHONE #: (_____)_____ E-MAIL: _____

HEIGHT: _____ WEIGHT: _____ BMI: _____

Participation in Athletics/Exercise:

ACTIVITY	HOW OFTEN?	HOW LONG? (HOURS)

Do you usually eat breakfast? ☐ **YES** ☐ **NO**

If YES, what do you usually eat? _____

Health Status:

Do you have diabetes? ☐ **YES** ☐ **NO**

Do you have any other major disease or condition? ☐ **YES** ☐ **NO**

If YES, please specify: _____

Are you taking any medications? ☐ **YES** ☐ **NO**

If YES, please specify: _____

Do you have reactions to any foods? ☐ **YES** ☐ **NO**

If YES, please specify: _____

Are you lactose-intolerant? ☐ **YES** ☐ **NO**

Are you on a special diet? ☐ **YES** ☐ **NO**

If YES, please specify: _____

Have you recently lost or gained weight? ☐ **YES** ☐ **NO**

If YES, please specify: _____

Do you smoke? ☐ **YES** ☐ **NO**

How many alcoholic beverages do you consume per day? _____ Per week? _____

5.2. Sleep Habits Questionnaire

1. **At what time do you normally wake up in the morning?**

   During the week: _____

   Weekends/days off: _____

2. **At what time do you normally get out of bed? (If different from above)**

   During the week: _____

   Weekends/days off: _____

3. **What is the earliest time you would get up in a normal week?**

   During the week: _____

   Weekends/days off: _____

4. **What is the latest time you would get up in a normal week?**

   During the week: _____

   Weekends/days off: _____

5. **How long do you wait for, to eat after rising?**

   During the week: _____

   Weekends/days off: _____

5.3. Eating Habits Questionnaire

Please choose the appropriate answer to best describe your personal situation (last 6 months).

1. **How often are you dieting?**

   never ☐ rarely ☐ sometimes ☐ often ☐ always ☐

2. **What is the maximum amount of weight (in pounds) that you have ever lost within 1 month?**

   1 – 4 ☐ 5 – 9 ☐ 10 – 14 ☐ 15 – 19 ☐ 20 + ☐

3. **What is your maximum weight gain within 1 week?**

   0 – 1 ☐ 1.1 – 2 ☐ 2.1 – 3 ☐ 3.1 – 5 ☐ 5.1 + ☐

4. **In a typical week, how much does your weight fluctuate (in pounds)?**

   0 – 1 ☐ 1.1 – 2 ☐ 2.1 – 3 ☐ 3.1 – 5 ☐ 5.1 + ☐

5. **Would a weight fluctuation of 5 lbs. affect the way you live your life?**

   not at all ☐ slightly ☐ moderately ☐ very much ☐

6. **Do you eat sensibly in front of others and splurge alone?**

   never ☐ rarely ☐ often ☐ always ☐

7. **Do you give too much time and thought to food?**

   never ☐ rarely ☐ often ☐ always ☐

8. **Do you have feelings of guilt after overeating?**

   never ☐ rarely ☐ often ☐ always ☐

9. **How conscious are you of what you are eating?**

   not at all ☐ slightly ☐ moderately ☐ very much ☐

10. **How many pounds over your desirable weight were you at your maximum weight?**

    0 – 1 ☐ 2 – 5 ☐ 6 – 10 ☐ 11 – 20 ☐ 21 + ☐

5.4. Food Acceptability Questionnaire

Please indicate with a rating between 1 and 10 how much you enjoy the following foods (1 = not at all, 10 = very much) and how often you eat them (never, daily, weekly, monthly).

	Enjoyment?	**How often?**
1. Pasta	_____	_____
2. Rice	_____	_____
3. Potatoes (mashed, roasted)	_____	_____
4. French fries	_____	_____
5. Pizza	_____	_____
6. Bread, bagels, dinner rolls	_____	_____
7. Sandwiches, subs	_____	_____
8. Cereal	_____	_____
9. Cake, donuts, cookies	_____	_____
10. Tomato/vegetable juice	_____	_____
11. Milk/chocolate milk	_____	_____

Will you be able to drink a milk or milk substitute (e.g. soy beverage)?

☐ **YES** ☐ **NO**

At the end of each session, you will be provided with pizza. In order to provide you with a meal that you will enjoy, we ask that you rank the following pizzas according to your **personal preferences (i.e. 1st, 2nd, 3rd choice)** in the space provided. If you **do NOT** like a particular type of pizza, then do not rank it but instead place an **"X"** in the space provided.

Pepperoni (cheese, pepperoni) _____

Deluxe (cheese, pepperoni, peppers, mushrooms) _____

Three-cheese (mozzarella, cheddar, parmesan) _____

APPENDIX 6. Study Day Questionnaires

Sleep Habits and Stress Factor Questionnaire

Recent Food Intake and Activity Questionnaire

Motivation to Eat VAS

Physical Comfort VAS

Energy and Fatigue VAS

Treatment and Test Palatability

Test Meal Record

6.1. Sleep Habit and Stress Factor Questionnaire

DATE: _____
ID: _____
Session: _____

1. Did you have a normal night's sleep last night?

Yes_____ No_____

2. How many hours of sleep did you have?

_____

3. What time did you go to bed last night?

_____

4. What time did you wake up this morning? _____

5. Recount your activities since waking:

Time	Activity
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____

6. Are you experiencing any feelings of illness or discomfort, other than those from hunger?

Today: Yes ____ No_____
Past 24 hours: Yes ____ No_____

If yes, please describe briefly:

_____

7. Are you under any unusual stress? Exams/reports/work deadlines, personal, etc.

Today: Yes ____ No_____
Past 24 hours: Yes ____ No_____

If yes, please describe briefly:

_____

_____

8. Have you been involved in any physical activity within the past 24 hours that is unusual to your normal routine?

Yes_____ No_____

If yes, please describe briefly:

_____

_____

9. Have you had anything to eat or drink, other than water and provided breakfast, for the past 11-12 hours?

Yes_____ No_____

If yes, please describe briefly:

_____

6.2. Recent Food Intake and Activity Questionnaire

At what time did you have dinner? _____

Please describe your dinner last night (list all food and drink and give an estimate of the portion size):

_____

_____

_____

The following three (3) questions relate to your food intake, activity and stress over the last 24 hours. Please rate yourself by placing a small "x" across the horizontal line at the point which best reflects your present feelings.

How would you describe your **food intake** over the past 24 hours?

Much LESS than usual _____ Much MORE than usual

How would you describe your **level of activity** over the last 24 hours?

Much LESS than usual _____ Much MORE than usual

How would you describe your **level of stress** over the last 24 hours?

Much LESS _____ Much MORE

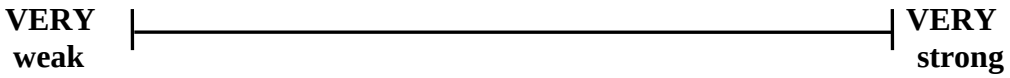
than usual than usual

6.3. Motivation to Eat VAS

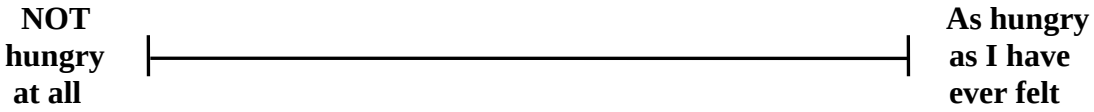
Visual Analogue Scales
Motivation to Eat

These questions relate to your **"motivation to eat"** at this time. Please rate yourself by placing a small **"x"** across the horizontal line at the point which best reflects your present feelings.

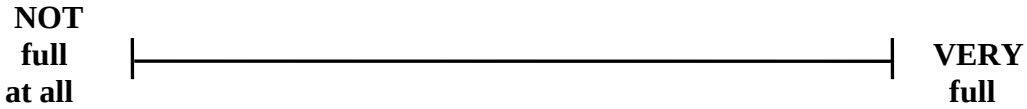
1. **How strong is your desire to eat?**



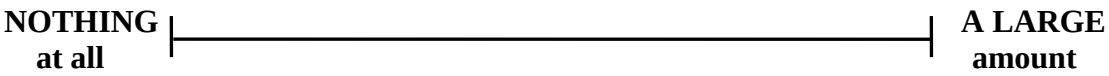
2. **How hungry do you feel?**



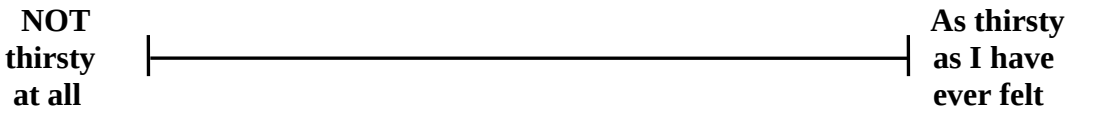
3. **How full do you feel?**



4. **How much food do you think you could eat?**



5. **How thirsty do you feel?**



6.4. Physical Comfort VAS

Visual Analogue Scales
Physical Comfort

These questions relate to your **"physical comfort"** at this time. Please rate yourself by placing a small **"x"** across the horizontal line at the point which best reflects your present feelings.

1. **Do you feel nauseous?**

NOT at all ——— **VERY much**

2. **Does your stomach hurt?**

NOT at all ——— **VERY much**

3. **How well do you feel?**

NOT well at all ——— **VERY well**

4. **Do you feel like you have gas?**

NOT at all ——— **VERY much**

5. **Do you feel like you have diarrhea?**

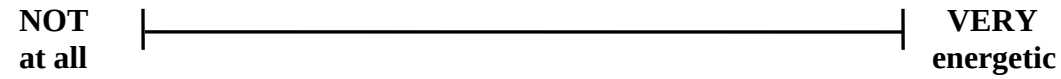
NOT at all ——— **VERY much**

6.5. Energy and Fatigue VAS

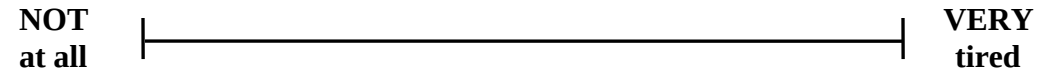
Visual Analogue Scales
Energy/Fatigue and Stress

These questions relate to your **"energy level and fatigue"** at this time. Please rate yourself by placing a small "**x**" across the horizontal line at the point which best reflects your present feelings.

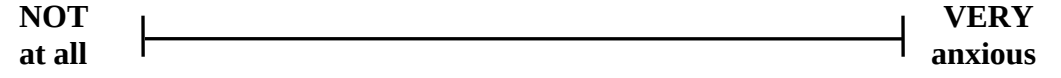
1. **How energetic do you feel right now?**



2. **How tired do you feel right now?**



3. **How anxious do you feel right now?**



6.6. Treatment and Test Palatability

Visual Analogue Scales
Palatability

This question relates to the **"palatability of the beverage/food"** you just consumed. Please rate yourself by placing a small "**x**" across the horizontal line at the point which best reflects your present findings.

1. How pleasant have you found the beverage/food?

NOT at all pleasant ——— **VERY pleasant**

2. How tasty have you found the beverage/food?

NOT at all tasty ——— **VERY tasty**

3. How did you like the texture of the beverage/food?

NOT at all ——— **VERY much**

APPENDIX 7. Blood Glucose and Pizza Test Meal Records

Chapter 4: Experiment 1

Subject ID: _____ Treatment: _____ Session: _____ **Study 1: Experiment 1**

Blood Glucose Measurements

Time (minutes)	Time on the timer	Blood Glucose (mmol/L)
0	0:00	
10	0:10	
20	0:20	
30	0:30	
50	0:50	
65	1:05	
80	1:20	
95	1:35	
110	1:50	
140	2:20	
170	2:50	

Chapter 4: Experiment 2

Subject ID: _____ Treatment: _____ Session: _____ **Study 1: Experiment 2**

Blood Glucose Measurements

Time (minutes)	Time on the timer	Blood Glucose (mmol/L)
0	0:00	
10	0:10	
20	0:20	
30	0:30	
50	0:50	
65	1:05	
80	1:20	
95	1:35	
110	1:50	
140	2:20	
170	2:50	
200	3: 20	
230	3: 50	
260	4: 20	

Chapter 5

Subject ID: _____ Session: ____ Treatment: ____ Date: _____

Blood Glucose Measurements

Time (minutes)	Time on the timer	Blood Glucose (mmol/L)
0	0:00	
20	0:20	
30	0:30	
45	0:45	
60	1:00	
75	1:15	
90	1:30	
105	1:45	
120	2:00	

Pizza Test Meal Record

ID: ______ Date: ________________________

Session: ______ Treatment: _______________

Tray 1	Before	After	**Number**
Pepperoni (g)			
Deluxe (g)			
3-cheese (g)			
Tray 2			
Pepperoni (g)			
Deluxe (g)			
3-cheese (g)			
Tray 3			
Pepperoni (g)			
Deluxe (g)			
3-cheese (g)			
Water 1 (g)			
Water 2 (g)			

ID: ______ Date: ________________________

Session: ______ Treatment: _______________

Tray 1	Before	After	Number
Pepperoni (g)			
Deluxe (g)			
3-cheese (g)			
Tray 2			
Pepperoni (g)			
Deluxe (g)			
3-cheese (g)			
Tray 3			
Pepperoni (g)			
Deluxe (g)			
3-cheese (g)			
Water 1 (g)			
Water 2 (g)			

ID: ______ Date: ________________________

Session: ______ Treatment: _______________

Tray 1	Before	After	Number
Pepperoni (g)			
Deluxe (g)			
3-cheese (g)			
Tray 2			
Pepperoni (g)			
Deluxe (g)			
3-cheese (g)			
Tray 3			
Pepperoni (g)			
Deluxe (g)			
3-cheese (g)			
Water 1 (g)			
Water 2 (g)			

ID: ______ Date: ________________________

Session: ______ Treatment: _______________

Tray 1	Before	After	Number
Pepperoni (g)			
Deluxe (g)			
3-cheese (g)			
Tray 2			
Pepperoni (g)			
Deluxe (g)			
3-cheese (g)			
Tray 3			
Pepperoni (g)			
Deluxe (g)			
3-cheese (g)			
Water 1 (g)			
Water 2 (g)			

ID: ______ Date: ________________________

Session: ______ Treatment: _______________

Tray 1	Before	After	Number
Pepperoni (g)			
Deluxe (g)			
3-cheese (g)			
Tray 2			
Pepperoni (g)			
Deluxe (g)			
3-cheese (g)			
Tray 3			
Pepperoni (g)			
Deluxe (g)			
3-cheese (g)			
Water 1 (g)			
Water 2 (g)			

ID: ______ Date: ________________________

Session: ______ Treatment: _______________

Tray 1	Before	After	Number
Pepperoni (g)			
Deluxe (g)			
3-cheese (g)			
Tray 2			
Pepperoni (g)			
Deluxe (g)			
3-cheese (g)			
Tray 3			
Pepperoni (g)			
Deluxe (g)			
3-cheese (g)			
Water 1 (g)			
Water 2 (g)			