DIFFERENTIAL PLASMA AMINO ACID RESPONSES FOLLOWING THE CONSUMPTION OF ISONITROGENOUS DOSES OF GREEK YOGURT AND SKIM MILK

JOSEPH K. BROWN

A THESIS SUBMITTED TO THE FACULTY OF GRADUATE STUDIES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

GRADUATE PROGRAM IN KINESIOLOGY AND HEALTH SCIENCE
YORK UNIVERSITY
TORONTO, ONTARIO

APRIL 2022

Abstract

This thesis examined rested postprandial plasma branched-chain amino acid (BCAA) responses after ingesting Greek yogurt (GY) and skim milk (Milk). Utilizing a cross over design, 10 healthy males consumed isonitrogenous boluses of GY and Milk (20 g protein each) at baseline and 60 minutes. Blood was collected at baseline, 30-, 60-, 90-, 120-, 180- and 240-minutes. Leucine, and total BCAA concentrations, maximal concentration (C_{max}), time to maximal concentration (T_{max}), and total area under the curve (tAUC) were assessed. Significant interactions demonstrated that GY ingestion elicited higher leucine concentration at 60- and 120-minutes and BCAA concentrations at 60-, 90- and 180-minutes vs. Milk. GY exceeded Milk for leucine and BCAA C_{max}, with no differences for T_{max}. Leucine and BCAA tAUCs were greater for GY vs. Milk. These data demonstrate that GY elicits a greater blood leucine/BCAA response compared to Milk over 4 hours.

Dedication

To my late mother and father, may your presence within never fade.

Acknowledgements

I am deeply indebted to **Dr. Andrea Josse** for her patience, mentorship, guidance, and expertise that made my masters the most enjoyable learning experience. When times were tough, she believed in my work ethic and devotion to succeed, and I am forever grateful. Her past and present expertise has allowed me to be connected and involved with numerous high-level researchers... experiences I will never forget. Thank you to my committee member, **Dr. Christopher Perry**, for his expertise and critical thinking, which has allowed me to be well versed with my work.

A huge thanks to my former colleagues and current lab mates. **Hayden**, a good friend who played a part in my drive toward science, and who provided a huge hand with data collection and analysis. **Aaron**, who also played a part in my involvement in research through his own scientific work and offered a huge hand with data collection and analysis. To **Emily**, for her continuous positive attitude in the lab and assistance in shared grad courses that has made our schooling most enjoyable. To **Joel**, for guidance in research and shared grad courses. Finally, to **Dr. Lauren Skelly**, for all her expertise and mentorship that has allowed me to become a better graduate student.

To **Todd Prior**, for his extensive expertise and help with data analysis. This thesis would not be possible without his assistance, along with others from the EMRG lab at McMaster University. To **Dr. Stuart Phillips**, for kindly allowing our research group to conduct data analysis out of his laboratory space.

To my brothers, **Geoffrey**, **Kyle**, and **Tyler**, for filling the void of fatherhood, for being lifelong role models and for your continuous support throughout my academic endeavors.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

AA	Amino acids
ANOVA	Analysis of variance
AUC	Area under the curve
BMI	Body mass index
BCAA	Branched chain amino acids
carb	Carbohydrate
CHO	Carbohydrate placebo
DIAAS	Digestible indispensable amino acid scale
EAA	Essential amino acids
GY	Greek yogurt
HCL	Hydrochloric acid
Ile	Ileucine
iAUC	Incremental area under the curve
IQR	Interquartile Range
Leu	Leucine
C_{max}	Maximal concentration
min	Minutes
MPC	Milk protein concentrate
MPI	Milk protein isolate
MPS	Muscle Protein Synthesis
NEAA	Non-essential amino acids
PEPT1	Peptide transporter 1
Phen	Phenylalanine
Pro	Proline
pro	Protein
PDCAAS	Protein digestibility amino acid score
RE	Resistance exercise
RT	Resistance training
M	Skim milk
SI	Small intestine
subsp	Subspecies
T_{max}	Time to maximal concentration
T_{min}	Time to minimal concentration
tAUC	Total area under the curve
Tyr	Tyrosine
Val	Valine
WPI	Whey protein isolate
β-Lg	β-lactoglobulin

CHAPTER 1:

1.1 Introduction

Protein is an essential nutrient that drives tissue protein synthesis throughout the body (1,2). Among tissues, human skeletal muscle is receptive to protein feeding, as illustrated by elevations in muscle protein synthesis (MPS) following protein consumption (3–5). Prior to initiating a muscle synthetic response, dietary protein must be digested into its constituent parts and absorbed as amino acids (AAs) before becoming bioavailable (in the blood stream) for tissue utilization (6,7). Consequently, analyzing the AA digestion and absorption kinetics of a multitude of popular dietary protein sources could aid in establishing the practical anabolic application of these products.

A variety of protein sources can be consumed and utilized to stimulate MPS including; wholefood animal proteins (e.g., beef, eggs, dairy) (8–12), plant-based proteins (e.g., soy, wheat, rice, potato and corn) (13–18) and isolated protein sources (e.g., whey and casein) (19–22); however, each offer slightly different and thus unique AA digestion and absorption kinetic responses (10,22–24) which are based on individual properties of the protein food/isolate. Amidst these sources, dairy products such as milk, yogurt and cheese render a combination of high quality proteins (whey and casein) that have top scores on the digestible indispensable amino acid score (DIAAS) scale (25–27). After consumption, whey stimulates MPS and whole body AA oxidation more than casein, in part, because of the faster rate of the appearance of essential AAs in circulation, namely leucine, following whey consumption (23). Although leucine is contained in both whey and casein, whey has a greater amount per a given volume of protein than casein (28,29). This is likely because a bulk of the whey component in milk consists

of β-lactoglobulin (β-Lg), a soluble protein that limits interaction with the acidic gastric environment, resulting in a more intact appearance in the upper jejunum, where pancreatic proteases act quickly to begin breakdown (30). Thus, due to its much faster appearance in the small intestine (SI), and its quick SI breakdown, whey results in a rapid AA absorption and AA appearance in circulation (30). On the other hand, casein protein shows a slower/dampened but more prolonged plasma AA profile that may reduce muscle protein breakdown beyond the initial postprandial period because it facilitates a more sustained uptake of essential AAs at the muscle due to its delayed appearance in the circulation (23). The delayed postprandial response has been attributed to casein curdling when exposed to acidic gastric conditions which requires greater time for protein hydrolysis and thus slower transit time (22,30). Further, among casein types, micellar casein (found in dairy protein), is a less soluble protein form that contributes to this delayed plasma AA bioavailability compared to processed caseinate forms such as sodium caseinate, potassium caseinate and calcium caseinate (processed in a laboratory/factory), which are more soluble, and display a greater plasma AA appearance (22,31).

With a protein composition of ~80% casein to ~20% whey, milk is a popular dairy product that has been commonly shown to enhance MPS in a variety of different contexts, including healthy, young, trained and untrained individuals (10,11,32–34). Typically, studies involving acute protein metabolism and MPS responses also detail the postprandial AA kinetics as secondary outcomes to inform their results. Milk is often compared in these studies to other protein sources such as protein blends (whey, casein and soy), soy beverage, beef, eggs and cheese at rest (24,35–37) and after resistance exercise (RE) (10,32,33,38). Following ingestion, milk displays measurable increases in AAs, essential AAs (EAAs) and leucine within a range of 20-45 minutes after consumption, and remains elevated above baseline concentrations toward

later postprandial timepoints (120-300 minutes) (24,35). This initial elevation, and prolonged delivery of AAs after ingestion likely reflects the combination of its constituent proteins, whey and casein, as both together would contribute to the initial and sustained rise of milk's AA response. Indeed, milk has been shown to stimulate MPS with RE acutely, and increase lean body mass and strength with resistance training (RT; chronically) in both male (32,33) and female (34) populations, younger (32–34) and older (39–43) at protein dosages ranging from 15-40 g.

Apart from milk, the majority of other dairy products exist in semi-solid or solid food forms that may influence the postprandial gastrointestinal digestion and absorption of macronutrients, including protein (44). Greek yogurt (GY) has become increasingly popular as a dairy food owing to its dense micronutrient profile (e.g., calcium, magnesium, vitamin D, potassium) (27,45) and high amount of protein (predominantly casein) (46). Yogurt also contains bacterial cultures which may offer additional health benefits beyond milk (45,47). Recently, our laboratory demonstrated greater training-induced increases in strength, muscle thickness and lean mass after daily GY consumption compared to a carbohydrate pudding of similar consistency to GY following 12 weeks of RT in young lean males (48). To date, this is the only randomized controlled trial comparing GY in this regard. The postprandial plasma AA response of GY has yet to be examined to accompany these findings.

GY is a semi-solid, fermented food product much like regular yogurt which involves the acidification of milk by common yogurt starter cultures; *Streptococcus salivarius* subsp. *thermophilies* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, leading to a coagulated food form (49). Additional processing is needed to formulate GY, which involves further straining of the whey protein fraction, leading to a thicker, higher protein casein-based product (46). The AA

kinetics of absorption of GY have yet to be studied; however, *in vivo* animal literature using mini-pig models assessing the plasma AA and leucine kinetics of dairy products subjected to gelation, such as cheese and yogurt, compared to liquid milk, revealed a more rapid and intense increase in AA from liquid milk compared to other dairy products with gel food matrices (50). Further, a review by Dupont and colleagues (51) comparing the plasma AA kinetics of various simulated dairy product matrices including liquid, acid gel, stirred acid gel and rennet gel, demonstrated that the liquid matrix had a more rapid and elevated (i.e., greater maximal concentration [C_{max}] and time to maximal concentration [T_{max}]) AA blood pattern than the gel matrices, which showed lower yet more prolonged AA responses. Unfortunately, area under the curves (AUCs) were not assessed in this investigation. Worth noting, however, the stirred acid gel (most indicative of yogurt), displayed an AA pattern intermediate and more prolonged to milk and the other gel matrices indicating that this may be the behavior of GY (51). Thus, the above data provide a glimpse into the effects of dairy food processing on the postprandial plasma AA response; however, evidence to validate these findings in humans is scarce, and non-existent for GY.

1.2 Rationale for investigating postprandial plasma aminoacidemia

In order to induce a synthetic response in target tissues, protein must first be digested and absorbed into AA constituents before being dispersed into circulation (1,6,7). In recent review, Burd and colleagues (52) highlighted the importance of measuring plasma AA availability in addition to MPS rates to provide a complete understanding of the anabolic potential of different protein sources. Given that AAs, especially EAAs are the main fuel source that activates human muscle synthetic machinery (53), and the fact AA digestion, absorption (6,7), and uptake into skeletal muscle (54–56) all influence MPS, it is logical to speculate that the plasma AA response

has anabolic significance.

Currently, a bulk of the protein metabolism research involves the analysis of isolated protein sources such as: whey, casein and soy (14,20,23,28,57). However, the utilization of wholefood protein sources such as animal and plant-based protein foods to augment MPS (58) is becoming increasingly popular because the majority of the general population obtains their dietary protein from wholefoods in meals and snacks (52). Consequently, wholefood protein sources, like milk, have taken a front seat in protein metabolism research (10,11,32,33,59). Owing also to its unique combination of whey and casein protein, dairy sources may induce an advantageous postprandial aminoacidemic response compared to other wholefood protein sources. For example, milk post-exercise promotes a more sustained rise in plasma AA concentration over an absorption period of 4 hours compared to an isonitrogenous soy protein beverage (18g of protein in each) (32). When comparing milk to other wholefoods such as beef (30g of protein in each), data show a more rapid peak in blood AAs after milk consumption; however, a greater amplitude (C_{max}) of aminoacidemia after beef consumption (10). Despite these varying kinetic responses between protein wholefoods, acute milk consumption augmented myofibrillar MPS to greater extent than soy (32), but similarly to beef over a 5 hour duration (10). Together, the above findings reveal milk to exhibit different and favourable postprandial plasma AA patterns that could pose anabolic significance with respect to MPS when comparing to other dietary proteins. It is possible that GY would demonstrate an intermediate response between milk and beef given its AA content and concentration (similar to milk and beef) and its semi-solid consistency (i.e., between milk and beef). A solid food generally takes longer to digest than a liquid food.

Noting these findings in response to milk ingestion, it is prudent to investigate other dairy products like GY. Although yogurt is a dairy wholefood, it does have different textural and nutrient properties/constituents compared to milk. In response to our laboratory's recent investigation demonstrating that the post-resistance exercise consumption of GY compared to a carbohydrate pudding induced lean mass and strength gains in 12 weeks (48), a commentary was published in the Journal (*Frontiers in Nutrition*) alongside our paper. In this commentary, researchers acknowledged our novel results and called for further analyses of GY because of its unique wholefood matrix (60). Further, assessing the blood AA patterns (postprandial concentration and duration) of these popular dairy foods, will help to establish the effectiveness of high protein, fermented milk products in addition to regular milk in an anabolic context (60). These were cited as important areas for future research in the commentary and lend further credence to our research herein. Given this direction, this thesis endeavoured to assess the postprandial AA responses of GY and milk within the same individuals using a cross-over design. It is evident that both GY and milk are nutrient-dense dairy foods that contain high quality protein with a complete spectrum of EAAs (26,27,61). However, due to the potential subtle differences in the ratios of whey to casein (GY is more casein-based) (46), along with their unique food matrices (GY is semi-solid and fermented) (45), the plasma AA response and delivery of nutrients from GY may differ to milk.

1.3 Rationale for investigating postprandial insulinemia and glycemia

Blood metabolites such as insulin and glucose are commonly measured in analyses of acute protein metabolism due to the effects they have on muscle tissue (62–64). Postprandial increases in insulin after protein consumption may benefit the MPS response by allowing the AAs to get into skeletal muscle quicker. That is, insulin may be helping to drive AA uptake

(62,64). However, in order to further enhance MPS, a synchronous elevation in muscle blood flow is necessary (62,63), which can be achieved through exercise. Additional research suggests that rises in circulating insulin are loosely associated with the stimulation of MPS and are rather more permissive in conditions of enhanced aminoacidemia (65,66). It is more likely that increases in circulating postprandial insulin during exercise recovery better serve to facilitate an anabolic environment by limiting muscle protein breakdown (thus contributing to net synthesis) (65,67), which plays an essential role in muscle tissue remodeling (58). Dairy products are considered to be insulinogenic (68,69). They contain a full complement of EAAs, especially branched-chain amino acids (BCAAs), that trigger insulin release from pancreatic beta cells (68,69). This aids in attenuating muscle protein breakdown and maintaining an anabolic environment (58,65). Accordingly, examination of the postprandial insulin response is necessary to further compliment the circulating AA response pattern following consumption of different protein foods. The examination of insulin in combination with AAs has yet to be assessed with GY.

Examining the postprandial blood glucose response in studies of protein metabolism is also important due to the fact that skeletal muscle, by virtue of its mass, is the largest site for glucose uptake, metabolism and storage in the human body (70). Although the current investigation involves a healthy, young population, assessing the acute postprandial glycemic (and insulinemic) responses of different (predominantly) protein wholefoods may highlight their capacity to regulate postprandial glucose homeostasis (71), and could ultimately provide context regarding their utility in populations with metabolic diseases. Research has investigated the postprandial plasma responses of insulin and glucose following the consumption of wholefood dairy products such as milk and cheese in mixed meals (24,68); however, only 2 studies, carried

out in heterogenous samples of people (young, old, men and women together), compared a single dairy food like GY to other dairy products (with varying protein contents), such as skim milk (72,73). Given the lack of specificity in the participant samples (72) and the varying dosages of protein within the dairy products (73), further examination is needed to assess these outcomes in a single population with isonitrogenous protein servings using a crossover design. Consequently, this thesis was designed to assess the effects of isonitrogenous doses of plain GY and skim milk on the postprandial plasma insulin and glucose responses in healthy young men.

1.4 Literature Review

1.4.1 Amino Acids (AA)

Dietary proteins are broken down into their amino acid (AA) constituents that are considered a critical tool for creating and sustaining muscular tissue across the lifespan (53,74–76). Twenty AAs are utilized by the body to synthesize protein, each unique in structure and sequence, which is essential for their functional capabilities (77). AAs can be further categorized into essential amino acids (EAA), which the body is incapable of synthesizing and are therefore obtained through diet, and non-essential amino acids (NEAA), which are synthesized by the body (77). With respect to MPS, research has established that an ensuing response is indicative of EAA administration, whether it is through infusion (6,78) or feeding (53,74,79,80). After an infusion of mixed AAs, Bohe et al. (6), demonstrated that plasma increases in EAAs of 50-80%, corresponded to similar increases in MPS. To better showcase the anabolic utility of EAAs, Volpi et al. (53) demonstrated that lower doses of EAAs can elicit a comparable increase in MPS to those of a higher dose balanced AA meal. In particular, ingesting 18 g of EAAs compared to 40 g of total AAs (EAA [18 g] + NEAA) induced similar MPS responses among groups of healthy elderly individuals (53). Given the non-additive effects of the NEAAs, these data confirm that EAAs are the primary drivers for activating MPS (53).

Further, among the EAAs, *in vivo* and *in vitro* research has established that the branch-chain amino acid (BCAA) leucine has significant anabolic effects on MPS stimulation (81–84). After feeding young and elderly individuals 7 g of EAA with either 1.7 or 2.8 g of leucine, Katsonos et al. (82) found that the additional leucine was able to induce MPS to a greater extent in elderly but not young individuals. Additional investigations have assessed the anabolic utility of leucine by combining it with suboptimal doses of protein in comparison to optimal doses of

protein (80,85). Churchward-Venne et al. (80) found that combining a high dose of leucine (5 g) with 6.25 g of whey protein, was able to elicit similar rates of MPS compared to a 25 g dose of whey protein (3 g of leucine) in healthy young adults. Later, the same authors demonstrated that leucine (3 g) combined with 6.25 g of whey protein, shows similar rates of MPS to a 25 g dose of whey protein (also with 3 g of leucine) (85). The above studies demonstrate collectively, the importance of leucine for the stimulation of MPS.

1.4.2 Amino Acid Digestion and Absorption

The stomach and small intestine are essential organs for protein digestion and subsequent absorption (86). However, prior to gastric and small intestine activity, the protein bolus is mechanically broken down by mastication, then it is swallowed and shuttled down the gastrointestinal tract (87,88). Once in the stomach, the protein is exposed to gastric proteases (pepsin), secreted by gastric chief cells, and hydrochloric acid through mechanical churning, which provides further protein breakdown (86,88–90). Gastric emptying rate into the small intestine (duodenum) is highly indicative of the above process (88,89,91). The products of gastric digestion are then emptied into the duodenum of the small intestine, where protein digestion continues, through proteolytic enzymatic activity from the pancreas (86,90). In particular, the pancreatic protease trypsinogen is activated by an enterokinase enzyme secreted by intestinal mucosal cells and converted into trypsin, which in turn, activates additional pancreatic proteases chymotrypsin and carboxypeptidase, all working to break down the protein constituents into smaller AA fragments and peptides (oligo-, di- and tri-peptides) (86,88,90). Further activity of aminopeptidases secreted by intestinal mucosal cells work to break down these oligopeptides into smaller peptides and free AAs, allowing for enhanced intestinal absorption (86,90).

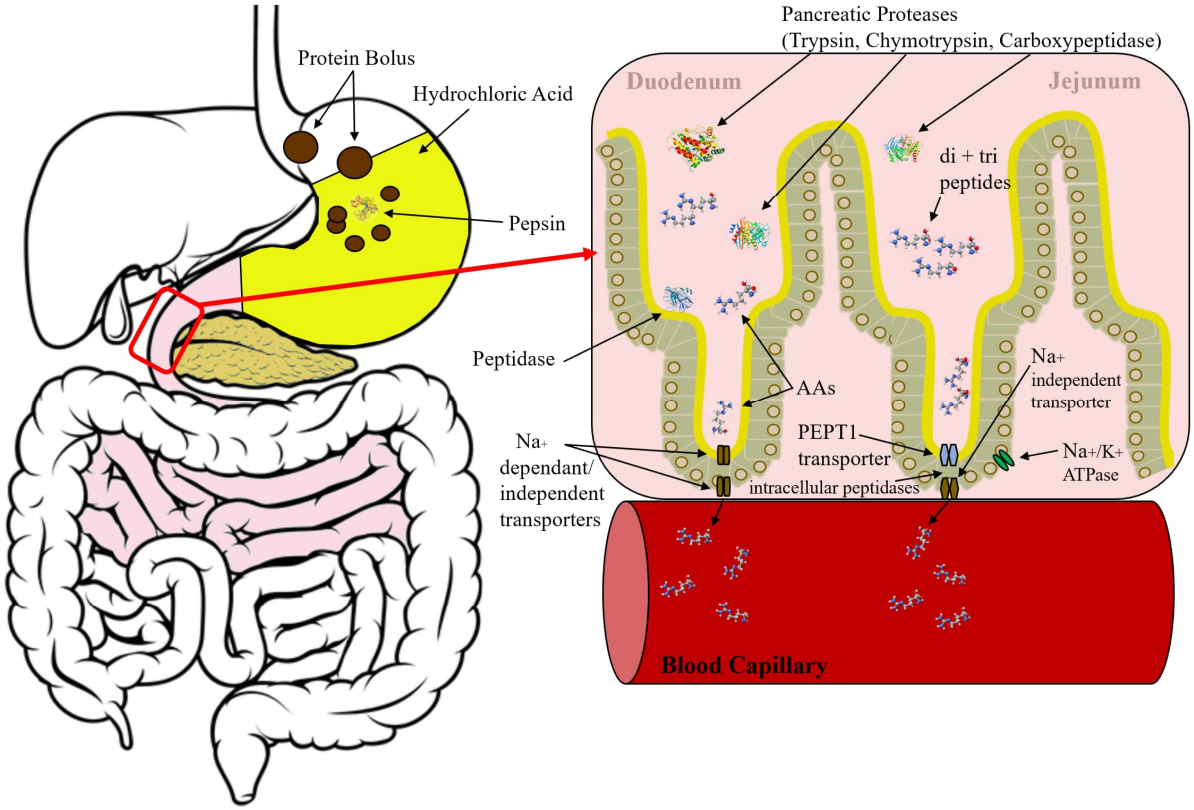


Figure 1. Schematic of protein digestion and absorption. AAs (amino acids), Sodium dependent and independent AA transporters, PEPT1 (di/tri peptide transporter), Pancreatic proteases (trypsin, chymotrypsin and carboxypeptidase) and intestinal brush border peptidase. Adapted from Goodman et al. (90).

Once broken down, AAs and small peptides in the lumen of the duodenum and jejunum must undergo absorption in order to enter the blood *via* intestinal transporters (86,90). Typically, in order to effectively pass through the intestinal enterocytes, AAs require membrane proteins (AA transport systems), to move through the intestinal brush border (86,90). AA transport systems vary in function (facilitated diffusion or secondary active transport) and in the types of AAs that cross these membrane proteins, which is dependent on the structural AA components (86,90). For example, neutral AAs such as the BCAAs, tend to migrate toward intestinal membrane proteins that utilize secondary active transport (Na+ dependent), leaving the intestinal epithelial cell across the basolateral membrane *via* facilitated diffusion (Na+ independent) (86,90). Though numerous transport systems are involved in AA delivery and departure from intestinal cells, sodium dependent/independent transporters perform the bulk of intracellular AA

transport (90). Conversely, small peptides (di/tri and oligopeptides) utilize a different form of transport system for intestinal absorption known as the di/tri peptide transporter (PEPT1) (90). AA peptides cross the intestinal brush border *via* PEPT1, and some are further hydrolyzed by intracellular peptidases prior to being shuttled into the blood *via* Na+ independent transporters (90). Coupled with peptide transport across the intestinal brush border is an electrochemical H+ gradient, removing H+ from the intestinal cell in exchange for Na+ (90,92). Na+/K+ ATPases work to remove the sodium across the basolateral membrane in exchange for potassium, while AA fragments are absorbed into the blood (90,92).

1.4.3 Protein Quality

Protein quality can be assessed predominantly by utilizing these two estimation methods: the protein digestibility corrected amino acid score (PDCAAS) and the digestible indispensable amino acid score (DIAAS) (25). PDCAAS utilizes total tract fecal digestibility in rat models to exemplify EAA efficiency in the body, whereas for DIAAS, true ileal digestibility (digestion to small intestine) in pig models is taken into consideration, incorporating the indigestible AA content within protein sources (25). Though both estimation methods depict the protein quality of food sources, DIAAS is more commonly accepted given that the bulk of true protein digestion occurs in the small intestine (25,86,90). When referring to protein quality, it is evident that animal-derived protein sources render a greater DIAAS compared to plant proteins, strictly based on the abundance of bioavailable EAAs offered, in which most plant protein sources lack. Among animal-derived protein, dairy proteins such as whey and casein are the highest quality proteins, offering DIAAS of 1.07 and 1.09, respectively (with 1.00 being the high reference score) (52,93,94). Given bovine milk is composed of a combination of whey and casein, it offers a DIAAS of 1.16, posting as one of the highest quality protein sources among animal and plant

derived proteins (95). Like milk, other dairy products are also comprised of these high-quality proteins. Table 1 depicts the DIAAS and PDCAAS for a wide range of animal and plant-derived isolated and wholefood protein sources.

Table 1. DIAAS and PDCAAS for isolated and wholefood animal and plant-derived protein sources**.**

Animal-products	DIAAS[a]	PDCAAS (nontruncated)[b]	References[c]	Plant-products	DIAAS[a]	PDCAAS (nontruncated)[b]	References[c]
Goat milk[e]	1.24		(95)	Soya flour[e]	0.89	0.98	(93)
MPC[e]	1.20	1.0 (1.21)	(93)	SPI[e]	0.84	0.93	(93)
Cow milk[e]	1.16		(95)	OPC[e]	0.67	0.69	(96)
WMP[e]	1.16	1.0 (1.16)	(95)	Peas[e]	0.65	0.79	(97)
Pork[e]	1.14	1.0	(95)	PPC[e]	0.62	0.75	(93)
WE, B[e]	1.13	1.0 (1.05)	(95)	CKB[f]	0.59	0.65	(98)
Beef[e]	1.12	1.0 (1.14)	(97)	Cooked peas[f]	0.58	0.60	(98)
Sheep milk[e]	1.09		(95)	Cooked rolled oat[f]	0.54	0.67	(98)
Casein[e,f]	1.09	1.0 (1.2)	(94)	Rye[e]	0.48	0.59	(95)
Chicken breast[e]	1.08	1.0 (1.01)	(95)	Barley[e]	0.47	0.59	(95)
WPC[e]	1.07	1.0 (1.07)	(93)	Wheat[e]	0.45	0.50	(93)
SMP[e]	1.05	1.0 (1.12)	(93)	Roasted peanuts[f]	0.43	0.51	(98)
WPI[e]	1.00	0.99	(93)	RPC	0.37	0.42	(98)
Tilapia (fish)[d]	1.00		(97)	Corn cereal[f]	0.01	0.08	(93)

[a] DIAAS were calculated from the ileal digestibility of AAs. [b] PDCAAS were calculated from the total tract digestibility of crude protein. [c] DIAAS and PDCAAS in humans, if available, growing pigs, or in growing rats. [d] Human measurement. [e] Pig measurement. [f] Rat measurement. MPC, milk protein concentrate. WMP, whole milk powder. WE, B, whole-eggs boiled. WPC, whey protein concentrate. SMP, skimmed milk protein. WPI, whey protein isolate. SPI, soy protein isolate. OPC, oat protein concentrate. PPC, pea protein concentrate. CKB, cooked kidney beans. RPC, rice protein concentrate. This table has been modified to present a comparison of animal vs. plant-based protein sources with ascending DIAAS. This table was adapted from Burd et al. (52).

1.4.4 Dairy Protein

Dairy products can be classified as 'functional foods' due to their nutrient-rich composition of protein, calcium, potassium, magnesium, phosphorus and zinc, which are essential for the development and maintenance of the musculoskeletal system (42,99–101). In particular, dairy protein is composed of a combination of whey and casein, both of which are high quality proteins (52,93) that enhance AA bioavailability (28), and ability to effectively stimulate MPS (56,102). Though both milk proteins display anabolic characteristics, each offer unique gastric emptying kinetics and thus AA delivery to the periphery (23,30,103). Overall, milk protein has complex physiochemical properties that can be further broken down into 78% caseins, 17% whey (12% lactalbumin, 5% lactoglobulin), and 5% minor proteins (2% proteose peptones and 3% non-protein nitrogen) (104). Given that casein and whey are the predominant proteins in dairy products, the physiochemical properties of these proteins will be addressed in further detail herein as they pertain to digestion and absorption. Within the milk complex, casein protein has a micellar form, which details a large fragmented, open structure that is insoluble in acidic conditions (low pH) (105,106). Owing to this, casein tends to coagulate under gastric conditions, due to a combination of interactions with gastric HCl, pepsin, and the low pH (107) (Figure 2). This results in a longer gastric retention time and thus delivery of AA to the blood plasma (23,30,108). Contrary to casein, whey protein exists within a tight, globular form, which is soluble in acidic conditions (105,106). When exposed to gastric conditions, whey protein tends to resist proteolytic activity by HCl and pepsin (107,109), moving intact through the stomach into the small intestine, where it is rapidly digested causing a quick delivery of AA into the blood plasma (23,30).

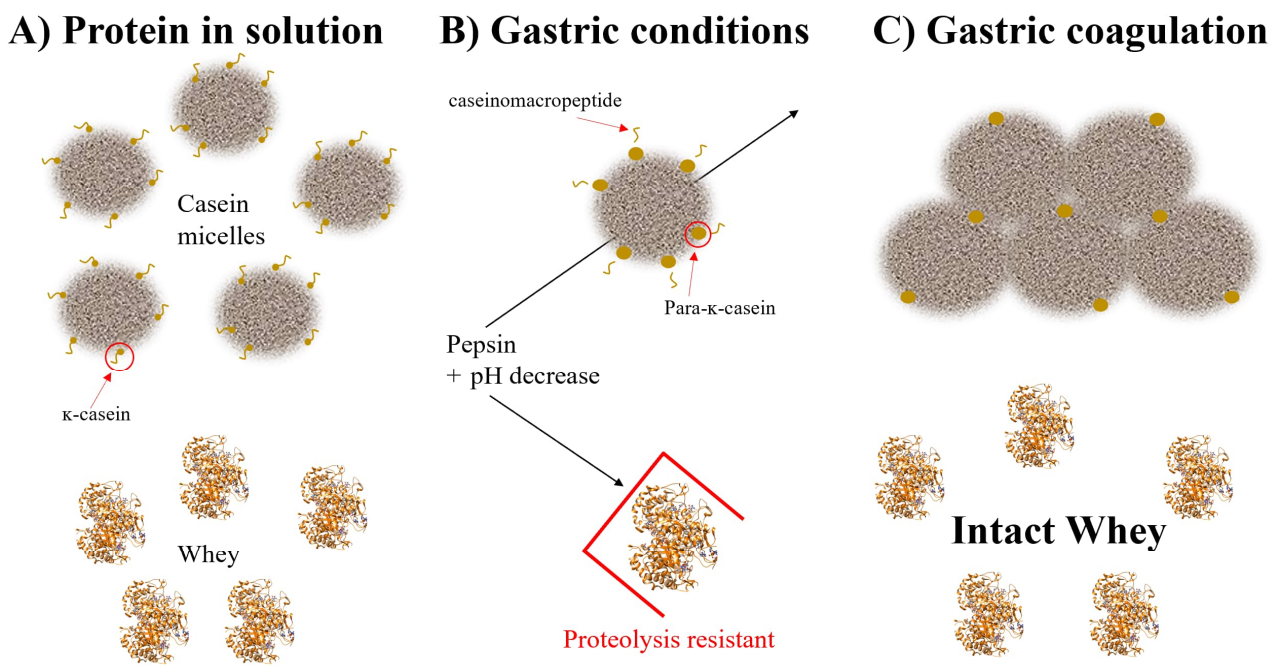


Figure 2. Schematic of casein and whey gastric digestion. A) Casein is gathered in micelles, with k-casein on the surface. Whey is gathered in a compact globular structure. B) During gastric digestion, pepsin is secreted and there is a reduction in pH. Pepsin interacts with k-casein on the surface of micelle, breaking the bond between caseinmacropeptide and para-k-casein. Whey is soluble during gastric conditions, resistant to gastric protein breakdown and remains intact. C) Casein micelle destabilization leads coagulation while whey remains intact. Adapted from Mulet-Cabero et al. (106), Wang et al. (107) and Reddy et al. (109).

1.4.5 Postprandial plasma aminoacidemia following dairy protein consumption

The postprandial plasma AA profile of dairy proteins is highly indicative of the physiochemical structure of the proteins (23,30), the protein amount administered (21,43,110,111), and the food matrix it exists in (36,52,112). Isolated dairy proteins (casein and whey), have been investigated within this context, quite extensively (14,20,23,28,36,57,103,113). For example, feeding studies in healthy young men comparing whey and casein at dosages of 30-43 g, illustrate greater peak concentrations (C_{max}) of leucine from 0-2 hours following whey consumption (~205-290%) compared to casein (~40-90%) (23,103). Interestingly, casein demonstrated greater leucine concentrations ~5 hours following

consumption (i.e., at a later timepoint), relative to whey (23,103). Similarly, research has demonstrated greater initial increases (30-90 minutes) in plasma leucine and EAAs after consumption of isonitrogenous 20 g boluses of whey compared to casein in both young (male and female) (14,113), and older men (20,28); however, no between condition differences were evident after 120 minutes (14,20,28,113). More recently, Traylor and colleagues (57) compared an isonitrogenous 25 g dose of whey and casein in young healthy men, finding whey protein to render a C_{max} of 1.54x, 1.33x and 1.31x greater than casein, for plasma leucine, BCAA and EAAs, respectively. These results were replicated in elderly men who consumed 25 g of various dairy protein sources (whey, casein, milk, yogurt and cheese), with whey protein showing a C_{max} 1.4x greater than that of casein, reaching peak concentrations around 65 minutes as opposed to 117 minutes (36). In contrast, contradictory evidence exists, suggesting little to no differences among isolated dairy proteins in postprandial aminoacidemia (114,115). In these investigations, whey protein was compared to modified caseinate forms (115) and casein hydrolysate (114), both showing similar AA AUC's to whey protein. These findings relate to the fact that modified caseinate protein (as opposed to micellar casein found naturally in dairy) is soluble in the stomach and displays a more rapid increase in postprandial blood AAs that more closely mimics whey (31). A recent investigation by Trommelen et al. (22) supports this notion after showing modified cross-linked sodium caseinate to have a greater postprandial BCAA, EAA and total AA response compared to micellar casein and calcium caseinate. These findings suggest sodium caseinate acts differently from micellar casein (22,31), and sheds light on the importance of physiochemical protein structure and its utility in relation to protein metabolism research (58,111).

Milk is a widely investigated dairy product due to its advantageous composition of both slow and fast protein constituents (35,58,111). However, it is important to note that the postprandial behavior of these dairy protein constituents may vary within the different food matrices in which they reside (116). To further explain, Churchward-Venne et al. (116) demonstrated greater plasma leucine concentrations after feeding healthy elderly men a 25 g dose of isolated micellar casein + water compared to micellar casein + bovine milk serum (i.e., casein + other milk-containing nutrients including carbohydrates). Isolated casein exceeded the casein + bovine milk serum condition from 30-180 minutes (116) indicating that additional matrix components (i.e., nutrients like carbohydrates), could attenuate postprandial aminoacidemia. When comparing postprandial plasma aminoacidemia (EAA, BCAA and leucine) after two isocaloric doses of milk and whey protein (matched for protein-20 g, fat-6 g and carbohydrate-40 g), consumed at 0 and 120 minutes, Hamarsland et al. (117) found whey to exceed milk for EAA, BCAA and leucine from 45-75 minutes, then again from 160-200 minutes. Data from Wilkinson and colleagues (32) incorporating acute RE and post-exercise protein supplementation (18 g), showed that skim milk increased TAA by 39% compared to baseline at 30 minutes, exceeding an isonitrogenous soy beverage, which exhibited a 26% change at the same postprandial timepoint. Hartman and colleagues (33) found milk to show a 26% and 50% change in plasma leucine and EAAs, respectively, after 60 minutes, with no difference when comparing to the soy condition. This may relate to the fact that milk contains whey protein, and soy beverage similarly contains fast-absorbing soy proteins so they may share similar kinetics and they are both liquids (14). Despite similar plasma kinetics between whey and soy, the fate of their AAs in the periphery have been shown to be different, whereby whey AAs preferentially go to muscle (35,118), while soy AAs are preferentially used for splanchnic (gut) protein turnover.

This finding was demonstrated using stable isotope tracer methodology (119,120). Additional research by Burd and colleagues (10) demonstrated that 30 g of protein from skim milk can rapidly deliver plasma leucine in as quick as 30 minutes, exceeding an isonitrogenous dose of beef which did not reach maximal AA concentrations until 90 minutes. This timing may relate to milk being liquid and beef being solid. Beef also showed a greater plasma AA bioavailability (64%) compared to milk (57%), and a more elevated leucine response (277%), compared to milk (231%) (10). The latter finding by Burd et al. (10) seems appropriate, given that beef has a higher DIAAS compared to skim milk (1.12 vs. 1.05) (52,93,97). Interestingly, rates of MPS reflected the postprandial AA bioavailability, with milk eliciting a greater response initially (0-2 hours), though no differences were apparent over the entire 5 hour protocol (10). Within the dairy matrix and other wholefood protein sources, other macronutrients exist, such as fat, which could also influence protein kinetics (121). In particular, Horstman et al. (36) explored the postprandial serum EAA response following consumption of 694 mL (25 g protein) of low-fat milk compared to full fat milk in elderly men, finding the low-fat condition to display a more rapid (51 vs. 91.4 minutes) and greater maximal concentration of EAAs (1.61 vs. 1.42 $mmol \cdot L^{-1}$) compared to the full fat milk. In contrast, Elliot et al. (11), found no differences when comparing blood AA concentrations (phenylalanine and threonine) among 237 g of fat-free milk and whole-milk. The latter finding could be a result of the overall small doses of protein (8 g) and fat (8.2 g vs. 0.6 g) administered (11), compared to Horstman et al. (36), which provided a 25 g protein bolus with 0.6 g fat (low fat milk), compared to 25 g protein with 25 g fat (full-fat milk). Therefore, the above findings demonstrate that fat could have an effect on postprandial aminoacidemia.

The form in which milk protein exists (i.e., milk protein concentrate-MPC or milk protein isolate-MPI) also affects postprandial AA kinetics. MPI (35,108) and MPC (59,111) are both essentially processed skim milk that is mainly casein and whey, with minimal other nutrients (122,123). In a study by Lacroix and colleagues, compared to isolated casein (23 g-protein), MPI displayed a greater maximal BCAA concentration during the initial 2 hour postprandial period, returning to baseline concentrations and below casein by 4 hours (108). When compared to soy protein, the elevated response of MPI was dampened (33% vs. 56%), with soy showing a greater maximal BCAA concentration during the first 2 hours, and MPI exceeding soy at the 4 hour timepoint (35). As mentioned above, this initial elevation, and prolonged delivery of AA to the plasma can be attributed to the combination of fast and slow proteins in MPI (122). Research assessing MPC typically displays a similar response to MPI, with rapid spikes in leucine (158%), phenylalanine (61%) and threonine (110%) 30 minutes after bolus consumption (59). These leucine, phenylalanine and threonine spikes remained elevated 34%, 15%, 52%, respectively, above baseline values at 300 minutes (59). Interestingly, this rapid yet sustained elevation in postprandial AAs led to a 2.9% incorporation of AA into the muscle from 0-120 minutes, and an additional 4.2% from 120-300 minutes (59). The above response has been compared to isolated whey and casein with MPI/MPC demonstrating an intermediate blood AA pattern to whey (with whey being the greatest), and casein with the lowest blood AA delivery pattern (111,124). In recent review by Gorissen et al. (111), when assessing plasma AA appearance (%), whey tends to exceed MPC from 60-120 minutes, with MPC having a more elevated response from the 240-300 minute timepoints (111). Taken together, evidence suggests that milk (and MPI, MPC) offers a rapid, yet sustained release of dietary AAs, a response that could be driven by the unique protein composition of its casein and

whey constituents. The underlying question then becomes: can other dairy products with similar protein constituents yet different food matrices (e.g., GY), elicit a similar postprandial plasma AA response?

Numerous studies in animal models (50,51,125,126), and humans (36–38,127–130) have been carried out assessing the postprandial AA patterns following the consumption of milk and other solid dairy food structures (i.e., cheese and yogurt-like semi-solid foods). However, there are currently limited data assessing fermented dairy products, including Greek yogurt (36,126,129). Barbé et al (50) studied the postprandial plasma AA response in mini-pigs after feeding a gelled vs liquid milk structure, demonstrating a more profound and rapid increase in plasma leucine concentrations from the liquid compared to gel matrices (205% steeper slope). Another study by the same group demonstrated marked differences in AA kinetics between different dairy gel types; acid gel (yogurt-like) and rennet gel (cheese) (125). They found similar times to peak concentration of 60 minutes for both sources, but 55% greater EAA concentrations in the acid gel condition compared to the rennet gel condition, which remained elevated 420 minutes after ingestion (125). Dupont et al. (51) conducted a review on the above studies concluding that liquid skim milk reached higher leucine concentrations initially, but stirred gels (yogurts) demonstrated more prolonged responses. Thøgersen et al. (130) investigated the effects of dairy food structure (cheddar cheese, homogenized cheese, micellar casein isolate + cream [MCI drink] or MCI gel) on postprandial blood BCAA in healthy young men. The authors found no difference in AA kinetics (leucine and BCAA) between the MCI drink compared to the gelled (yogurt-like) structure (130), which is different from the above animal data (50,51,125). Of note, the acid gels represented in these experimental models may be different from the lactic acid fermentation process utilized to create yogurt (131), therefore,

caution is warranted when drawing conclusions from these studies about the AA kinetics of yogurt. More recently, Sumi et al. (126) found different results with fermented liquid yogurt and acidified skim milk compared to skim milk, showing greater postprandial AA concentrations from fermented liquid yogurt and milk at 30-60 minutes compared to the regular skim milk, yet no differences towards the latter postprandial period (60-240 minutes). Interestingly, the free AA content of the fermented liquid yogurt was much greater than that of the acidified milk or skim milk prior to consumption, which could be a result of the fermentation process (132–135).

Early investigations in healthy adult humans comparing nitrogen kinetics after yogurt vs. milk consumption showed a delayed nitrogen gastric emptying (stomach to small intestine) in the yogurt compared to the milk condition (127,128). Though blood AAs were not assessed, this delayed response and longer gastric retention time could suggest a delayed blood AA response from yogurt. More recently, a crossover study assessed the postprandial plasma AA response of select free AAs using probiotic yogurt and acidified milk in young healthy men (129). After consuming 800 g (35 g protein) of each supplement, plasma AA levels for both showed an elevated incremental AUC response from 60-120 minutes, which returned to baseline from 240-300 minutes, with no between condition differences (129). Additionally, the impact of the dairy matrix on postprandial serum aminoacidemia was recently assessed by Horstman and colleagues in older adults (36), in which a direct comparison between low-fat regular yogurt (not Greek) and milk (25 g protein each) was assessed. Authors found yogurt to show a greater EAA response (C_{max} = 1.82 vs. 1.61 mmol·L^{-1}) during the initial 30-90 minute timepoints, with no difference from 120-300 minutes (36). Cheese was also assessed in this study showing a dampened blood AA response compared to yogurt from 30-120 minutes (36). These data are contradictory to some of the animal data (50,51,125) assessing postprandial AA responses amongst different

dairy matrices. Most recently in younger individuals, de Hart and colleagues (37) examined the acute postprandial AA kinetics after cheddar cheese and milk consumption (20g protein each). The authors found milk to exceed cheese in plasma leucine, BCAA and EAA during the initial 20-60 minutes, with cheese exceeding milk from 120-180 minutes (37). Similar findings by Hermans et al. (38) further showcase this response among cheese and milk (30 g protein each) after RE. Yogurt was not investigated in these studies. These data corroborate earlier work by Barbe et al. (50) suggesting dairy existing in a solid food form displays a dampened and more prolonged aminoacidemia compared to a liquid form. Taken together, there is limited human data assessing the plasma AA fate after yogurt compared to milk consumption, and no comparative data on Greek yogurt in healthy young people; thus, this thesis aids in addressing this gap in the literature.

1.4.6 The effects of food processing on dairy protein digestion and absorption

Most dairy products have undergone some form of processing, therefore, understanding the effects of this milk processing in order to achieve a given dairy matrix is imperative when addressing dairy protein digestion and absorption kinetics (44,136). Data suggests different dairy processing techniques/features affect the physiochemical and structural properties of protein within these foods (136–139), which likely relates to the proteins existing in different dairy matrices offering differential postprandial AA kinetic responses. The following section of the literature review discusses dairy food processing, with particular attention to acid coagulation (gel vs. liquid dairy structures), protein structural remodeling, bacterial fermentation and pH changes, and evidence for their effects on postprandial blood aminoacidemia.

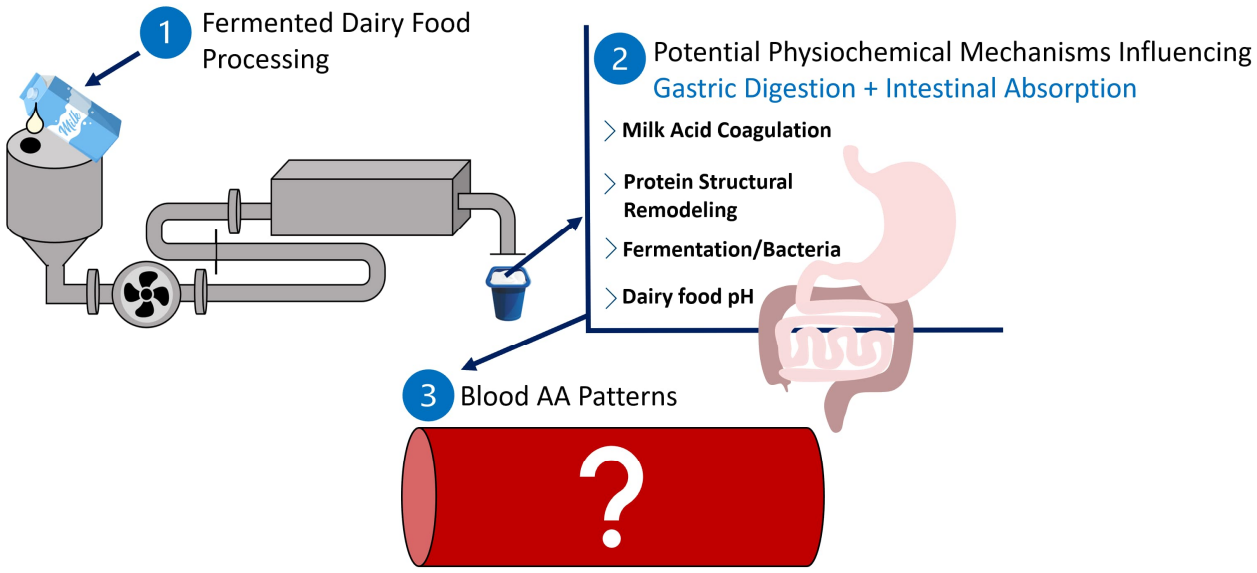


Figure 3: Overview of Physiochemical Mechanisms of Dairy Food Processing that affect postprandial blood aminoacidemia.

Milk acid coagulation is a common processing technique utilized in the dairy food industry to obtain cultured dairy products such as yogurt and cheese (140–142). Studies assessing yogurt and the effects that acid coagulation may have on protein kinetics are limited, with most being carried out in growing mini-pigs (50,125,143,144). In these studies, it is evident that yogurt displays a slower delivery of protein from the stomach to the SI (143), thus resulting in a reduction in AA appearance in the peripheral blood, when compared to liquid milk (50,125,144). More specifically, after feeding pigs yogurt or milk (18 g protein in each), Rychen et al. (144) found significantly less protein absorbed at 60 minutes in the yogurt condition compared to milk, with no difference among any other timepoint over the 240 minute study. This study shows a greater initial nitrogen delivery from milk compared to yogurt; however, a similar delivery pattern toward the later postprandial timepoints (144). Data from Barbé et al. (50) better showcased these differences among dairy matrices when comparing gelled vs. liquid milk, as the postprandial plasma leucine response was greater in the liquid milk compared to gelled matrices over a 420 minute duration. Of note, the gelled structured utilized in this investigation

was rennet formulated (a harder cheese gel), which was later compared by the same group, to an acid coagulated softer yogurt gel. The acid gel displayed a much more rapid and greater postprandial leucine response compared to the rennet gel (50,125). To contrast, data from Scanff et al. (145) in calves suggest that acid coagulated yogurt may resist casein coagulation in the stomach, and thus render a faster delivery of nitrogen components from the stomach to the SI in comparison to the milk conditions. The latter was supported by sodium dodecyl sulphate electrophoresis, in which intact casein from the yogurt was detected longer, suggesting little coagulation in the stomach compared to casein in milk, which tended to coagulate and cause protein retention (145). Additional analyses by Scanff et al. (146) indicate that hydrophobic AAs (Tyr, Phe, Ile, Leu, Val, Pro) may exhibit more gastric retention in milk compared to yogurt, which may contribute to their delayed appearance in the blood. This may relate to the consistent emptying of AAs into the duodenum after yogurt consumption and consequently, a consistently greater stimulation of pancreatic secretions (147,148), compared to the milk conditions, which experienced a different gastric emptying pattern (146). In terms of human studies assessing how acid coagulation utilized in yogurt formation may influence the rate of protein digestion, a study by Mackie et al. (149), assessed gastric retention in middle-aged men after feeding isonitrogenous 25 g doses of a semi-solid meal (cheese and low fat yogurt) and a liquid meal (caseinate solution). In this study, the liquid meal had a much faster emptying rate from 0-30 minutes; however, the semi-solid meal exceeded the liquid meal later on, from 50-70 minutes (149). Despite the fact that liquids empty faster than solids (150), this may not accurately reflect the comparison of yogurt to liquid milk, given that the liquid meal was comprised of caseinate, a processed form of casein that displays faster protein kinetics compared to milk micellar casein (22). In addition, the active meal was compromised of both cheese and yogurt, and research

suggests that cheese displays a dampened postprandial AA response due to its physical structure and rennet processing (36,51,125). Regarding gastric protein breakdown, an *in vitro* study utilizing an artificial stomach model and the same test meals with 9 gastric timepoints, found the semi-solid matrix to undergo a greater initial (G1 and G2) and later (G7 and G8) proteolysis compared to the liquid matrix, which had a constant breakdown across all timepoints (151). More recent simulated gastric yogurt and milk digestion studies show rapid casein and whey digestion in both dairy matrices from 15-30 minutes; however interestingly, yogurt displayed a greater whey hydrolysis compared to milk (152). The authors attribute this difference to the *lower pH in yogurt* allowing for greater pepsin activity and thus protein hydrolysis (152), though, there is limited evidence to validate this (125). Additional data suggest the whey component in higher viscosity dairy products (i.e., yogurt) are more easily hydrolyzed when compared to less viscous (i.e. liquid yogurt/milk) potentially due to matrix reorganization under gastric conditions (153). Given that GY possesses more casein protein than whey compared to milk (as more of the whey is removed in processing), and this casein component has been shown to be more rapidly hydrolyzed due to matrix reorganization, food pH and gastric enzyme accessibility (152,153), one could speculate yogurt to provide more bioavailable AAs for intestinal absorption when compared to milk.

Further, the post-processing dairy microstructure between milk and yogurt may also affect dairy protein digestion and absorption. Yogurt processing, in particular acidification, causes *structural modifications to dairy protein* (137–139), leading to large, porous, clustered-chained networks of casein micelles and denatured whey protein (Figure 4) (138,139,154,155). This may affect the way in which gastric enzymes interact with the protein leading to increased absorption and detection of AAs in the blood (152,156). Research suggests the size of these

pores can vary based on the yogurt processing technique, with heated and higher protein yogurt having smaller and more condensed chains (154). Further, the size of these protein aggregate microparticles can range from 1-800 μm (139). In a recent review, one study found a high protein yogurt similar to GY, to display particle sizes of ~315-520 μm, depending on the pH level (157), and this may improve AA availability. To compare, research suggests casein micelles in milk (that are prone to coagulation in the stomach) have a diameter of ~150-200 nm (137). Accordingly, this suggests yogurt protein particles may effectively pass through the ~1 mm gastric sieve pore size (158) into the small intestine at a similar rate to milk (36).

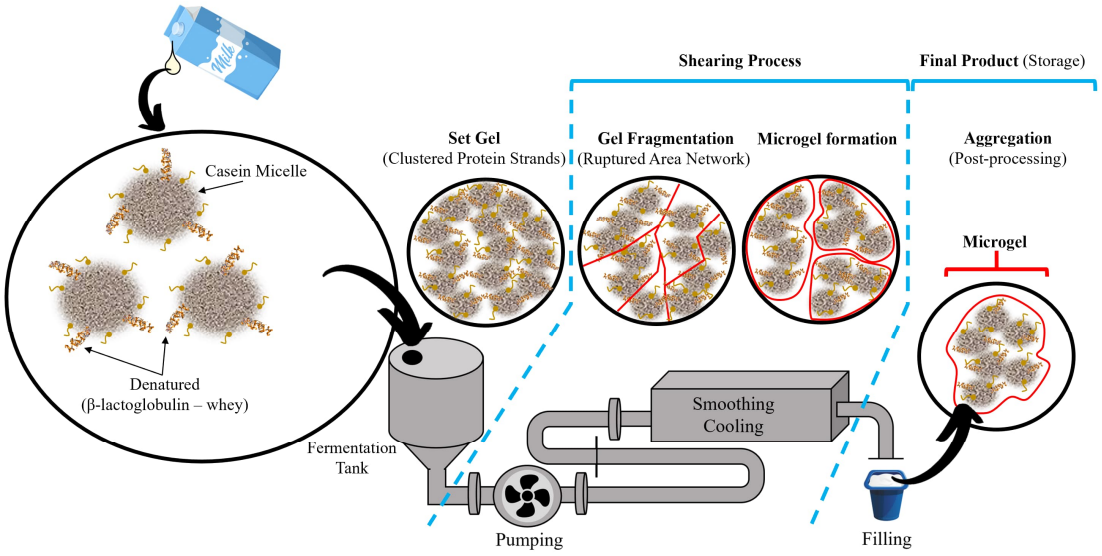


Figure 4. Schematic representation of dairy protein microstructure subjected to heat treatment and gelation via stirred yogurt processing. Heat treated milk may lead to loosely formed casein micelle coagulation and denatured whey protein interaction. To obtain a stirred yogurt structure, milk is fermented, causing loosely, clustered protein strands in the form of set gel. Post-fermentation pumping leads to a ruptured, fragmented structure, followed by smoothing and cooling, that causes the protein to take on a microgel form. Post-processing renders a reorganized microgel protein structure. These unique protein structures may affect the behaviour of the protein and clot dynamic under gastric conditions. Adapted from Gilbert et al. (139), Ye et al. (159) and Mokoonlall et al. (160).

In addition to food structure, some data indicate that bacterial cultures in fermented foods may contribute to a more efficient/rapid breakdown of the milk-protein in comparison to the milk-protein breakdown in non-fermented dairy (132–135). *Bacterial fermentation* by starter

cultures *Streptococcus salivarius* subsp. *thermophilies* and *Lactobacillus delbrueckii* subsp. *bulgaricus* acidifies the liquid milk into a coagulated semi-solid form (49); however, less is known about the proteolytic function of these cultures. Interestingly, enhanced AA profiles have been related to yogurt production and yogurt shelf-life, leading to increased amount of certain AAs prior to consumption (133–135). For example, *in vitro* data by Beshkova et al. (133) found a significant increase ($mg \cdot 100\ g^{-1}$) in leucine, valine and lysine after 168 hours of inoculation with *L. bulgaricus* strain, 120x, 14x, 15.8x, respectively, in reference to the base milk. Also, Germani et al. (135) found yogurt shelf life to have an impact on free AA production/content. Natural (plain) yogurt displayed a 97% increase in free AAs from prepackaging to a shelf life of 45 days. AAs such as histidine, aspartic acid, threonine, serine, cystine, methionine, phenylalanine and tryptophan rendered a 150% increase (135). The above data highlight the proteolytic effect of bacterial strains during fermentation and shelf life, potentially harvesting more free AAs and thus enhancing bioavailability in comparison to milk (133–135). Regarding postprandial protein metabolism, bacterial proteolysis may improve yogurt protein digestion and absorption (132,161). Specifically, during *in vitro* digestion, Matar and colleagues (132) found a greater AA availability of proline, alanine and valine after fermented milk digestion in comparison to regular/unfermented milk. More recently, a multi-strain probiotic, *L. paracasei* in combination with 20 g of pea protein, was found to yield a 22%, 16% and 23% greater plasma BCAA, EAA and leucine appearance, respectively, compared to isolated pea protein in young healthy men (161). The latter human *in vivo* finding may be specific to probiotic yogurts; whether this proteolytic effect pertains to common yogurt starter cultures; *Streptococcus salivarius* subsp. *thermophilies* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, remains unknown.

Evidence for the potential effects of bacterial proteolytic activity during simulated dairy protein digestion can be seen in findings by Lamothe et al. (162), in which greater protein breakdown (TCA soluble protein fraction %) during the initial stages of digestion (t=0-60 min) was demonstrated with semi-solid (GY-4 g) compared to liquid dairy matrices at different temperatures (milk-12 g, 65 °C and 95 °C), 38.3%, 33.1% and 34.8%, respectively. Additionally, Nguyen et al. (163) found a greater amount of smaller peptides in yogurt vs. milk toward the end of intestinal digestion compared to the early gastric phase in an *in vitro* model enhancing the bioavailability of peptides for intestinal absorption. Given the variability of simulated digestion models and *in vivo* animal models, and lack of human data, it is challenging to draw definitive conclusions; however, the above findings suggest that bacterial fermentation may impact protein digestion both before and after yogurt consumption.

1.4.7 Insulin and glucose responses to protein digestion and absorption

Both milk and yogurt contain nutrients that can affect the postprandial glucose and insulin responses, including carbohydrate in the form of lactose and certain AAs that have been shown to be insulinogenic (68,72,73). Further, incorporating postprandial assessments of insulin and glucose in combination with aminoacidemia serve to support the anabolic functionality of various dairy protein sources. An investigation by Pennings et al. (28) demonstrated in healthy older men, a more rapid insulinemia at 30-60 minutes following whey and casein hydrolysate compared to micellar casein, yet no differences in plasma glucose concentrations between conditions. These differences displayed by whey and casein hydrolysate compared to micellar casein could be a result of the enhanced delivery of insulinogenic AAs from these already hydrolyzed proteins to the blood plasma that was not evident in the micellar casein condition (28). These findings align with the majority of assessments in healthy adults (men and women),

which reveal a greater initial insulin concentration at 30-60 minutes following whey compared to casein (18-25g protein) ingestion with no difference in glucose concentrations (20,36,113,164,165). To contrast, an investigation in healthy young men comparing isonitrogenous doses of whey, casein and a blend (casein+whey) found no difference in plasma insulin and glucose concentrations (C_{max}, T_{max} and AUC), with spikes of 12.9 mU $\cdot L^{-1}$ at 46 minutes for insulin and 5.5 mmol$\cdot L^{-1}$ at 99 minutes for blood glucose (57). This lack of difference between protein beverages could be due to their similar carbohydrate content and near identical BCAA content, which are known to be insulinogenic in nature (68,69). The above data demonstrate, in some instances, that whey protein displays a greater postprandial insulin response compared to casein, and this may be due to the differing protein kinetics, processing of protein (hydrolyzed), and greater amount of BCAAs delivered to the blood from whey (68,69).

Some investigations have compared the postprandial insulin and glucose responses among multiple dairy products/constituents such as yogurt, milk, cheese, and/or whey (36,68,72,73,166). For example, in healthy individuals, Nilsson et al. (68) demonstrated that dairy protein sources, whey and milk, ingested in a mixed meal, showed similar peak insulin concentrations at 30 minutes; however, whey relative to cheese (which contains more casein) produced a more elevated response. The response between milk and cheese was similar (milk was intermediate to whey and cheese at 30 min). With respect to insulin area under the curve ($AUC_{0\text{-}90\ min}$), a noticeable difference was observed in which whey induced the greatest AUC with cheese and milk posing similar lesser responses to each other (68). The differences in blood insulin between whey and the other dairy products could be a result of whey containing a greater amount of BCAAs that get into circulation faster compared to cheese (predominantly casein) and milk, and the lack of difference between milk and cheese AUC could be because they both

contain less of a whey fraction, resulting in a delayed appearance into circulation compared to isolated whey protein (164,165). Additional investigations of wholefoods (milk, soy beverage, beef and eggs) and liquid protein isolate beverages (whey, caseinate and soy), revealed liquid foods and isolated proteins to have a more elevated plasma insulin $AUC_{0\text{-}180min}$ compared to solid foods (24). Further, skim milk showed a significantly elevated insulin response compared to other wholefoods such as beef, eggs and soy beverage at 20 minutes post consumption (24), a result which likely relates to the AA composition and state of the food. For plasma glucose, liquid foods (skim milk and soy beverage) displayed greater glucose concentrations at 20-40 minutes post consumption compared to the solid foods; however, this pattern more closely reflected the amount of carbohydrate in each wholefood, for which there is very little/none in beef and eggs. Literature in healthy adults (male and female) examining doses (17-19 g protein) of regular and fermented (i.e., yogurt) milk products in a mixed meal setting (similar kcals), found no difference in postprandial glucose and insulin over 120 minutes, both peaking around 30 minutes (166). This response was also shown by Horstman et al. (36) in older men, in which no differences were detected for serum glucose (5.5 $mmol \cdot L^{-1}$) and insulin (55 ± 3 $mU\ L^{-1}$), between low-fat yogurt and skim-milk (both 25 g protein), peaking at 30 minutes for both blood analytes. The above response was likely related to the lactose content within each dairy product, further evidenced by both yogurt and milk exceeding cheese for serum insulin and glucose (as cheese contains no carbohydrate) (36,72). Another investigation conducted in healthy young men evaluated the postprandial glycemic and insulinemic responses of isovolumetric servings (i.e., equated to ~250 g) of skim milk, orange juice and different GYs with varying protein to carbohydrate ratios (e.g., pro:carb: 2.3 [23 g pro], 1.24 [22 g pro], 0.79 [18 g pro]) (73). Results demonstrated similar incremental $iAUC_{0\text{-}120\ min}$ for insulin and total glucose following

consumption of plain GY (pro:carb: 2.3) and skim milk, although, there was a greater peak in blood glucose at 20-30 minutes with skim milk compared to plain GY, with no differences for the remainder of the 120 min protocol (73). Strawberry GY (pro:carb: 0.79) demonstrated a significantly greater insulin $iAUC_{0\text{-}120\ min}$ compared to skim milk; however, did not differ amongst the other GY conditions and orange juice (Figure 5) (73). No differences were reported in terms of total serum glucose over the 2 hours; however, glucose peaks were lower in plain GY compared to strawberry GY and orange juice (likely reflecting their carbohydrate content) (73).

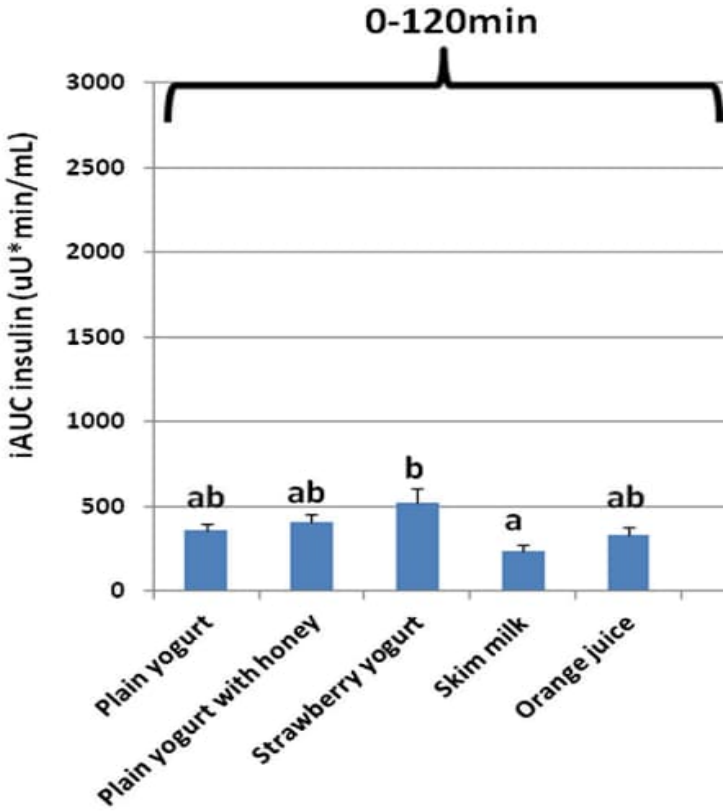


Figure 5. Incremental area under the curve (iAUC) for insulin amongst GYs with varying (carb:pro), skim milk and orange juice. Conditions without common letters are statistically different from each other. Reprinted from El Khoury D, Brown P, Smith G, Berengut S, Panahi S, Kubant R, Anderson H G. Increasing the protein to carbohydrate ratio in yogurts consumed as a snack reduces post-consumption glycemia independent of insulin. Clin Nutr [Internet]. Elsevier Ltd; 2014;33:29–38; Page 33., Copyright (2014), with permission from Elsevier (Appendix B).

More recently, an investigation of wholefood dairy products in healthy younger and older adults compared GY (175 g), whole milk and cheese to a reference serving of skim milk (250 mL) and found no differences in serum insulin between milk variations and GY, but all were higher than cheese at 30 minutes (72). Interestingly, the insulin response for GY remained elevated above skim milk from 30-90 minutes, while serum glucose concentrations were significantly lower in GY and cheese from 15-45 minutes compared to the milk conditions, yet more elevated from 90-120 minutes (72). These kinetic differences between dairy products could be attributed to the greater initial carbohydrate content in the milks compared to GY and cheese (milk-13 g and -12 g vs. GY-4 g and cheese-1 g) and the concomitant insulinogenic effects of these foods (72). Despite these postprandial differences in insulin and glucose, overall, iAUC for milk variations and GY was similar (72). Collectively, the above findings suggest minor differences in blood insulin and glucose responses between milk and GY which provides interesting insight to the responses that may be seen in the current investigation.

1.5 Objectives

Primary:

The primary objective of this thesis was to investigate the acute (4 hour), postprandial (i.e., fed state) plasma BCAA (leucine, isoleucine and valine) response following the consumption of plain GY compared to an isonitrogenous bolus of skim milk in young healthy males.

Secondary:

To investigate the acute (4 hour), postprandial (i.e., fed state) plasma insulin and glucose responses following the consumption of plain GY compared to an isonitrogenous bolus of skim milk in young healthy males.

1.6 Hypotheses

Primary:

We hypothesized that, relative to isonitrogenous skim milk, plain GY will induce a similar postprandial plasma leucine and total BCAA AUC over 4 hours, but the response will be characterized by a lower peak plasma leucine and total BCAA and a more prolonged increase relative to skim milk.

Secondary:

We hypothesize that plain GY will induce a similar postprandial plasma glucose AUC over 4 hours, as well as insulin peak concentrations and total AUC over 3 hours compared to an isonitrogenous bolus of skim milk.

CHAPTER 2: MATERIALS AND METHODS

2.1 Participants

Ten healthy, recreationally active, university-aged males (age 23.1±1.1 y; body mass 79.9±9.6 kg; BMI 25.6±2.4 kg/m^2) were recruited from the Brock University (Ontario, Canada) student population for this acute investigation. This population was selected because it was the same as was previously used by Bridge and colleagues (2019) (48). Subjects were required to have no known allergy to dairy protein and/or intolerance to lactose and were to be recreationally active. Upon satisfying the appropriate inclusion criteria and prior to participating in the study, written informed consent was obtained from each subject indicating that they understood the risks and experimental procedures associated with this investigation. This study was approved by the research ethics board of Brock University (BREB) File #: 16-295. Data collection for this study was carried out in the summer of 2018.

2.2 Experimental Design

The current investigation utilized an acute, randomized, within-subject (crossover) protocol in which participants completed 2 or 3 conditions (Greek Yogurt – GY, Skim Milk – Milk, and carbohydrate-based pudding – CHO). All ten participants underwent the two main trials (GY and milk separated by a one-week washout period. Five participants also underwent a third trial of the isoenergetic carbohydrate control that was used as the control pudding in the long-term intervention study (48). The CHO pudding trial was for proof-of-principle only and was to demonstrate that this supplement was void of protein/AAs (hence why not all participants did it). It was not used in the statistical analysis of the study. On experimental days, participants arrived at the laboratory after at least an 8-hour overnight fast (no consumption of food or liquid containing calories, water was permitted *ad libitum*). In addition, participants were asked to

abstain from any strenuous exercise and alcohol consumption 24 hours prior to each experimental trial. Each acute experimental trial involved fasted venous blood samples taken at baseline (0 min), 30, 60, 90, 120, 180 and 240 min. Amongst the blood sample collections, study supplements were ingested at two feeding points (0 and 60 min: Figure 6), to mimic the supplement protocol of our 12-week training intervention.

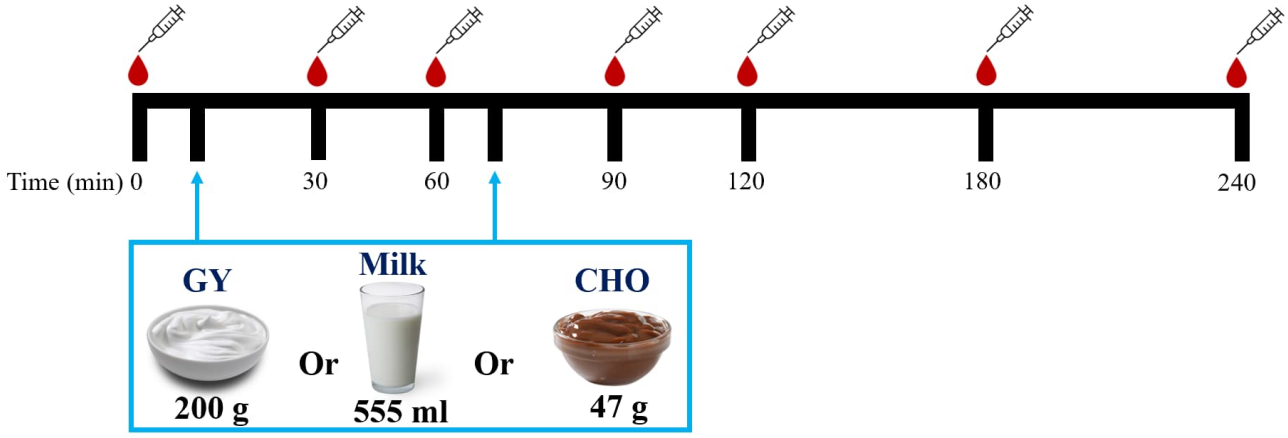


Figure 6. Sample collection and supplement consumption protocol.

2.3 Supplements

On different occasions/trials, participants consumed either 2 boluses of plain GY (200 g, 110 kcals, 20 g protein, 10 g carbohydrate) or 2 boluses of skim milk (555 ml, 200 kcal, 20 g protein, 29 g carbohydrate). Some participants also completed a third trial (n=5) where they consumed 2 boluses of an isoenergetic (to GY) carbohydrate based semi-solid pudding (47 g, 110 kcal, 0 g protein, 28 g carbohydrate). Within each trial, one bolus was consumed at baseline (following the first blood sample) and the other at 60 minutes. Supplements were ingested within 10 minutes. The rationale for utilizing a 200 g serving of plain GY was to provide participants with an experimental dose of 20 g of protein at one time, which is an amount of protein reported

to maximally stimulate MPS following exercise (4,110) and at rest (56) in young adult males. Importantly, as mentioned above, the GY and the CHO pudding were the exact same supplements that were utilized in Bridge et al. (48) and the supplements were provided using the same post-exercise paradigm (split into to 2 bolus doses separated by 1 hour). The Milk dosage was designed to also provided 20 g protein to be isonitrogenous to the GY, and was based on previous research (33,34). Table 2 depicts the macronutrient and AA composition of each dairy supplement. The complete AA composition analysis and nitrogen determination (using the Kjeldahl method) was conducted by an external company (Merieux NutriSciences, Markam, ON).

Table 2. Supplement Macronutrient Composition

	GY	Milk
Serving size	200 g	555 mL
Total Energy (kcal)	110	200
Carbohydrate (g)	10	29.4
Fat (g)	0	0
Protein (g)	20	20
Total BCAA (g)	4.76	3.60
Leucine (g)	2.24	1.72
Valine (g)	1.40	0.99
Isoleucine (g)	1.12	0.89

2.4 Sample Collection and Analysis

Blood samples were collected from superficial veins in the antecubital fossa utilizing a standard venipuncture technique and *BD Vacutainer Safety Lok* Blood Collection Sets. Blood

was collected into heparinized evacuated containers. Approximately 10 ml of blood was collected at every timepoint for each experimental trial. After collection, blood was centrifuged at 1000 g for 10 minutes at 4 °C. Plasma was then aliquoted and stored at - 80 °C until further analysis upon study completion.

The quantitative assessment of plasma BCAA concentrations was conducted utilizing a proprietary EZ:faast GC/MS analysis kit (KG0-7166) supplied by Phenomenex Inc. (Torrance, Calif., USA). The EZ:faast kit included a series of reagents and materials used for sample preparation, derivatization and quantification of BCAAs in plasma. The gas chromatographic analysis was carried out utilizing a model 6890 GC oven (Agilent, Santa Clara, CA) coupled to a mass selective detector (MSD) model 5975C (Agilent, Santa Clara, CA) in Dr. Stuart Phillips' laboratory at McMaster University. Sample preparation involved pipetting blood plasma into a preparation vial, followed by additional pipetting of the internal standard into separate preparation vials, different from the blood (167). The combined sample was then pipetted with a solid phase extraction (SPE) sorbent tip followed by a wash solution drawn through the tip (167). AAs in combined sample were then expelled utilizing an eluting medium and then the sample was ready for the addition of the derivatizing reagent which was added to each vial (167). AA derivatives were then extracted from the aqueous layer of the sample marking the completion of sample preparation (167). The GC/MS utilized electron impact ionization and was calibrated to the appropriate mode as per the Phenomenex column supplied (Phenom cgo-7169ZB-AAA; 325 °C; 10 m x 250 μm x 0.25 μm). The GC/MS system was calibrated to run a 15:1 split mode injection at a temperature of 250 °C, with aliquots of a 2 μL portion of the derivatized sample extract distributed into the multi-mode inlet via the autosampler. The gas chromatography component of the system was set at a constant pressure mode (2.9 psi), yielding a starting flow

rate of 1.4 $mL \cdot min^{-1}$ at an initial oven temperature of 110 °C. The oven program reflected as follows: 110 °C with no hold, 30 °C/min ramp to a final temperature of 320 °C and held for 1 min post run. The transfer line to the mass spectrometer was set at a fixed value of 310 °C and the ion source and quadrupole were set at 240 °C and 180 °C, respectively. Total run time was 8 minutes not including the post run. Plasma BCAAs were identified using both their retention time and by comparison of their characteristic m/z ions. The identification and quantification was carried out utilizing the scan mode of the mass spectrometer (45-450 mz) with analysis of the resulting data performed with Agilent Chemstation software (E.02.02.1431), and the provided EZ:Faast spectral database. Unknown concentrations were determined using a standard curve and calculations were made utilizing Norvaline (200 uM) as an internal standard.

Whole blood glucose concentrations were measured utilizing a *One Touch Verio Flex* Blood Glucose Monitoring System (Lifescan Canada, Inc) at the same time the blood samples were obtained. A droplet of blood was taken from each blood tube during each sample timepoint to measure glucose concentrations in the blood over the span of the 4-hour study. The droplet was transferred using a disposable transfer pipette onto the glucose strip which was inserted into the Glucometer device.

For the plasma insulin analyses, a Human Insulin ELISA kit (Crystal Chem USA, Elk Grove Village, IL) was utilized. This insulin kit is a sandwich assay that entails the immobilization of an antibody onto microplate wells with an HRP enzyme labeled antibody (168). To begin the protocol, 25 μL of sample was transferred into a microplate well. The sample was then be combined with 100 μL of diluent and incubated for 2 hours at a temperature of 37°C. The microplate was then washed, and 100 μL of substrate solution was added to the sample. The sample was then incubated for another 15 minutes at room temperature before the

microplate was washed again. To finish the assay protocol, 100 μL of stop solution was added to the sample. Optical density of insulin was measured at 450/630 nm and concentrations were determined utilizing colour intensity (168).

2.5 Statistical Analysis

All statistical analyses were conducted utilizing IBM SPSS Statistics (version 27; IBM Corp., Armonk, N.Y., USA). Significance level was set to $P < 0.05$. The primary analyses were carried out between the GY and Milk condition arms. The CHO condition was not included in the primary statistical analyses (and represents a proof-of-principle analysis to demonstrate the lack of BCAAs in the CHO supplement). Prior to all statistical analyses, data were checked, and normality was verified. A two-way repeated measures analysis of variance (RMANOVA) was utilized to assess differences between GY and Milk conditions over time for plasma leucine, total BCAAs, glucose and insulin concentration curves. Post hoc analyses (paired t-tests) using a Bonferroni corrected p values for plasma leucine, total BCAAs and glucose (P=0.0071), and for insulin (P=0.0083), were conducted if a significant time effect or interaction was found in the main RMANOVA to further explore the responses within a condition over time and between conditions at particular timepoints. The trapezoid method was implemented to calculate total AUC (tAUC) over the 240 minutes study duration. C_{max} was determined for leucine, BCAA and insulin. T_{max} was determined for leucine, BCAA and insulin as the timepoint that corresponded with C_{max}. For leucine, BCAA and insulin, a paired t-test was used to identify differences between GY and Milk conditions for C_{max} and tAUC, and a nonparametric related samples Wilcoxon signed ranked test was utilized to assess differences in T_{max}.

2.6 COVID-19: Implications on Research

The initial AA data analysis was completed by J. Brown in the late Fall/early Winter of 2019/2020 in collaboration with Dr. Stuart Phillips, Todd Prior (senior research associate) and the Exercise Metabolism Research Group (EMRG), Department of Kinesiology, McMaster University. However, some of the data were not appropriate to utilize because of unforeseen technical difficulties and issues with the GC/MS system at McMaster. In consultation with Dr. Phillips and Mr. Prior, data for some AAs were deemed unusable. Given these issues, it was decided that we would try to re-analyze the data once the machine was fixed. Then the first COVID-19 pandemic lockdown happened (March 2020) and we were unable to reanalyze any of the remaining samples we had. Unfortunately, since then, the lab at McMaster has remained closed to outside users/collaborators, and as such, we have been unable to continue with the re-analysis of our samples.

Consequently, and since the closure, we evaluated the BCAA (leucine, isoleucine and valine) data as they were suitable and usable in most participants. Thus, this thesis presents pilot AA data for BCAAs from GY and Milk, as well as their respective insulin and glucose responses.

CHAPTER 3: RESULTS

3.1 Participants

Ten participants were randomized to this acute crossover trial, all completing both experimental arms of the study. Due to technical difficulties with the GC/MS system, BCAA data for 3 participants were deemed unusable. Consequently, postprandial BCAA data from 7 participants were analyzed. Plasma insulin was analyzed from the remaining samples after the primary AA analysis. Due to a lack of remaining sample from some participants, 7 participants were incorporated in this insulin analysis. Three of these 7 participants had 1 or more missing data points, 2 from the GY trial and 1 from the Milk trial. Missing data points for individual participants were replaced with the timepoint condition mean. Condition means were added to 1 timepoint for one GY participant, and 2 timepoints for another GY participant, and to 2 timepoints for one Milk participant. Glucose data were collected and analyzed from all 10 participants, with no missing data.

3.2 Plasma Amino Acid Concentrations (Leucine and BCAAs)

Figures 7 and 8 depict the plasma leucine and total BCAA (leucine, isoleucine and valine) concentrations following the consumption of both GY and Milk, respectively. According to the main RMANOVA, there were significant main effects of time ($P<0.001$), condition ($P=0.001$) and time x condition interactions ($p<0.01$) for both the leucine and total BCAA responses (Figures 7 and 8). For plasma leucine (Figure 7), the GY condition showed a significantly elevated postprandial response above baseline concentrations, spanning 30 to 180 minutes ($P<0.0071$). For the Milk condition, plasma leucine was significant elevated from baseline at 30 minutes and trended to be elevated at 90 minutes ($P=0.058$; Figure 7). A similar prolonged elevated response by GY was also evident for BCAAs (Figure 8) from 30 to 180

minutes, and Milk demonstrated a significant increase above baseline concentrations at the 30- and 90- minute timepoints (Figure 8). In terms of differences between conditions at particular timepoints, plasma leucine increased to a greater extent after GY ingestion compared to Milk at the 60- and 120-minute timepoints ($P<0.0071$; Fig. 7), with no significant differences between conditions at other timepoints. Plasma total BCAAs showed a significantly greater response after GY ingestion compared to Milk at the 60-, 90- and 180-minute timepoints ($P<0.0071$; Fig. 8).

The plasma leucine and BCAA excursions following the ingestion of the CHO pudding supplement are also depicted in Figures 7 and 8, respectively. These results were not used in the main statistical analysis which compared the responses between GY and Milk only. The CHO supplement did not contain any protein (i.e., devoid of protein but isoenergetic to GY) which supports the inclusion of this supplement as the 'active-control' in our previously published exercise intervention (48).

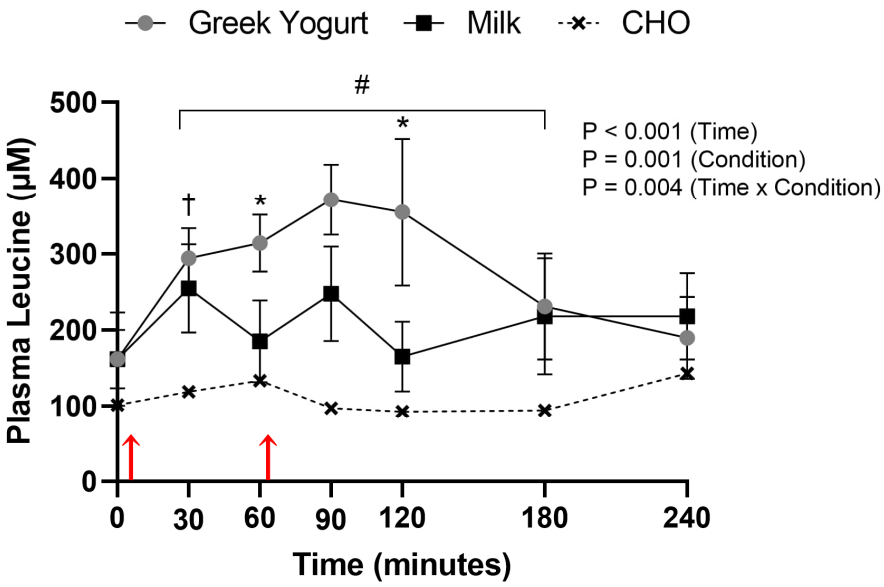


Figure 7. Plasma leucine concentrations (µM) in a fasted state (baseline) and postprandially (up to 4 hours) after consumption of two boluses of 20 g protein as either Greek Yogurt (GY-200 g) or Milk (M-

555 mL). Boluses were consumed following the first blood sample at baseline and after the 60-minute blood sample (as indicated by the red arrows). Values are expressed as mean ± SD, n = 7 per condition. Post-hoc pairwise results are indicated using different symbols indicating significance after using a Bonferroni correction (P<0.0071). * indicates GY condition was significantly different from Milk at 60 and 120 minutes. # indicates GY was significantly different from its baseline value from 30 – 180 minutes. † indicates Milk condition was significantly different from its baseline value at the 30- minute timepoint. The dashed line indicates the response from the carbohydrate condition (n=2).

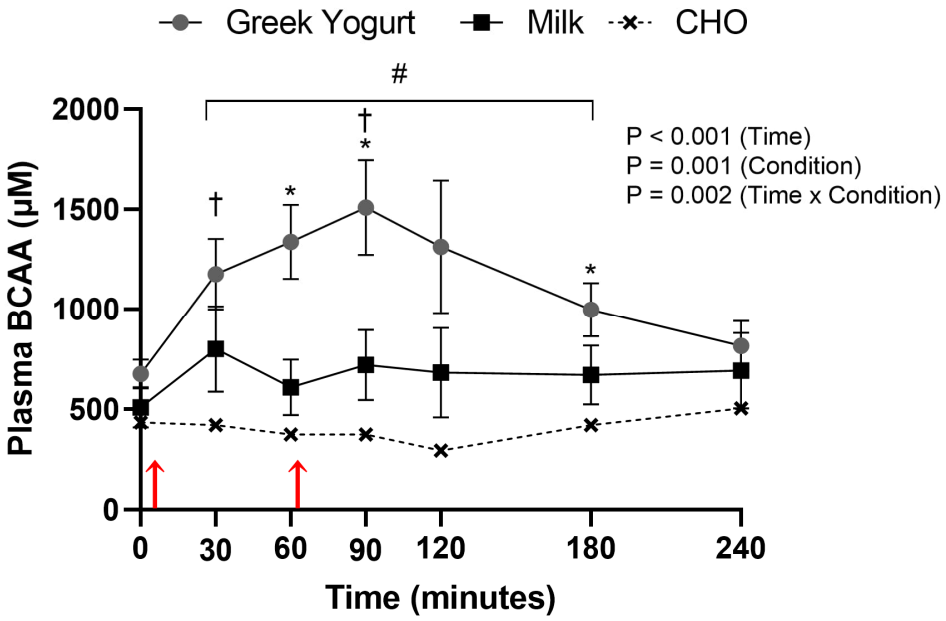


Figure 8. Plasma branched-chain amino acid (BCAA) concentrations (µM) in a fasted state (baseline) and postprandially (up to 4 hours) after consumption of two boluses of 20 g protein as either Greek Yogurt (GY-200 g) or Milk (M- 555 mL). Boluses were consumed following the first blood sample at baseline and after the 60-minute blood sample (as indicated by the red arrows). Values are means ± SD, n = 7 per condition. Post-hoc pairwise results are indicated using different symbols indicating significance after using a Bonferroni correction (P<0.0071). * indicates GY condition is statistically different from Milk at 60, 90 and 180 minutes. # indicates GY was statistically different from its baseline values from 30 – 180 minutes. † indicates Milk condition was statistically different from its baseline values at 30 and 90 minutes. The dashed line indicates the response from the carbohydrate condition (n=2).

Peak postprandial plasma leucine and total BCAA concentrations were significantly greater in the GY condition compared to Milk (C_{max}; P<0.05; Table 3). Time to maximal concentration (T_{max}) for both leucine and total BCAAs did not differ among conditions (Table 3). The AUC for the entire 4-hour period (tAUC) for plasma leucine was greater after the consumption of GY compared to milk (Table 3, Figure 9). This response was consistent for total BCAAs, with GY showing significantly greater responses (Table 3, Figure 10).

Table 3. Plasma Amino Acid Kinetics

	GY	Milk	P-value
Leucine			
C_{max} (μmol·L^{-1})	372±46	255±58	0.004
T_{max} (min)	90 (30)	90 (150)	1.00
tAUC (μmol·L^{-1}·4h)	67406±12418	48506±6362	0.004
Total BCAA			
C_{max} (μmol·L^{-1})	1509±237	800±213	0.003
T_{max} (min)	90 (30)	30 (150)	0.83
tAUC (μmol·L^{-1}·4h)	273911±34135	162269±33518	0.001

Values are means ± SD, n=7 per condition for C_{max}, maximal concentration and tAUC, total area under curve; Values are medians [inter quartile range (IQR)] for T_{max}, time to maximal concentration; BCAA, branched-chain amino acids

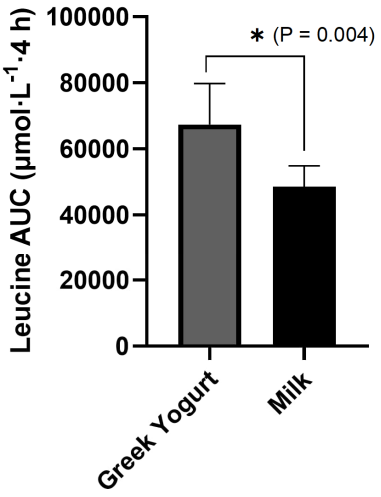


Figure 9. Plasma leucine total area under curve (tAUC, μmol·L^{-1}·4 h) over the 4-hour study period. Values are mean ± SD, n = 7 per condition. * indicates that GY was significantly different from Milk, assessed by a paired t-test (P=0.004).

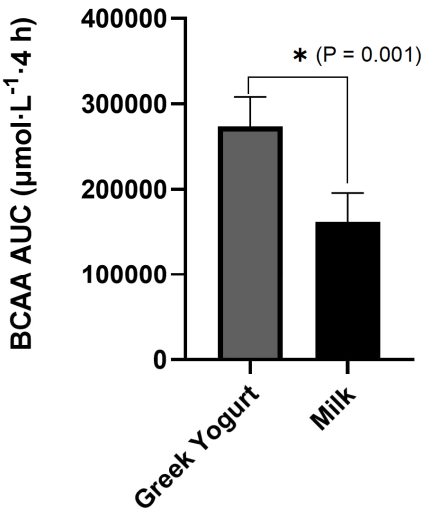


Figure 10. Plasma branched-chain amino acid (BCAA) total area under curve (tAUC, µmol·L^{-1}·4 h) over the 4-hour study period. Values are mean ± SD, n = 7 per condition. * indicates that GY was significantly different from Milk, assessed by a paired t-test (P=0.001).

3.3 Plasma Insulin and Glucose Concentrations

Figure 11 depicts the plasma insulin concentrations following the consumption of both GY and Milk. According to the main RMANOVA, there was a significant main effect of time (P<0.001) for the insulin response. There was no main effect of condition (P=0.122) or interaction (P=0.120). Post-hoc analyses reveal that plasma insulin was significantly elevated above baseline concentrations from 30 to 90 minutes after test product consumption (P < 0.00833; Figure 11). Both conditions returned to baseline concentrations from 120 minutes onwards.

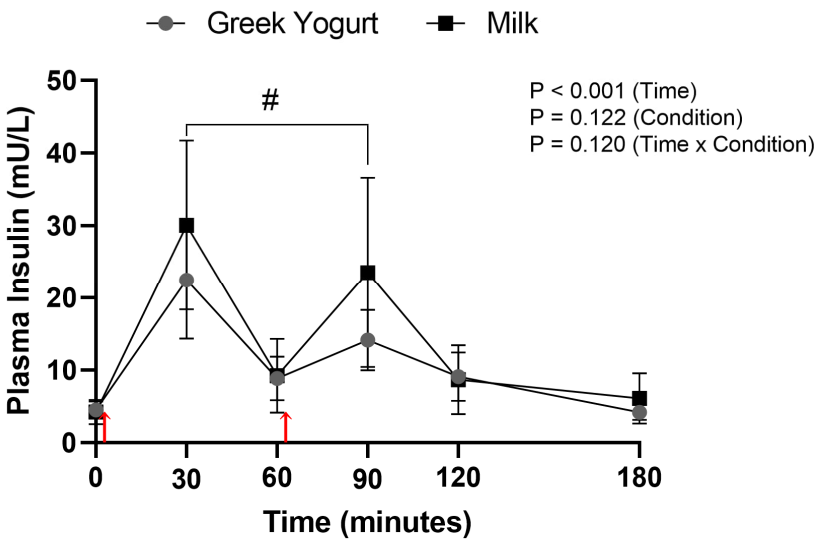


Figure 11. Plasma insulin concentrations (mU/L) in a fasted state (baseline) and postprandially (up to 3 hours) after consumption of two boluses of 20 g protein as either Greek Yogurt (GY-200 g) or Milk (M-555 mL). Boluses were consumed following the first blood sample at baseline and after the 60-minute blood sample (as indicated by the red arrows). Values are means ± SD, n = 7 per condition. Post-hoc pairwise results are indicated using symbols indicating significance after Bonferroni correction (P<0.00833). # indicates conditions are significantly different from baseline values from 30 to 90 minutes.

There was no significant difference in the peak insulin concentration between Milk and GY (Table 4; Figure 11), and both conditions shared a similar time to maximal concentration of insulin (Table 4; Figure 11). The AUC over the 3-hour postprandial period for plasma insulin also revealed no significant differences between conditions (Table 4).

Figure 12 depicts the blood glucose concentrations following the consumption of both GY and Milk. According to the main RMANOVA, there was no significant main effect of condition or interaction (P>0.05), but there was a main effect of time (P=0.003). Post hoc analyses revealed that glucose levels were significantly lower compared to baseline values from timepoints 60 to 120 minutes after test product consumption (P<0.0071; Figure 12). The AUC over the 4-hour postprandial period for blood glucose also revealed no significant differences between conditions (Table 4).

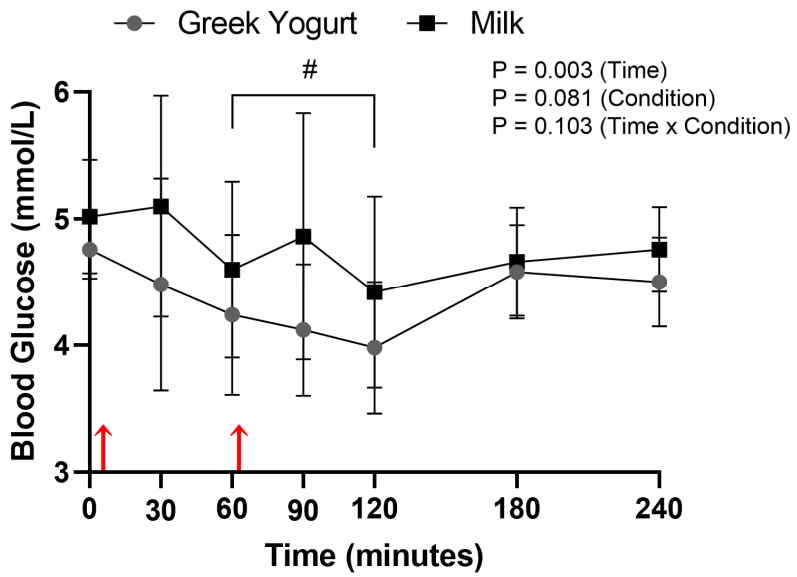


Figure 12. Blood glucose concentrations (mmol/L) in a fasted state (baseline) and postprandially (up to 4 hours) after consumption of two boluses of 20 g protein as either Greek Yogurt (GY-200 g) or Milk (M-555 mL). Values are means ± SD, n = 10 per condition. Post-hoc pairwise results are indicated using symbols indicating significance after Bonferroni correction (P<0.0071). # indicates conditions are significantly different from baseline values at the 60 to 120 minutes.

Table 4. Plasma insulin maximal concentration and time to maximal concentration, and plasma insulin and glucose total area under the curve

	GY	Milk	P-value
Insulin			
C_{max} (mU·L^{-1})	22.46±8.13	30.06±11.67	0.17
T_{max} (min)	30 (0)	30 (0)	0.32
tAUC (mU·L^{-1}·4h)	1943.16±429.07	2521.85±948.12	0.11
Glucose			
C_{max} (µmol·L^{-1})	--	--	--
T_{max} (min)	--	--	--
tAUC (µmol·L^{-1}·4h)	1106.85±140.19	1160.4±104.05	0.17

Values are means ± SD n=7 per condition for insulin C_{max}, maximal concentration and tAUC, total area under curve and n=10 per condition for glucose. tAUC, total area under curve; Values are medians [inter quartile ranges (IQR)] for T_{max}, time to reach maximal concentration.

CHAPTER 4: DISCUSSION

4.1 Overall Discussion

This thesis demonstrates that the ingestion of identical protein dosages (2 x 20 g), through different dairy foods (GY and Milk) with different food matrices and AA contents lead to different postprandial plasma BCAA patterns during a 4-hour experimental feeding protocol. Our data reveal that the consumption of plain 0% fat GY renders a significantly greater postprandial plasma leucine and total BCAA response compared to an isonitrogenous amount of 0% fat skim milk. This investigation is the first to directly compare postprandial plasma aminoacidemia following the consumption of commercially available GY and milk in young healthy males.

The postprandial influx of AAs into the blood/plasma after dietary protein consumption provides a glimpse into protein digestion and absorption, two processes that can influence the subsequent muscle protein synthetic response (6,7). The current investigation demonstrates that dairy protein, existing in the semi-solid, fermented, acidic, food matrix of GY, can effectively provide crucial EAAs (specifically leucine, valine, and isoleucine [BCAAs]) for muscle adaptation and recovery, in the blood, to a greater extent than the more often investigated liquid milk.

4.1.1 Plasma AA concentrations

Though the anabolic utility (i.e., ability to stimulate muscle protein synthesis [MPS]) of these two foods was not directly assessed, our data provide an interesting look at the delivery pattern of BCAAs to the blood stream after ingestion of different wholefood dairy products with different food matrices. We demonstrate plasma responses for both foods that are analogous to what others have shown to effectively stimulate MPS in healthy young men (10). Specifically,

Burd and colleagues (10), demonstrated that minced beef (30 g protein) displayed a greater maximal leucine concentration compared to isonitrogenous milk (C_{max}; 277 vs. 231 μmol/L, respectively), with both stimulating MPS to a similar extent post-exercise over 5 hours. Our data demonstrate a postprandial leucine maximal concentration of 372 umol/L after the consumption of 2 boluses of GY (2x20 g of protein; 25% more protein than Burd et al), which may suggest a similar (or even greater) MPS response between GY and beef (10). Given that the postprandial AA response and AA bioavailability is implicated in the MPS response and muscle utilization of AAs, our data provided important context to our recent intervention study demonstrating that supplemental GY consumption over 12 weeks resulted in greater training-induced increases in lean mass compared to the carbohydrate pudding (48).

When addressing blood AA concentrations among dairy products, our data is congruent with recent work by Horstman et al. (36), who demonstrated that 532 mL (25 g protein) of low fat regular yogurt, displays a greater postprandial AA response compared to an isonitrogenous dose of 676 mL skim milk in healthy elderly men. Interestingly, with a smaller serving size of GY (200 g), we demonstrated a similar response in healthy young men, whereby GY exceeded Milk in maximal AA concentration (i.e., C_{max}). Both Horstman et al. (36) and the current investigation shed light on the effects of the dairy food matrix and postprandial AA handling but at different stages across the lifespan. Also, both studies exemplify the utility of yogurt for supplying both young and elderly populations with an adequate amount of AAs to the blood. Indeed, elderly individuals have been shown to display dampened blood AA concentrations after ingestion of dairy protein (111). The current investigation displayed an elevated blood AA appearance in healthy young men that was similar to what Horstman et al. saw in the elderly (albeit with a much larger intake of regular yogurt) (36). Thus, when consuming wholefoods, it

may be important to ensure that older adults consume more AAs and/or a more concentrated source of AAs (i.e., GY) in order to achieve adequate blood AAs for metabolism and anabolism; GY may help attenuate the effects of age-related sarcopenia (76). While protein supplementation alone (i.e., whey protein powder) may be an important strategy for older people to ensure adequate daily protein intakes, it is important to note that wholefood dairy products also offer additional bone supporting nutrients which contribute greatly to enhanced musculoskeletal health and overall diet quality compared to protein alone (27). Nevertheless, despite age-related differences in blood AA levels following consumption of semi-solid yogurt, both investigations found that isonitrogenous boluses of yogurt displayed greater amounts of blood leucine, total BCAA, and total EAA (in Horstman only) compared to skim milk (36). The question then becomes, what components of these different food matrices contribute to the differences in AA delivery to the blood?

In addition to assessing the postprandial plasma BCAA concentrations following the consumption of dairy products, we also determined the total nitrogen/protein and individual AA content of each dairy product *via* the Kjeldahl method (169). This analysis (done by an outside company) was essential to our understanding of our results as it provides information on the exact AA composition of each dairy product, which we used to inform the postprandial blood response seen in the current investigation. More specifically, within an isonitrogenous bolus of each food, a complete AA analysis revealed that GY had a greater leucine (2.24 g) and total BCAA (4.27 g) content compared to Milk (1.72 g and 3.60 g, respectively; Table 2). These findings corroborate our postprandial plasma AA responses, in which GY showed greater plasma leucine and total BCAA concentrations overall. It has been previously shown by Nakayama et al. (170) that the food product AA composition reflects the postprandial plasma AA response

following ingestion. Specifically, they investigated blood aminoacidemia after ingestion of a whey protein hydrolysate (WPH) and an EAA mixture in healthy young men. WPH with varying leucine contents (3.0, 4.5 and 6.8 mmol) showed a stepwise blood leucine concentration, and when compared to the EAA mixture (4.1 mmol leucine), the WPH with 4.5 and 6.8 mmol, significantly exceeded the mixture for both blood leucine concentrations and tAUC over 2 postprandial hours (170). Despite this stepwise increase in blood AA with increased leucine concentration, using AA content as the only factor to infer AA delivery may be not accurate since varying protein/AA formulations and matrices may play a role. Again, looking to Burd and colleagues (10), they found beef to display a much greater blood leucine concentration over time compared to an isonitrogenous dose of milk, despite milk having a slightly greater leucine content (2.71 g) compared to beef (2.48 g). Thus, despite being composed of similar total protein and EAA content, different protein foods render different AA bioavailabilities (52) likely through factors and mechanisms relating to altered protein digestion and absorption (10).

One such factor is bacterial fermentation. It plays a pivotal role during the processing of milk to achieve semi-solid acidified yogurt (49); however, extending beyond this altered food form, is the proteolytic function of these bacterial cultures which could provide a further reason for the enhanced blood AA response after yogurt consumption (132,152). Indeed, bacterial fermentation displays enhanced proteolytic activity during food processing that is further extended during yogurt's shelf life, leading to an increased amount of free AAs in the food prior to consumption/digestion (133–135). Also, the bacterial proteolytic function of GY further contributes to the breakdown of larger casein and whey peptides into smaller di/tri peptides (i.e., the pre-digestion of protein) also leading to a more potent/elevated aminoacidemia (132,152). Dong and colleagues (152) demonstrated, using an artificial stomach system, that yogurt

produced a greater number of smaller peptides compared to milk 60 minutes after simulated gastric digestion, which could be attributed to yogurt's bacterial proteolytic function. Given that AAs can be absorbed in both free AA form *via* AA transporters and in small peptide (di/tripeptide) form *via* peptide transporters followed by subsequent intestinal intracellular peptidase activity (see Figure 1) (90), it is likely that the enhanced small peptide content in yogurt following digestion could also help promote a greater AA response in the blood (vs. Milk) (170). Thus, the above findings by Dong and colleagues (152) may help explain, in part, the elevated response in plasma leucine and BCAAs seen in the current investigation.

Further justification for the elevated blood leucine and BCAA response with yogurt demonstrated in the current investigation comes from *in vivo* animal work by Scanff et al. (146), that showed greater gastric retention of hydrophobic AAs (including leucine and BCAAs) in calves after consumption of pasteurized compared to fermented milk (i.e., liquid yogurt). These data suggest that a greater amount of leucine and BCAAs make it to the duodenum after consumption of fermented milk compared to non-fermented (regular) milk (146), which would result in a greater concentration of these AAs and small peptides in the blood following intestinal absorption. Further evidence for this comes from the notion that milk casein tends to coagulate in gastric (i.e., acidic) conditions; whereas, yogurt (predominantly casein) experiences much less gastric coagulation, and consequently, minimal gastric retention (145). This could be attributed to its already lower pH (compared to milk) (Figure 13) (125,126,152,171). Minimal gastric structural changes to the protein in yogurt may partially explain the enhanced delivery of protein to the duodenum. Specifically, the limited conformational changes to the protein during the exposure of yogurt to gastric conditions may be a result of an impaired ability of pepsin to break down the k-casein-casein micelle complex, thus inhibiting gastric coagulation (Figure 13) (171).

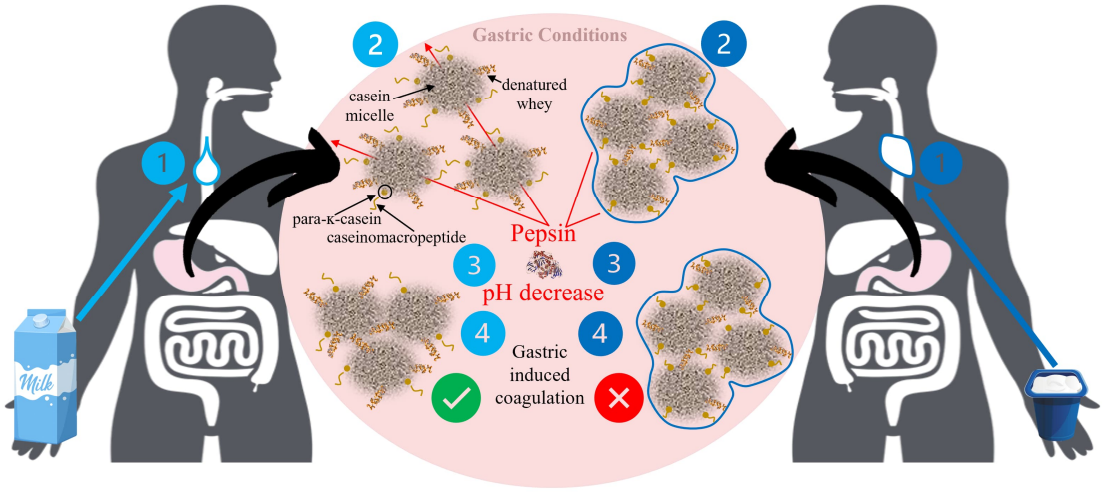


Figure 13. Schematic representation of potential dairy protein restructuring under human gastric conditions. 1) Buccal phase of protein digestion with dairy food moving toward the stomach. 2) Gastric phase of protein digestion with dairy products subjected to the gastric environment of the stomach. 3) Gastric pepsin and pH reductions induce structural changes to casein micelle, causing instability (para-κ-casein cleavage) to casein in milk, due to higher initial pH level. 4) Gastric-induced coagulation of milk protein, with little alteration to the protein structure of yogurt. Figure adapted from Mulet-Cabero et al. (106), Ye et al. (159,171), Gilbert et al. (139) and Mokoonlall et al. (160).

Sumi et al. (126) assessed the effects of gastric pH on postprandial aminoacidemia. They fed rats either fermented milk, acidified milk or skim milk, and implemented a proton pump inhibitory treatment (PPI), which blocked the function of gastric-induced coagulation of skim milk. In doing so, they displayed a greater leucine content in the blood compared to normal gastric conditions. In both cases (PPI present or absent), fermented milk exceeded skim milk in postprandial blood leucine and EAAs (126), which may, in part, help to explain the findings in the current investigation. Further, Menard and colleagues (153) suggest that the AAs of viscous yogurts may populate a fixed proximal portion of the SI as opposed to less viscous yogurt, which was found to leave the duodenum and populate different areas of the SI. The above may be true for the test products in the current investigation, with milk exhibiting a less viscous food form

compared to GY and potentially, a lower bioavailability of AAs in the duodenum (the primary point for AA absorption), leading to a dampened AA response in the blood. Collectively, the above data provide support to the current findings suggesting that GY would provide a greater amount of blood leucine and BCAAs compared to milk because a) greater bioavailability of hydrophobic AAs (BCAAs) for intestinal absorption, b) minimal structural changes and less gastric-induced coagulation in yogurt, c) more bioavailable AAs directed to the primary point of intestinal absorption (the duodenum).

The current investigation demonstrated greater concentrations of blood leucine and BCAAs after consumption of GY compared to Milk. When assessing the absorption kinetics, we demonstrated no differences in the time to maximal concentration between test products. The above finding seems plausible when comparing to a recent investigation by Horstman et al. (36), which displayed increases in blood AAs within 60 minutes of yogurt and milk consumption, with no differences in T_{max} among dairy products. This elevation lasted until the 120-minute postprandial timepoint after only one baseline feeding (36). In the current investigation, due to the consumption of the second bolus of food at the 60-minute timepoint, the concentration of AAs continued to rise toward the later postprandial timepoints (i.e., producing a cumulative effect), which rendered a later maximal concentration (C_{max}) compared to Horstman et al. (36). Additionally, data suggest that dairy macrostructure (food form) may influence the rate of protein digestion among gelled (yogurt) and liquid (milk) dairy matrices (50,51). Specifically, Barbé and colleagues (50) demonstrated a greater and faster postprandial plasma leucinemia after feeding mini-pigs liquid dairy compared to gelled dairy. These data differ from our current findings, suggesting that a gelled dairy macrostructure (yogurt) may offer a later peak plasma leucinemia compared to a liquid milk matrix (50,51). In addition to a longer T_{max}, the above data

found a dampened leucine bioavailability in the gelled matrix compared to the liquid (50) which is contradictory to the findings of the current investigation, and suggests that food form is only one of several concomitant components affecting kinetics. The same group also compared the effect of an acidified dairy gel (yogurt) compared to a rennet gel (cheese) and found that the acidified dairy matrix displayed a significantly greater blood leucinemia compared to the rennet (125). This highlights the importance of acidification to postprandial AA kinetics, whereby it contributes to a greater AA bioavailability vs. similar non-acidic foods (125). Importantly, acidification in the above investigation was done utilizing glucono-δ-lactone (125); a process that is different from bacterial fermentation, nevertheless, both act to acidify the food matrix, but in addition to protein conformational changes, fermentation also has a proteolytic function. Thus, our current findings of a greater (C_{max}) and similar (T_{max}) of AAs when comparing GY to Milk are likely influenced by elements of the dairy macrostructure (i.e., food form), microstructure (i.e., fermentation and matrix restructuring), as well as protein composition (i.e., more leucine and BCAAs in GY vs. Milk). These collectively serve to best explain the findings of the current investigation.

4.1.2 Blood Insulin and Glucose

In the current investigation, plasma insulin significantly increased after the consumption of both dairy products, with no between condition differences. These findings are interesting given the varying carbohydrate contents within the foods (Milk 29.4 g CHO vs. GY 10 g CHO), which is often associated with rises in postprandial insulin (72,73). Indeed, greater rises 30 min post-ingestion of both boluses were apparent for Milk versus GY, but these differences did not reach statistical significance. Nonetheless, GY contained more leucine and BCAAs which are insulinogenic (68,69), which could explain the plasma insulin responses of both foods despite

different carbohydrate contents. Horstman and colleagues (36) displayed similar findings where skim milk and regular yogurt showed no significant differences in postprandial plasma insulin concentrations, yet the CHO content of the milk (33.8 g) exceeded that of the yogurt (21.3 g). Other research investigating postprandial insulinemia following GY compared to skim milk in young and elderly adults showed no differences 30 minutes after consumption, but GY remained elevated until 90 minutes compared to skim milk (72).

In the current investigation, blood glucose significantly decreased after the consumption of both dairy products, with no between condition differences. For both products, blood glucose significantly decreased compared to baseline levels from 60 to 120 minutes and then returned to baseline and there were no differences in the tAUC for glucose between GY and Milk. The glucose responses among dairy products could relate to the carbohydrate content of each product with Milk exceeding GY, and the quicker bioavailability of carbohydrate from liquid milk compared to semi-solid GY. Vien and colleagues (72) similarly demonstrated no differences in iAUC between GY and skim milk when glucose was assessed in young individuals only; however, in a combination of young and old persons, skim milk exceeded GY at 30-45 minutes post-consumption, with no differences from 60 minutes onward (72).

4.2 Implications

The utility of dairy as a muscle-supporting food has been established given its enhanced AA bioavailability (28), and rich composition of leucine and EAAs (52,93). The results from the current investigation not only showcase GY in this regard, but also demonstrate the anabolic utility of dairy in general, as a functional food group. The anabolic utility of GY in healthy young men has been demonstrated in our recent resistance training intervention (48) where young males gained more lean mass following 12 weeks of exercise and GY consumption

compared to CHO. Our current results contribute to a deeper understanding of this response. Our data further indicate that GY, a dairy product that is not the often-investigated liquid milk, can provide a greater amount of dietary leucine and BCAAs to the blood for a given isonitrogenous bolus. These are essential key nutrients for the growth and maintenance of muscle tissue. Investigating a variety of dairy foods (and other protein foods including meat and plant-based sources) regarding protein/AA delivery to the bloodstream is important, given that the different properties of the food can implicate the conditions surrounding how AAs get to the blood and thus their bioavailability for anabolism (at the skeletal muscle and elsewhere). Indeed, research shows that the postprandial blood AA response relates to the muscle protein synthetic response (6,7,37). Further, given the current dietary intake trends in our population, suggesting that milk consumption is decreasing while other milk products (including yogurt) are increasing (172), it remains important to investigate other high-quality dairy protein sources beyond milk. GY is comprised of almost double the amount of protein per serving compared to milk (173), with a greater postprandial aminoacidemia that has been found to sufficiently stimulate MPS (10). For the above reasons, GY should be deemed an effective wholefood alternative to milk for meeting human dietary protein needs, and for providing advantageous nutrients to best support our muscles. This is particularly important to elderly populations who are more susceptible to age-related sarcopenia (27,76) and suboptimal nutrient intakes (172) and perhaps also to other populations with muscular diseases (174,175). Additional data suggest that elderly individuals display a dampened postprandial AA response and AA bioavailability compared to their younger counterparts, potentially leading to muscle loss if an adequate amount of AAs are not consumed (111). It is therefore prudent that older individuals consume high-protein wholefoods such as GY (that also provide additional supporting micronutrients), to compensate

for this attenuated AA bioavailability (27). Lastly, individuals who wish to obtain exercise-induced gains in muscle mass and strength may also benefit from GY consumption as demonstrated in our recent investigation assessing GY supplementation in young healthy males (48). In particular, and according to a recent systematic review and meta-analysis, individuals who are seeking such gains should consume between ~1.6-2.2 g/kg of protein daily (176,177), amounts that are attainable through ingestion of high protein wholefoods, including GY, as we have demonstrated (48).

Extending beyond protein, GY may offer additional healthful benefits that stem from its unique food form and physiochemical formulation (45,72). For example, some data suggest consuming GY may be a beneficial strategy for weight management due to the semi-solid food form enhancing satiety (compared to liquid milk) (45). Specifically, Vien and colleagues (72) demonstrated that GY displayed greater postprandial appetite suppression when compared to skim milk. Also, GY may offer further utility in the diet due to its fermentation properties and bacterial composition. Some bacteria strains in certain GYs can promote enhanced AA bioavailability (161), and serve to maintain a healthy gut microbiome through microbiota alterations (45). The consumption of yogurt for all these reasons is also an appropriate strategy to enhance overall diet quality. This has been demonstrated by Panahi and colleagues (178) in younger and older female populations.

4.3 Strengths and Limitations

4.3.1 Strengths

The findings from this thesis add to protein metabolism research. They corroborate and strengthen the results of our 12-week GY and resistance training intervention study, given that the supplement consumption protocol was the same. The current investigation utilized a

crossover design, whereby each participant completed both experimental trials/conditions. Implementing this approach allowed for effective and best practice comparisons of blood AAs between conditions, given that each participant served as their own control. This mitigated some of the inter-subject variability typically seen with parallel study designs (179). Further, all supplements were prepared by the same individual and this helped maintain consistency and reduce variability. Lastly, our results using wholefoods as opposed to isolated nutrients are more translatable to the general public (172,180) since diets typically involve the consumption of wholefoods/meals that supply nutrients within a wholefood matrix rather than as isolated nutrient sources (112).

4.3.2 Limitations

The most evident limitation to our dataset is that we were unable to analyze all AAs or even all the EAAs (despite being most interested in the BCAA response) due to technical difficulties with the GC/MS system (mentioned in the Methods section). Though leucine is considered the most potent stimulator of MPS (81–84,181), all EAAs have a role in this response (53). We also had to reduce our sample size from n=10 to n=7, because of issues with the GC/MS. Nonetheless, we still demonstrated a robust response to both supplements with only 7 individuals. Additionally, although AA digestion and absorption influence the response in the muscle (6,7), MPS was not an endpoint in the current investigation; thus, we are unable to say that our findings in the blood directly relate to responses in the muscle. Finally, we only studied young healthy males. Although evidence suggests that there are no sex differences in postprandial protein metabolism following equated protein ingestion (182), future research should incorporate both sexes and a wider age range to see if similar findings can be revealed.

4.4 Future Directions

Further research is needed to more intricately assess the differences between the anabolic responses of GY and Milk. More specifically, obtaining muscle biopsies after the consumption of these wholefood protein sources would be optimal. Burd and colleagues (10) displayed notable differences in postprandial aminoacidemia after a single bout of exercise with beef or milk consumption; however, no differences between foods were evident in MPS over the entire 5 hour protocol. In contrast, de Hart et al. (37) found cheddar cheese to display a dampened blood AA response compared to microfiltered milk, and this response was replicated at the muscle level *via* lower mTOR signaling with cheese. Thus, it would be prudent to compliment blood AA patterns (leucine, BCAAs, EAAs and TAAs) with measures of MPS and signaling to represent the entire acute anabolic effect of these dairy products (52). Recently, a study in rodents assessed the anabolic response of regular yogurt compared to skim milk demonstrating that fermented dairy renders a greater portal vein leucinemia and subsequent rate of MPS (126); however no such data exist in humans utilizing GY. Furthermore, fermented dairy products may contain certain bacterial cultures (some of which are specifically probiotic) which not only enhance gut health (45,183), but also AA bioavailability (161). Additional research should assess probiotic GY vs. non-probiotic GY (i.e., GY with cultures that are not specifically probiotic), and analyze the postprandial blood AA bioavailability and subsequent response in the muscle, which would allow us to determine the potential effects of differentially fermented dairy on AA kinetics and muscle anabolism.

The majority of protein metabolism research, including the current investigation, examines postprandial aminoacidemia acutely. Assessing the effects of how chronic consumption of a protein food can influence/change the postprandial blood aminoacidemia could

be interesting and applicable to regular consumers of certain protein sources. This could be done through the implementation of a baseline acute feeding challenge, followed by a chronic feeding intervention with a specific wholefood, then finishing with another acute feeding challenge. With yogurt, for example, chronic feeding could alter the gut microbiome leading to altered acute metabolism. More specifically, chronic yogurt consumption improves gut barrier function (184,185), which may in turn maintain efficient villi function and protein absorption. Further, due to the rising consumption of GY in the population (and milk becoming less popular) (172), investigating a wider spectrum of dairy foods and protein food matrices (both animal- and plant-derived) in regard to postprandial aminoacidemia and MPS would be prudent. The above has been assessed in the blood (24,36,186); however building upon these data with muscle analyses would be optimal (41,187). Finally, as suggested by Jakobsson in a commentary regarding our recent intervention study (60), it would be interesting to conduct an additional resistance exercise intervention comparing GY and Milk as this would relate most to the current findings of this thesis and shed light on the effects that the food matrix and food composition have on post-exercise protein metabolism leading to longer-term lean mass gains.

4.5 Conclusions

This thesis demonstrated that isonitrogenous doses of dairy protein existing in different food matrices renders different blood leucine and BCAA patterns over a 4-hour postprandial period. Plasma leucine and total BCAA concentrations were greater after the consumption of GY compared to Milk, with no differences in time to maximal concentration. GY exceeded milk in tAUC over the entire experimental duration for both leucine and total BCAAs. It is likely that the greater plasma concentration of leucine was, in part, a result of the greater leucine concentration within GY compared to Milk, but also the different physicochemical properties of

the foods. Indeed, technological food processing to obtain a given food matrix has an impact on postprandial aminoacidemia. These findings support our recent intervention study findings using GY and support the role of GY in eliciting an appropriate and enhanced delivery (vs. Milk) of important AAs to the blood, which when consumed post-RT, may compliment exercise-induced increases in lean mass. Further research is needed to better understand the mechanisms that contribute to the different leucine and total BCAA patterns, including whether gastrointestinal (i.e., digestion/absorption) kinetics differ between GY and milk.

CHAPTER 5: REFERENCES

1. Trommelen J. Symposium two : Novel methods for assessing protein metabolism Assessing the whole-body protein synthetic response to feeding in vivo in human subjects Proceedings of the Nutrition Society. 2021;1–9.
2. Burd NA, Hamer HM, Pennings B, Pellikaan WF, Senden JMG, Gijsen AP, van Loon LJC. Substantial Differences between Organ and Muscle Specific Tracer Incorporation Rates in a Lactating Dairy Cow. PLoS One. 2013;8.
3. Biolo G, Tipton KD, Klein S, Wolfe RR. An abundant supply of amino acids enhances the metabolic effect of exercise on muscle protein. Am J Physiol - Endocrinol Metab. 1997;273.
4. Morton RW, McGlory C, Phillips SM. Nutritional interventions to augment resistance training-induced skeletal muscle hypertrophy. Front Physiol. 2015;6:245.
5. McGlory C, van Vliet S, Stokes T, Mittendorfer B, Phillips SM. The impact of exercise and nutrition on the regulation of skeletal muscle mass. J Physiol. 2019;597:1251–8.
6. Bohé J, Low A, Wolfe RR, Rennie MJ. Human muscle protein synthesis is modulated by extracellular, not intramuscular amino acid availability: A dose-response study. J Physiol. 2003;552:315–24.
7. West DWD, Burd NA, Coffey VG, Baker SK, Burke LM, Hawley JA, Moore DR, Stellingwerff T, Phillips SM. Rapid aminoacidemia enhances myofibrillar protein synthesis and anabolic intramuscular signaling responses after resistance exercise. Am J Clin Nutr. 2011;94:795–803.
8. Robinson MJ, Burd NA, Breen L, Rerecich T, Yang Y, Hector AJ, Baker SK, Phillips SM. Dose-dependent responses of myofibrillar protein synthesis with beef ingestion are

enhanced with resistance exercise in middle-aged men. Appl Physiol Nutr Metab. 2013;38:120–5.

9. Pennings B, Groen BBL, Van Dijk JW, De Lange A, Kiskini A, Kuklinski M, Senden JMG, Van Loon LJC. Minced beef is more rapidly digested and absorbed than beef steak, resulting in greater postprandial protein retention in older men. Am J Clin Nutr. 2013;98:121–8.
10. Burd NA, Gorissen SH, Van Vliet S, Snijders T, Van Loon LJC. Differences in postprandial protein handling after beef compared with milk ingestion during postexercise recovery: A randomized controlled trial. Am J Clin Nutr. 2015;102:828–36.
11. Elliot TA, Cree MG, Sanford AP, Wolfe RR, Tipton KD. Milk ingestion stimulates net muscle protein synthesis following resistance exercise. Med Sci Sports Exerc. 2006;38:667–74.
12. Van Vliet S, Shy EL, Sawan SA, Beals JW, West DWD, Skinner SK, Ulanov A V., Li Z, Paluska SA, Parsons CM, et al. Consumption of whole eggs promotes greater stimulation of postexercise muscle protein synthesis than consumption of isonitrogenous amounts of egg whites in young men. Am J Clin Nutr. 2017;106:1401–12.
13. Brown EC, DiSilvestro RA, Babaknia A, Devor ST. Soy versus whey protein bars: Effects on exercise training impact on lean body mass and antioxidant status. Nutr J. 2004;3:1–5.
14. Tang JE, Moore DR, Kujbida GW, Tarnopolsky MA, Phillips SM. Ingestion of whey hydrolysate, casein, or soy protein isolate: effects on mixed muscle protein synthesis at rest and following resistance exercise in young men. J Appl Physiol. 2009;107:987–92.
15. Gorissen SHM, Horstman AMH, Franssen R, Crombag JJR, Langer H, Bierau J, Respondek F, van Loon LJC. Ingestion of wheat protein increases in vivo muscle protein

synthesis rates in healthy older men in a randomized trial. J Nutr. 2016;146:1651–9.

16. Joy JM, Lowery RP, Wilson JM, Purpura M, De Souza EO, Wilson SM, Kalman DS, Dudeck JE, Jäger R. The effects of 8 weeks of whey or rice protein supplementation on body composition and exercise performance. Nutr J. 2013;12:1–7.

17. Oikawa SY, Bahniwal R, Holloway TM, Lim C, Mcleod JC, Mcglory C, Baker SK, Phillips SM. Potato Protein Isolate Stimulates Muscle Protein Synthesis at Rest and with Resistance Exercise in Young Women. 2020;1:1–13.

18. Pinckaers PJM, Weijzen MEG, Houben LHP, Zorenc AH, Kouw IWK, Groot LC de, Verdijk LB, Snijders T, Loon LJ van. The Muscle Protein Synthetic Response Following Ingestion of Corn Protein, Milk Protein and Their Protein Blend in Young Males. Curr Dev Nutr. 2020;4:651–651.

19. Koopman R, Walrand S, Beelen M, Gijsen AP, Kies AK, Boirie Y, Saris WHM, van Loon LJC. Dietary Protein Digestion and Absorption Rates and the Subsequent Postprandial Muscle Protein Synthetic Response Do Not Differ between Young and Elderly Men. J Nutr. 2009;139:1707–13.

20. Burd NA, Yang Y, Moore DR, Tang JE, Tarnopolsky MA, Phillips SM. Greater stimulation of myofibrillar protein synthesis with ingestion of whey protein isolate v. micellar casein at rest and after resistance exercise in elderly men. Br J Nutr. 2012;108:958–62.

21. Witard OC, Jackman SR, Breen L, Smith K, Selby A, Tipton KD. Myofibrillar muscle protein synthesis rates subsequent to a meal in response to increasing doses of whey protein at rest and after resistance exercise. Am J Clin Nutr. 2014;99:86–95.

22. Trommelen J, Weijzen MEG, van Kranenburg J, Ganzevles RA, Beelen M, Verdijk LB,

van Loon LJC. Casein protein processing strongly modulates post-prandial plasma amino acid responses in vivo in humans. Nutrients. 2020;12:1–12.

23. Boirie Y, Dangin M, Gachon P, Vasson M-P, Maubois J-L, Beaufrere B. Slow and fast dietary proteins differently modulate postprandial protein accretion. Proc Natl Acad Sci. 1997;94:14930–5.

24. Burke LM, Winter JA, Cameron-Smith D, Enslen M, Farnfield M, Decombaz J. Effect of intake of different dietary protein sources on plasma amino acid profiles at rest and after exercise. Int J Sport Nutr Exerc Metab. 2012;22:452–62.

25. Nations F and AO of the U. Dietary protein quality evaluation in human nutrition: report of an FAO expert consultation, 31 March-2 April, 2011, Auckland, New Zealand. FAO food and nutrition paper. 2013. 64 p.

26. Phillips SM, Fulgoni VL, Heaney RP, Nicklas TA, Slavin JL, Weaver CM. Commonly consumed protein foods contribute to nutrient intake, diet quality, and nutrient adequacy. Am J Clin Nutr. 2015;101:1346S-1352S.

27. Phillips SM, Martinson W. Nutrient-rich, high-quality, protein-containing dairy foods in combination with exercise in aging persons to mitigate sarcopenia. Nutr Rev. 2019;77:2.16-2.29.

28. Pennings B, Boirie Y, Senden JMG, Gijsen AP, Kuipers H, Van Loon LJC. Whey protein stimulates postprandial muscle protein accretion more effectively than do casein and casein hydrolysate in older men. Am J Clin Nutr. 2011;93:997–1005.

29. Devries MC, Phillips SM. Supplemental protein in support of muscle mass and health: Advantage whey. J Food Sci. 2015;80:A8-15.

30. Mahé S, Roos N, Benamouzig R, Davin L, Luengo C, Gagnon L, Gaussergès N,

Rautureau J, Tomé D. Gastrojejunal kinetics and the digestion of [15N]β-lactoglobulin and casein in humans: The influence of the nature and quantity of the protein. Am J Clin Nutr. 1996;63:546–52.

31. Phillips SM. A comparison of whey to caseinate. Am J Physiol - Endocrinol Metab. 2011;300:2021.

32. Wilkinson SB, Tarnopolsky MA, MacDonald MJ, MacDonald JR, Armstrong D, Phillips SM. Consumption of fluid skim milk promotes greater muscle protein accretion after resistance exercise than does consumption of an isonitrogenous and isoenergetic soy-protein beverage. Am J Clin Nutr. 2007;85:1031–40.

33. Hartman JW, Tang JE, Wilkinson SB, Tarnopolsky MA, Lawrence RL, Fullerton A V., Phillips SM. Consumption of fat-free fluid milk after resistance exercise promotes greater lean mass accretion than does consumption of soy or carbohydrate in young, novice, male weightlifters. Am J Clin Nutr. 2007;86:373–81.

34. Josse AR, Tang JE, Tarnopolsky MA, Phillips SM. Body composition and strength changes in women with milk and resistance exercise. Med Sci Sports Exerc. 2010;42:1122–30.

35. Bos C, Metges CC, Gaudichon C, Petzke KJ, Pueyo ME, Morens C, Everwand J, Benamouzig R, Tomé D. Postprandial kinetics of dietary amino acids are the main determinant of their metabolism after soy or milk protein ingestion in humans. J Nutr. 2003;133:1308–15.

36. Horstman AMH, Ganzevles RA, Kudla U, Kardinaal AFM, van den Borne JJGC, Huppertz T. Postprandial blood amino acid concentrations in older adults after consumption of dairy products: The role of the dairy matrix. Int Dairy J.

2021;113:104890.

37. de Hart NMMP, Mahmassani ZS, Reidy PT, Kelley JJ, McKenzie AI, Petrocelli JJ, Bridge MJ, Baird LM, Bastian ED, Ward LS, et al. Acute effects of cheddar cheese consumption on circulating amino acids and human skeletal muscle. Nutrients. 2021;13:614.

38. Hermans WJH, Fuchs CJ, Hendriks FK, Houben LHP, Senden JM. Cheese ingestion increases muscle protein synthesis rates both at rest and during recovery from exercise in healthy, young males: A randomized parallel-group trial. 2022;

39. Leenders M, Verdijk LB, Van Der Hoeven L, Van Kranenburg J, Nilwik R, Wodzig WKWH, Senden JMG, Keizer HA, Van Loon LJC. Protein supplementation during resistance-type exercise training in the elderly. Med Sci Sports Exerc. 2013;45:542–52.

40. Dirks ML, Tieland M, Verdijk LB, Losen M, Nilwik R, Mensink M, de Groot LCPGM, van Loon LJC. Protein Supplementation Augments Muscle Fiber Hypertrophy but Does Not Modulate Satellite Cell Content During Prolonged Resistance-Type Exercise Training in Frail Elderly. J Am Med Dir Assoc. Elsevier Inc.; 2017;18:608–15.

41. Gorissen SHM, Witard OC. Characterising the muscle anabolic potential of dairy, meat and plant-based protein sources in older adults. Proc Nutr Soc. 2018;77:20–31.

42. Du Y, Oh C, No J. Advantage of Dairy for Improving Aging Muscle. J Obes Metab Syndr. 2019;28:167–74.

43. Yang Y, Breen L, Burd NA, Hector AJ, Churchward-Venne TA, Josse AR, Tarnopolsky MA, Phillips SM. Resistance exercise enhances myofibrillar protein synthesis with graded intakes of whey protein in older men. Br J Nutr. 2012;108:1780–8.

44. Turgeon SL, Rioux LE. Food matrix impact on macronutrients nutritional properties. Food Hydrocoll. Elsevier Ltd; 2011;25:1915–24.

45. Panahi S, Tremblay A. The Potential Role of Yogurt in Weight Management and Prevention of Type 2 Diabetes. J Am Coll Nutr. 2016;35:717–31.

46. Chandan RC. An overview of yogurt production and composition. Yogurt in Health and Disease Prevention. 2017.

47. Kok CR, Hutkins R. Yogurt and other fermented foods as sources of health-promoting bacteria. Nutr Rev. 2018;76:4–15.

48. Bridge A, Brown J, Snider H, Nasato M, Ward WE, Roy BD, Josse AR. Greek yogurt and 12 weeks of exercise training on strength, muscle thickness and body composition in lean, untrained, university-aged males. Front Nutr. 2019;55.

49. De Brabandere AG, De Baerdemaeker JG. Effects of process conditions on the pH development during yogurt fermentation. J Food Eng. 1999;41:221–7.

50. Barbé F, Ménard O, Le Gouar Y, Buffière C, Famelart MH, Laroche B, Le Feunteun S, Dupont D, Rémond D. The heat treatment and the gelation are strong determinants of the kinetics of milk proteins digestion and of the peripheral availability of amino acids. Food Chem. Elsevier Ltd; 2013;136:1203–12.

51. Dupont D, Le S, Marze S, Souchon I. Structuring food to control its disintegration in the gastrointestinal tract and optimize nutrient bioavailability. Innov Food Sci Emerg Technol. Elsevier; 2018;46:83–90.

52. Burd NA, Beals JW, Martinez IG, Salvador AF, Skinner SK. Food-First Approach to Enhance the Regulation of Post-exercise Skeletal Muscle Protein Synthesis and Remodeling. Sport Med. Springer International Publishing; 2019;49:59–68.

53. Volpi E, Kobayashi H, Sheffield-Moore M, Mittendorfer B, Wolfe RR. Essential amino acids are primarily responsible for the amino acid stimulation of muscle protein anabolism

in healthy elderly adults. Am J Clin Nutr. 2003;78:250–8.

54. Burd NA, West DWD, Moore DR, Atherton PJ, Staples AW, Prior T, Tang JE, Rennie MJ, Baker SK, Phillips SM. Enhanced amino acid sensitivity of myofibrillar protein synthesis persists for up to 24 h after resistance exercise in young men. J Nutr. 2011;141:568–73.

55. Dickinson JM, Rasmussen BB. Amino acid transporters in the regulation of human skeletal muscle protein metabolism. Curr Opin Clin Nutr Metab Care. 2013;16:638.

56. Groen BBL, Horstman AM, Hamer HM, De Haan M, Van Kranenburg J, Bierau J, Poeze M, Wodzig WKWH, Rasmussen BB, Van Loon LJC. Post-prandial protein handling: You are what you just ate. PLoS One. 2015;10:e0141582.

57. Traylor DA, Gorissen SH, Hopper H, Prior T, McGlory C, Phillips SM. Aminoacidemia following ingestion of native whey protein, micellar casein, and a whey-casein blend in young men. Appl Physiol Nutr Metab. 2018;44:103–6.

58. Trommelen J, Betz MW, van Loon LJC. The Muscle Protein Synthetic Response to Meal Ingestion Following Resistance-Type Exercise. Sport Med. Springer International Publishing; 2019;49:185–97.

59. van Vliet S, Beals JW, Holwerda AM, Emmons RS, Goessens JP, Paluska SA, De Lisio M, Van Loon LJC, Burd NA. Time-dependent regulation of postprandial muscle protein synthesis rates after milk protein ingestion in young men. J Appl Physiol. 2019;127:1792–801.

60. Jakobsson J. Commentary: Greek Yogurt and 12 Weeks of Exercise Training on Strength, Muscle Thickness and Body Composition in Lean, Untrained, University - Aged Males. Front Nutr. 2019;137.

61. Panahi S, El Khoury D, Kubant R, Akhavan T, Luhovyy BL, Goff HD, Anderson GH. Mechanism of action of whole milk and its components on glycemic control in healthy young men. J Nutr Biochem. Elsevier Inc.; 2014;25:1124–31.

62. Fujita S, Rasmussen BB, Cadenas JG, Grady JJ, Volpi E. Effect of insulin on human skeletal muscle protein synthesis is modulated by insulin-induced changes in muscle blood flow and amino acid availability. Am J Physiol - Endocrinol Metab. 2006;291.

63. Trommelen J, Groen BBL, Hamer HM, Groot LCPGM De. Exogenous insulin does not increase muscle protein synthesis rate when administered systemically : a systematic review. 2015;173:R25-34.

64. Groen BBL, Horstman AMH, Hamer HM, Haan M De, Kranenburg J Van, Bierau J, Poeze M, Wodzig WKWH, Rasmussen BB, Loon LJC Van. Postprandial Muscle Protein Synthesis Rates in Healthy Young and Older Men. 2016;101:3978–88.

65. Greenhaff PL, Karagounis LG, Peirce N, Simpson EJ, Hazell M, Layfield R, Wackerhage H, Smith K, Atherton P, Selby A, et al. Disassociation between the effects of amino acids and insulin on signaling, ubiquitin ligases, and protein turnover in human muscle. Am J Physiol - Endocrinol Metab. 2008;295:E595-604.

66. Phillips SM. Insulin and muscle protein turnover in humans: Stimulatory, permissive, inhibitory, or all of the above? Am J Physiol - Endocrinol Metab. 2008;295:E731.

67. Abdulla H, Smith K, Atherton PJ, Idris I. Role of insulin in the regulation of human skeletal muscle protein synthesis and breakdown : a systematic review and meta-analysis. 2016;44–55.

68. Nilsson M, Stenberg M, Frid AH, Holst JJ, Björck IME. Glycemia and insulinemia in healthy subjects after lactose-equivalent meals of milk and other food proteins: The role of

plasma amino acids and incretins. Am J Clin Nutr. 2004;80:1246–53.

69. Nilsson M, Holst JJ, Björck IME. Metabolic effects of amino acid mixtures and whey protein in healthy subjects : studies using glucose-equivalent drinks 1 – 3. 2007;996–1004.

70. Thiebaud D, Jacot E, DeFronzo RA, Maeder E, Jequier E, Felber JP. The effect of graded doses of insulin on total glucose uptake, glucose oxidation, and glucose storage in man. Diabetes. 1982;31:957–63.

71. Heer M, Egert S. Nutrients other than carbohydrates: Their effects on glucose homeostasis in humans. Diabetes Metab Res Rev. 2015;31:14–35.

72. Vien S, Fabek H, Yamagishi Y, Lee YT, Luhovyy BL, Anderson GH. Role of single serving form of dairy on satiety and postprandial glycaemia in young and older healthy adults. Appl Physiol Nutr Metab. 2019;44:1289–96.

73. El Khoury D, Brown P, Smith G, Berengut S, Panahi S, Kubant R, Anderson GH. Increasing the protein to carbohydrate ratio in yogurts consumed as a snack reduces post-consumption glycemia independent of insulin. Clin Nutr. Elsevier Ltd; 2014;33:29–38.

74. Paddon-Jones D, Sheffield-Moore M, Zhang XJ, Volpi E, Wolf SE, Aarsland A, Ferrando AA, Wolfe RR. Amino acid ingestion improves muscle protein synthesis in the young and elderly. Am J Physiol - Endocrinol Metab. 2004;286:E321–8.

75. Pillai RR, Kurpad A V. Amino acid requirements in children and the elderly population. Br J Nutr. 2012;108:S44–9.

76. McKendry J, Currier BS, Lim C, McLeod JC, Thomas ACQ, Phillips SM. Nutritional supplements to support resistance exercise in countering the sarcopenia of aging. Nutrients. 2020;12:2057.

77. Wu G. Amino acids: Metabolism, functions, and nutrition. Amino Acids. 2009;37:1–17.

78. Smith K, Reynolds N, Downie S, Patel A, Rennie MJ. Effects of flooding amino acids on incorporation of labeled amino acids into human muscle protein. Am J Physiol - Endocrinol Metab. 1998;275:73–8.

79. Mitchell WK, Phillips BE, Williams JP, Rankin D, Lund JN, Smith K, Atherton PJ. A Dose- rather than Delivery Profile–Dependent Mechanism Regulates the "Muscle-Full" Effect in Response to Oral Essential Amino Acid Intake in Young Men. J Nutr. 2015;145:207–14.

80. Churchward-Venne TA, Burd NA, Mitchell CJ, West DWD, Philp A, Marcotte GR, Baker SK, Baar K, Phillips SM. Supplementation of a suboptimal protein dose with leucine or essential amino acids: Effects on myofibrillar protein synthesis at rest and following resistance exercise in men. J Physiol. 2012;590:2751–65.

81. Anthony JC, Yoshizawa F, Anthony TG, Vary TC, Jefferson LS, Kimball SR. Leucine Stimulates Translation Initiation in Skeletal Muscle of Postabsorptive Rats via a Rapamycin-Sensitive Pathway. J Nutr. 2000;130:2413–9.

82. Katsanos CS, Kobayashi H, Sheffield-Moore M, Aarsland A, Wolfe RR. A high proportion of leucine is required for optimal stimulation of the rate of muscle protein synthesis by essential amino acids in the elderly. Am J Physiol - Endocrinol Metab. 2006;291:E381–7.

83. Atherton PJ, Smith K, Etheridge T, Rankin D, Rennie MJ. Distinct anabolic signalling responses to amino acids in C2C12 skeletal muscle cells. Amino Acids. 2010;38:1533–9.

84. Devries MC, McGlory C, Bolster DR, Kamil A, Rahn M, Harkness L, Baker SK, Phillips SM. Leucine, Not Total Protein, Content of a Supplement Is the Primary Determinant of Muscle Protein Anabolic Responses in Healthy Older Women. J Nutr. 2018;148:1088–95.

85. Churchward-Venne TA, Breen L, Di Donato DM, Hector AJ, Mitchell CJ, Moore DR, Stellingwerff T, Breuille D, Offord EA, Baker SK, et al. Leucine supplementation of a low-protein mixed macronutrient beverage enhances myofibrillar protein synthesis in young men: A double-blind, randomized trial1-3. Am J Clin Nutr. 2014;99:276–86.
86. Matthews DM. Protein absorption. J Clin Pathol. 1971;S3-5:29–40.
87. Rémond D, Machebeuf M, Yven C, Buffière C, Mioche L, Mosoni L, Mirand PP. Postprandial whole-body protein metabolism after a meat meal is influenced by chewing efficiency in elderly subjects. Am J Clin Nutr. 2007;85:1286–92.
88. Trommelen J, Tomé D, van Loon LJC. Gut amino acid absorption in humans: Concepts and relevance for postprandial metabolism. Clin Nutr Open Sci. 2021;36:43–55.
89. Bornhorst GM, Singh RP. Gastric digestion in vivo and in vitro: How the structural aspects of food influence the digestion process. Annu Rev Food Sci Technol. 2014;5:111–32.
90. Goodman BE. Insights into digestion and absorption of major nutrients in humans. Am J Physiol - Adv Physiol Educ. 2010;34:44–53.
91. Hellström PM, Grybäck P, Jacobsson H. The physiology of gastric emptying. Best Pract Res Clin Anaesthesiol. 2006;20:397–407.
92. Steel A, Hediger MA. The molecular physiology of sodium-and proton-coupled solute transporters. News Physiol Sci. 1998;13:123–31.
93. Mathai JK, Liu Y, Stein HH. Values for digestible indispensable amino acid scores (DIAAS) for some dairy and plant proteins may better describe protein quality than values calculated using the concept for protein digestibility-corrected amino acid scores (PDCAAS). Br J Nutr. 2017;117:490–9.

94. Cervantes-Pahm SK, Stein HH. Ileal digestibility of amino acids in conventional, fermented, and enzyme-treated soybean meal and in soy protein isolate, fish meal, and casein fed to weanling pigs. J Anim Sci. 2010;88:2674–83.

95. Ertl P, Steinwidder A, Schönauer M, Krimberger K, Knaus W, Zollitsch W. Net food production of different Livestock: A national analysis for Austria including relative occupation of different land categories. Bodenkultur. 2016;67:91–103.

96. Abelilla JJ, Liu Y, Stein HH. Digestible indispensable amino acid score (DIAAS) and protein digestibility corrected amino acid score (PDCAAS) in oat protein concentrate measured in 20- to 30-kilogram pigs. J Sci Food Agric. 2018;98:410–4.

97. Ertl P, Knaus W, Zollitsch W. An approach to including protein quality when assessing the net contribution of livestock to human food supply. Animal. 2016;10:1883–9.

98. Rutherfurd SM, Fanning AC, Miller BJ, Moughan PJ. Protein digestibility-corrected amino acid scores and digestible indispensable amino acid scores differentially describe protein quality in growing male rats. J Nutr. 2015;145:372–9.

99. Caroli A, Poli A, Ricotta D, Banfi G, Cocchi D. Invited review: Dairy intake and bone health: A viewpoint from the state of the art1. J Dairy Sci. Elsevier; 2011;94:5249–62.

100. Bonjour JP, Kraenzlin M, Levasseur R, Warren M, Whiting S. Dairy in Adulthood: From Foods to Nutrient Interactions on Bone and Skeletal Muscle Health. J Am Coll Nutr. 2013;32:251–63.

101. Geiker NRW, Mølgaard C, Iuliano S, Rizzoli R, Manios Y, van Loon LJC, Lecerf JM, Moschonis G, Reginster JY, Givens I, et al. Impact of whole dairy matrix on musculoskeletal health and aging–current knowledge and research gaps. Osteoporos Int. 2020;31:601–15.

102. Mitchell CJ, McGregor RA, D'Souza RF, Thorstensen EB, Markworth JF, Fanning AC, Poppitt SD, Cameron-Smith D. Consumption of milk protein or whey protein results in a similar increase in muscle protein synthesis in middle aged men. Nutrients. 2015;7:8685–99.

103. Dangin M, Boirie Y, Garcia-Rodenas C, Gachon P, Fauquant J, Callier P, Ballèvre O, Beaufrère B. The digestion rate of protein is an independent regulating factor of postprandial protein retention. Am J Physiol Metab. 2001;280:E340–8.

104. Ribadeau-Dumas B, Grappin R. Milk protein analysis. Lait. 1989;69:357–416.

105. Morr C V. Chemistry of Milk Proteins in Food Processing. J Dairy Sci. 1975;58:977–84.

106. Mulet-Cabero AI, Mackie AR, Brodkorb A, Wilde PJ. Dairy structures and physiological responses: a matter of gastric digestion. Crit Rev Food Sci Nutr. 2020;60:3737–52.

107. Wang X, Ye A, Lin Q, Han J, Singh H. Gastric digestion of milk protein ingredients: Study using an in vitro dynamic model. J Dairy Sci. 2018;101:6842–52.

108. Lacroix M, Bos C, Léonil J, Airinei G, Luengo C, Daré S, Benamouzig R, Fouillet H, Fauquant J, Tomé D, et al. Compared with casein or total milk protein, digestion of milk soluble proteins is too rapid to sustain the anabolic postprandial amino acid requirement. Am J Clin Nutr. 2006;84:1070–9.

109. Mohan Reddy I, Kella NKD, Kinsella JE. Structural and Conformational Basis of the Resistance of β-Lactoglobulin to Peptic and Chymotryptic Digestion. J Agric Food Chem. 1988;36:737–41.

110. Moore DR, Robinson MJ, Fry JL, Tang JE, Glover EI, Wilkinson SB, Prior T, Tarnopolsky MA, Phillips SM. Ingested protein dose response of muscle and albumin protein synthesis after resistance exercise in young men. Am J Clin Nutr. 2009;89:161–8.

111. Gorissen SHM, Trommelen J, Kouw IWK, Holwerda AM, Pennings B, Groen BBL, Wall BT, Churchward-Venne TA, Horstman AMH, Koopman R, et al. Protein type, protein dose, and age modulate dietary protein digestion and phenylalanine absorption kinetics and plasma phenylalanine availability in humans. J Nutr. 2020;150:2041–50.

112. Thorning TK, Bertram HC, Bonjour J, Groot L De, Dupont D, Feeney E, Ipsen R, Lecerf JM, Mackie A, Mckinley MC, et al. Whole dairy matrix or single nutrients in assessment of health effects : current evidence and knowledge gaps. 2017;105:1033–45.

113. Tipton KD, Elliott TA, Cree MG, Wolf SE, Sanford AP, Wolfe RR. Ingestion of casein and whey prosteins result in muscle anabolism after resistance exercise. Med Sci Sports Exerc. 2004;36:2073–81.

114. Calbet JAL, Holst JJ. Gastric emptying, gastric secretion and enterogastrone response after administration of milk proteins or their peptide hydrolysates in humans. Eur J Nutr. 2004;43:127–39.

115. Reitelseder S, Agergaard J, Doessing S, Helmark IC, Lund P, Kristensen NB, Frystyk J, Flyvbjerg A, Schjerling P, van Hall G, et al. Whey and casein labeled with L-[1-13C]leucine and muscle protein synthesis: effect of resistance exercise and protein ingestion. Am J Physiol Endocrinol Metab. 2011;300:E231–42.

116. Churchward-Venne TA, Snijders T, Linkens AM, Hamer HM, van Kranenburg J, van Loon LJ. Ingestion of Casein in a Milk Matrix Modulates Dietary Protein Digestion and Absorption Kinetics but Does Not Modulate Postprandial Muscle Protein Synthesis in Older Men. J Nutr. 2015;145:1438–45.

117. Hamarsland H, Nordengen AL, Nyvik Aas S, Holte K, Garthe I, Paulsen G, Cotter M, Børsheim E, Benestad HB, Raastad T. Native whey protein with high levels of leucine

results in similar post-exercise muscular anabolic responses as regular whey protein: A randomized controlled trial. J Int Soc Sports Nutr. 2017;14.

118. Bos C, Mahé S, Gaudichon C, Benamouzig R, Gausserès N, Luengo C, Ferrière F, Rautureau J, Tomé D. Assessment of net postprandial protein utilization of 15N-labelled milk nitrogen in human subjects. Br J Nutr. 1999;81:221–6.

119. Fouillet H, Gaudichon C, Bos C, Mariotti F, Tomé D. Contribution of plasma proteins to splanchnic and total anabolic utilization of dietary nitrogen in humans. Am J Physiol - Endocrinol Metab. 2003;285:88–97.

120. Phillips SM, Hartman JW, Wilkinson SB. Dietary Protein to Support Anabolism with Resistance Exercise in Young Men. J Am Coll Nutr. 2005;24:134S-139S.

121. Mulet-Cabero AI, Mackie AR, Wilde PJ, Fenelon MA, Brodkorb A. Structural mechanism and kinetics of in vitro gastric digestion are affected by process-induced changes in bovine milk. Food Hydrocoll. 2019;86:172–83.

122. Huffman LM, Harper WJ. Maximizing the value of milk through separation technologies. J Dairy Sci. 1999;82:2238–44.

123. Ryan G, Nongonierma AB, O'Regan J, FitzGerald RJ. Functional properties of bovine milk protein isolate and associated enzymatic hydrolysates. Int Dairy J. 2018;81:113–21.

124. Churchward-Venne TA, Pinckaers PJM, Smeets JSJ, Peeters WM, Zorenc AH, Schierbeek H, Rollo I, Verdijk LB, Van Loon LJC. Myofibrillar and Mitochondrial Protein Synthesis Rates Do Not Differ in Young Men Following the Ingestion of Carbohydrate with Milk Protein, Whey, or Micellar Casein after Concurrent Resistance- and Endurance-Type Exercise. J Nutr. 2019;149:198–209.

125. Barbé F, Ménard O, Gouar Y Le, Buffière C, Famelart MH, Laroche B, Feunteun S Le,

Rémond D, Dupont D. Acid and rennet gels exhibit strong differences in the kinetics of milk protein digestion and amino acid bioavailability. Food Chem. 2014;143:1–8.

126. Sumi K, Osada K, Ashida K, Nakazato K. Lactobacillus-fermented milk enhances postprandial muscle protein synthesis in Sprague-Dawley rats. J Funct Foods. Elsevier; 2020;66:103789.

127. Mahé S, Marteau P, Huneau J-F, Thuillier F, Tomé D. Intestinal nitrogen and electrolyte movements following fermented milk ingestion in man. Br J Nutr. 1994;71:169–80.

128. Gaudichon C, Mahé S, Roos N, Benamouzig R, Luengo C, Huneau J-F, Sick H, Bouley C, Rautureau J, Tome D. Exogenous and endogenous nitrogen flow rates and level of protein hydrolysis in the human jejunum after [15 N]milk and [15 N]yoghurt ingestion . Br J Nutr. 1995;74:251–60.

129. Pimentel G, Burton KJ, von Ah U, Bütikofer U, Pralong FP, Vionnet N, Portmann R, Vergères G. Metabolic footprinting of fermented milk consumption in serum of healthy men. J Nutr. 2018;148:851–60.

130. Thøgersen R, Egsgaard KL, Kjølbæk L, Jensen KJ, Astrup A, Hammershøj M, Raben A, Bertram HC. Effect of dairy matrix on the postprandial blood metabolome. Nutrients. 2021;13:4280.

131. Renan M, Arnoult-Delest V, Pâquet D, Brulé G, Famelart MH. Changes in the rheological properties of stirred acid milk gels as induced by the acidification procedure. Dairy Sci Technol. 2008;88:341–53.

132. Matar C, Amiot J, Savoie L, Goulet J. The Effect of Milk Fermentation by Lactobacillus helveticus on the Release of Peptides during in Vitro Digestion. J Dairy Sci. Elsevier; 1996;79:971–9.

133. Beshkova DM, Simova ED, Frengova GI, Simov ZI, Adilov EF. Production of amino acids by yogurt bacteria. Biotechnol Prog. 1998;14:963–5.

134. Settachaimongkon S, Nout MJR, Antunes Fernandes EC, Hettinga KA, Vervoort JM, van Hooijdonk TCM, Zwietering MH, Smid EJ, Van Valenberg HJF. Influence of different proteolytic strains of Streptococcus thermophilus in co-culture with Lactobacillus delbrueckii subsp. bulgaricus on the metabolite profile of set-yoghurt. Int J Food Microbiol. Elsevier B.V.; 2014;177:29–36.

135. Germani A, Luneia R, Nigro F, Donini LM, del Balzo V. The yogurt amino acid profile ' s variation during the shelf-life. 2014;26:205–12.

136. Turgeon SL, Brisson G. Symposium review: The dairy matrix—Bioaccessibility and bioavailability of nutrients and physiological effects. J Dairy Sci. 2020;103:6727–36.

137. Dalgleish DG, Corredig M. The structure of the casein micelle of milk and its changes during processing. Annu Rev Food Sci Technol. 2012;3:449–67.

138. Tamime AY, Hassan A, Farnworth E, Toba T. Structure of Fermented Milks. Structure of Dairy Products. 2007.

139. Gilbert A, Turgeon SL. Studying stirred yogurt microstructure and its correlation to physical properties: a review. Food Hydrocoll. 2021;121:106970.

140. Lucey JA, Singh H. Formation and physical properties of acid milk gels: A review. Food Res Int. 1997;30:529–42.

141. Lucey JA. Formation and physical properties of milk protein gels. J Dairy Sci. 2002;23:1127–36.

142. Horne DS. Formation and structure of acidified milk gels. Int Dairy J. 1999;9:261–8.

143. Gaudichon C, Roos N, Mahé S, Sick H, Bouley C, Tomé D. Gastric Emptying Regulates

the Kinetics of Nitrogen Absorption from 15N-Labeled Milk and 15N-Labeled Yogurt in Miniature Pigs. J Nutr. 1994;124:1970–7.

144. Rychen G, Mpassi D, Jurjanz S, Mertes M, Lenoir-Wijnkoop I, Antoine JM, Laurent F. 15N as a marker to assess portal absorption of nitrogen from milk, yogurt and heat-treated yogurt in the growing pig. J Dairy Res. 2002;69:95–101.

145. Scanff P, Savalle B, Miranda G, Pelissier JP, Guilloteau P, Toullec R. In Vivo Gastric Digestion of Milk Proteins. Effect of Technological Treatments. J Agric Food Chem. 1990;38:1623–9.

146. Scanff P, Yvon M, Pelissier JP, Guilloteau P, Toullec R. Effect of Some Technological Treatments of Milk on Amino Acid Composition of in Vivo Effluents During Gastric Digestion. J Agric Food Chem. 1991;39:1482–7.

147. Meyer JH, Kelly GA, Jones RS. Canine pancreatic response to intestinally perfused oligopeptides. Am J Physiol. 1976;231:678–81.

148. Niederau C, Sonnenberg A, Erckenbrecht J. Effects of intravenous infusion of amino acids, fat, or glucose on unstimulated pancreatic secretion in healthy humans. Dig Dis Sci. 1985;30:445–55.

149. Mackie AR, Rafiee H, Malcolm P, Salt L, van Aken G. Specific food structures supress appetite through reduced gastric emptying rate. Am J Physiol - Gastrointest Liver Physiol. 2013;304:G1038–43.

150. Zhu Y, Hsu WH, Hollis JH. The Impact of Food Viscosity on Eating Rate, Subjective Appetite, Glycemic Response and Gastric Emptying Rate. PLoS One. 2013;8:6–11.

151. Mulet-Cabero AI, Rigby NM, Brodkorb A, Mackie AR. Dairy food structures influence the rates of nutrient digestion through different in vitro gastric behaviour. Food Hydrocoll.

2017;67:63–73.

152. Dong L, Wu K, Cui W, Fu D, Han J, Liu W. Tracking the digestive performance of different forms of dairy products using a dynamic artificial gastric digestive system. Food Struct. 2021;29:100194.

153. Ménard O, Famelart MH, Deglaire A, Le Gouar Y, Guérin S, Malbert CH, Dupont D. Gastric emptying and dynamic in vitro digestion of drinkable yogurts: Effect of viscosity and composition. Nutrients. 2018;10:1308.

154. Kalab M, Allan-Wojtas P, Phipps-Todd BE. Development of Microstructure in Set-Style Nonfat Yogurt -A Review. J Food Struct. 1983;2:7.

155. Lucey JA. Cultured dairy products: An overview of their gelation and texture properties. Int J Dairy Technol. 2004;57:77–84.

156. Floury J, Bianchi T, Thévenot J, Dupont D, Jamme F, Lutton E, Panouillé M, Boué F, Le Feunteun S. Exploring the breakdown of dairy protein gels during in vitro gastric digestion using time-lapse synchrotron deep-UV fluorescence microscopy. Food Chem. 2018;239:898–910.

157. Körzendörfer A, Hinrichs J. Manufacture of high-protein yogurt without generating acid whey – Impact of the final pH and the application of power ultrasound on texture properties. Int Dairy J. 2019;99:104541.

158. Gao Z, Guo B, Gao R, Zhu Q, Wu W, Qin H. Probiotics modify human intestinal mucosa-associated microbiota in patients with colorectal cancer. Mol Med Rep. 2015;12:6119–27.

159. Ye A, Liu W, Cui J, Kong X, Roy D, Kong Y, Han J, Singh H. Coagulation behaviour of milk under gastric digestion: Effect of pasteurization and ultra-high temperature treatment. Food Chem. 2019;286:216–25.

160. Mokoonlall A, Nöbel S, Hinrichs J. Post-processing of fermented milk to stirred products: Reviewing the effects on gel structure. Trends Food Sci Technol. 2016;54:26–36.

161. Jäger R, Zaragoza J, Purpura M, Iametti S, Marengo M, Tinsley GM, Anzalone AJ, Oliver JM, Fiore W, Biffi A, et al. Probiotic Administration Increases Amino Acid Absorption from Plant Protein: a Placebo-Controlled, Randomized, Double-Blind, Multicenter, Crossover Study. Probiotics Antimicrob Proteins. 2020;12:1330–9.

162. Lamothe S, Rémillard N, Tremblay J, Britten M. Influence of dairy matrices on nutrient release in a simulated gastrointestinal environment. Food Res Int. Elsevier Ltd; 2017;92:138–46.

163. Nguyen HTH, Gathercole JL, Day L, Dalziel JE. Differences in peptide generation following in vitro gastrointestinal digestion of yogurt and milk from cow, sheep and goat. Food Chem. Elsevier; 2020;317:126419.

164. Gunnerud U, Holst JJ, Stman E, Björck I. The glycemic, insulinemic and plasma amino acid responses to equi-carbohydrate milk meals, A pilot- study of bovine and human milk. Nutr J. 2012;11:1–9.

165. Mcgregor RA, Poppitt SD. Milk protein for improved metabolic health : a review of the evidence. 2013;1–13.

166. Östman EM, Liljeberg Elmståhl HGM, Björck IME. Inconsistency between glycemic and insulinemic responses to regular and fermented milk products. Am J Clin Nutr. 2001;74:96–100.

167. Phenomenex. EZ:faast Amino Acid Information [Internet]. Available from: https://www.phenomenex.com/Products/AminoAcidDetail/EZfaast

168. Kit, Insulin Elisa CCU. Insulin ELISA Kit. p. 90095.

169. Mæhre HK, Dalheim L, Edvinsen GK, Elvevoll EO, Jensen IJ. Protein determination—method matters. Foods. 2018;7:5.

170. Nakayama K, Sanbongi C, Ikegami S. Effects of whey protein hydrolysate ingestion on postprandial aminoacidemia compared with a free amino acid mixture in young men. Nutrients. 2018;10:507.

171. Ye A. Gastric colloidal behaviour of milk protein as a tool for manipulating nutrient digestion in dairy products and protein emulsions. Food Hydrocoll. 2021;115:106599.

172. Tugault-Lafleur CN, Black JL. Differences in the quantity and types of foods and beverages consumed by Canadians between 2004 and 2015. Nutrients. 2019;11:526.

173. America DN. Oikos [Internet]. Available from: https://www.oikosyogurt.com/

174. Kitagawa M, Haji S, Amagai T. High Serum Essential Amino Acids as a Predictor of Skeletal Muscle Depletion in Patients with Cachexia and Advanced Gastrointestinal Cancers. Nutr Clin Pract. 2017;32:645–51.

175. Holeček M. Branched-chain amino acids in health and disease: Metabolism, alterations in blood plasma, and as supplements. Nutr Metab. 2018;15:1–12.

176. Stokes T, Hector AJ, Morton RW, McGlory C, Phillips SM. Recent perspectives regarding the role of dietary protein for the promotion of muscle hypertrophy with resistance exercise training. Nutrients. 2018;10:180.

177. Morton RW, Murphy KT, McKellar SR, Schoenfeld BJ, Henselmans M, Helms E, Aragon AA, Devries MC, Banfield L, Krieger JW, et al. A systematic review, meta-analysis and meta-regression of the effect of protein supplementation on resistance training-induced gains in muscle mass and strength in healthy adults. Br J Sports Med. 2017;52:376–84.

178. Panahi S, Fernandez MA, Marette A, Tremblay A. Yogurt, diet quality and lifestyle

factors. Eur J Clin Nutr. 2017;71:573–9.

179. Lim CY, In J. Considerations for crossover design in clinical study. Korean J Anesthesiol. 2021;74:293–9.

180. Auclair O, Han Y, Burgos SA. Consumption of milk and alternatives and their contribution to nutrient intakes among Canadian adults: Evidence from the 2015 Canadian Community Health Survey-Nutrition. Nutrients. 2019;11:1948.

181. Lynch HM, Buman MP, Dickinson JM, Ransdell LB, Johnston CS, Wharton CM. No significant differences in muscle growth and strength development when consuming soy and whey protein supplements matched for leucine following a 12 week resistance training program in men and women: A randomized trial. Int J Environ Res Public Health. 2020;17:3871.

182. Burd NA, Tang JE, Moore DR, Phillips SM. Exercise training and protein metabolism: Influences of contraction, protein intake, and sex-based differences. J Appl Physiol. 2009;106:1692–701.

183. Beena Divya J, Kulangara Varsha K, Madhavan Nampoothiri K, Ismail B, Pandey A. Probiotic fermented foods for health benefits. Eng Life Sci. 2012;12:377–90.

184. Luo X, Sui J, Birmann BM, Ivey KL, Tabung FK, Wu Y, Yang W, Wu K, Ogino S, Liu H, et al. Association between yogurt consumption and plasma soluble CD14 in two prospective cohorts of US adults. Eur J Nutr. 2021;60:929–38.

185. Putt KK, Pei R, White HM, Bolling BW. Yogurt inhibits intestinal barrier dysfunction in Caco-2 cells by increasing tight junctions. Food Funct. 2017;8:406–14.

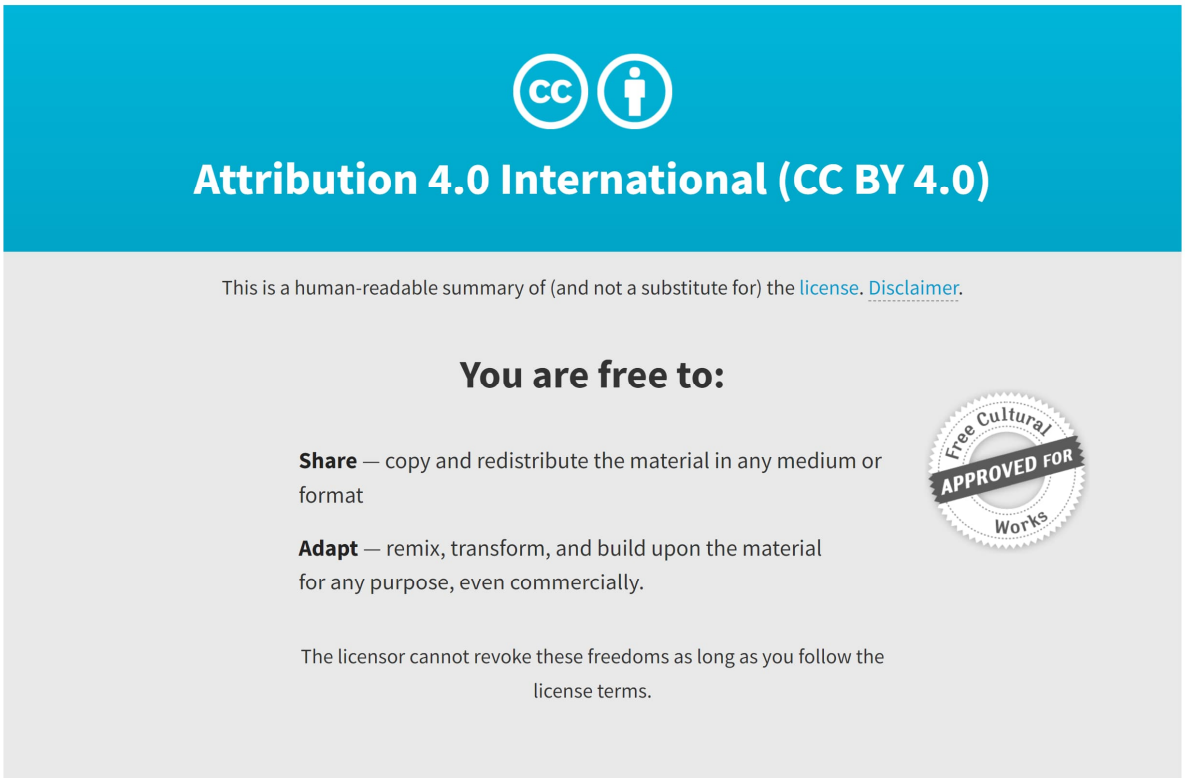
186. Liu J, Klebach M, Visser M, Hofman Z. Amino acid availability of a dairy and vegetable protein blend compared to single casein, whey, soy, and pea proteins: A double-blind,

cross-over trial. Nutrients. 2019;11:2613.

187. vanVliet S Van, Burd NA, van Loon LJ. The Skeletal Muscle Anabolic Response to Plant- versus. J Nutr Crit Rev. 2015;145:1981–91.

APPENDICES

Appendix A: Permission to recreate Table 1



Attribution 4.0 International (CC BY 4.0)

This is a human-readable summary of (and not a substitute for) the license. Disclaimer.

You are free to:

Share — copy and redistribute the material in any medium or format

Adapt — remix, transform, and build upon the material for any purpose, even commercially.

The licensor cannot revoke these freedoms as long as you follow the license terms.

Appendix B: Permissions to reuse Figure 5

ELSEVIER LICENSE
TERMS AND CONDITIONS

Feb 02, 2022

This Agreement between York University -- Joe Brown ("You") and Elsevier ("Elsevier") consists of your license details and the terms and conditions provided by Elsevier and Copyright Clearance Center.

License Number	5240941291437
License date	Feb 02, 2022
Licensed Content Publisher	Elsevier
Licensed Content Publication	Clinical Nutrition
Licensed Content Title	Increasing the protein to carbohydrate ratio in yogurts consumed as a snack reduces post-consumption glycemia independent of insulin
Licensed Content Author	Dalia El Khoury,Peter Brown,Gary Smith,Shari Berengut,Shirin Panahi,Ruslan Kubant,G. Harvey Anderson
Licensed Content Date	Feb 1, 2014
Licensed Content Volume	33

Licensed Content Issue	1
Licensed Content Pages	10
Start Page	29
End Page	38
Type of Use	reuse in a thesis/dissertation
Portion	figures/tables/illustrations
Number of figures/tables/illustrations	1
Format	both print and electronic
Are you the author of this Elsevier article?	No
Will you be translating?	No
Title	DIFFERENTIAL PLASMA AMINO ACID RESPONSES FOLLOWING THE CONSUMPTION OF ISONITROGENOUS DOSES OF GREEK YOGURT AND SKIM MILK
Institution name	York University

Expected presentation date	Mar 2022
Portions	Figure 1 on page 33. Effect of treatments on pre-meal and post-meal incremental area under the curve (iAUC) for insulin (mU min/mL).
Requestor Location	York University 4700 Keele Steet Toronto, ON M3J 1P3 Canada Attn: York University