Bioactive peptides in hydrolysates of bovine and camel milk proteins: A review of studies on peptides that reduce blood pressure, improve glucose homeostasis, and inhibit pathogen adhesion

Rami M. Althnaibat, Heather L. Bruce, Jianping Wu, Michael G. Gänzle*)

University of Alberta, Dept. of Agricultural, Food and Nutritional Science, Edmonton, AB, Canada.

*) Corresponding author footnote

Michael Gänzle,

University of Alberta, Dept. of Agricultural, Food and Nutritional Science

4-10 Ag/For Centre

Edmonton, AB

Canada, T6G 2P5

mail; mgaenzle@ualberta.ca

Abstract

The prevalence of diet-related chronic conditions including hypertension and cardiovascular disease, and diabetes mellitus has increased worldwide. Research regarding the use of food-derived bioactive peptides as an alternative strategy to mitigate chronic diseases is on the rise. Milk is recognized as one of the main dietary protein sources for health beneficial bioactive compounds. Hundreds of *in vitro* studies have suggested that milk-derived bioactive peptides offer multiple biological and physiological benefits, and some but not all were confirmed *in vivo* with animal models for hypertension, hyperglycemia, and pathogen adhesion. However, only a limited number of health benefits have been confirmed by randomized clinical trials. This review provides an overview of the current clinical studies that target hypertension, postprandial hyperglycemic, and adhesion of enteric pathogen with bioactive peptides derived from bovine and camel milk, with a focus on the factors affecting the efficacy of orally ingested products.

Keywords: Protein hydrolysate, bioactive peptides, caseinmacropeptide, chronic diseases, hypertension, hyperglycemia, pathogen adhesion, randomized clinical trials.

1. Introduction

Protein-derived bioactive peptides have health-beneficial properties (Layman et al., 2003). Bioactive peptides can be derived from dietary proteins by enzymatic hydrolysis during food processing or food fermentation, and / or during intestinal transit (Park, 2009). The health promoting properties of bioactive peptides depend on the peptide sequence, which determines size, charge and hydrophobicity (Park, 2009). Pepsin hydrolysis in the stomach is the first step of food protein digestion; proteins are then further hydrolyzed by the pancreatic proteases trypsin and chymotrypsin, and by brush border peptidases that are expressed in the mucosal membrane of the small intestine (Hooton et al., 2015). Specifically, brush border enzymes that contribute to peptide hydrolysis include the aminopeptidase N, dipeptidyl aminopeptidase IV, aminopeptidase A, peptidyl dipeptidase, γ-glutamyltranspeptidase, and carboxypeptidase. Of these peptidases, the γ-glutamyltranspeptidase is specifically active on γ-glutamyl-peptides of plant or microbial origin. The only enzymes with activity on peptide bonds adjacent to proline are aminopeptidase and carboxypeptidase (Hooton et al., 2015; Mentlein, 2004; Yoshioka et al., 1988).

Some bioactive peptides act locally in the gastro-intestinal tract (GIT), examples particularly include peptides that inhibit starch digestion and antihypertensive peptides, which are hypothesized to act on the intestinal renin-angiotensin system, or are active after transfer to the blood stream (Miner-Williams et al., 2014; Xu et al., 2019). The bioavailability of peptides is affected by digestive enzymes in the gastrointestinal tract, absorption in the small intestine, and distribution or degradation in the blood stream. The bioavailability of ingested bioactive peptides depends on the composition, number, and sequence of amino acids (Xu et al., 2019; Yoshioka et al., 1988; Zambrowicz et al., 2015). These characteristics of peptides determine the pathway that may be used to cross the intestinal epithelial cell (Xu et al., 2019).

The bioavailability of bioactive peptides and their transfer to the bloodstream is a major hurdle to health beneficial effects in humans and animals (Miner-Williams et al., 2014; Xu et al., 2017). Proteins with high content of proline are resistant to gastric and pancreatic peptidases, and proline-rich peptides are thus most likely to escape the digestion and to reach the intestinal membrane in relatively intact sequence and reach the brush border enzyme (Mentlein, 2004; Yoshioka et al., 1988). The bioactive peptides IPP (Nongonierma & FitzGerald, 2016), VPP (Ten Have et al., 2015), PG (Shigemura et al., 2012), IP (Foltz et al., 2007), HLPLP (Sánchez-Rivera et al., 2014) have been detected in the plasma of human and animals. However, the peptide concentrations in the blood serum are substantially lower than the concentrations that are required for *in vitro* activity (Bouglé & Bouhallab, 2016). For example, angiotensin converting enzyme (ACE) inhibitory activity of the tripeptide IPP is observed at 10 µmol/L (Y. Nakamura et al., 1995; Ohsawa et al., 2008) while the concentration in blood was reported to be 10,000 times lower, 0.90 ± 0.16 nmol / L (Foltz et al., 2007).

Many studies document a favorable effect of bioactive peptides in humans and animals after oral administration but even for peptides that were shown to be effective in animal models including pigs and rats and in randomized clinical trials (RCT) in humans, the role of bioavailability, effects, pharmacokinetics, and plasma concentrations of bioactive peptides are not fully understood (Bouglé & Bouhallab, 2016; Xu et al., 2019). The maximum concentrations (C_{max}) and the elimination half-lives ($t_{1/2}$) of absorbed bioactive peptides in the blood plasma reflect their bioavailability, and the potential for *in vivo* activity (Chabance et al., 1998; Horner et al., 2016). Most of the bioactive peptides have achieved their C_{max} in the micromolar range (µM), and $t_{1/2}$ ranged between a few minutes to a few hours. The variation in C_{max} and $t_{1/2}$ of the bioactive peptides

in the human plasma are determined by sex, age, diseases and interaction with the food matrix (Cicero et al., 2010, 2017).

Hundreds of *in vitro* studies have suggested that bioactive peptides have a favorable effect on the functions of various organs and that they offer multiple biological and physiological benefits with a wide range of biological activities (Figure 1) (Chakrabarti et al., 2018). Some but not all of these promising *in vitro* data were confirmed *in vivo* with animal models, however, and the susceptibility of orally ingested peptides to GIT and brush border peptidases is a relevant hurdle for reproducing *in vitro* data *in vivo* (Figure 1) (Daroit & Brandelli, 2021). Even for those favorable effects that were demonstrated *in vivo*, e.g. by using rodent models, only a limited number of clinical outcomes in humans have been reported (Figure 1). To date, RCTs that have shown a health beneficial effect of dietary bioactive peptides are limited to antihypertensive activity and anti-hyperglycemic effects of peptides (Figure 1) (Duffuler et al., 2022; Zhang et al., 2015).

For some peptides, however, animals are not only relevant models for human health but are the actual target for the activity of bioactive peptides. This specifically pertains to glycopeptides that aim to prevent adhesion of enterotoxigenic *E. coli* (ETEC) to the intestinal mucosa of swine and calves (Yan et al., 2017). These peptides were shown to reduce the cell numbers of ETEC in post-weaning piglets (Hermes et al., 2013; Yan et al., 2017). However, the strains of ETEC that cause diarrheal disease in humans differ in the glycan specificity, and successful interventions in swine does not necessarily translate to potential applications in humans (Hermes et al., 2013; Mouricout et al., 1990).

Milk is recognized as a main dietary sources for health beneficial bioactive compounds. Many of the dairy products are fermented and thus include the fermentation microbes that release peptides

by proteolysis (García-Burgos et al., 2020; Pasolli et al., 2020). Whey is a by-product of cheese and casein production. Bovine whey contains approximately 20% of the original milk protein and therefore represents an inexpensive source of high-nutritional quality protein and bioactive peptides for the food and health industries (Luhovyy et al., 2007). Sweet whey contains about 5% caseinmacropeptide (CMP), which prevents adhesion of ETEC to the porcine intestinal mucosa (Althnaibat et al., 2022; Hermes et al., 2013; Yan et al., 2017). Whey is processed into relatively low-value commodities such as whey protein concentrates or galacto-oligosaccharides for use as a food additive, or to purify specific whey proteins as food ingredient. However, the full potential of this resource has not yet been fully explored (Onwulata & Huth, 2008).

A large number of studies on milk-derived bioactive peptides and most review articles focused on bovine milk (Chakrabarti et al., 2018; Cicero et al., 2017; Daroit & Brandelli, 2021; Duffuler et al., 2022; El-Agamy, 2009; García-Burgos et al., 2020; Horner et al., 2016; Luhovyy et al., 2007; Nongonierma & FitzGerald, 2016; Park, 2009). Only few studies have investigated bioactive peptides from sheep, goats, horse, or camel milk (El-Agamy, 2009), reflecting that milk from these animals makes only relatively small contribution to the consumption of dairy products (Shahbandeh, 2022). Camel milk is widely consumed in many countries of the Middle East and in North Africa (Jafar et al., 2018; Rafiq et al., 2015). The proportion of casein and whey proteins in camel milk is different than that in bovine milk. The ratio of casein and whey proteins in bovine milk is about 80%:20% of the total milk protein, meanwhile, this ratio in camel milk is 75%:25% (El-Agamy, 2009). RCTs for bioactive peptides from camel milk are not yet available. Therefore, this review aims to summarize the possible bioactivities like antihypertension activities, antihyperglycemic activities, and antiadhesion activities against bacteria of peptides derived from

bovine and camel milk, with a focus on those activities that were confirmed by RCTs, or animal trials where farm animals are the therapeutic target of the intervention (Figure 1).

2. Antihypertensive peptides

In 2019, about 23% of Canadian adults (20 - 79 years old) were diagnosed with hypertension (DeGuire, J., Clarke, J., Rouleau, K., Roy, J., & Bushnik, 2019). The blood pressure in the body is mainly regulated by the renin angiotensin aldosterone system. The angiotensin I-converting enzyme (ACE) plays a key role in blood pressure regulation as well as water and electrolytes balance. ACE increases blood pressure by conversion of angiotensin I (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu) to angiotensin II (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe) by hydrolyzing the peptide bond between Phe and His (Donoghue et al., 2000). Angiotensin II receptor blockers and ACE inhibitors are the main types of antihypertensive drugs (Whelton et al., 2018). The mechanism for most of the antihypertensive bioactive peptides derived from milk are based on the inhibition of ACE (Aihara et al., 2004; Boelsma & Kloek, 2010; Cicero et al., 2016; de Leeuw et al., 2009; Hata et al., 1996; Jauhiainen et al., 2005; Martin et al., 2020; Mizuno et al., 2005; T. Nakamura et al., 2011; Townsend et al., 2004; Turpeinen et al., 2011, 2012; van der Zander et al., 2008; Yamasue et al., 2010). Few studies proposed other mechanisms for antihypertensive action such as decreasing aldosterone (Cadée et al., 2007), increasing endothelial vascular function (Hirota et al., 2007), diminishing arterial stiffness (Jauhiainen et al., 2010), and increasing endothelial dilation (Vieira de Oliveira et al., 2020). Because the concentration of ACE inhibitory peptides in the plasma is well below their active concentration, dietary ACE inhibitory peptides are thought to act on the intestinal rather than the circulating renin-angiotensin system (Miner-Williams et al., 2014).

Several *in vitro* and *in vivo* studies demonstrated that the milk products hydrolysates are a good sources of ACE inhibitory peptides or vasodilators (T. Nakamura et al., 2013; Y. Nakamura et al., 1995). ACE inhibitor peptides that are shown to be effective *in vivo* usually have a short sequence (2-12 amino acids) (Hata et al., 1996; Jauhiainen et al., 2005; Mizuno et al., 2005). Clinical randomized, single and/or double-blind, placebo-controlled human trials that documented the effect of milk-derived dietary peptides on the blood pressure are summarized in Table 1. These studies used fermented dairy products with a known concentration of VPP and IPP (Aihara et al., 2004; Hata et al., 1996; Mizuno et al., 2005), fermented dairy products supplemented with VPP and IPP (Jauhiainen et al., 2005; Turpeinen et al., 2011; Yamasue et al., 2010), or pure VPP and IPP (Boelsma & Kloek, 2010; Turpeinen et al., 2012). Studies that employed fermented milk products used yoghurt-type products fermented with *L. helveticus* (Romero et al., 2020), a dairy starter culture with a well-characterized proteolytic system. The dose of VPP and IPP ranged from 1.5 and 1.1 mg / person and day to 30 and 23.2 mg/ person and day, respectively (Table 1). The selection of study participants included normotensive, moderately hypertensive, and hypertensive subjects and the treatment time ranged from single dose to 24 weeks (Table 1).

For example, consuming the fermented milk products Calpis supplemented with 1.5 mg and 1.1 mg VPP and IPP, respectively, per day reduced in the systolic and diastolic blood pressure by 14.1 ± 3.1 mmHg and 6.6 ± 2.5 mmHg after 8 weeks of treatments with medication in hypertensive subjects, respectively (Hata et al., 1996). The blood pressure lowering effect of *Lactobacillus helveticus* LBK-16H fermented milk fortified with 30 mg VPP and 22.5 mg IPP was determined through a randomized, double blinded placebo-controlled study on 94 mildly hypertensive patients for 10 weeks. The systolic blood pressure dropped from 148.4 ± 8.1 mmHg to 132.6 ± 9.9 mmHg,

168 whereas diastolic blood pressure dropped from 93.5 ± 6.2 to 83 ± 8.0 mmHg for the *L. helveticus*
169 fermented milk group (Jauhiainen et al., 2005).

170 A clinical, randomized, single-blind, placebo-controlled trial evaluated the dose-dependent
171 antihypertensive effect of casein hydrolysate tablets supplemented with VPP and IPP on 131 high-
172 normal blood pressure and mild hypertension participants for 6 weeks. Four doses, 0, 1.8, 2.5, and
173 3.6 mg of VPP and IPP mixture were supplemented through tablets. After 6 weeks of treatment, a
174 dose-dependent decrease in the systolic blood pressure for the active group receiving 1.8, 2.5, and
175 3.6 mg compared to the baseline and placebo group was observed (Mizuno et al., 2005). The role
176 of a low salt diet in conjunction with dietary VPP and IPP was also evaluated (Yamasue et al.,
177 2010). Ambulatory blood pressure measurements were taken after ingestion of 5.32 mg VPP and
178 2.76 mg IPP per day for 8 weeks. This study demonstrated that the systolic blood pressure was
179 affected by tripeptides and decreased during nighttime sleep after 4 and 8 weeks. Therefore, the
180 low intake of salt could support the antihypertensive activity of VPP and IPP (Yamasue et al.,
181 2010).

182 Most of the studies were performed to evaluate the antihypertensive effect of casein and whey
183 hydrolysate supplemented with VPP and IPP as beverages or tablets (Table 1). The impact of
184 dietary intervention with VPP and IPP on the systolic blood pressure differs in magnitude but is
185 largely consistent across the different studies. In contrast, an inconsistent effect on the diastolic
186 blood pressure is observed (Table 1). Only a few studies reported outcomes that are related to
187 blood pressure such as endothelial vascular function or arterial stiffness (Table 1).

188 The modest dose-dependent effects were also observed for the systolic blood pressure. The
189 magnitude of the decrease in systolic blood pressure was 14.1 ± 3.1 mg Hg. Most of the studies

showed that the antihypertensive effects were greater in normotensive and mildly hypertensive subjects in compared to more severely hypertensive patients. The duration of the dietary intervention does not seem to impact the outcomes related to blood pressure (Table 1).

None of the studies summarized in Table 1 reported any adverse effects of consuming milk hydrolysates and / or purified peptides on human health. This is an optimistic point for using milk bioactive peptides in human studies because safety of nutraceuticals is a necessary feature for regulatory acceptance and successful commercialization. Protein hydrolysates that are obtained by food grade enzymes are generally considered as safe; however, it was indicated that purified peptide fractions or purified peptides may require a safety assessment for approval as novel food (Duffuler et al., 2022).

Regarding the antihypertensive activity of bioactive peptides derived from camel milk, camel caseins have a similar frequency of the IPP motif as bovine caseins but the VPP motif is lacking (Table 1). Camel casein has more proline than bovine milk proteins. Since an N-terminal proline is a key structural determinant of ACE-inhibitory peptides (Wu et al., 2006) and peptide bonds adjacent to prolines are more resistant to proteolysis (Hu et al., 2011), camel milk may include ACE inhibitory peptides that are not present in bovine milk. As only few *in vivo* studies are available (Yahya et al., 2017), data for ACE inhibitory activity of camel milk derived peptides are mostly based on *in vitro* studies (Ayyash et al., 2018). One RCT showed no significant differences between fermented camel milk and diluted yogurt from bovine milk on blood pressure and obesity measures on 24 healthy adolescents with mild metabolic syndrome (13.77 ± 1.87 years old) (Fallah et al., 2019).

3. Peptides that inhibit starch digestion or improve glucose homeostasis.

Diabetes mellitus, a dominant chronic disease in developed countries, is characterized by an innate insulin secretion deficiency in type 1 diabetes or a defect in the insulin action in type 2 diabetes. Type 2 diabetes accounts for about 90% of the diabetes cases, which cause an insufficiency in conveying glucose from the bloodstream into cells, thus increase the glucose level in blood (Yu et al., 2012). Persistent hyperglycemia can lead to the development of insulin resistance, and then diabetes mellitus (Gangoiti et al., 2018). Delaying carbohydrate digestion is indispensable for the most beneficial treatment of type 2-diabetes. Peptides can delay starch digestion by inhibiting the starch digesting enzymes such as α-amylase and α-glucosidase. Potential inhibitors of starch digestion should have the capacity to bind to the target enzyme's active sites (catalytic sites) via hydrophobic interactions to impede the enzymes arrival to substrates (Figure 2) (Gangoiti et al., 2018; Miao & Hamaker, 2021; Yu et al., 2012). In addition, dietary peptides can improve glucose homeostasis by inhibition of the dipeptidyl peptidase IV, which increases insulin secretion and slows gastric emptying (Jao et al., 2015; Lacroix & Li-Chan, 2012). Bioactive peptides or bovine whey proteins also improve blood glucose homeostasis after ingestion of glucose (Gunnerud et al., 2013; Lan-Pidhainy & Wolever, 2010), indicating that additional mechanisms are likely to play a role.

Several milk-derived bioactive peptides were reported to exhibit an anti-hyperglycemic property that decrease postprandial glucose level in the blood. Numerous *in silico, in vitro,* and *in vivo* studies demonstrated that the peptides from milk product hydrolysates are a good source of anti-hyperglycemic agents. Clinical randomized, single and/or double-blind, partial and/or complete cross-over, placebo-controlled human trials that documented the effect of milk-derived dietary peptides on hyperglycemia are summarized in Table 2. These studies used milk protein hydrolysates (Sartorius et al., 2019), casein hydrolysates (Geerts et al., 2011), whey protein

hydrolysates with a known concentration of milk bovine minerals (Y. C. Chen et al., 2020), or bovine casein hydrolysates supplemented with leucine (Geerts et al., 2011; Manders et al., 2006). The doses of bovine milk protein hydrolysates, whey protein hydrolysates, and casein hydrolysates were 1.4 and 2.8 g (Sartorius et al., 2019), 50 g (Y. C. Chen et al., 2020), and 0.3 g / kg body weight (Manders et al., 2006) and 17.6 g (Geerts et al., 2011) / person per day, respectively (Table 2). The study participants included were normal healthy, prediabetic, and type 2- diabetes subjects and the treatment time ranged from single dose to 6 weeks (Table 2).

Co-ingestion of bovine casein hydrolysate beverage (0.3g / 4 mL water/kg body weight) enriched with leucine (L) (0.1g / 4 mL water/ kg body weight) after each main standardized meal reduced the prevalence of hyperglycemia significantly with a substantial reduction in the average of 24-h blood glucose concentrations in type 2 diabetes patients compared to placebo group. The 24-h blood glucose concentrations of the test group and placebo group were 9.6 ± 0.6 and 10.8 ± 0.5 mmol/L, respectively ($P < 0.05$) (Manders et al., 2006).

Only one study assessed the effect of bovine casein hydrolysates compared to intact casein protein and placebo (Geerts et al., 2011). Casein hydrolysates or casein were provided as a single meal replacement and the biological markers postprandial glucose concentration, serum glucagon, and insulin were measured in type 2 diabetes. Each patient received four types of treatment, specifically, placebo (control), casein hydrolysates (17.61 g), casein hydrolysates plus leucine (17.61 g and 5 g respectively), and unhydrolyzed casein (15 g). The results showed that both casein hydrolysates and casein hydrolysates with leucine supplementation had a similar postprandial glucose concentration reduction of 4.7% compared to 1.7% and 1.6% for casein and placebo, respectively. Glucagon concentrations increased by 14% for all treatments compared to the

placebo. The casein hydrolysates plus leucine treatment achieved the highest increase in insulin (Geerts et al., 2011).

A monocentric, three-way-cross-over, randomized, placebo-controlled, and double-blind study was performed on prediabetic subjects to determine the α-glucosidase inhibitory activity of whey protein hydrolysates rich in arginine-proline (AP) dipeptide. The tested products were provided in capsules, and each capsule had 350 mg whey protein hydrolysate (include ~ 0.96 mg of AP dipeptide) (Sartorius et al., 2019). In a single dose experiment, after 10 h overnight fasting, participants received a single dose of placebo, a low dose of whey protein hydrolysate peptides (1400 mg), or a high dose of whey protein hydrolysate peptides (2800 mg) 15 minutes before having a challenge meal rich in carbohydrates (standardized to 75 g of carbohydrates). After a one-week washout period, an open-label single arm design was applied in the experiment of 6 weeks, and participants received a low dose of whey protein hydrolysate peptides (1400 mg) daily 15 minutes before having a challenge meal rich in carbohydrates. The incremental areas under the concentration–time curves of glucose were significantly reduced by the low dose of whey protein hydrolysate peptides (1400 mg) compared to placebo. However, the longer period of treatment did not have any additional postprandial glycemic effect (Sartorius et al., 2019).

Bovine whey protein hydrolysates plus bovine milk minerals beverage elevated glucagon-like peptide-1 (GLP-1) approximately ninefold compared to other beverages (Y. C. Chen et al., 2020). Whey protein hydrolysates plus bovine milk minerals produced ~25% of GLP-1 more than whey protein hydrolysate only. No significant differences between bovine milk minerals beverage compared to the placebo was observed (Y. C. Chen et al., 2020).

Table 2 shows the studies that were carried out to evaluate the beneficial anti-hyperglycemic effect of bovine casein hydrolysates supplemented with leucine (Geerts et al., 2011; Manders et al.,

2006), whey protein hydrolysates with a known concentration of AP (Sartorius et al., 2019), calcium-enriched bovine milk minerals supplemented with whey protein hydrolysates as beverages or tablets (Y. C. Chen et al., 2020), or casein hydrolysates compared to intact casein and placebo (Geerts et al., 2011). These studies determined the anti-hyperglycemic effect based on different parameters like, postprandial glucose concentration, serum insulin, glucacon-like-peptides-1 (GLP-1), gastric inhibitory polypeptide (GIP), and peptide YY (PYY). The degree of effects of bovine casein and whey protein hydrolysates on the postprandial hyperglycemia differs but is largely consistent across the different studies. However, the effect of whey protein hydrolysates is much higher than the effect of casein protein hydrolysates and is due to the high content of leucine and proline amino acids in whey protein hydrolysates.

Collectively, the studies compiled in Table 2 demonstrate that co-ingestion of bovine whey protein hydrolysates clearly stimulates plasma GLP-1, increases the insulin concentration, and decreases the blood glucose concentration. Addition of calcium-enriched bovine milk to whey protein hydrolysates remarkably support high plasma GLP-1 concentrations. Enriched casein protein hydrolysates with leucine increase plasma insulin level. None of the studies that are summarized in Table 2 reported any significant adverse effects of bovine milk protein hydrolysates consumption on human health, except only a few subjects claimed gastrointestinal-related abdominal cramps with or without diarrhea. There was no linear dose-response relationship and there were no minimum effective doses of the hydrolysates. However, there was a clear impact of hydrophobic amino acids leucine and proline availability on anti-hyperglycemic activity of hydrolysates.

The inhibitory effect relates to the amino acid composition of the peptides itself. However, the inhibitory effects on dipeptidyl peptidase-4, α-amylase, and α-glucosidase enzymes depend on

303 competitive direct interaction to the active binding sites of hydrophobic enzymes and catalytic
304 triad against the substrate (Figure 2) (Gangoiti et al., 2018; Miao & Hamaker, 2021). Clinical
305 studies listed in table 2 emphasized that the proposed mechanisms for anti-hyperglycemic activity
306 are reduced starch digestion through inhibition of α-glucosidase activity by milk protein
307 hydrolysates rich in leucine. Only few studies, however, identified specific milk protein derived
308 peptides with inhibitory activity on intestinal glycosyl-hydrolases (Althnaibat et al., 2023).
309 Moreover, bovine whey proteins also reduced postprandial glucose levels when glucose rather than
310 starch was provided (Gunnerud et al., 2013; Lan-Pidhainy & Wolever, 2010). Other mechanisms
311 including amino acid-induced secretion of gut hormones that regulate glucose homeostasis such
312 as insulin and GLP-1 thus likely also play a role.
313 Because information on specific sequences of the peptides with anti-hyperglycemic activity is
314 limited, it is very difficult to predict which peptides in camel milk protein hydrolysates are
315 responsible for inhibiting the digestion of starch. However, camel milk proteins have more
316 hydrophobic amino acids such as leucine and proline in their sequence compared to bovine milk
317 proteins. Several peptides obtained by hydrolysis of camel milk proteins were shown to inhibit
318 amylase and glucosyl-hydrolases *in vitro* (Althnaibat et al., 2023), however, *in vivo* studies with
319 rodent models for diabetes used camel milk proteins rather than protein hydrolysates or defined
320 peptides (Muthukumaran et al., 2022; Shori, 2015). Likewise, the RCTs that investigated camel
321 milk efficacy in diabetic patients used an experimental design that does not allow conclusions as
322 to whether the observed effects are attributable to bioactive peptides that are released during
323 digestion or not. In addition, studies with camel milk that claimed the anti-hyperglycemic activity
324 of camel milk used different volumes of whole camel milk with various treatment times as doses
325 (Muthukumaran et al., 2022; Shori, 2015).

4. Antiadhesion activity of caseinmacropeptide (CMP).

The casein macropeptides (CMP) is the third most abundant protein in cheese whey, constituting about 15-20% of the total whey proteins. CMP represents the C-terminal of κ-casein obtained by the hydrolysis of milk protein by rennet (Nakano et al., 2018). Hydrolysis of the bovine milk κ-casein at Phe^{105}-Met^{106} linkage releases a polar polypeptide and a non-polar polypeptide. The former is the para-κ-casein, which consists of 105 amino acids and stays in the cheese curd. The latter is the CMP, which consists of 64 amino acids (Met^{106} - Val^{169} residue) and remains in the whey (Silva-Hernandez et al., 2002). CMP is produced commercially from bovine whey (Córdova-Dávalos et al., 2019; Neelima et al., 2013). It was reported that sialic acid linked to κ-casein of bovine milk supported growth of *Bifidobacterium* species (Delfour et al., 1965; György et al., 1954). CMP is a very good source of sialic acid which constitutes 7% - 9% of the total GMP (O'Riordan et al., 2014).

CMP has a unique chemical structure and functional properties. CMP is rich in amino acids such as proline, serine, glutamine, and threonine. However, CMP does not have any aromatic amino acids (tyrosine, phenylalanine, and tryptophan) or cysteine (Brody, 2000; Córdova-Dávalos et al., 2019). CMP is also rich in the branched-chain and hydrophobic amino acids leucine, isoleucine, valine (Figure 3). The presence of two aspartic acid and 7-8 glutamic acids makes CMP an acidic peptide (Brody, 2000; Neelima et al., 2013). Non-glycosylated CMP from bovine milk represents the two isoforms of κ-casein, A and B with molecular weights 6.75 kDa and 6.78 kDa, respectively (Figure 3). The average molecular weight of glycosylated CMP is 7.500 kDa (Córdova-Dávalos et al., 2019). CMP characteristics are affected by glycosylation and phosphorylation modifications. The glycosylation and phosphorylation of GMP occurs at serine and/or threonine residues at multiple positions (Figure 3) (Brody, 2000; Eigel et al., 1984; Nakano & Ozimek, 2000).

CMP has multiple biological functions that are conferred by its glycosylation. In addition to the composition of the oligosaccharides, frequency and spacing of glycan on the peptide backbone (i.e., increase glycosylation sites) affect the biological activity (Lewallen et al., 2009; O'Riordan et al., 2014; Oyelaran et al., 2009; Oyelaran & Gildersleeve, 2009).

Bovine CMP contains galactose (Gal), N-acetylgalactosamine (GalNAc), and N-acetylneuraminic acid (NeuNAc). These constituent monosaccharides give rise to the oligosaccharide structures that are linked by *O*-glycosylation to the peptide backbone: monosaccharide: (GalNAc), disaccharide: Gal-β-(1→3)-GalNAc), trisaccharides: NeuAc α2 – 3Gal β 1 – 3 GalNAc) and (Gal β 1 – 3 (NeuAc α2 – 6 GalNAc), and tetrasaccharide: (NeuAc α2- 3 Gal β 1 - 3 (NeuAc α2 – 6 GalNAc) (Figure 3) (Huth et al., 2004; O'Riordan et al., 2014; Saito & Itoh, 1992). Glycosylation with oligosaccharides that additionally include fucose and N-acetylglucosamine (GlcNAc) are present in CMP from bovine colostrum (O'Riordan et al., 2014).

In addition to providing a dietary source of sialic acid, biological activities of CMP include substrate for intestinal bacteria including bifidobacteria and the inhibition of pathogen adhesion (Brück et al., 2006).

The inhibition of pathogen adhesion is well supported by *in vitro* and *in vivo* studies (Figure 4). Most of enteric pathogens including *Salmonella*, enterotoxigenic *Escherichia coli* (ETEC), Shiga toxin-producing *E. coli* (STEC), *Shigella flexneri*, *Helicobacter pylori*, enterotoxins LT-I and LT-II derived from *E. coli.*, and the cholera toxin adhere by glycan recognition to infect or invade the host cells (Isoda et al., 1990; Nakajima et al., 2005; Sauvé et al., 2021; Wang et al., 2010; Yan et al., 2017). ETEC are a major cause of childhood diarrhea in developing countries and cause traveler's diarrhea. ETEC K88 cause watery diarrhea in newborn and post-weaning piglets and calves (DuPont, 1995; Jin & Zhao, 2000; Qadri et al., 2005). ETEC adhere to the small intestinal

epithelial cells and to the mucosal tissue through receptors of glycoprotein the host cells. Specifically, specific fimbriae mediate host-specific adhesion and support colonization of microvilli, followed by secretion of heat stable or heat labile enterotoxins that lead to electrolytes imbalance and water loss (Figure 4) (X. Y. Chen et al., 2014; Nagy & Fekete, 2005).

Glycan receptor analogues bind to bacterial lectins and thus inhibit the initial stages of infection and bacterial colonization (Figure 4) (Kulkarni et al., 2010; Shoaf-Sweeney & Hutkins, 2008). For example, K88 fimbriae mediate the binding of *E. coli* ECL13795 to porcine glycan receptors (Jin & Zhao, 2000). Porcine aminopeptidase N is a receptor for K88 fimbriae; in addition, surface glycan oligosaccharides composed of GalNAc, GlcNAc, galactosamine, and N-acetylmannosamine were proposed as receptors for ETEC K88 adhesion (Jin & Zhao, 2000; Moonens et al., 2015).

Molecules that act as glycan receptor analogues and thus prevent adhesion are a promising alternative to antibiotics. Anti-adhesion agents are not antibacterial agents and thus do not lead to the development of antimicrobial resistance (Krachler & Orth, 2013). Inhibition of the adhesion of enteric pathogens is one of the main biological properties of CMP. *In vitro* studies demonstrated anti-adhesion activity of CMP against enteric pathogens to the intestinal mucosa including enterohemorrhagic *E. coli* (EHEC) O157, ETEC K88, *Salmonella* Enteritidis, *Salmonella* Typhimurium, *Helicobacter pylori*, and *Shigella flexneri* (Isoda et al., 1990; Nakajima et al., 2005; Sauvé et al., 2021; Wang et al., 2010; Yan et al., 2017). Several *in vivo* studies confirmed the anti-adhesion activity of CMP against enteric pathogens in farm animals (Hermes et al., 2013; Isoda et al., 1990; Mouricout et al., 1990; Rong et al., 2015) .

For example, the anti-adhesion activity of glycoprotein glycans against the ETEC K99 in calves was confirmed *in vivo*. Directly after birth, colostrum was administered to calves, at age of 2-8

hours, and then 10^7-10^{10} CFU of ETEC K99 was administered orally to the calves. When the first sign of diarrhea appeared, 250 mg of oligosaccharides was ingested orally every day for three days. The adhesion of ETEC K99 to the small intestine was significantly reduced in the calves treated with oligosaccharides compared to control (Mouricout et al., 1990). Moreover, the anti-adhesion activity of CMP against the ETEC K88 was confirmed by inclusion of CMP in the diet of weaning piglets challenged with ETEC K88 (1 and/or 2%; 10 (Rong et al., 2015) and/or 20 (Hermes et al., 2013) g/ Kg dry matter of diet). A significant reduction in ETEC adhesion to the intestine epithelial cells and reduced overgrowth of ETEC in digestive tract was observed in the challenged treated group (Hermes et al., 2013; Rong et al., 2015).

The differences in the topological spacing of glycans and glycan content are recognized as important factors affecting the anti-adhesion activity of glycopeptides (Lewallen et al., 2009; O'Riordan et al., 2014; Oyelaran et al., 2009; Oyelaran & Gildersleeve, 2009). Camel milk is not as well studied as bovine milk. Camel milk CMP was about 10 times more effective than bovine CMP in preventing adhesion of ETEC K88 to porcine blood cells (Althnaibat et al., 2022). The chemical composition of camel milk CMP indicate that the altered glycan composition and the higher degree of glycosylation are responsible for the higher activity when compared to bovine CMP (Althnaibat et al., 2022).

5. Conclusions.

In summary, numerous *in vitro* and *in vivo* studies confirmed the efficacy of milk-derived bioactive peptides against blood hypertension, postprandial hyperglycemia, and anti-adhesion activity against enteric pathogens. However, research on camel milk is also very limited in compared to that involving bovine milk. Most of the research has focused on bovine milk and whey protein from camel milk has been overlooked. There are no active structures of peptide, and only a few

clinical studies have addressed the antihyperglycemic activity of camel milk. Therefore, to explore the potential bioactive peptides from camel milk, well-designed *in vivo* studies on camel milk-derived protein hydrolysates are required. Further, additional RCT trials are required to evaluate the full potentials of bioactive peptides derived from milk, and to determine the bioavailability of ingested bioactive peptides.

Acknowledgements

We acknowledge the Natural Sciences and Engineering Research Council of Canada (NSERC) and Canada Research Chairs (CRC) for funding.

References

Aihara, K., Nakamura, Y., Kajimoto, O., Kaneko, K., Mizutani, J., Ikeda, N., Nishimura, A., & Kajimoto, Y. (2004). Effects of the liquid yogurts containing "lactotripeptide (VPP, IPP)" on high-normal blood pressure. *Journal of Nutritional Food*, *7*(1), 123–137. https://www.researchgate.net/publication/255578225

Althnaibat, R. M., Bruce, H. L., & Gänzle, M. G. (2023). Identification of peptides from camel milk that inhibit starch digestion. *International Dairy Journal*, *141*, 105620. https://doi.org/10.1016/J.IDAIRYJ.2023.105620

Althnaibat, R. M., Koch, M., Bruce, H. L., Wefers, D., & Gänzle, M. G. (2022). Glycomacropeptide from camel milk inhibits the adhesion of enterotoxigenic *Escherichia coli* K88 to porcine cells. *International Dairy Journal*, *134*(105448), 1–9. https://doi.org/10.1016/j.idairyj.2022.105448

Asano, M., Nio, N., & Ariyoshi, Y. (1992). Inhibition of prolyl endopeptidase by synthetic beta-casein peptides and their derivatives with a C-terminal prolinol or prolinal. *Bioscience,*

Biotechnology, and Biochemistry, *56*(6), 976–977. https://doi.org/10.1271/BBB.56.976

Ayyash, M., Al-Nuaimi, A., Al-Mahadin, S., & Liu, S. (2018). In vitro investigation of anticancer and ACE-inhibiting activity, α-amylase and α-glucosidase inhibition, and antioxidant activity of camel milk fermented with camel milk probiotic: A comparative study with fermented bovine milk. *Food Chemistry*, *239*, 588–597. https://doi.org/10.1016/J.FOODCHEM.2017.06.149

Babkova, K., Korabecny, J., Soukup, O., Nepovimova, E., Jun, D., & Kuca, K. (2017). Prolyl oligopeptidase and its role in the organism: attention to the most promising and clinically relevant inhibitors. *Future Medicinal Chemistry*, *9*(10), 1015–1038. https://doi.org/10.4155/FMC-2017-0030

Badr, G. (2013). Camel whey protein enhances diabetic wound healing in a streptozotocin-induced diabetic mouse model: The critical role of β-Defensin-1, -2 and -3. *Lipids in Health and Disease*, *12*(1), 1–11. https://doi.org/10.1186/1476-511X-12-46

Bashir, S., & Al-Ayadhi, L. Y. (2014). Effect of camel milk on thymus and activation-regulated chemokine in autistic children: double-blind study. *Pediatric Research*, *75*(4), 559–563. https://doi.org/10.1038/PR.2013.248

Boelsma, E., & Kloek, J. (2010). IPP-rich milk protein hydrolysate lowers blood pressure in subjects with stage 1 hypertension, a randomized controlled trial. *Nutrition Journal*, *9*(1), 1–7. https://doi.org/10.1186/1475-2891-9-52/FIGURES/2

Bouglé, D., & Bouhallab, S. (2016). Dietary bioactive peptides: Human studies. *Http://Dx.Doi.Org/10.1080/10408398.2013.873766*, *57*(2), 335–343. https://doi.org/10.1080/10408398.2013.873766

462 Brody, E. P. (2000). Biological activities of bovine glycomacropeptide. *British Journal of*
463 *Nutrition*, *84*(SUPPL. 1). https://doi.org/10.1017/s0007114500002233

464 Brück, W. M., Redgrave, M., Tuohy, K. M., Lönnerdal, B., Graverholt, G., Hernell, O., &
465 Gibson, G. R. (2006). Effects of bovine α-lactalbumin and casein glycomacropeptide-
466 enriched infant formulae on faecal microbiota in healthy term infants. *Journal of Pediatric*
467 *Gastroenterology and Nutrition*, *43*(5), 673–679.
468 https://doi.org/10.1097/01.MPG.0000232019.79025.8F

469 Cadée, J. A., Chang, C.-Y., Chen, C.-W., Huang, C.-N., Chen, S.-L., & Wang, C.-K. (2007).
470 Casein Hydrolysate (C12 Peptide) Reduces Blood Pressure in Prehypertensive Subjects.
471 *AJH*, *20*(1), 1–5. https://doi.org/10.1016/j.amjhyper.2006.06.005

472 Chabance, B., Marteau, P., Rambaud, J. C., Migliore-Samour, D., Boynard, M., Perrotin, P.,
473 Guillet, R., Jollès, P., & Fiat, A. M. (1998). Casein peptide release and passage to the blood
474 in humans during digestion of milk or yogurt. *Biochimie*, *80*(2), 155–165.
475 https://doi.org/10.1016/S0300-9084(98)80022-9

476 Chakrabarti, S., Guha, S., & Majumder, K. (2018). Food-Derived Bioactive Peptides in Human
477 Health: Challenges and Opportunities. *Nutrients*, *10*(11), 1738–1755.
478 https://doi.org/10.3390/NU10111738

479 Chen, X. Y., Woodward, A., Zijlstra, R. T., & Gänzle, M. G. (2014). Exopolysaccharides
480 synthesized by *Lactobacillus reuteri* protect against enterotoxigenic *Escherichia coli* in
481 piglets. *Applied and Environmental Microbiology*, *80*(18), 5752–5760.
482 https://doi.org/10.1128/AEM.01782-14

483 Chen, Y. C., Smith, H. A., Hengist, A., Chrzanowski-Smith, O. J., Mikkelsen, U. R., Carroll, H.

A., Betts, J. A., Thompson, D., Saunders, J., & Gonzalez, J. T. (2020). Co-ingestion of whey protein hydrolysate with milk minerals rich in calcium potently stimulates glucagon-like peptide-1 secretion: an RCT in healthy adults. *European Journal of Nutrition*, *59*(6), 2449–2462. https://doi.org/10.1007/S00394-019-02092-4

Cicero, A. F. G., Colletti, A., Rosticci, M., Cagnati, M., Urso, R., Giovannini, M., Borghi, C., & D'addato, S. (2016). Effect of lactotripeptides (isoleucine-proline-proline/valine-proline-proline) on blood pressure and arterial stiffness changes in subjects with suboptimal blood pressure control and metabolic syndrome: A double-blind, randomized, crossover clinical rrial. *Metabolic Syndrome and Related Disorders*, *14*(3), 161–166. https://doi.org/10.1089/met.2015.0093

Cicero, A. F. G., Fogacci, F., & Colletti, A. (2017). Potential role of bioactive peptides in prevention and treatment of chronic diseases: a narrative review. *British Journal of Pharmacology*, *174*(11), 1378–1394. https://doi.org/10.1111/BPH.13608

Cicero, A. F. G., Gerocarni, B., Laghi, L., & Borghi, C. (2010). Blood pressure lowering effect of lactotripeptides assumed as functional foods: a meta-analysis of current available clinical trials. *Journal of Human Hypertension 2011 25:7*, *25*(7), 425–436. https://doi.org/10.1038/jhh.2010.85

Córdova-Dávalos, L. E., Jiménez, M., & Salinas, E. (2019). Glycomacropeptide Bioactivity and Health: A Review Highlighting Action Mechanisms and Signaling Pathways. *Nutrients*, *11*(3), 598–620. https://doi.org/10.3390/NU11030598

Dalgleish, D., & Corredig, M. (2012). The structure of the casein micelle of milk and its changes during processing. *Annual Review of Food Science and Technology*, *3*(1), 449–467.

https://doi.org/10.1146/ANNUREV-FOOD-022811-101214

Daroit, D. J., & Brandelli, A. (2021). In vivo bioactivities of food protein-derived peptides – a current review. *Current Opinion in Food Science*, *39*, 120–129. https://doi.org/10.1016/J.COFS.2021.01.002

de Leeuw, P. W., van der Zander, K., Kroon, A. A., Rennenberg, R. M. W., & Koning, M. M. G. (2009). Dose-dependent lowering of blood pressure by dairy peptides in mildly hypertensive subjects. *Http://Dx.Doi.Org/10.1080/08037050902761209*, *18*(1–2), 44–50. https://doi.org/10.1080/08037050902761209

DeGuire, J., Clarke, J., Rouleau, K., Roy, J., & Bushnik, T. (2019). *Blood pressure and hypertension*. https://doi.org/82-003-X

Delfour, A., Jolles, J., & Alais, C. (1965). Caseino-glycopeptides: Characterization of a methionine residue and of the N-terminal sequence. *Elsevier*, *19*(4), 452–455. https://www.sciencedirect.com/science/article/pii/0006291X65901452

Donoghue, M., Hsieh, F., Baronas, E., Godbout, K., Gosselin, M., Stagliano, N., Donovan, M., Woolf, B., Robison, K., Jeyaseelan, R., Breitbart, R. E., & Acton, S. (2000). A Novel Angiotensin-Converting Enzyme–Related Carboxypeptidase (ACE2) Converts Angiotensin I to Angiotensin 1-9. *Circulation Research*, *87*(5), 1–9. https://doi.org/10.1161/01.RES.87.5.E1

Duffuler, P., Bhullar, K. S., de Campos Zani, S. C., & Wu, J. (2022). Bioactive Peptides: From Basic Research to Clinical Trials and Commercialization. *Journal of Agricultural and Food Chemistry*, *70*(12), 3585–3595. https://doi.org/10.1021/ACS.JAFC.1C06289

DuPont, H. (1995). Pathogenesis of traveler's diarrhea. *Chemotherapy*, *41 Suppl 1*, 33–39.

https://doi.org/10.1159/000239395

Eigel, W. N., Butler, J. E., Ernstrom, C. A., Farrell, H. M., Harwalkar, V. R., Jenness, R., & Whitney, R. M. L. (1984). Nomenclature of proteins of cow's milk: Fifth revision. *Journal of Dairy Science*, *67*(8), 1599–1631. https://doi.org/10.3168/JDS.S0022-0302(84)81485-X

El-Agamy, E. I. (2009). *Bioactive components in camel milk. Bioactive components in milk and dairy products* . (pp. 159–192). Wiley-Blackwell.

Fallah, Z., Feizi, A., Hashemipour, M., Namazi, N., Azarbayejani, L., & Kelishadi, R. (2019). Effect of Fermented Camel Milk on Obesity Measures and Blood Pressure of Adolescents With Metabolic Syndrome. *Journal of Pediatrics Review*, *7*(3), 181–189. https://doi.org/10.32598/JPR.7.3.181

Foltz, M., Meynen, E. E., Bianco, V., Van Platerink, C., Koning, T. M. M. G., & Kloek, J. (2007). Angiotensin Converting Enzyme Inhibitory Peptides from a Lactotripeptide-Enriched Milk Beverage Are Absorbed Intact into the Circulation. *The Journal of Nutrition*, *137*(4), 953–958. https://doi.org/10.1093/JN/137.4.953

Gangoiti, J., Corwin, S. F., Lamothe, L. M., Vafiadi, C., Hamaker, B. R., & Dijkhuizen, L. (2018). Synthesis of novel α-glucans with potential health benefits through controlled glucose release in the human gastrointestinal tract. *Critical Reviews in Food Science and Nutrition, in press*. https://doi.org/10.1080/10408398.2018.1516621

García-Burgos, M., Moreno-Fernández, J., Alférez, M. J. M., Díaz-Castro, J., & López-Aliaga, I. (2020). New perspectives in fermented dairy products and their health relevance. *Journal of Functional Foods*, *72*(104059). https://doi.org/10.1016/J.JFF.2020.104059

Geerts, B. F., Van Dongen, M. G. J., Flameling, B., Moerland, M. M., De Kam, M. L., Cohen, A.

F., Romijn, J. A., Gerhardt, C. C., Kloek, J., & Burggraaf, J. (2011). Hydrolyzed casein decreases postprandial glucose concentrations in T2DM patients irrespective of leucine content. *Taylor & Francis*, *8*(3), 280–292. https://doi.org/10.3109/19390211.2011.593617

Guha, S., Sharma, H., Deshwal, G. K., & Rao, P. S. (2021). A comprehensive review on bioactive peptides derived from milk and milk products of minor dairy species. *Food Production, Processing and Nutrition 2021 3:1*, *3*(1), 1–21. https://doi.org/10.1186/S43014-020-00045-7

Gunnerud, U. J., Östman, E. M., & Björck, I. M. E. (2013). Effects of whey proteins on glycaemia and insulinaemia to an oral glucose load in healthy adults; a dose–response study. *European Journal of Clinical Nutrition 2013 67:7*, *67*(7), 749–753. https://doi.org/10.1038/ejcn.2013.88

György, P., Norris, R. F., & Rose, C. S. (1954). Bifidus factor. I. A variant of Lactobacillus bifidus requiring a special growth factor. *Archives of Biochemistry and Biophysics*, *48*(1), 193–201. https://doi.org/10.1016/0003-9861(54)90323-9

Hafeez, Z., Benoit, S., Cakir-Kiefer, C., Dary, A., & Miclo, L. (2021). Food protein-derived anxiolytic peptides: their potential role in anxiety management. *Food & Function*, *12*(4), 1415–1431. https://doi.org/10.1039/D0FO02432E

Hata, Y., Yamamoto, M., Ohni, M., Nakajima, K., Nakamura, Y., & Takano, T. (1996). A placebo-controlled study of the effect of sour milk on blood pressure in hypertensive subjects. *The American Journal of Clinical Nutrition*, *64*(5), 767–771. https://doi.org/10.1093/AJCN/64.5.767

Hermes, R. G., Molist, F., Francisco Pérez, J., De Segura, A. G., Ywazaki, M., Davin, R.,

572 Nofrarías, M., Korhonen, T. K., Virkola, R., & Martín-Orúe, S. M. (2013). Casein
573 glycomacropeptide in the diet may reduce *Escherichia coli* attachment to the intestinal
574 mucosa and increase the intestinal *lactobacilli* of early weaned piglets after an
575 enterotoxigenic *E. coli* K88 challenge. *British Journal of Nutrition*, *109*(6), 1001–1012.
576 https://doi.org/10.1017/S0007114512002978

577 Hirota, T., Ohki, K., Kawagishi, R., Kajimoto, Y., Mizuno, S., Nakamura, Y., & Kitakaze, M.
578 (2007). Casein Hydrolysate Containing the Antihypertensive Tripeptides Val-Pro-Pro and
579 Ile-Pro-Pro Improves Vascular Endothelial Function Independent of Blood Pressure–
580 Lowering Effects: Contribution of the Inhibitory Action of Angiotensin-Converting
581 Enzyme. *Hypertension Research 2007 30:6*, *30*(6), 489–496.
582 https://doi.org/10.1291/hypres.30.489

583 Holland, J. W., Deeth, H. C., & Alewood, P. F. (2006). Resolution and characterisation of
584 multiple isoforms of bovine κ-casein by 2-DE following a reversible cysteine-tagging
585 enrichment strategy. *PROTEOMICS*, *6*(10), 3087–3095.
586 https://doi.org/10.1002/PMIC.200500780

587 Hooton, D., Lentle, R., Monro, J., Wickham, M., & Simpson, R. (2015). The secretion and action
588 of brush border enzymes in the mammalian small intestine. In *Reviews of Physiology,*
589 *Biochemistry and Pharmacology* (Vol. 168, pp. 59–118).
590 https://doi.org/10.1007/112_2015_24

591 Horner, K., Drummond, E., & Brennan, L. (2016). Bioavailability of milk protein-derived
592 bioactive peptides: a glycaemic management perspective. *Nutrition Research Reviews*,
593 *29*(1), 91–101. https://doi.org/10.1017/S0954422416000032

Hu, Y., Stromeck, A., Loponen, J., Lopes-Lutz, D., Schieber, A., & Gänzle, M. G. (2011). LC-MS/MS quantification of bioactive angiotensin I-converting enzyme inhibitory peptides in rye malt sourdoughs. *Journal of Agricultural and Food Chemistry*, *59*(22), 11983–11989. https://doi.org/10.1021/jf2033329

Hua, S., Nwosu, C. C., Strum, J. S., Seipert, R. R., An, H. J., Zivkovic, A. M., German, J. B., & Lebrilla, C. B. (2011). Site-specific protein glycosylation analysis with glycan isomer differentiation. *Analytical and Bioanalytical Chemistry 2011 403:5*, *403*(5), 1291–1302. https://doi.org/10.1007/S00216-011-5109-X

Huth, P. J., Layman, D. K., & Brown, P. H. (2004). The emerging role of dairy proteins and bioactive peptides in nutrition and health: foreword. *Journal of Nutrition*, *134*(4), 989–995. https://academic.oup.com/jn/article/134/4/989S/4757182

Isoda, H., Kawasaki, Y., Tanimoto, M., Dosako, S., & Idota, T. (1990). *Use of compounds containing or binding sialic acid to neutralize bacterial toxins - European Patents* (Patent No. EP0385118A2). 385. https://patents.google.com/patent/EP0385118B1/un

Jafar, S., Kamal, H., Mudgil, P., Hassan, H. M., & Maqsood, S. (2018). Camel whey protein hydrolysates displayed enhanced cholesteryl esterase and lipase inhibitory, anti-hypertensive and anti-haemolytic properties. *LWT*, *98*, 212–218. https://doi.org/10.1016/J.LWT.2018.08.024

Jao, C. L., Hung, C. C., Tung, Y. S., Lin, P. Y., Chen, M. C., & Hsu, K. C. (2015). The development of bioactive peptides from dietary proteins as a dipeptidyl peptidase IV inhibitor for the management of type 2 diabetes. *BioMedicine (Taiwan)*, *5*(3), 9–15. https://doi.org/10.7603/S40681-015-0014-9/TABLES/2

Jauhiainen, T., Rönnback, M., Vapaatalo, H., Wuolle, K., Kautiainen, H., Groop, P.-H., & Korpela, R. (2010). Long-term intervention with *Lactobacillus helveticus* fermented milk reduces augmentation index in hypertensive subjects. *European Journal of Clinical Nutrition*, *64*, 424–431. https://doi.org/10.1038/ejcn.2010.3

Jauhiainen, T., Vapaatalo, H., Poussa, T., Kyrönpalo, S., Rasmussen, M., & Korpela, R. (2005). *Lactobacillus helveticus* fermented milk lowers blood pressure in hypertensive subjects in 24-h ambulatory blood pressure measurement. *American Journal of Hypertension*, *18*(12), 1600–1605. https://doi.org/10.1016/J.AMJHYPER.2005.06.006/2/AJH.1600.F1.JPEG

Jin, L. Z., & Zhao, X. (2000). Intestinal receptors for adhesive fimbriae of enterotoxigenic *Escherichia coli* (ETEC) K88 in swine - a review. *Applied Microbiology and Biotechnology*, *54*(3), 311–318. https://doi.org/10.1007/s002530000404

Kondrashina, A., Brodkorb, A., & Giblin, L. (2020). Dairy-derived peptides for satiety. *Journal of Functional Foods*, *66*, 1–14. https://doi.org/10.1016/J.JFF.2020.103801

Krachler, A. M., & Orth, K. (2013). Targeting the bacteria–host interface. *Virulence*, *4*(4), 284–294. https://doi.org/10.4161/VIRU.24606

Kulkarni, A. A., Weiss, A. A., & Iyer, S. S. (2010). Glycan-based high-affinity ligands for toxins and pathogen receptors. *Medicinal Research Reviews*, *30*(2), 327–393. https://doi.org/10.1002/MED.20196

Kumar, K. A., Venu, L., & Manchala, R. (2016). Anti-Hyperglycemic Activity of Camel Milk in Rat Models of Diabetes / Insulin Resistance. *International Journal of Research Studies in Medical and Health Sciences*, *1*(1), 7–14. https://doi.org/10.22259/ijrsmhs.0101002

Lacroix, I. M. E., & Li-Chan, E. C. Y. (2012). Dipeptidyl peptidase-IV inhibitory activity of

638 dairy protein hydrolysates. *International Dairy Journal*, *25*(2), 97–102.
639 https://doi.org/10.1016/J.IDAIRYJ.2012.01.003

640 Lan-Pidhainy, X., & Wolever, T. M. S. (2010). The hypoglycemic effect of fat and protein is not
641 attenuated by insulin resistance. *The American Journal of Clinical Nutrition*, *91*(1), 98–105.
642 https://doi.org/10.3945/AJCN.2009.28125

643 Layman, D. K., Shiue, H., Sather, C., Erickson, D. J., & Baum, J. (2003). Increased Dietary
644 Protein Modifies Glucose and Insulin Homeostasis in Adult Women during Weight Loss.
645 *The Journal of Nutrition*, *133*(2), 405–410. https://doi.org/10.1093/JN/133.2.405

646 Lewallen, D., Siler, D., & Iyer, S. (2009). Factors affecting protein-glycan specificity: effect of
647 spacers and incubation time. *Chembiochem : A European Journal of Chemical Biology*,
648 *10*(9), 1486–1489. https://doi.org/10.1002/CBIC.200900211

649 Luhovyy, B., Akhavan, T., & Anderson, G. (2007). Whey proteins in the regulation of food
650 intake and satiety. *Journal of the American College of Nutrition*, *26*(6), 704S-712S.
651 https://doi.org/10.1080/07315724.2007.10719651

652 Manders, R. J. F., Praet, S. F. E., Meex, R. C. R., Koopman, R., De Roos, A. L., Wagenmakers,
653 A. J. M., Saris, W. H. M., & Van Loon, L. J. C. (2006). Protein hydrolysate/leucine co-
654 ingestion reduces the prevalence of hyperglycemia in type 2 diabetic patients. *Diab. Care*,
655 *29*, 2721–2722. https://doi.org/10.2337/dc06-1424

656 Martin, M., Hagemann, D., Nguyen, T. T., Schwarz, L., Khedr, S., Moskopp, M. L., Henle, T., &
657 Deussen, A. (2020). Plasma concentrations and ACE-inhibitory effects of tryptophan-
658 containing peptides from whey protein hydrolysate in healthy volunteers. *European Journal*
659 *of Nutrition*, *59*(3), 1135–1147. https://doi.org/10.1007/S00394-019-01974-X

660 Mentlein, R. (2004). Cell-surface peptidases. *International Review of Cytology*, *235*, 165–213.
661 https://doi.org/10.1016/S0074-7696(04)35004-7

662 Miao, M., & Hamaker, B. R. (2021). Food matrix effects for modulating starch bioavailability.
663 *Annual Review of Food Science and Technology*, *12*(1). https://doi.org/10.1146/annurev-
664 food-070620-013937

665 Miner-Williams, W. M., Stevens, B. R., & Moughan, P. J. (2014). Are intact peptides absorbed
666 from the healthy gut in the adult human? *Nutrition Research Reviews*, *27*(2), 308–329.
667 https://doi.org/10.1017/S0954422414000225

668 Mizuno, S., Matsuura, K., Gotou, T., Nishimura, S., Kajimoto, O., Yabune, M., Kajimoto, Y., &
669 Yamamoto, N. (2005). Antihypertensive effect of casein hydrolysate in a placebo-controlled
670 study in subjects with high-normal blood pressure and mild hypertension. *British Journal of*
671 *Nutrition*, *94*, 84–91. https://doi.org/10.1079/BJN20051422

672 Moonens, K., Van Den Broeck, I., De Kerpel, M., Deboeck, F., Raymaekers, H., Remaut, H., &
673 De Greve, H. (2015). Structural and functional insight into the carbohydrate receptor
674 binding of F4 fimbriae-producing enterotoxigenic *Escherichia coli*. *Journal of Biological*
675 *Chemistry*, *290*(13), 8409–8419. https://doi.org/10.1074/JBC.M114.618595

676 Mouricout, M., Petit, J. M., Carias, J. R., & Julien, R. (1990). Glycoprotein glycans that inhibit
677 adhesion of *Escherichia coli* mediated by K99 fimbriae: Treatment of experimental
678 eolibacillosis. *Infection and Immunity*, *58*(1), 98–106. https://journals.asm.org/journal/iai

679 Muthukumaran, M. S., Mudgil, P., Baba, W. N., Ayoub, M. A., & Maqsood, S. (2022). A
680 comprehensive review on health benefits, nutritional composition and processed products of
681 camel milk. *Food Reviews International*. https://doi.org/10.1080/87559129.2021.2008953

Nagy, B., & Fekete, P. Z. (2005). Enterotoxigenic *Escherichia coli* in veterinary medicine. *International Journal of Medical Microbiology*, *295*(6–7), 443–454. https://doi.org/10.1016/J.IJMM.2005.07.003

Nakajima, K., Tamura, N., Kobayashi-Hattori, K., Yoshida, T., Hara-Kudo, Y., Ikedo, M., Sugita-Konishi, Y., & Hattori, M. (2005). Prevention of intestinal infection by glycomacropeptide. *Bioscience, Biotechnology, and Biochemistry*, *69*(12), 2294–2301. https://doi.org/10.1271/BBB.69.2294

Nakamura, T., Hirota, T., Mizushima, K., Ohki, K., Naito, Y., Yamamoto, N., & Yoshikawa, T. (2013). Milk-Derived Peptides, Val-Pro-Pro and Ile-Pro-Pro, Attenuate Atherosclerosis Development in Apolipoprotein E–Deficient Mice: A Preliminary Study. *Https://Home.Liebertpub.Com/Jmf*, *16*(5), 396–403. https://doi.org/10.1089/JMF.2012.2541

Nakamura, T., Mizutani, J., Ohki, K., Yamada, K., Yamamoto, N., Takeshi, M., & Takazawa, K. (2011). Casein hydrolysate containing Val-Pro-Pro and Ile-Pro-Pro improves central blood pressure and arterial stiffness in hypertensive subjects: A randomized, double-blind, placebo-controlled trial. *Atherosclerosis*, *219*, 298–303. https://doi.org/10.1016/j.atherosclerosis.2011.06.007

Nakamura, Y., Yamamoto, N., Sakai, K., Okubo, A., Yamazaki, S., & Takano, T. (1995). Purification and characterization of angiotensin I-converting enzyme inhibitors from sour milk. *Journal of Dairy Science*, *78*(4), 777–783. https://doi.org/10.3168/JDS.S0022-0302(95)76689-9

Nakano, T., & Ozimek, L. (2000). Purification of glycomacropeptide from caseinate hydrolysate by gel chromatography and treatment with acidic solution. *Journal of Food Science*, *65*(4),

588–590.

Nakano, T., Ozimek, L., & Betti, M. (2018). Separation of bovine κ-casein glycomacropeptide from sweet whey protein products with undetectable level of phenylalanine by protein precipitation followed by anion exchange chromatography. *Journal of Dairy Research*, *85*(1), 110–113. https://doi.org/10.1017/S0022029917000838

Neelima, Sharma, R., Rajput, Y. S., & Mann, B. (2013). Chemical and functional properties of glycomacropeptide (GMP) and its role in the detection of cheese whey adulteration in milk. *Dairy Science and Technology*, *93*(1), 21–43. https://doi.org/10.1007/S13594-012-0095-0

Nongonierma, A. B., & FitzGerald, R. J. (2016). Strategies for the discovery, identification and validation of milk protein-derived bioactive peptides. *Trends in Food Science and Technology*, *50*, 26–43. https://doi.org/10.1016/J.TIFS.2016.01.022

Nongonierma, A. B., Paolella, S., Mudgil, P., Maqsood, S., & FitzGerald, R. J. (2017). Dipeptidyl peptidase IV (DPP-IV) inhibitory properties of camel milk protein hydrolysates generated with trypsin. *Journal of Functional Foods*, *34*, 49–58. https://doi.org/10.1016/J.JFF.2017.04.016

O'Riordan, N., Kane, M., Joshi, L., & Hickey, R. M. (2014). Structural and functional characteristics of bovine milk protein glycosylation. *Glycobiology*, *24*(3), 220–236. https://doi.org/10.1093/GLYCOB/CWT162

Ohsawa, K., Satsu, H., Ohki, K., Enjoh, M., Takano, T., & Shimizu, M. (2008). Producibility and digestibility of antihypertensive beta-casein tripeptides, Val-Pro-Pro and Ile-Pro-Pro, in the gastrointestinal tract: analyses using an in vitro model of mammalian gastrointestinal digestion. *Journal of Agricultural and Food Chemistry*, *56*(3), 854–858.

https://doi.org/10.1021/JF072671N

Onwulata, C., & Huth, P. (Peter J. . (2008). *Whey processing, functionality and health benefits* (C. Onwulata & P. (Peter J. . Huth (eds.)). Wiley-Blackwell.

Oyelaran, O., & Gildersleeve, J. C. (2009). Glycan arrays: recent advances and future challenges. *Current Opinion in Chemical Biology*, *13*(4), 406–413. https://doi.org/10.1016/J.CBPA.2009.06.021

Oyelaran, O., Li, Q., Farnsworth, D., & Gildersleeve, J. C. (2009). Microarrays with varying carbohydrate density reveal distinct subpopulations of serum antibodies. *Journal of Proteome Research*, *8*(7), 3529–3538. https://doi.org/10.1021/PR9002245

Park, Y. W. (2009). *Bioactive components in milk and dairy products*. Wiley-Blackwell.

Pasolli, E., De Filippis, F., Mauriello, I. E., Cumbo, F., Walsh, A. M., Leech, J., Cotter, P. D., Segata, N., & Ercolini, D. (2020). Large-scale genome-wide analysis links lactic acid bacteria from food with the gut microbiome. *Nature Communications*, *11*, 2610. https://doi.org/10.1038/s41467-020-16438-8

Qadri, F., Svennerholm, A.-M., Faruque, A. S. G., & Sack, R. B. (2005). Enterotoxigenic *Escherichia coli* in developing countries: epidemiology, microbiology, clinical features, treatment, and prevention. *CLINICAL MICROBIOLOGY REVIEWS*, *18*(3), 465–483. https://doi.org/10.1128/CMR.18.3.465-483.2005

Rafiq, S., Huma, N., Pasha, I., Sameen, A., Mukhtar, O., & Khan, M. I. (2015). Chemical composition, nitrogen fractions and amino acids profile of milk from different animal species. *Asian-Australasian Journal of Animal Sciences*, *29*(7), 1022–1028. https://doi.org/10.5713/AJAS.15.0452

748 Romero, D. A., Magill, D., Millen, A., Horvath, P., & Fremaux, C. (2020). Dairy lactococcal and
749 streptococcal phage–host interactions: An industrial perspective in an evolving phage
750 landscape. *FEMS Microbiology Reviews*, *44*(6), 909–932.
751 https://doi.org/10.1093/femsre/fuaa048

752 Rong, Y., Lu, Z., Zhang, H., Zhang, L., Song, D., & Wang, Y. (2015). Effects of casein
753 glycomacropeptide supplementation on growth performance, intestinal morphology,
754 intestinal barrier permeability and inflammatory responses in *Escherichia coli* K88
755 challenged piglets. *Animal Nutrition*, *1*(2), 54–59.
756 https://doi.org/10.1016/j.aninu.2015.05.006

757 Saito, T., & Itoh, T. (1992). Variations and distributions of O-glycosidically linked sugar chains
758 in bovine kappa-casein. *Journal of Dairy Science*, *75*(7), 1768–1774.
759 https://doi.org/10.3168/JDS.S0022-0302(92)77936-3

760 Sánchez-Rivera, L., Ares, I., Miralles, B., Gómez-Ruiz, J. Á., Recio, I., Martínez-Larrañaga, M.
761 R., Anadón, A., & Martínez, M. A. (2014). Bioavailability and kinetics of the
762 antihypertensive casein-derived peptide HLPLP in rats. *Journal of Agricultural and Food*
763 *Chemistry*, *62*(49), 11869–11875. https://doi.org/10.1021/jf5035256

764 Sartorius, T., Weidner, A., Dharsono, T., Wilhelm, M., Boulier, A., & Brown, C. S. (2019).
765 Postprandial effects of a proprietary milk protein hydrolysate containing bioactive peptides
766 in prediabetic subjects. *Nutrients*, *11*(7), 1700–1717. https://doi.org/10.3390/NU11071700

767 Sauvé, M. F., Spahis, S., Delvin, E., & Levy, E. (2021). Glycomacropeptide: A Bioactive Milk
768 Derivative to Alleviate Metabolic Syndrome Outcomes. *Antioxidants and Redox Signaling*,
769 *34*(3), 201–222. https://doi.org/10.1089/ars.2019.7994

Shahbandeh, M. (2022). • *Cattle/cow population worldwide 2012-2022 | Statista*. https://www.statista.com/statistics/263979/global-cattle-population-since-1990/

Shigemura, Y., Nakaba, M., Shiratsuchi, E., Suyama, M., Yamada, M., Kiyono, T., Fukamizu, K., Park, E. Y., Nakamura, Y., & Sato, K. (2012). Identification of food-derived elastin peptide, prolyl-glycine (pro-gly), in human blood after ingestion of elastin hydrolysate. *Journal of Agricultural and Food Chemistry*, *60*(20), 5128–5133. https://doi.org/10.1021/JF300497P/ASSET/IMAGES/MEDIUM/JF-2012-00497P_0006.GIF

Shoaf-Sweeney, K. D., & Hutkins, R. W. (2008). Chapter 2 Adherence, anti-adherence, and oligosaccharides: preventing pathogens from sticking to the host. In *Advances in Food and Nutrition Research* (Vol. 55, pp. 101–161). Academic Press. https://doi.org/10.1016/S1043-4526(08)00402-6

Shori, A. B. (2015). Camel milk as a potential therapy for controlling diabetes and its complications: A review of in vivo studies. *Journal of Food and Drug Analysis*, *23*(4), 609–618. https://doi.org/10.1016/J.JFDA.2015.02.007

Silva-Hernandez, E., Nakano, T., & Ozimek, L. (2002). Isolation and analysis of kappa-casein glycomacropeptide from goat sweet whey. *Journal of Agricultural and Food Chemistry*, *50*(7), 2034–2038.

Ten Have, G. A. M., Van Der Pijl, P. C., Kies, A. K., & Deutz, N. E. P. (2015). Enhanced lacto-tri-Peptide bio-availability by co-ingestion of macronutrients. *PLOS ONE*, *10*(6), e0130638. https://doi.org/10.1371/JOURNAL.PONE.0130638

Townsend, R. R., Mcfadden, C. B., Ford, V., & Cadé, J. A. (2004). A randomized, double-blind,

placebo-controlled trial of casein protein hydrolysate (C12 peptide) in human essential hypertension. *AJH*, *17*(11), 1056–1058. https://doi.org/10.1016/j.amjhyper.2004.06.018

Turpeinen, A. M., Ehlers, P. I., Kivimäki, A. S., Järvenpää, S., Filler, I., Wiegert, E., Jähnchen, E., Vapaatalo, H., Korpela, R., & Wagner, F. (2011). Ile-Pro-Pro and Val-Pro-Pro tripeptide-containing milk product has acute blood pressure lowering effects in mildly hypertensive subjects. *Taylor & Francis*, *33*(6), 388–396. https://doi.org/10.3109/10641963.2010.549267

Turpeinen, A. M., Ikonen, M., Kivim€, A. S., Kautiainen, H., Vapaatalo, H., & Korpela, R. (2012). A spread containing bioactive milk peptides Ile-Pro-Pro and Val-Pro-Pro, and plant sterols has antihypertensive and cholesterol-lowering effects. *Food Funct.*, *3*, 621–627. https://doi.org/10.1039/c2fo10286b

van der Zander, K., Jäkel, M., Bianco, V., & Koning, M. M. G. (2008). Fermented lactotripeptides-containing milk lowers daytime blood pressure in high normal-to-mild hypertensive subjects. *Journal of Human Hypertension* , *22*(11), 804–806. https://doi.org/10.1038/jhh.2008.59

Vieira de Oliveira, G., Volino-Souza, M., Mendes Cordeiro, E., Adam Conte-Junior, C., Silveira Alvares, T., & Volino-Souza, onica. (2020). Effects of fish protein hydrolysate ingestion on endothelial function compared to whey protein hydrolysate in humans. *Taylor & Francis*, *71*(2), 242–248. https://doi.org/10.1080/09637486.2019.1635090

Wang, Y., Gänzle, M., & Schwab, C. (2010). Exopolysaccharide synthesized by *Lactobacillus reuteri* decreases the ability of enterotoxigenic *Escherichia coli* to bind to porcine erythrocytes. *Appl Environ Microbiol*, *76*(14), 4863–4866.

https://doi.org/10.1128/aem.03137-09

Whelton, P. K., Carey, R. M., Aronow, W. S., Casey, D. E., Collins, K. J., Himmelfarb, C. D., DePalma, S. M., Gidding, S., Jamerson, K. A., Jones, D. W., MacLaughlin, E. J., Muntner, P., Ovbiagele, B., Smith, S. C., Spencer, C. C., Stafford, R. S., Taler, S. J., Thomas, R. J., Williams, K. A., … Hundley, J. (2018). 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA guideline for the prevention, detection, evaluation, and management of high blood pressure in adults a report of the American College of Cardiology/American Heart Association Task Force on Clinical practice guidelines. *Hypertension*, *71*(6), E13–E115. https://doi.org/10.1161/HYP.0000000000000065/-/DC2

Wu, J., Aluko, R. E., & Nakai, S. (2006). Structural requirements of angiotensin I-converting enzyme inhibitory peptides: Quantitative qtructure−activity relationship study of di- and tripeptides. *Journal of Agricultural and Food Chemistry*, *54*(3), 732–738. https://doi.org/10.1021/JF051263L

Xu, Q., Fan, H., Yu, W., Hong, H., & Wu, J. (2017). Transport study of egg-derived antihypertensive peptides (LKP and IQW) using Caco-2 and HT29 coculture nonolayers. *Journal of Agricultural and Food Chemistry*, *65*(34), 7406–7414. https://doi.org/10.1021/ACS.JAFC.7B02176

Xu, Q., Hong, H., Wu, J., & Yan, X. (2019). Bioavailability of bioactive peptides derived from food proteins across the intestinal epithelial membrane: A review. *Trends in Food Science & Technology*, *86*, 399–411. https://doi.org/10.1016/J.TIFS.2019.02.050

Yahya, M. A., Alhaj, O. A., & Al-Khalifah, A. S. (2017). Efecto antihipertensivo de la leche de camello fermentada (*Camelus dromedarius*) en ratas hipertensas. *Nutricion Hospitalaria*,

34(2), 416–421. https://doi.org/10.20960/NH.1163

Yamasue, K., Morikawa, N., Mizushima, S., & Tochikubo, O. (2010). The blood pressure lowering effect of lactotoripeptides and salt intake in 24-h ambulatory blood pressure measurements. *Clinical and Experimental Hypertension*, *32*, 214–220. https://doi.org/10.3109/10641963.2010.491885

Yan, Y. L., Hu, Y., Simpson, D. J., & Gänzle, M. G. (2017). Enzymatic synthesis and purification of galactosylated chitosan oligosaccharides reducing adhesion of enterotoxigenic *Escherichia coli* K88. *Journal of Agricultural and Food Chemistry*, *65*(25), 5142–5150. https://doi.org/10.1021/acs.jafc.7b01741

Yoshioka, M., Erickson, R. H., & Kim, Y. S. (1988). Digestion and assimilation of proline-containing peptides by rat intestinal brush border membrane carboxypeptidases. Role of the combined action of angiotensin-converting enzyme and carboxypeptidase P. *The Journal of Clinical Investigation*, *81*(4), 1090–1095. https://doi.org/10.1172/JCI113421

Yu, Z., Yin, Y., Zhao, W., Liu, J., & Chen, F. (2012). Anti-diabetic activity peptides from albumin against α-glucosidase and α-amylase. *Food Chemistry*, *135*(3), 2078–2085. https://doi.org/10.1016/J.FOODCHEM.2012.06.088

Zambrowicz, A., Pokora, M., Setner, B., Dąbrowska, A., Szołtysik, M., Babij, K., Szewczuk, Z., Trziszka, T., Lubec, G., & Chrzanowska, J. (2015). Multifunctional peptides derived from an egg yolk protein hydrolysate: isolation and characterization. *Amino Acids*, *47*(2), 369. https://doi.org/10.1007/S00726-014-1869-X

Zhang, G., Hasek, L. Y., Lee, B. H., & Hamaker, B. R. (2015). Gut feedback mechanisms and food intake: a physiological approach to slow carbohydrate bioavailability. *Food &*

858 *Function*, *6*(4), 1072–1089. https://doi.org/10.1039/C4FO00803K

859

Figure legends.

Figure 1. *In vitro, in vivo,* and randomized controlled trails confirmed studies for bioactive peptides derived from milk. The information for the draw from (Asano et al., 1992; Ayyash et al., 2018; Babkova et al., 2017; Badr, 2013; Bashir & Al-Ayadhi, 2014; Y. C. Chen et al., 2020; Guha et al., 2021; Hafeez et al., 2021; Huth et al., 2004; Kondrashina et al., 2020; Kumar et al., 2016; Nongonierma et al., 2017; Ohsawa et al., 2008). For peptides that prevent pathogen adhesion, food animals serve not only as animal model for human disease but as the actual target for intervention.

Figure 2. Starch digestion in gastrointestinal tract and potential mechanism of milk derived bioactive peptides for inhibition of starch digestion.

Figure 3. Amino acid sequence and glycan structure of caseinmacropeptide (CMP) derived from bovine milk (variant A and B). The differences between variant A and B only in two amino acid residues at ^{136} and ^{148} and variant B shown between brackets. Potential glycosylation sites are printed in bold. Bold blue-colored correspond to reported glycosylation sites, while red-colored correspond to reported phosphorylation sites. The red arrow and underline indicate the chymosin cleave sites between P^{105}-M^{106}. Glycans are indicated as follows: orange squares, N-acetylgalactosamine; orange diamonds, galactose; green circle, N-acetylneuraminic acid (sialic acid); black dotted lines, β-(1→3) glycosidic linkage; black solid lines; α-(2→3) or α-(2→6) glycosidic linkages; blue lines, link to threonine or serine residues on the peptide backbone. The peptide sequence of bovine CMP is drawn based on the UniProt (https://www.uniprot.org/uniprot/) accession numbers P02668, the numbering of residues is based on the sequence of the κ-casein without precursor. Drawn with information from (Dalgleish & Corredig, 2012; Eigel et al., 1984; Holland et al., 2006; Hua et al., 2011).

882 **Figure 4.** Adhesion of bacteria to the epithelial cells and antiadhesion activity for glycopeptides.

Table 1. Antihypertensive activity of milk-derived bioactive peptides in randomized clinical trials.

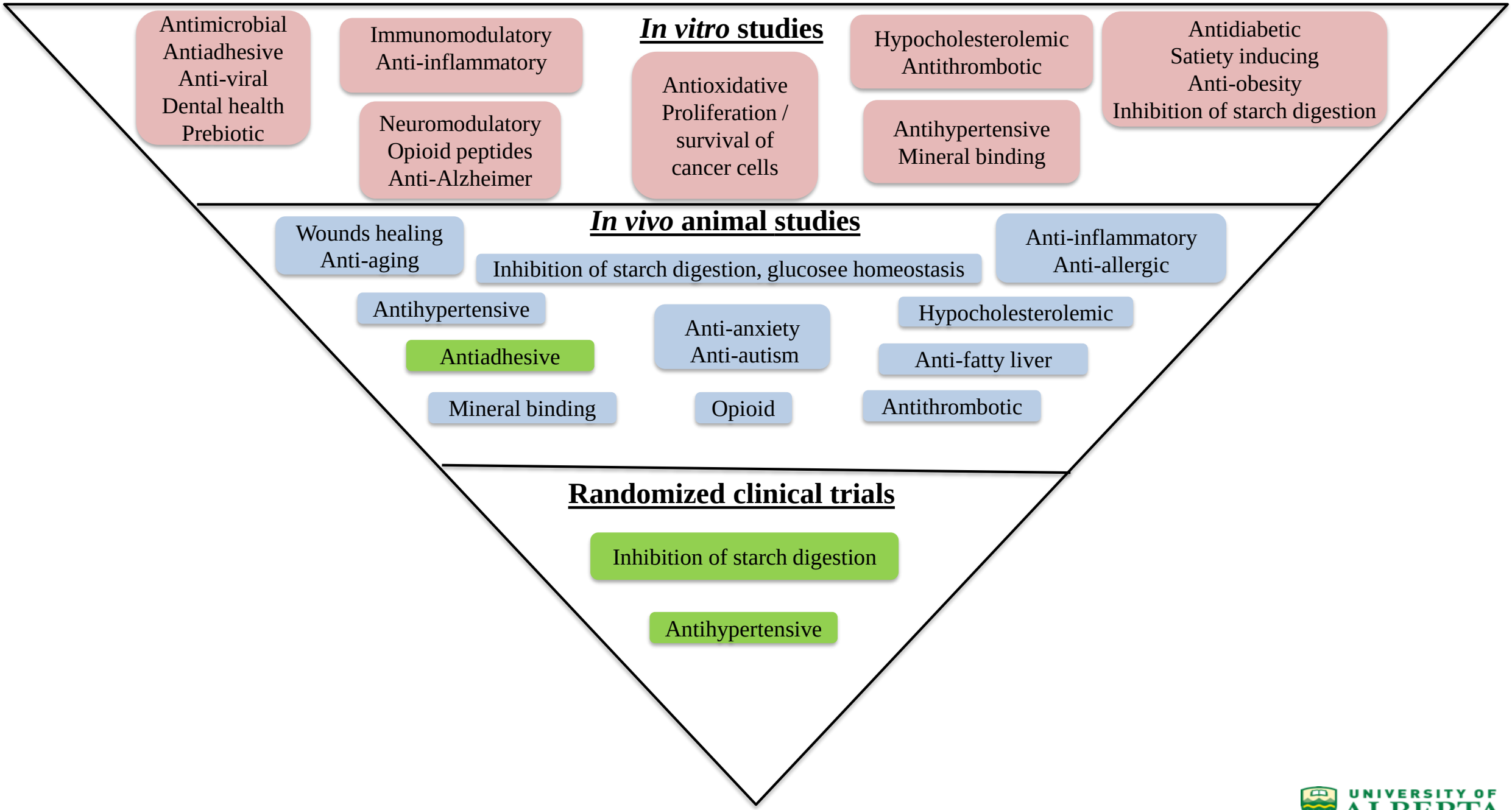
Protein/ Peptide (Source)	# In bovine protein	# In camel protein	Treatment period [week] (dose [mg/day])	Effects on blood pressure (▼in SBP/DBP compared to placebo) [ref]
FFVAPFPEVFGK (Bovine)	1(αs1 casein)	0	Single dose (200 mg & 3.51 g alginic acid)	▼(–9.2 ± 3.2/–6.0 ± 2.0) (Townsend et al., 2004)
			4 (3.8 g)	▼(–10.7 ± 1.6 /–6.9 ± 1.2 mm Hg), ▼plasma angiotensin II and aldosterone (Cadée et al., 2007)
Bovine casein hydrolysate with (VPP + IPP)	VPP 1(β-casein) IPP 1(β-casein) 1(κ-casein)	VPP 0 IPP 1(β-casein) 1(κ-casein)	6 (0, 1.8, 2.5, & 3.6 mg VPP+IPP)	▼ (0, –5.8/0, –6.2/0, & –9.3/0 mmHg) (Mizuno et al., 2005)
			8 (2.3, 4.6, & 9 mg VPP+IPP)	▼(+0.1/–1.3, –1.5/–1.4, & –2.5/–1.9 mmHg) (de Leeuw et al., 2009)
			10 (30 mg VPP & 22.5 mg IPP)	▼ (–4. 1± 0.9/ –1.8 ± 0.7) (Jauhiainen et al., 2005)
			4 (18.7 mg VPP & 15.9 mg IPP)	▼ (~ –5.0/0) (van der Zander et al., 2008)
			8 (1.5 mg VPP & 1.1 mg IPP)	▼ (–14.1 ± 3.1/–6.6 ± 2.5 mm Hg) (Hata et al., 1996)
			4 (4.1 mg VPP & 6 mg IPP)	▼ (– 3.4 ± 4.4/–3.1 ± 3.2 mmHg) (Cicero et al., 2016)
			8 (5.32 mg VPP & 2.76 mg IPP)	▼(~ –5.0/–2.0 night sleeping) (Yamasue et al., 2010)
			12 (2.26 mg VPP & 1.48 mg IPP)	▼(– 6.1 ± 5.7/–3.8 ± 6.3 mmHg) (Aihara et al., 2004)
			8 (3.4 mg VPP+IPP)	▼(−11.0 ± 11.0/ 0) (T. Nakamura et al., 2011)
			1 (3.42 mg VPP & 3.87 mg IPP)	▲ endothelial vascular function, (▼0/0) (Hirota et al., 2007)
			24; 12 (2.6 mg VPP & 2.4 mg IPP) +12 (26.4 mg VPP & 23.2 mg IPP)	▼ arterial stiffness, especially in metabolic syndrome patients, ▼ (− 4.6/− 2.7 mmHg) (Jauhiainen et al., 2010)
VPP + IPP (+ plant sterol esters)			10 (4.2 mg VPP+IPP & 2 g plant sterols)	▼ (− 4.1/0 mmHg),▼total and LDL cholesterol (Turpeinen et al., 2012)
			Single dose (25 mg VPP+IPP & 2 g plant sterols)	▼ (−2.1/−1.6 mm Hg) (Turpeinen et al., 2011)
IPP (Bovine)			4 (15 mg IPP)	▼ (−3.8/−2.3 mm Hg) (Boelsma & Kloek, 2010)
Bovine whey hydrolysate	–	—	Single dose (20 g Whey hydrolysate)	▲ endothelial dilation (Vieira de Oliveira et al., 2020)
Bovine whey hydrolysate (IW+WL)	IW 1(α-LA) 1(LF) WL 2(α-LA)	IW 1(α-LA) 1(LF) WL 1 (Ig) 1(α-LA)	Single dose (250.5 mg IW & 47.5 mg LW)	▼ plasma ACE activity (0/0) (Martin et al., 2020)

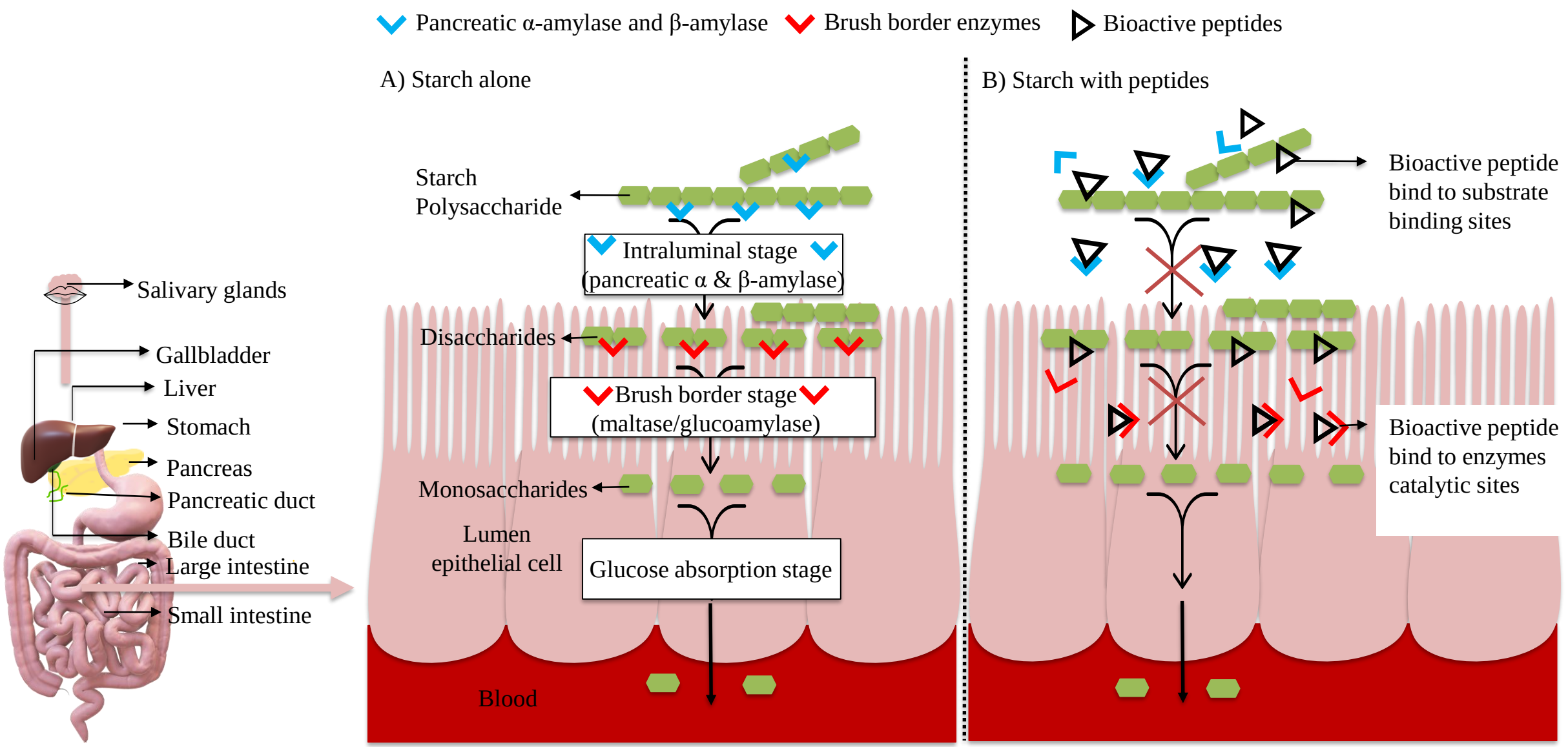
α-LA; α-lactalbumin, LF; lactoferrin, Ig; immunoglobulin, L; leucine, I; isoleucine, and W; tryptophan.

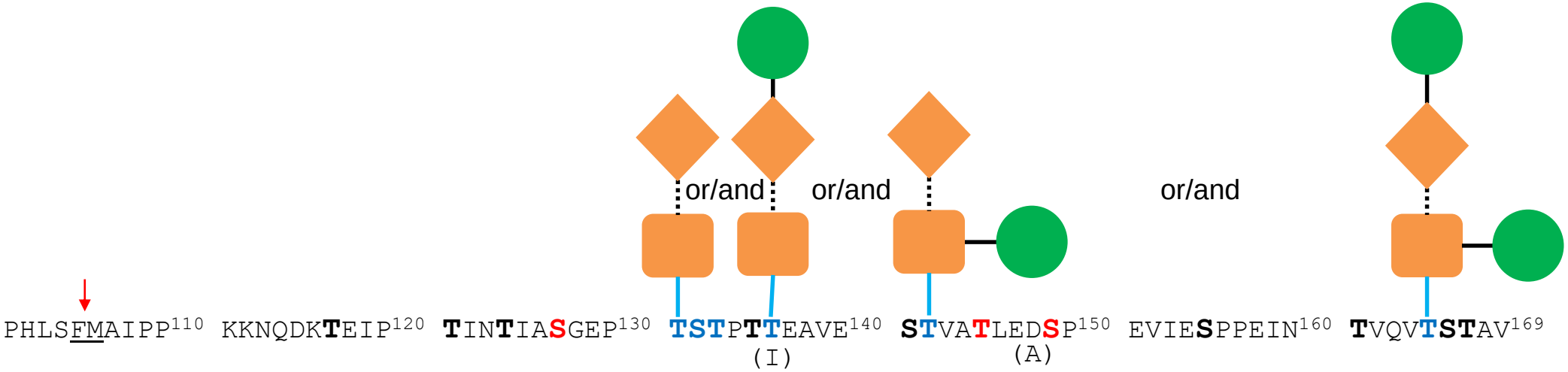
Table 2. Antihyperglycemic activity of bioactive peptides in randomized clinical trials.

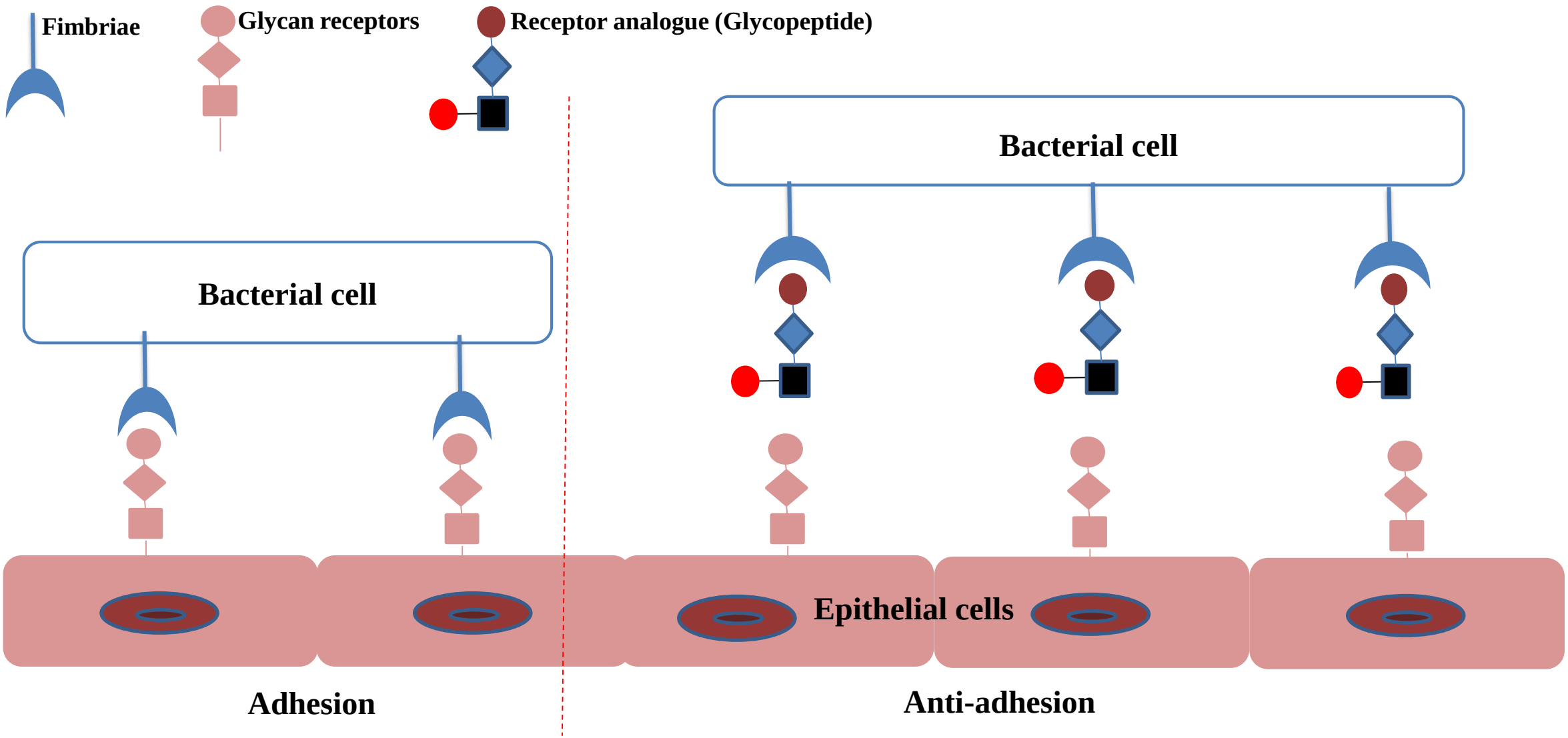
Protein/ Peptide (Source)	Treatment period (dose)	Effects [ref]
Casein hydrolysate (Bovine)	Single dose (17.61 g ca. hy.)	▼ postprandial glucose values and ▲postprandial insulin response (Geerts et al., 2011)
Casein hydrolysate + Leucine (Bovine)	3 doses/ day ((0.3 g ca. hy. & 0.1 g L)/ kg body weight)	▼ hyperglycemia in T2D patients over 24 h (Manders et al., 2006)
	Single dose (17.61 g ca. hy. & 5 g L)	▼ postprandial glucose values and ▲postprandial insulin response (Geerts et al., 2011)
Bovine milk hydrolysate	6 weeks (1.4 wp. hy.)	▼ plasma glucose after high carbohydrate meal and HbA1c (Sartorius et al., 2019)
	Single dose (1.4 & 2.8 g wp. hy.)	▼ plasma glucose after high carbohydrate meal (Sartorius et al., 2019)
Bovine whey hydrolysate + milk minerals	Single dose (50 g wp. hy. & 1000mg Ca.)	▲ plasma GLP (Y. C. Chen et al., 2020)

GLP-1; Glucagon-like peptide-, GIP; Glucose-dependent insulinotropic polypeptide, PYY; Peptide tyrosine–tyrosine, HbA1c; glycated hemoglobin, supp.; supplementary drink, ca. hy.; casein hydrolysate, wp. Hy.; whey protein hydrolysate, and Ca; calcium.


Antimicrobial
Antiadhesive
Anti-viral
Dental health
Prebiotic
Immunomodulatory
Anti-inflammatory
Neuromodulatory
Opioid peptides
Anti-Alzheimer
In vitro studies
Antioxidative
Proliferation / survival of cancer cells
Hypocholesterolemic
Antithrombotic
Antihypertensive
Mineral binding
Antidiabetic
Satiety inducing
Anti-obesity
Inhibition of starch digestion
In vivo animal studies
Wounds healing
Anti-aging
Inhibition of starch digestion, glucosee homeostasis
Anti-inflammatory
Anti-allergic
Antihypertensive
Antiadhesive
Anti-anxiety
Anti-autism
Hypocholesterolemic
Anti-fatty liver
Mineral binding
Opioid
Antithrombotic
Randomized clinical trials
Inhibition of starch digestion
Antihypertensive


Pancreatic α-amylase and β-amylase
Brush border enzymes
Bioactive peptides
A) Starch alone
B) Starch with peptides
Salivary glands
Gallbladder
Liver
Stomach
Pancreas
Pancreatic duct
Bile duct
Large intestine
Small intestine
Starch
Polysaccharide
Intraluminal stage
(pancreatic α & β-amylase)
Disaccharides
Brush border stage
(maltase/glucoamylase)
Monosaccharides
Lumen
epithelial cell
Glucose absorption stage
Blood
Bioactive peptide
bind to substrate
binding sites
Bioactive peptide
bind to enzymes
catalytic sites


or/and
or/and
or/and
PHLSFMAIPP110 KKNQDKTEIP120 TINTIASGEP130 TSTPTTEAVE140 STVATLEDSP150 EVIESPPEIN160 TVQVTSTAV169
(I)
(A)


Fimbriae
Glycan receptors
Receptor analogue (Glycopeptide)
Bacterial cell
Bacterial cell
Epithelial cells
Adhesion
Anti-adhesion