Bioactive properties of milk proteins in humans: A review

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Abstract

Many studies have demonstrated that milk protein consumption has benefits in terms of promoting human health. This review assesses the intervention studies which have evaluated potential health enhancing effects in humans following the ingestion of milk proteins. The impact of milk protein ingestion has been studied to assess their satiating, hypotensive, antimicrobial, anti-inflammatory, anticancer, antioxidant, insulinotropic properties as well as their impact on morphological modifications (e.g., muscle and fat mass) in humans. Consistent health promoting effects appear to have been observed in certain instances (i.e., muscle protein synthesis, insulinotropic and hypotensive activity). However, controversial outcomes have also been reported (i.e., antimicrobial, anti-inflammatory, anticancer and antioxidant properties). Several factors including interindividual differences, the timing of protein ingestion as well as the potency of the active components may explain these differences. In addition, processing conditions have been reported, in certain instances, to affect milk protein structure and therefore modify their bioactive potential. It is thought that the health promoting properties of milk proteins are linked to the release of bioactive peptides (BAPs) during gastrointestinal digestion. There is a need for further research to develop a more in-depth understanding on the possible mechanisms involved in the observed physiological effects. In addition, more carefully controlled and appropriately powered human intervention studies are required to demonstrate the health enhancing properties of milk proteins in humans.

Key words: milk proteins; human studies; bioactive peptides; processing.
1. Introduction

Milk proteins are recognized for their high nutritional quality. In addition, intact milk proteins have been associated with a wide range of *in vitro* bioactivities, such as satiating, antimicrobial, mineral binding, anti-lipidaemic and anticancer properties [2, 28, 30, 93]. During digestion in humans, milk proteins are cleaved by various proteinases and peptidases in the gastrointestinal tract (GIT), resulting in the generation of free amino acids and peptides [131]. Specific peptide fragments, also known as bioactive peptides (BAPs), have been shown *in vitro* to positively affect markers associated, for instance, with inflammation, hypertension, diabetes and osteoporosis [41, 52, 66, 96]. These BAPs may be released during gastrointestinal (GI) digestion of milk proteins in humans [15]. Several studies have reported that milk protein-derived BAPs may also play a beneficial *in vivo* role in humans [for reviews: see 7, 51, 84, 100].

To date, most studies in humans appear to have been conducted with hydrolysed milk proteins while intact milk proteins may also have some potential in human health [76]. Human intervention studies with intact milk proteins have focused on their satiating, hypotensive, antimicrobial, anti-inflammatory, anticancer, antioxidant and insulino-tropic properties [44, 47, 81, 102, 119, 130]. Milk protein consumption has also been shown to have potential in increasing muscle protein synthesis in humans [32, 135]. However, certain human intervention studies have failed to demonstrate health promoting effects following milk protein consumption [48, 103].

The aim of this review was to specifically investigate the potential link between milk protein consumption and their health promoting effects in humans. Therefore, human intervention studies which have been conducted with milk proteins were studied for a wide range of bioactive (i.e., satiating, hypotensive, antimicrobial, anti-inflammatory, anticancer, antioxidant and insulino-tropic and muscle protein synthesis) properties. The outcomes of these human intervention studies as well as the parameters (e.g., dose, duration, health status of the subjects, etc.) which generally yielded health enhancement were studied. The scientific literature related to the bioactive properties of milk proteins in humans was assessed for the time period ranging from 1985 to 2015. Current evidence on the release of BAP sequences following milk protein ingestion in humans was collated. The impact of processing on milk protein structure and the
effect on the bioactivity was also assessed to better understand how these may modulate peptide release and in turn impact on human health.

2. Satiating and weight management effects of milk proteins in humans

It has been suggested that a significant increase in protein intake, over habitual intake, could help in weight management and positively alter body composition [2, 77, 128]. Significant differences in anthropometric parameters were reported in healthy and obese humans when the protein concentration in the test group was on average 58.4% (g/kg/day) higher than in the control group. In contrast, no effect on food intake was observed with differences of up to 38.8% (g/kg/day) between the test and control groups [14]. Milk proteins, which have been described for their satiating properties, may have potential for use as natural dietary components to reduce food intake in humans [2, 36, 77, 115, 128]. Numerous human intervention studies have focused on the satiating properties of milk proteins and their effect on the reduction of food intake [for reviews: see 13, 77]. A strong link between protein intake and weight loss has been suggested, highlighting the role of their texturizing properties, amino acid composition and rate of absorption in increasing satiety. However, to date, a direct link between satiety and food intake reduction followed by weight loss has proved difficult to establish in humans.

Owing to differences between the rate of digestion, amino acid and peptide appearance in the plasma, whey proteins (WPs) have been postulated as being more satiating than caseins (CNs). A faster gastric emptying for WPs compared to CNs has been shown [2]. Differences were found with intact CN solutions, which showed a slower rate of intestinal absorption compared to the WPs [22]. This was linked to the fact that part of the CN may coagulate at the acidic gastric pH, thereby delaying CN protein breakdown [56]. On a time scale basis, it has been suggested that WPs are more satiating in the short term and CN in the long term [13].

Table 1 summarizes the outcomes of human intervention studies conducted with intact milk proteins. A positive role of WPs in reducing food intake has been shown in human studies involving obese subjects [9, 122]. Recently, a 12 week study was conducted with obese subjects with a body mass index (BMI) between 25 and 40 kg m⁻² who received a preload of whey protein concentrate (WPC, 65 g) before an ad libitum meal [122]. At the end of the intervention, there
was a significant effect of the WPC preload on appetite (-41%), calorie intake (-50%), anthropometry (reduction in body weight and waist circumference) and body composition (reduction in fat and increase in lean muscle mass) relative to the baseline. A 23 week intervention with WPs carried out with obese subjects showed the same outcomes with a reduction in weight (-2.5%), fat mass (-2.3 kg), waist circumference (-2.4 cm) and fasting ghrelin (-13.6%) compared to a control group receiving an isocaloric maltodextrin drink [9]. In contrast, with overweight adolescents, the intake of skimmed milk, CN and WP (30 [72] or 35 g/day [6]) over 12 weeks was shown to negatively affect anthropometric parameters. The 3 milk proteins caused an increase in BMI, BMI-for-age Z-scores and weight. Fasting insulin, homeostatic model assessment (HOMA, a predictor of insulin resistance) and fasting C-peptide (a surrogate marker of insulin resistance) increased in the WP and CN groups compared to baseline. However, these changes were not seen in the control group receiving water instead of the milk protein drinks [6]. A significant increase in lean and fat mass was seen with the 3 milk proteins. In addition, there was a 30 and 15% increase in leptin (an orexigenic hormone) levels in the CN and WP groups, respectively. In contrast, consumption of water instead of the milk proteins resulted in a significant increase in lean mass but not in fat mass. These studies suggested that in obese adolescents, water intake instead of milk protein drinks may be more beneficial to positively alter body composition [6, 72].

α-Lactalbumin (α-La) is rich in Trp, the precursor of serotonin, which has been linked to cognitive function and in certain instances to satiating effects in humans [for review, see: 99]. α-La has been shown to increase free plasma Trp in humans, which may have resulted in an increase in hypothalamic serotonin [83]. Supplementation of α-La in a custard-style breakfast reduced subsequent energy intake by 20% in an ad libitum lunch in healthy humans with a BMI between 22 and 32 kg m⁻² [130]. The reduction in food intake was directly correlated with a reduction in appetite (-40%). This effect was not observed with other milk proteins including CN, whey, and whey with caseinomacropeptide (CMP) [130]. Similarly, with other milk proteins, no significant differences in energy intake and lean body mass were seen. No subsequent reduction in food intake was found with 18 lean subjects ingesting a preload of different WP ingredients (25 g of WPC, CMP, β-lactoglobulin (β-Lg) or colostrum WPC) 90 min before an ad libitum lunch [111]. However, β-Lg induced a greater feeling of subjective fullness. Different WPs (whey protein isolate (WPI), whey without CMP and CMP isolate) were
consumed as a preload (25 g) 75 min before an *ad libitum* lunch [21]. The CMP preload induced a higher pre-meal subjective satiety and a lower compensatory food intake on the study day. However, these effects, which were linked with a higher cholecystokinin (CCK) release, were only seen within the female and not the male group. Similarly, the consumption of CMP and WPI with a high and low CMP content as a preload (60 g) drink 120 min before an *ad libitum* lunch was studied with 22 healthy females with a BMI of 20-25 kg m⁻². Only the WPIs induced a reduction in food intake by more than 15% in comparison to CMP [27].

In the context of exercise, milk proteins have been described for their positive role as recovery drinks in humans [61]. Reduction of caloric intake (-25.2%) has been shown post-exercise (30 min cycling) in females following the consumption of skim milk (600 mL) vs. orange juice drink [115]. Similarly, anthropometric modifications (increased lean mass and decreased fat mass) were reported while combining exercise and fat free milk consumption in male (500 mL for 12 weeks [49] and female (1000 mL for 12 weeks) [58] subjects.

While food protein digestion is affected by its structure, several studies have not taken into consideration the impact of the food structure on food satiety eventhough it is known to be an important component in satiety. An effect of food structure (liquid milk vs. yoghurt) in delaying gastric emptying in humans has been reported [45, 79]. It has been proposed that food structure may play a greater role in satiety than the protein type itself [60]. For example, modification of CN by enzymatic cross-linking with transglutaminase (EC 2.3.2.13) increased the feeling of fullness and induced a lower postprandial glucose response along with lower insulin and CCK concentrations in healthy humans [60]. These effects were explained by the differential accessibility of the enzymes to the CN proteins, which affected the kinetics of release of free amino acids and peptides in the GIT and in the circulation.

In summary, the effect of milk proteins on satiety has not been consistently demonstrated in humans. However, several studies appear to indicate that milk protein administered as a preload positively affect subjective satiety. This effect seems to be protein dependent, with selected WPs (i.e., α-La and β-Lg) generally having a greater impact than CN. Sex also appeared to affect the response to milk protein ingestion, which is possibly linked to hormonal differences between males and females. There did not appear to be a link between subjective satiety and a reduction of food intake in humans as in many instances, satiety ratings did not translate into a reduction in food intake. Milk proteins were shown to have an impact on food intake when consumed in

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amounts $\geq 20$ g as a meal preload both in healthy and overweight/obese adults. Morphological changes, including a reduction in weight, fat mass and an increase in lean mass were reported in obese subjects consuming milk proteins ($\geq 50$ g/day) for periods $\geq 12$ weeks. In contrast, in obese adolescents, the opposite was reported with a negative effect of milk protein intake ($\geq 30$ g/day) on BMI. Overall, the inclusion of milk proteins in the diet may be used as an alternative to reduce weight and improve body composition of adults. However, with overweight/obese adolescents, the opposite strategy consisting in no further supplementation of the diet with milk proteins may be suggested.

3. Hypotensive properties in humans following the ingestion of milk proteins

A link between the consumption of dairy products and a reduced risk of heart diseases has been suggested in several human intervention studies [for review, see: 46]. Following the intake of a WPC80 (28 g/day) beverage for 6 weeks, a significant reduction of diastolic blood pressure (DBP; -8.6 mm Hg) and systolic blood pressure (SBP; -8.0 mm Hg) were reported in stage 1 prehypertensive young adults. In contrast, no effects of the WPC80 on blood pressure (BP) were reported in healthy young adults [42]. Consumption of $2 \times 27$ g WPI and CN administered daily over 12 weeks was shown to induce a reduction in SBP (-4%) and DBP (-3%) compared to the control group [102]. The augmentation index (AI) decreased by 14% for WP and was not significantly modified by CN intake. Interestingly, it has been shown that the hypotensive effects were not linked with serum ACE inhibition [102]. However, ACE has been identified in the gastrointestinal tract of humans [19, 38]. Therefore, it is likely that direct effects at the GIT level may be involved in the hypotensive activity seen following milk protein intake in humans. Supplementation of WP and CN (30 g/day for 4 weeks) to obese females combined with resistance exercise was shown to have a positive effect on reducing BP. At the end of the intervention, there was a significant reduction in SBP (-7 and -6 mm Hg for WP and CN, respectively) and AI (-34 and -31% for WP and CN, respectively). In addition, it was demonstrated for the first time that arterial stiffness measured by brachial-ankle pulse wave velocity could be significantly reduced (-4 cm s$^{-1}$ in both groups) by ingestion of intact WP and CN. In contrast, DBP was not modified compared to baseline [40].
To date, most studies which have investigated the effects of milk protein intake on hypertension have been conducted in adults. The effect of skim milk, WP or CN (35 g/day for 12 weeks) intake on BP has recently been investigated in obese adolescents [5]. No effect on SBP and arterial stiffness could be seen at the end of the dietary intervention. However, reductions in DBP of -1.8 and -2.2 mm Hg in the CN and the control group, respectively, were reported. The hypotensive effects reported in certain studies may be linked to the release of angiotensin converting enzyme (ACE) inhibitory peptides in the GIT of humans following the ingestion of milk proteins (Table 2). In summary, dietary intervention studies with milk proteins have only shown a modest reduction in SBP and DBP or no effect. The largest effect seen (-8.6 and -8.0 mm Hg in DBP and SBP, respectively) was observed with pre-hypertensive subjects who received 28 g WPC80/day for 6 weeks. These effects may be similar to those previously seen with ACE inhibitory lactotripeptides (LTPs – i.e., Ile-Pro-Pro and Val-Pro-Pro), also reporting higher hypotensive effects in human subjects having a higher basal BP [for reviews, see: 39, 100].

4. Antimicrobial role of milk proteins in humans

Particular attention has to date been given to lactoferrin (LF), a minor WP, as it is able in its intact format to display antimicrobial activities. The mechanisms involved are thought to relate to binding to the lipopolysaccharide membrane of microorganisms. LF has been shown to induce membrane disruption, to penetrate into dendritic cells, to sequester iron and to display prebiotic activities preventing pathogen growth [17, 24, 50, 90, 118, 123]. Several human studies are found in the literature evaluating the antimicrobial properties of intact LF [104]. A human recombinant LF solution (600 mL at 5 mg mL⁻¹) was administered via gastrostomy tubes for 56 days to nursing-home residents at the start of an antibiotic treatment [70]. This resulted in a decreased incidence of antibiotic-associated diarrhea, which has in certain instances been linked with the presence of Clostridium difficile. Diarrhea was seen with 44% of the subjects receiving the LF solution as opposed to 92% in the control group administered with a placebo. Bovine LF has also been used as an adjunct in the triple therapy against Helicobacter pylori in randomized control trials (RCTs) [33, 34]. There was a higher rate of H. pylori eradication in the group receiving the clarithromycin/tinidazole/rabeprazole 7 day triple therapy combined with bovine LF (92.2 [34] and 90% [33] H. pylori eradication) than in the group
receiving the triple therapy alone (71.2% [34] and 77% [33] H. pylori eradication). The positive effect of LF may be antibiotic specific as no additional benefit of bovine LF on H. pylori eradication was seen in another study using an esomeprazole/clarithromycin/amoxicillin 7 day triple therapy [140]. In addition, human recombinant LF (250 or 1 000 mg doses, 5 times over a 24 h period) when ingested on its own did not allow eradication of H. pylori in 12 healthy subjects infected with the bacteria [101]. Similar results were reported, also showing no effect of human recombinant LF administration (1 000 mg, 5 times for 5 or 14 days) on H. pylori in humans [48]. Consumption of fermented milk has been linked with a reduction in H. pylori infection in humans. However, this effect may arise from the contribution of different factors including other milk components, BAPs and probiotic organisms [117].

The beneficial role of bovine LF in delaying the onset of late-sepsis in very low-birth-weight (VLBW) neonates has also been evaluated in different studies. The frequency of occurrence of bacterial and fungal late-onset of sepsis was decreased from 17.3% in the control group fed with a placebo to 5.9% in the test group receiving LF (100 mg/day until age 30 days) [81]. Similarly, a reduction in the incidence of fungal infection (0.7 and 7.7% for the LF and control group, respectively [82]) and necrotizing enterocolitis (NEC, 2.0 and 5.4% for the LF and control group, respectively [80]) were also seen in VLBW neonates and infants.

Overall, the antimicrobial effects following milk protein ingestion appear to mainly arise from LF. While several studies have concluded in a positive effect of LF in controlling conditions associated with human infections (e.g., diarrhea), the use of LF on its own has not been shown to correlate with a complete removal of pathogens in various human intervention studies. The combination of LF with other agents such as antibiotics or probiotics, however, seems to yield promising results in several instances (NEC and H. pylori). Co-ingestion of quantities as low as 100 mg LF over 7 days were shown to enhance antimicrobial activity. These results suggest that milk proteins have potential as adjuncts to antimicrobial drugs and other natural alternatives.

5. Anti-inflammatory effects of milk proteins in humans

Studies have demonstrated a post-prandial reduction in low-grade inflammatory biomarkers (monocyte chemotactic protein-1 (MCP-1) and CC chemokine ligand-5 regulated on activation in normal T cell expressed and secreted (CCL5/RANTES), a biomarker of atherosclerosis) in obese non-diabetic human subjects following the consumption of CN and particularly WPs [53].
After consumption of a high fat meal and 45 g (on a protein basis) of WPI, a decrease in CCL5/RANTES (36% below the baseline) was seen [53]. Contradictory results are also found in the literature showing that WPI or caseinate intake (45 g at breakfast over 3 weeks) in post-menopausal overweight women had no effect on inflammatory markers, i.e., C-reactive protein (CRP), IL-6 and tumor necrosis factor (TNF)-α [103]. Similar results were reported during an intervention study with 27 × 2 g daily of WPI or caseinate at breakfast for 12 weeks in obese/overweight individuals [102]. Interestingly, several immunomodulatory peptides such as β-casomorphin 7 (β-CN (f 603-66)) have been identified in the GIT of humans following milk protein intake (Table 2). Therefore, these may have potential to act at least at the gut level as anti-inflammatory/immunomodulatory agents. To date, the anti-inflammatory properties of milk proteins appear to have mainly been studied in the context of obesity in humans. Overall, the results on the anti-inflammatory properties in humans caused by the ingestion intact milk proteins appear to be inconclusive.

While some effects were reported in a post-prandial study on markers of low-grade inflammation, others studies have failed in demonstrating a benefit of milk protein consumption on the inflammatory status of overweight subjects.

6. Anticancer effects of milk proteins in humans

The majority of studies demonstrating the anticancer activity of milk proteins appear to have been carried out with LF. In the context of colorectal cancer, for example, a study was conducted in 104 participants (40-75 y) with polyps ≤ 5 mm diameter [67]. Bovine LF was supplemented for a 12 month period at a daily intake of 1.5 (6×0.25) or 3 (6×0.5) g. Overall, there was no significant effect of LF on polyp growth. However, the 3 g bovine LF intake significantly retarded colorectal adenomatous polyp growth in subjects ≤ 63 y, with a higher effect being observed in females than in males. The mechanisms of action are not yet understood, but they may involve immunomodulatory effects, an increase in human plasma LF concentration (causing a reduced infiltration of polymorphonuclear leukocytes in the polyps) and possibly the iron chelating activities of LF at the polyp sites [67]. A second study was recently conducted with colorectal cancer patients undergoing chemotherapy who were supplemented with 250 mg/day bovine LF for 3 months [87]. The effects seen with bovine LF supplementation were not...
significantly different from the control, with the exception of an increased anti-inflammatory and immunomodulatory effect. In addition, several side-effects (liver and kidney toxicity and mucositis) associated with the chemotherapy were alleviated in the bovine LF treated group and certain symptoms such as anemia were decreased [87].

Complexes of the Ca-depleted apo form of α-La in the molten globule state stabilized by a fatty acid cofactor (oleic acid), named human (or bovine) α-La made lethal to tumors (HAMLET/BAMLET), have been reported for their antitumor properties. A small number of human studies have been conducted to demonstrate the antitumor activity of HAMLET [43]. Following a 3 week application of HAMLET (0.7 mM in 0.9% saline on each lesion) to skin papillomas, a reduction in lesion volume by ≥ 75% was seen in the test group (20 subjects). A complete resorption of the lesions was reported after treatment and the subjects remained free of lesions after a 2 y follow-up [47]. A positive effect of HAMLET in 9 bladder cancer patients has also been demonstrated. HAMLET was instilled (5 × 25 mg mL⁻¹) daily in the bladder for 5 days. Tumor cell apoptosis together with a reduction in tumor size and a change in tumor character such as surface atrophy were observed [92].

Two peptides sequences (Val-Glu-Asn-Leu-His-Leu-Pro-Leu-Pro-Leu-Leu and Asn-Leu-His-Leu-Pro-Leu-Pro-Leu-Leu, β-CN (f 130-140) and β-CN (f 132-140), respectively) previously reported for their anticancer activity in cell cultures [59] have been found in the jejunum of humans following milk protein ingestion (Table 2). While their in vivo effects are unknown, they may be relevant to gut related cancers. In summary, utilization of a dietary strategy to prevent/manage cancer is very interesting, given the deleterious side-effects of chemotherapy for cancer patients. Milk proteins and particularly LF and HAMLET have shown various positive effects on the resorption of lesions/polyps, or cancerous cell development (e.g., apoptosis). Furthermore, results showing the alleviation of chemotherapy side-effects or a reduction of inflammatory markers were reported. These effects could be seen at relatively low LF doses < 3 g daily. While milk proteins cannot replace drugs in cancer treatment, the current scientific evidence suggests that they may be utilized to complement anticancer drugs as well as reducing their side-effects in humans. In addition, the role of HAMLET as a carrier of anticarcinogenous agents may be further investigated to improve possible synergies between milk proteins and drugs in the management of cancer.
7. Antioxidant effects of milk proteins in humans

Antioxidant species (such as reactive nitrogen and oxygen species (ROS)) are naturally found within the human body. However, high levels of antioxidant species are detrimental to human health as they may lead to cell damage [109]. The ingestion of milk proteins by humans has, in certain instances, been reported to reduce oxidative stress. Supplementation of healthy males participating in a resistance training program with WPI induced a significant increase in plasma total antioxidant capacity (TAC; +4%) and glutathione (GSH; +12%) level [119]. It was suggested that combining resistance training with WP consumption could help reduce the oxidant status in humans [119]. However, a different trend was reported in a similar study where WP supplementation combined with resistance training did not decrease the oxidant status in humans [18]. The impact of a Cys-rich WPI (20 g daily for 12 weeks) on plasma oxidative status (GSH and TAC) was evaluated in subjects with non-alcoholic steatohepatitis [26]. Along with an average weight reduction and other physiological improvements, the treatment led to an increased plasma TAC (+61%) and GSH (+28%) levels [26].

Interestingly, a CN-derived peptide (Val-Leu-Pro-Val-Pro-Gln-Lys, β-CN (f 170-176)) with in vitro antioxidant properties has been identified in the jejunum of humans following the digestion on milk proteins (Table 2). The possible role of this peptide in humans is still unknown. In summary, the effect of milk proteins on reducing oxidative status in humans is still unclear based on the scientific evidence to date. The antioxidant effects which have been reported in humans following dietary intervention with milk proteins are quite modest. There does not seem to be a clear scientific basis to recommend an increase of intact milk protein intake as a means of decreasing oxidative status in humans following exercise. The positive effect reported in the Cys-rich WPI is likely due to the Cys residue which is a well-known antioxidant amino acid and also a GSH building block.

8. Insulin secretory and serum glucose regulatory role of milk proteins in humans

Consumption of milk proteins has been linked with serum glucose regulatory properties in humans [for review: see 105]. In acute human studies, it was suggested that WPs had a higher
insulinotropic activity than CN, owing to their faster rate of digestion [94, 95]. However, in certain studies conducted with type 2 diabetic subjects, no significant differences between WP and CN ingestion were evidenced on insulin secretion and serum glucose levels [91].

WPs (55 g) ingestion 30 min before or during a high carbohydrate meal by type 2 diabetic subjects led to an increase in insulin secretion and a reduction in glycaemia [78]. Other studies also showed that supplementation of a high carbohydrate lunch with 27.6 [44] and 50 g [57] WPs induced insulinotropic effects and a post-prandial reduction in serum glucose in type 2 diabetics. Similar results were reported following the consumption of WPC administered 30 min before a meal, yielding a reduction of post meal blood glucose and insulin levels in a dose-dependent manner in healthy young adults [1]. The blood glucose regulatory effects of WPs were explained by a faster gastric emptying in the presence of WPs, which had incretin (glucagon like peptide-1 (GLP-1), CCK and glucose inhibitory polypeptide (GIP)) secretagogue activities [1, 44, 57, 64, 78]. The increase in plasma concentration of specific amino acids, including Leu, Ile, Thr, Val, Phe, Arg and Lys, and possibly peptides, which are thought to act as insulin secretagogues, has also been reported to correlate with the insulinotropic/hypoglycaemic activity of milk proteins [23, 89, 94, 95]. Other mechanisms of action may include inhibition of the activity of different metabolic enzymes also involved in the regulation of serum glucose such as dipeptidyl peptidase IV (DPP-IV) and α-glucosidase [68, 97]. However, in a recent study [57], plasma DPP-IV activity was measured in the post-prandial phase with (test group) or without (placebo) a WP preload (50 g) [57]. No significant effect of WP was seen even though higher levels of active GLP-1 were observed in the test compared to the placebo group.

While other studies have evaluated the role of intact milk proteins on insulin secretion and blood glucose regulation, it has been suggested that hydrolysed proteins generally display higher insulinotropic effects than unhydrolysed proteins [23, 89, 112]. This effect was attributed to a greater increase in plasma amino acids and di-peptides seen with pre-hydrolysed milk proteins [64, 89, 127].

To sum up, in several instances, the consumption of milk proteins and more particularly WPs has been linked to improved serum glucose regulation (higher insulinemia and lower glycaemia) in the post-prandial phase. The effects were acute and could be observed in healthy and type 2 diabetic subjects with doses as low as 10 and 28 g milk proteins, respectively. In a few instances, positive effects of WP preload on the level of incretin hormones have been demonstrated. Within
the peptides which have been identified in the gastrointestinal tract of humans subsequent to milk protein digestion, a number of the peptide fragments have previously been reported to possess DPP-IV inhibitory activity (Table 2). Although no human study appears to have linked milk protein ingestion to DPP-IV inhibition, it may be possible that DPP-IV inhibition occurs directly in the gastro-intestinal tract. This could in part explain the incretin effect observed in dietary intervention studies with milk proteins and therefore their insulinotropic activity.

9. Muscle protein synthesis action of milk proteins in humans

It is well accepted that athletes have a higher requirement for dietary proteins than sedentary individuals. Milk proteins have been used as ingredients in the formulation of sport nutrition products for their positive role in myofibrillar protein synthesis (MPS) [135]. MPS, will in turn, repair damaged muscle proteins, maintain lean mass and induce muscle hypertrophy. The timing of protein ingestion after exercise is thought to be important as a “window of opportunity” (or anabolic window) during which MPS is optimum has been suggested [107]. However, recent studies suggest that the quality/amount of the protein rather than the timing is the important determinant in MPS following exercise [for review, see: 8]. The digestibility and high level of branched chain amino acids (BCAA) in WPs, and particularly Leu, confer them with a good nutritional profile for sports nutrition [107, 110, 124]. In addition to building up muscle mass, oxidative status is another parameter which athletes aim to control in order to improve performances. Antioxidant status is compromised during physical exercise due to an increase in the respiratory metabolism which yields an increase in ROS in muscle (see section 7). The increase in ROS can result in an inflammatory reaction in muscle which is generally manifested by an increase in phagocytes in muscle following physical exercise [4]. This increase in oxidative and inflammatory status can have adverse effects as it may lead to increased fatigue during exercise.

The consumption of milk proteins (CN and WPs) associated or not with resistance training has been shown to correlate with an increase in MPS in young adults [114, 124, 125]. MPS is also highly relevant to the elderly population. Elderly people sometimes face malnutrition or undernutrition issues which may cause several complications including sarcopenia. Sarcopenia is defined as the loss of skeletal muscle mass. Sarcopenia may result in a reduction in mobility, and in certain instances in elevated fracture risk [16]. It is thought that in the elderly population, an
“anabolic resistance” following protein ingestion may prevail, which is characterized by less efficient muscle protein synthesis [62, 88]. An increased protein intake combined with an exercise regimen may help increase MPS and decrease body fat in elderly people [29, 32, 106]. Differences between WPs and CN have been found for post-prandial MPS. MPS was higher (+60%) following WPs than CN intake [20]. This was linked with the faster digestion of WPs compared to CN, resulting in higher levels of free amino acids in the circulation [31]. Furthermore, a Leu threshold, which corresponds to an intake of 2 g Leu, has been suggested as a rate limiting step in MPS, which may explain the development of sarcopenia in the elderly population [133, 136]. Therefore, Leu fortification in foods has been suggested as a means to increase MPS in the elderly population [133].

In summary, a positive role of milk proteins and more particularly WPs on MPS appear to have been consistently reported in human intervention studies at doses ≥ 20 and ≥ 7 g in healthy adults and elderly subjects, respectively. These effects were seen in acute studies and were enhanced in the context of an exercise regime. Milk proteins, notably WPs show promise for muscle repair and synthesis in the context of sports and elderly nutrition.

10. Influence of food processing on the release of bioactive peptides

Various technological processes may be applied to milk proteins which can affect their bioactive properties. These processes include enzymatic hydrolysis, fermentation, thermal and pressure treatments. Processing treatments such as enzymatic hydrolysis of milk proteins can affect their susceptibility to subsequent digestion. The impact of enzymatic hydrolysis on the bioactive properties of milk proteins in humans has recently been reviewed by Nongonierma and FitzGerald [100]. Therefore, this section will mainly deal with the possible effects of thermal and pressure treatments on the digestibility of milk proteins.

Thermal treatment is often applied to milk, which can result in alteration of the conformation of milk proteins which subsequently affect their digestibility [121]. Heat-treatment of milk proteins has been shown to positively affect the susceptibility of β-Lg to in vitro and in vivo (rat pup model) digestion with pepsin [65, 132]. This may be linked to modifications in β-Lg conformation (disulfide bond cleavage and Maillard reaction) following heat-treatment [65, 113, 132]. Although protein digestion is facilitated following heat-treatment, Wada and Lönnederl...
have shown that specific peptide bonds could not be cleaved. This was linked to the formation of Amadori products such as lactulosyllysine and cross-linked products (lysinoalanine, lanthionine and aggregates via disulfide bonds), which could not be recognized by digestive enzymes [132]. When CN and WPs are present in the same sample, ultra-high temperature (UHT) treatment may result in an increase in the interactions between CN micelles and WPs. This results in a weakening of the CN micelle structure and the appearance of aggregates, often increasing accessibility of the proteins to digestive enzymes in humans [69].

Application of high pressure treatments can induce milk protein denaturation, causing structural modifications of both CN and WPs [3, 10, 54, 63, 116, 134]. The effects of high pressure treatment on WPs have recently been reviewed by Piccolomini, Kubow and Lands [108]. The impact of high pressure treatments on milk protein digestibility has mainly been studied using SGID protocols [37, 55, 129, 138, 139]. However, a few human intervention studies have been conducted with high pressured milk proteins. For instance, the higher GSH lymphocyte concentration (+24%) seen in humans following the consumption of pressurized WPI (45 g/day for 2 weeks) has been linked with increased WPI digestibility and possibly a higher bioavailability of Cys [137]. In contrast, no effects of pressurized WPI on blood GSH were found in cystic fibrosis [71] or chronic obstructive pulmonary disease (COPD) sufferers [73].

Animal models have been used to better understand the impact of milk protein processing on the release of peptides during digestion [11, 12]. A trial with 18 month old mini-pigs fed with raw or heated (90°C for 10 min) milk, having similar compositions but different structures (liquid vs. rennet gel) was conducted [12]. Thermal treatment resulted in a faster digestion of milk proteins in contrast with gelation which slowed down gastric emptying and the rate of milk protein digestion [12]. In addition, identification of peptides released following the digestion of milk protein-based products was carried out in 18 month old mini-pigs [11]. The digestion of milk protein-based products with similar compositions but different structures (liquid milk, acid and rennet gels) prepared from raw or heated (90°C for 10 min) milk was studied. Sampling of the digesta was conducted in the duodenum to specifically study peptide release between gastric digestion and intestinal absorption. This allowed identification of a wide range of peptides (16000) which originated principally from the four CNs (αs1-, αs2-, β- and κ-CN) and β-Lg. For 29 of the peptides identified, in vitro bioactive properties including ACE inhibitory, anti-thrombotic, antimicrobial, anti-stress, antioxidant, opioid agonist, immunomodulating, protease/peptidase-
inhibitory and mineral-binding properties had previously been reported in the literature. It was shown that both the pH and matrix structure affected the kinetics of peptide release. Acid gels were digested more rapidly than liquid milk, followed by rennet gels. The effect of pH was explained by the fact that acid gels had a pH value closer to that of the optimum pH for pepsin (2.0) while liquid milk may have slowed down pepsin activity through its buffering capacity. In the case of rennet gels, their compact structure may have limited the access of digestive enzymes [11].

It is generally accepted that the structure of milk proteins affects their digestibility in humans [45, 79, 126]. In particular, the rate of nitrogen release from whey products has been reported to be faster than that of CN due to differences in the gastric emptying for both proteins. However, this is only observed with micellar CN as processed CN has lost its ability to form micelles [126]. The effect of the dairy matrix structure on the kinetics of digestion has recently been studied in a model of gastric emptying [74]. The kinetics of digestion was shown to be mostly influenced by the rate of gastric emptying, which was governed by the matrix structure. To our knowledge, there are a limited number of studies available in the scientific literature which have directly addressed the impact of food structure following processing on the kinetics of peptide release in the GIT of humans. The impact of liquid milk heat treatment (pasteurization and UHT processing) vs. no heat treatment (microfiltration) on digestion has been evaluated in humans [69]. The rate of protein digestion and therefore nitrogen incorporation in the plasma was higher with UHT than pasteurized and microfiltered milks.

In summary, the effect of processing on milk proteins is often studied from a nutritional point of view. However, processing also has major implications on the bioaccessibility (i.e., release of peptides in the intestinal lumen) of milk proteins, which can affect their bioactive properties in humans. This stresses the importance of evaluating milk proteins in the final food product in which they will be incorporated in order to properly assess their potential health enhancing properties.

11. Conclusions

Several reports link the ingestion of milk proteins with the maintenance of a favorable health status. While demonstration of the occurrence of milk protein-derived peptide sequences in the GIT tract of humans has been established in a small number of instances, some questions remain
on the potential physiological role of these peptides in humans. For instance, very little to no
information is available in terms of their stability, bioavailability and efficacy in humans. This
constitutes a major gap in knowledge to allow a better understanding on the role of milk protein-
derived peptides in human health. Technological advances have been made in terms of peptide
identification. These have allowed more accurate detection of peptide sequences within human
biological fluids and tissues. However, the scientific evidence for the biological relevance of
these peptides in humans is still lacking. The reasons for this may arise from the lack of
knowledge on the mechanism of action involved, the low potency and low bioavailability of the
milk protein-derived peptides and also the lack of knowledge on the time span (acute vs. chronic)
for their in vivo activity. Despite the lack of clear evidence for the role of milk proteins in human
health, selected bioactive properties such as muscle protein synthesis, insulinotropic and
hypotensive activity appear to be consistently observed in human intervention studies. An urgent
need exists for the development of integrated research platforms involving interdisciplinary skills
to address the role and mechanism of action of milk protein-derived peptides in humans.

Acknowledgements

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Author contributions

A.B. Nongonierma was responsible for writing the manuscript which was revised by R.J.
FitzGerald. All authors have approved the final article.

Conflicts of interests

The authors declare that they have no conflict of interest.
References


[21] Burton-Freeman BM. Glycomacropeptide (GMP) is not critical to whey-induced satiety, but may have a unique role in energy intake regulation through cholecystokinin (CCK). Physiol Behav. 2008;93:379-87.


Table captions

**Table 1.** Summary of the human intervention studies assessing the health enhancing properties of milk proteins.

**Table 2.** Peptide sequences detected in the gastrointestinal tract of humans following the ingestion of bovine milk and dairy products and their associated *in vitro* bioactive properties.
Table 1

<table>
<thead>
<tr>
<th>Bioactivity</th>
<th>Test sample</th>
<th>Study design¹</th>
<th>Biological outcome</th>
<th>Reference</th>
</tr>
</thead>
</table>
| Satiety     | WPC         | • Obese ♂ subjects (n=26)  
• 65 g/day meal preload  
• Duration: 12 weeks       | • Appetite: -44%  
• Calories intake: -51%  
• anthropometric changes: body weight (-10%), BMI (-19%) and waist circumference (-41%)  
• body composition: body fat mass (-9%) and lean muscle mass (+9%) | [122]     |
|             | WPC80       | • Overweight and obese adults subjects (n=19)  
• Treatment: WPC80  
• Dose: 56 g protein/day meal preload  
• Duration: 23 weeks       | • Weight: -1.8 kg  
• Fat mass: -2.3 kg  
• Waist circumference: -2.4 cm  
• Fasting ghrelin: -13.6% | [9]        |
|             | Skimmed milk, WP and CN | • Overweight adolescents (n=173)  
• Treatments (A): skimmed milk, (B): WP (C): CN and (D): water  
• Dose: 30 g/day  
• Duration: 12 weeks       | • Lean mass: increased in all groups  
• Fat mass: increased in all groups, except in (D)  
• Leptin: +30 and +15% in (C) and (B), respectively | [72]     |
|             | Skimmed milk, WP and CN | • Overweight adolescents (n=203)  
• Treatments (A): Skimmed milk, (B): WP (C): CN and (D): water  
• Dose: 35 g/day  
• Duration: 12 weeks       | • BMI, BMI-for-age Z-scores: increased in groups (A), (B) and (C)  
• Weight: increased in groups (A), (B) and (C) | [6]       |
α-La, CN, whey or WPC80 in a custard-style breakfast

- Healthy adults (n=24)
- Appetite assessment over 180 min post-breakfast
- Dose: 10/55/35 (normal) or 25/55/20 (high) energy% protein/carbohydrate/fat

-40% appetite at 180 min with α-La
- -20% food intake at lunch α-La
- No effect with CN, whey and WPC80 [130]

WPC, CMP, β-Lg and colostrum WPC

- Lean ♂ (n=18)
- Treatments (A): WPC, (B): CMP (C): β-Lg and (D): colostrum WPC
- Dose: 25 g preload 90 min before lunch

- Greater feeling of subjective fullness with (C) [111]
- No reduction in food intake at lunch with (B), (C) and (D) compared to (A)

WPC

- Healthy young ♂ adults (n=16)
- Treatment: ad libitum pizza meal vs. control (water)
- Dose: 10-40 g

- Subjective appetite: not affected by WP [1]
- Food intake: reduced in all WP groups with doses ≥ 20 g

WPI, whey without CMP and CMP isolate

- Healthy adults (n=20)
- Treatments (A): WPI, (B): whey without CMP and (C): CMP
- Dose: 25 g preload 75 min before lunch

- Pre-meal subjective satiety: higher in all groups [21]
- (C) group of ♀: +33% CCK concentration 30 min after lunch and lower compensatory food intake on the study day (-1.7 MJ)

CMP isolate, WPI with low and high CMP

- Healthy ♀ subjects (n=22)
- Treatments (A): CMP, (B): WPI low CMP and (C): WPI high CMP
- Dose: 60 g preload 120 min before lunch

- Food intake: > -15% with (B) and (C) compared to (A) [27]
- Appetite suppression and food intake: no effect of CMP
<table>
<thead>
<tr>
<th>Group</th>
<th>Details</th>
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</thead>
<tbody>
<tr>
<td><strong>Hypotensive</strong></td>
<td>WPC80</td>
</tr>
</tbody>
</table>
| Young normotensive and stage 1 pre-hypertensive adults (n=71) | - Dose: 28 g/day  
- Duration: 6 weeks |
| Pre-hypertensive group: DBP (-8.6 mm Hg) and SBP (-8.0 mm Hg) | - Normotensive group: No effect of WPC80 |

<table>
<thead>
<tr>
<th>Group</th>
<th>Details</th>
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</thead>
<tbody>
<tr>
<td><strong>WPI and CN</strong></td>
<td>Overweight and obese subjects (n=89)</td>
</tr>
</tbody>
</table>
| Treatments (A): CN and (B): WP | - Dose: 54 g/day  
- Duration: 12 weeks |
| SBP: -5 mm Hg for both (A) and (B) | - DBP: 5 mm Hg for both (A) and (B)  
- AI: -14% for (B) and no modification for (A) |

<table>
<thead>
<tr>
<th>Group</th>
<th>Details</th>
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<tbody>
<tr>
<td><strong>WPI and CN</strong></td>
<td>Post-menopausal overweight ♀ (n=20)</td>
</tr>
<tr>
<td>Dose: 45 g/day</td>
<td>- Duration: 3 weeks</td>
</tr>
<tr>
<td>No effect on AI, SBP and DBP</td>
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</table>

<table>
<thead>
<tr>
<th>Group</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>WP and CN</strong></td>
<td>Obese ♀ (n=33)</td>
</tr>
</tbody>
</table>
| Treatments (A): WP, (B): CN and (C): carbohydrate control | - Dose: 30 g/day and resistance exercise  
- Duration 4 weeks |
| SBP: -7 mm Hg for (A) and -6 mm Hg (B) | - DBP: no effect for (A) and (B)  
- AI: -34% for (A) and-31% for (B)  
- brachial-ankle pulse wave velocity: -4 cm s⁻¹ for (A) and (B) |
WP
- Obese adolescents (12-15 y, n=193)
- Treatments (A): skimmed milk, (B): WP, (C): CN and (D): water
- Dose: 35 g/day
- Duration: 12 weeks
- DBP: -1.8 and -2.2 mm Hg for (C) and (D), respectively
- SBP: no changes in all groups
- Arterial stiffness: no changes in all groups

Antimicrobial Human recombinant LF
- In tube fed long term patients (n=30)
- Treatments (A): LF and (B): placebo control
- Dose: LF (3 g) solution by gastrostomy administration
- Duration: 56 days
- Diarrhoea: 44% patients from (A) vs. 92% in (B)
- No significant difference in the rate of infection by *C. difficile* in (A) and (B)

Bovine LF
- Patients (n=150) infected with *H. pylori*
- Treatments (A): clarithromycin/tinidazole/rabeprazole+200 mg LF, (B): clarithromycin/tinidazole/rabeprazole for 7 days or (C): (B) for 10 days
- Duration: 7-10 days
- *H. pylori* eradication: 92, 71 and 70% in groups (A), (B) and (C), respectively

Bovine LF
- Patients (n=389) infected with *H. pylori*
- Treatments (A): resomeprazole clarithromycin/tinidazole, (B): 200 mg LF or (C): (A)+200 mg LF
- Duration: 7 days
- *H. pylori* eradication: 77, 73 and 90% in groups (A), (B) and (C), respectively

Bovine LF
- Patients (n=133) infected with *H. pylori*
- Treatments (A): esomeprazole/clarithromycin/amoxycillin or (B): (A)+200 mg LF
- Duration: 7 days
- *H. pylori* eradication: 80.3 and 78.1% in groups (A) and (B), respectively
- No significant difference between groups (A) and (B)
Human recombinant LF
- Healthy subjects (n=6) infected with *H. pylori*
- 1 g LF
- No clearance of *H. pylori* infection

Human recombinant LF
- Healthy subjects (n=9) infected with *H. pylori*
- 5 g LF daily
- Duration: 5-14 days
- No clearance of *H. pylori* infection

Bovine LF
- Preterm VLBW neonates (n=472)
  - Treatments (A): LF, (B): (A) + *Lactobacillus rhamnosus* GG or (C): placebo
  - Dose: 100 mg/day LF
  - Duration: 6 weeks
  - Fungal colonisation: no difference between treatments
  - Invasive fungal infection: 0.7, 2.0 and 7.7% in groups (A), (B) and (C), respectively
  - Rate colonization-infection: 3.7, 12 and 41.9% in groups (A), (B) and (C), respectively

Bovine LF
- VLBW neonates (n=743)
  - Treatments (A): LF, (B): (A) + *Lactobacillus rhamnosus* GG or (C): control
  - Dose: 100 mg/day LF
  - Duration: until age day 30
  - Incidence of NEC: 2.0, 0 and 5.4% in groups (A), (B) and (C), respectively

Anti-inflammatory WPI and CN
- Obese non diabetic subjects (n=11)
  - Treatments: high fat meal with (A): WPI, (B): CN, (C): cod or (D): gluten
  - Dose: 45 g protein
  - Reduction of post-prandial low grade inflammation (CCL5/RANTES and MCP-1): in all group and particularly in (A)
<table>
<thead>
<tr>
<th>Study Type</th>
<th>Description</th>
<th>Details</th>
</tr>
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<tbody>
<tr>
<td>WPI and CN</td>
<td>Overweight and obese subjects (n=89)</td>
<td>No modification in inflammatory markers [102]</td>
</tr>
<tr>
<td>WPI and CN</td>
<td>Post-menopausal overweight women (n=20)</td>
<td>No effect on inflammatory markers, i.e., C-reactive protein (CRP), IL-6 and tumour necrosis factor (TNF)-α [103]</td>
</tr>
<tr>
<td>Anticancer</td>
<td>Patients with adenomatous colorectal polyps (n=104)</td>
<td>Polyp growth: no significant effect overall [67]</td>
</tr>
<tr>
<td>Bovine LF</td>
<td>Colorectal cancer patients in chemotherapy (n=30)</td>
<td>No effect of LF supplementation [87]</td>
</tr>
</tbody>
</table>

- Dose: 54 g/day
- Duration: 12 weeks
- Dose: 45 g/day
- Duration: 3 weeks
- Dose: 1.5 or 3 g/day
- Duration: 12 months
- Dose: 250 mg/day
- Duration: 3 months
- Subjects ≤ 63 y, 3 g LF: retardation of polyp growth being more marked in ♀ than ♂.
HAMLET
- Patients with skin papillomas (n=40 & 34)
- Treatments (A): HAMLET and (B): placebo
- Dose: 0.7 mM HAMLET topical application on each lesion
- Duration: 3 weeks

- Reduction of lesions: -75% in (A)
- Time to resolution: 2.4 vs. 9.9 months in (A) and (B), respectively

HAMLET
- Patients with bladder cancer (n=9)
- Dose: daily bladder instillation of HAMLET 5 × 25 mg mL⁻¹
- Duration: 5 days

- Shedding of tumour cells in the urine increased from 10⁴ to 2.9 × 10⁵ cells mL⁻¹ post treatment
- Tumour cell apoptosis post treatment
- Tumour size reduced: -50 % post treatment
- Tumour surface atrophy post treatment

Antioxidant WPI
- Healthy ♂ (n=28)
- Treatments (A): resistance training + WPI, (B): resistance training + placebo or (C): control
- Dose: ~110 g/day
- Duration: 6 weeks

- Plasma total antioxidant capacity: +4% in group (A) compared to baseline
- Plasma GSH: +12% in group (A) compared to baseline

WP
- Healthy ♂ (n=27)
- Treatments (A): resistance training + WP, (B): resistance training + soy or (C): resistance training
- Dose: 33 g/day formulated in bars
- Duration: 9 weeks

- Plasma antioxidant status: no effect in (A); decreased in (B) and (C)

Cys-rich WPI
- Patients with non-alcoholic

- Plasma total antioxidant [26]
<table>
<thead>
<tr>
<th>Gluoregulatory</th>
<th>WP, CN</th>
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<tbody>
<tr>
<td>Treatment: Cys-rich WPI</td>
<td></td>
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<tr>
<td>Dose: 20 g/day</td>
<td></td>
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<tr>
<td>Duration: 12 weeks</td>
<td></td>
</tr>
<tr>
<td>Type 2 diabetes subjects (n=12)</td>
<td></td>
</tr>
<tr>
<td>Fat rich meal (100 g butter + 45 g carbohydrate) + Treatments (A): CN, (B): WP, (C): cod or (D): gluten</td>
<td></td>
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<tr>
<td>Dose: 45 g</td>
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</table>

- Plasma GSH: +28% compared to baseline
- Blood glucose: significantly lower for (B)

<table>
<thead>
<tr>
<th>WP</th>
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<tbody>
<tr>
<td>Type 2 diabetes patients (n=8)</td>
</tr>
<tr>
<td>Treatments: high carbohydrate meal with (A): WP preload, (B): WP, (C): no WP</td>
</tr>
<tr>
<td>Dose: 55 g</td>
</tr>
</tbody>
</table>

- Insulin and incretin levels: (A) and (B) > (C)
- Glycaemia: (A) and (B) < (C), 363.7, 406.3 and 734.9 mmol min$^{-1}$ L$^{-1}$, respectively

<table>
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<tr>
<th>WP</th>
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<tbody>
<tr>
<td>Type 2 diabetes subjects (n=14)</td>
</tr>
<tr>
<td>Treatments: high carbohydrate breakfast and lunch with (A): WP or (B): lean ham</td>
</tr>
<tr>
<td>Dose: 27.6 g</td>
</tr>
</tbody>
</table>

- Insulin: (A) > (B), +31 and +57% after breakfast and lunch, respectively
- Glycaemia: (A) < (B) -21 %
- GIP: (A) > (B)
- GLP-1: no difference between (A) and (B)

<table>
<thead>
<tr>
<th>WP</th>
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<tbody>
<tr>
<td>Type 2 diabetes patients (n=15)</td>
</tr>
<tr>
<td>Treatments: high carbohydrate breakfast with (A): WP preload or (B): water</td>
</tr>
<tr>
<td>Dose: 50 g</td>
</tr>
</tbody>
</table>

- Insulin: (A) > (B), +96%
- Glycaemia: (A) < (B), -28 %
- Intact and total GLP-1: (A) > (B), +141 and 298%, respectively
| WPC | (1) Healthy young♂ adults (n=16) and (2) healthy young♂ and♀ adults (n=21) | Insulin levels: reduced in all WP groups with doses ≥ 10 g. | [1] |
|     | Treatments: *ad libitum* pizza meal (1) or preset pizza meal (2) with WP preload vs. control (water) | Glycaemia: reduced in all WP groups with doses ≥ 10 g | |
|     | Dose: 10-40 g (1) and 5-40 g (2) | | |
| Muscle protein synthesis | Healthy elderly♂ (>70 y, n=72) | MPS: +65 and +60% higher in (A) than in (B) with and without resistance exercise, respectively | [20] |
| WP and CN | Treatments (A): WP and (B): micellar CN with or without resistance exercise. | | |
| | Dose: 20 g | | |
| WP and CN | Obese elderly subjects (n=12) | Muscle protein FSR: higher in (A) than (B) | [29] |
| | Treatments (A): WP and (B): CN in caloric restriction diets (1200 kcal/day) | Lean mass tissue: no difference in (A) and (B) | |
| | Dose: 7 g (WP) and 14 g (CN) | Fat loss: +30% higher in (A) than (B) | |
| | Duration: 8 weeks | | |
| WP and CN | Healthy elderly subjects (n=24) | FSR: no difference in (A) and (B) post exercise | [35] |
| | Treatments (A): WP and (B): CN before or after heavy resistance exercise and (C): placebo control | MPS: no difference in (A) and (B) post exercise | |
| | Dose: 7 g (WP) and 14 g (CN) | | |
| WP and CN | Healthy subjects (n=23) | amino acid balance: (A) and (B) > (C) with no difference between (A) and (B) | [125] |
| | Treatments (A): WP and (B): CN and (C): placebo control after resistance exercise | | |
| | Dose: 20 g | | |
WP and CN  
- Healthy young subjects (n=16)  
- Treatments (A): CN, (B): WP and (C): control followed by resistance exercise  
- Dose: 30-43 g  
- MPS: higher in (A) and (B) than in (C) \[114\]

CN  
- Healthy young ♂ (n=18)  
- Treatments (A): CN, (B): WP hydrolysate and (C): soy protein isolate followed by resistance exercise  
- Dose: 21-22 g  
- MPS: significant increase after exercise in (B) and (C) but not (A) \[124\]

\(^1\)No duration was indicated for acute studies.

AI: augmentation index; α-La: α-lactalbumin; β-Lg: β-lactoglobulin; BMI: body mass index; CCK: cholecystokinin; CCL5/RANTES: CC chemokine ligand-5 regulated on activation in normal T cell expressed and secreted; CMP: caseinomacropeptide; CN: casein; CRP: C-reactive protein; DBP: diastolic blood pressure; FSR: fractional synthesis rate; GLP-1: glucagon-like peptide 1; GIP: glucose-dependent insulinotropic polypeptide; GSH: glutathione; (HAMLET: human α-La made lethal to tumors; LF: lactoferrin; MCP-1: monocyte chemotactic protein-1; MPS: myofibriallar protein synthesis; NEC: necrotizing enterocolitis; SBP: systolic blood pressure; TNF-α: tumour necrosis factor-α; VLBW: very low birth weight; WPC: whey protein concentrate; WPI: whey protein isolate; ♂: male; ♀: female.
<table>
<thead>
<tr>
<th>Parent protein</th>
<th>Fragment</th>
<th>Peptide sequence</th>
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<tr>
<td>β-CN</td>
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<td>LNVPGEIVE</td>
<td>[15]</td>
<td>ACE inhibitor</td>
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<tr>
<td></td>
<td>7-14</td>
<td>NVPGEIVE</td>
<td>[15]</td>
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<td>DKIHPF</td>
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<td>59-66</td>
<td>VYPFPGPI</td>
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<td>YPFPGP</td>
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<td>YPFPGPI (β-casomorphin-7)</td>
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1CN: casein
2Peptide sequence with the one letter amino acid code; S(P) indicates phosphorylated serine residues.
3Bioactivity determined from Boutrou, Gaudichon, Dupont, Jardin, Airinei, Marsset-Baglieri et al. [15], Nongonierma and FitzGerald [98] and the BIOPEP database [86]; ACE: angiotensin converting enzyme; DPP-IV: dipeptidyl peptidase IV.