

REVIEWS: CURRENT TOPICS

Biochemical and metabolic mechanisms by which dietary whey protein may combat obesity and Type 2 diabetes[☆]Daniela Jakubowicz^{a,*}, Oren Froy^{b,*}^aDiabetes Unit E. Wolfson Medical Center, Tel Aviv University, Holon 58100, Israel^bInstitute of Biochemistry, Food Science and Nutrition, Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot 76100, Israel

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Abstract

Consumption of milk and dairy products has been associated with reduced risk of metabolic disorders and cardiovascular disease. Milk contains two primary sources of protein, casein (80%) and whey (20%). Recently, the beneficial physiological effects of whey protein on the control of food intake and glucose metabolism have been reported. Studies have shown an insulintropic and glucose-lowering properties of whey protein in healthy and Type 2 diabetes subjects. Whey protein seems to induce these effects via bioactive peptides and amino acids generated during its gastrointestinal digestion. These amino acids and peptides stimulate the release of several gut hormones, such as cholecystokinin, peptide YY and the incretins gastric inhibitory peptide and glucagon-like peptide 1 that potentiate insulin secretion from β -cells and are associated with regulation of food intake. The bioactive peptides generated from whey protein may also serve as endogenous inhibitors of dipeptidyl peptidase-4 (DPP-4) in the proximal gut, preventing incretin degradation. Indeed, recently, DPP-4 inhibitors were identified in whey protein hydrolysates. This review will focus on the emerging properties of whey protein and its potential clinical application for obesity and Type 2 diabetes.

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Keywords: Whey; Diabetes; Obesity; GLP-1; DPP-4; Milk**1. Introduction**

Obesity is associated with several metabolic and eating disorders, such as breakfast skipping and overeating at night, that result in poor adherence to most weight loss diets. Therefore, most attempts lead to initial weight loss followed by rapid weight regain [1]. In addition, diet-induced weight loss results in various changes, such as carbohydrate withdrawal, increased feeling of hunger and changes in gut hormone secretion [2] that may increase withdrawal from the diet. However, weight loss outcomes may be improved by strategies that lead to reduce hunger and/or increase satiety. One such strategy could be the use of anorectic drugs. However, safer alternatives, such as satiating foods, are preferred [3]. Recently, we have shown that meal timing and composition, namely, increased carbohydrate and

protein intake at breakfast, lead to successful weight loss and its maintenance by reducing compensatory changes in hunger, cravings and ghrelin levels [4]. These results are consistent with previous studies showing that weight loss was greater with high-protein diets leading to a high thermogenic effect, increased satiety and gut hormone secretion compared with isoenergetic intake of fat and carbohydrates [5–7]. Although protein consumption is satiating, certain sources of protein promote greater satiety [8]. Milk has received great attention as current data suggest that low-fat milk and dairy products consumption have beneficial effects on the prevention or treatment of obesity and Type 2 diabetes [9]. This positive association has prompted investigators to study the effects of milk components. One of milk components, whey protein, has recently received great attention because of its beneficial effects on energy balance, appetite and glucose metabolism and potential application for the treatment of obesity and Type 2 diabetes.

2. Whey protein composition

Whey protein accounts for only about 20% of of total milk protein, whereas casein comprises the most part, about 80% of total protein in milk [10]. During milk processing, the caseins are responsible for making curds, while whey remains soluble [10]. The various proteins in whey in order of abundance are β -lactoglobulin, α -lactalbumin, proteose peptone, immunoglobulins, bovine serum albumin,

Abbreviations: GMP, glycomacropeptide; DPP-4, dipeptidyl peptidase-4; GIP, glucose-dependent insulintropic polypeptide; GLP-1, glucagon-like peptide-1; BCAAs, branched-chain amino acids; PYY, peptide YY; CCK, Cholecystokinin.

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lactoferrin and lactoperoxidase [11]. Whey protein also contains glycomacropeptide (GMP), which is present in whey due to the action of chymosin (rennin) on casein in the first step of the enzymatic cheese making process. GMP is an excellent source of branched-chain amino acids (BCAAs) [10].

Not only is whey protein a good source of amino acids, but it is also a rich source of bioactive peptides generated during its digestion. Bioactive peptides of whey protein relay their effect by binding to specific receptors in the intestinal lumen before absorption or in target organs after absorption into the bloodstream [12]. Peptides shorter than four residues can cross intercellular junctions and reach the bloodstream, whereas larger peptides can be transported via peptide transporter-mediated transport system. The rate of transport is determined by their susceptibility to brush border peptidases [13]. Several bioactive peptides have been isolated, such as those that inhibit angiotensin converting enzyme or those with antimicrobial and immunomodulatory activities [11]. However, more work should be invested in identifying bioactive peptides with metabolic activities.

3. Effect of whey protein on thermogenesis

Dietary protein is considered to stimulate energy expenditure and to have a greater thermogenic effect in the postprandial period than either carbohydrates or fats. In human clinical trials, it was found that the energy cost of digesting, absorbing and metabolizing proteins is greater than that of carbohydrates or fat [14,15]. Interestingly, whey protein has recently been shown to elicit a greater thermogenic response than protein composed of either casein or soy [16]. Increased protein synthesis has been proposed as one possible mechanism responsible for the increased thermogenesis [17]. Indeed, the rate of protein synthesis after whey consumption was twofold greater than after casein consumption [18]. The high leucine content in whey protein (50–75% more than other protein sources) may be related to its ability to stimulate muscle protein synthesis, which may account for its thermogenic effect [19]. Leucine not only serves as a substrate for protein synthesis, but at high concentrations it also up-regulates mammalian target of rapamycin (mTOR) signaling [20,21]. mTOR is a serine/threonine kinase, whose pathway is activated, depending on both ribosomal protein S6 kinase 1 (S6K1) and eukaryotic initiation factor (eIF) 4E binding protein (4E-BP1) phosphorylation, two proteins involved in the initiation of protein synthesis [21]. In addition, it was shown that leucine-treated muscle cells had reduced adenosine monophosphate (AMP)/adenosine triphosphate (ATP) ratio and decreased activity of AMP-activated protein kinase (AMPK), a sensor of cellular energy status [22]. AMPK activation by 5-aminoimidazole-4-carboxamide 1- β -D-ribose nucleoside (AICAR), prevented the phosphorylation of 4E-BP1, S6K1, S6 and eIF4G in response to leucine suggesting decreased mTOR activity [23].

The postprandial rate of protein synthesis also depends on the speed of protein absorption. Fast absorbing protein has an anabolic effect [18]. Casein and whey have a different effect on plasma amino acid profiles [8]. Whereas whey protein passes quickly through the stomach without digestion, the release of casein from the stomach is delayed and degraded to peptides [18]. The acidic pH in the stomach leads to casein precipitation and exposure to gastric hydrolysis, whereas the soluble whey remains mostly intact [24]. As a result, whey protein is considered as fast digestible protein with a high and rapid amino acid profile, whereas casein has been characterized as slow protein leading to delayed gastric emptying and slower and lower amino acid profile [18]. Thus, plasma amino acid concentrations fall 100 min after whey ingestion but remain elevated even 300 min after casein ingestion [18]. The rapid absorption of whey protein, which leads to increased levels of leucine and other branched-chain amino acids and, as a result, mTOR signaling activation and protein

synthesis can account for the greater thermogenesis generated after its digestion.

4. Insulinotropic and glucose lowering effect of whey protein

It is generally accepted that low glycemic index (GI) diets may be protective against Type 2 diabetes, and that the addition of protein to foods decreases the GI [25]. Proteins vary in their ability to decrease postprandial hyperglycemia. Milk proteins have been shown to stimulate insulin secretion [26]. However, whey proteins prove to be more insulinotropic compared with caseins or other animal and plant proteins [27]. The addition of whey-based protein reduced postprandial hyperglycemia in a dose-dependent manner, when added to a drink of 50 g glucose [28]. This dose-dependent effect of whey protein was also achieved when acutely applying different amounts of whey protein before or together with high carbohydrate test meals. Quantities higher than 20 g per serving led to pronounced effects in lowering blood glucose and increased insulin levels [29]. Long-term effects in overweight/obese adults have shown that after 12 weeks of whey protein intake (54 g/day), fasting plasma insulin levels decreased by 11% and homeostasis model assessment of insulin resistance score by 10% compared with baseline [30] suggesting long-term improvement of insulin sensitivity. Indeed, it was shown in healthy subjects that ingestion of an amino acids mixture of leucine, isoleucine, valine, lysine and threonine resulted in blood glucose and insulin levels similar to those after whey ingestion though with a decreased magnitude [31]. The decrease in blood glucose and the insulinotropic effect of whey protein occurs not only in healthy people, but also in Type 2 diabetic patients [32,33]. Particularly in Type 2 diabetes, it was shown that the addition of whey to a meal containing rapidly digested and absorbed carbohydrates stimulated greater plasma insulin concentrations (+57% after lunch) and reduced postprandial blood glucose (–21% after 120 min) [33].

It is not known how whey protein leads to increased secretion of insulin. However, the high content of essential amino acids released after whey protein digestion could be the mediator of its insulinotropic response [34]. In particular, leucine, isoleucine, valine, lysine and threonine have been proposed as the most likely amino acids responsible for the increase seen in insulin concentrations. Leucine stimulates insulin secretion from pancreatic β cells either by its deaminated metabolite, α -ketoisocaproic acid (KIC) [35] or by enhancing the oxidation of glutamate by allosterically activating glutamate dehydrogenase [36,37]. Both pathways lead to increased ATP levels and inhibition of K_{ATP} channel activity. It is also believed that leucine or KIC inhibit K_{ATP} channel activity directly [38]. Either way, inhibition of K_{ATP} channels leads to depolarization of the β cell membrane, an increase of free cytosolic Ca^{2+} and release of insulin [39]. Elevated ATP levels can also be achieved by up-regulated expression of ATP synthase β subunit, shown to be mediated by leucine [40,41]. In addition, as mentioned above, leucine and other BCAA activate the mTOR pathway leading to increased protein synthesis and specifically β cell insulin. Some, yet unknown, mTOR-independent pathways possibly also play a role [37].

5. Effect of whey protein on the incretin system

In addition to the effect of whey amino acids on insulin secretion, incretin hormones released from the gut also seem to be involved, particularly, gastric inhibitory peptide (GIP) and glucagon-like peptide 1 (GLP-1).

5.1. Gastric inhibitory peptide

GIP, also known as glucose-dependent insulinotropic peptide, is released from K cells in the duodenum after food ingestion [42].

Recent studies show that a whey drink caused a significantly enhanced GIP response (+80%) in healthy subjects, while branched-chain amino acid mixtures did not [31]. Hydrolysates obtained from either whey or casein protein elicited about 50% more gastric secretion than whole protein solutions which was accompanied by increased GIP in plasma during the first 20 min of gastric emptying [43]. It is possible that bioactive peptides and/or other amino acids liberated during whey digestion are the primary stimulators of GIP synthesis and secretion, as was found for insulin [44]. Another possibility is that bioactive peptides released from whey protein may lead to increased half-life of GIP as will be explained later.

5.2. Glucagon-like peptide-1

Compared to casein or soy, whey triggered the strongest response of GLP-1 in healthy subjects [8,45]. However, plasma GLP-1 concentrations fell substantially 2 h after the administration of isoenergetic whey, whey hydrolysate and casein hydrolysate solutions, but continued to increase only after casein solution [43], reiterating the effect of casein slow digestion, as was discussed above. The stimulatory effect of whey protein is particularly important since it was shown that GLP-1 secretion after both mixed meal and oral glucose is reduced in Type 2 diabetes [46]. The impairment of GLP-1 secretion in Type 2 diabetes is related to the degree of hyperglycemia. Indeed, patients with more poorly regulated glycemic control exhibit reduced GLP-1 secretion, as is evident by the high glycosylated hemoglobin levels [46]. The stimulatory effect of whey protein on GLP-1 may have many beneficial effects not only on the increase of the glucose-induced insulin secretion and reduction in postprandial glycemia. Enhanced GLP-1 levels also increase the synthesis of pro-insulin and insulin stores in β -cells; promote differentiation of precursor cells into β -cells; lead to proliferation of β -cell lines resulting in increased β -cell mass; and reduce the rate of β -cell apoptosis [47–50]. GLP-1 effects are mediated via the signaling of GLP-1 receptor in β -cells through cAMP and protein kinase A (PKA) activation. Regarding insulin expression, GLP-1 is involved in regulation of PDX-1, the most studied insulin transcription factor, by increasing its protein levels and translocation to the nucleus, followed by its binding to the insulin promoter and increased transcription [49]. In addition, PKA phosphorylation of the K_{ATP} channel leads to its closure and subsequent depolarization of the β cell membrane, an increase of free cytosolic Ca^{2+} and release of insulin, as described above [49]. GLP-1 also slows gastric emptying leading to reduced appetite, increased satiety [51] and, as a result, weight loss [52]. It is noteworthy that when whey was included in the meal, glucose levels decreased by 21% after 180 min. This decline was in the same range as was reported for nateglinide, a novel rapid-acting non-sulfonylurea insulin secretagogue [53], and similar in the effect of the sulfonylureas glipizide and glyburide on postprandial plasma glucose reduction after several months of therapy [54]. These results demonstrate that whey protein is comparable in its glucose lowering effect to some prescribed medications. As with GIP, the increased GLP-1 levels seen after whey ingestion may result from bioactive peptides released from whey protein that increase GLP-1 half-life (see below).

5.3. Dipeptidyl peptidase-4 (DPP-4)

DPP-4 is the principal enzyme responsible for the rapid degradation of the incretins GLP-1 and GIP *in vivo*. DPP-4 exists as a cell membrane-spanning enzyme on numerous cell types, and as a soluble circulating form [55,56]. DPP-4 is highly expressed on endothelial cells directly adjacent to incretin-secreting cells in the gastrointestinal tract. This proximity leads to rapid cleavage of GLP-1 and GIP soon after their release [56]. High levels of the enzyme in the circulation

could indicate that the incretins might be degraded more rapidly in obese than in lean subjects, as was reported [57]. It was shown that administration of whey protein was associated with a significant reduction in DPP-4 activity in the proximal small bowel [58]. Digestion of whey protein generating high amounts of bioactive peptides and amino acids could act as competitive DPP-4 inhibitors. Indeed, a tripeptide (Ile-Pro-Ala) responsible for moderate DPP-4 inhibition has recently been identified in whey β -lactoglobulin hydrolysate [59]. The inhibitory effect of peptides released from whey protein could account for the increased half-life of the incretins GIP and GLP-1, and the consequent insulinotropic effect. Clearly, thorough analysis of whey protein, as a source of DPP-4 inhibitory peptides, is merited.

6. Effect of whey protein on appetite

Not only does protein increase energy expenditure, but it also decreases energy intake through mechanisms that influence appetite control [6]. Although there is inconsistent data from human studies, it is generally accepted that proteins are more satiating than carbohydrates and fats, but the source of protein may play a role in its satiating effect. Milk proteins have been considered to increase satiety, but the contribution of complete milk proteins vs. whey protein or casein is still unclear. One study found similar effects on satiety and food intake between whey protein and casein [60]. Other studies showed that whey protein has a stronger suppression of hunger and lower food intake compared to casein or soy and egg albumin [8,45]. Similarly, it was shown that mean energy intake was significantly lower with the whey meal than with tuna, egg and turkey meals in healthy subjects [27]. In a more recent long-term trial in overweight and obese participants it was also found that supplementation with whey protein led to increased satiety compared to supplementation with soy protein or carbohydrates [61]. It is plausible that whey is more satiating than casein due to increased plasma amino acids and levels of plasma incretins, as was described above. As was mentioned above, whey protein contains a high concentration of BCAAs, especially L-leucine [12,19]. Leucine enters the brain more rapidly than any other amino acid [62]. It has recently been shown that intracerebroventricular injection of leucine is important for food intake suppression for 24 h [63], suggesting that whey protein may exert a central effect on appetite. Elevation of dietary or brain leucine has been shown to suppress food intake via a mechanism involving mTOR, AMPK, and/or BCAA metabolism, as explained above. Leucine reduces food intake *via* promoting mTOR signaling pathway in hypothalamus, especially in the region containing orexigenic neurons expressing both neuropeptide Y and agouti-related protein [63]. Further research is needed to establish the effect of whey protein on long-term satiety.

Amino acids liberated from whey protein during its *in vivo* digestion may also stimulate the release of hormones [12]. Insulin secretion mediated by whey ingestion may directly affect food intake regulation by suppressing appetite and, as a consequence affect body weight. Indeed, insulin levels stimulated by the ingestion of whey, in addition to modifying the glycemic response, were strongly associated with satiety and decreased food intake [29,30]. Other hormones are also involved in the regulation of food intake either directly in the hypothalamus, such as ghrelin, or indirectly via the vagal nerve, such as cholecystokinin (CCK) and peptide YY (PYY).

6.1. Cholecystokinin

CCK is a well-established satiety hormone involved in protein-induced food intake suppression [64]. Whey protein increases CCK concentrations in plasma, peaking initially 15–20 min after the meal and remaining higher than basal levels for more than 3 h [8,60]. Whey

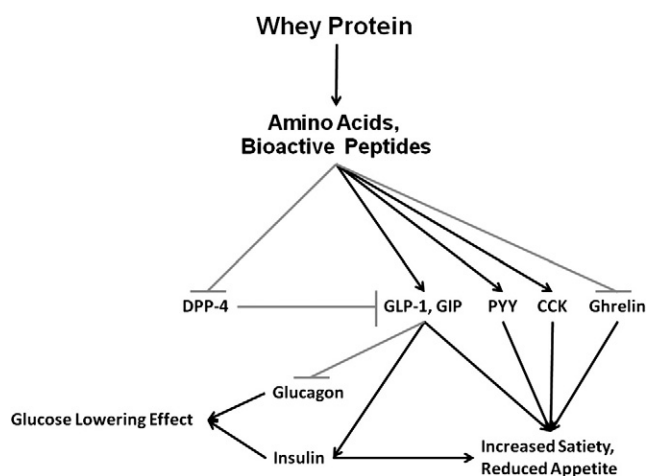


Fig. 1. Metabolic effects of whey protein.

increased CCK more than casein in one study [8], but not in another [60], due most likely to the degree of whey purification and possibly differences in the GMP content.

6.2. Peptide YY

PYY is a gut hormone secreted from L cells throughout the length of the gut, but at higher concentrations in the more distal parts [65]. It is secreted postprandially in proportion to caloric load and it depends on macronutrient composition. The concentration of plasma PYY increased after intragastric administration of whey or casein protein or their hydrolysates to healthy subjects [43]. The effect of whey compared with other proteins on PYY secretion requires further investigation.

6.3. Ghrelin

Ghrelin is the only orexigenic gut hormone known to date released into circulation from the stomach and its concentrations usually reach a peak just before meals and it is suppressed by food ingestion [66]. In humans, whey protein and calcium caseinate or other protein sources, i.e., soy and gluten, suppressed ghrelin concentrations similar to lactose, but more than glucose over 3 h [60,67]. However, in a recent study, it was found that fasting ghrelin was lower in participants consuming whey protein compared with soy or carbohydrate [61]. Reduced ghrelin secretion could account for the hunger suppression following whey or consumption of other proteins.

7. Conclusion

Whey protein, via bioactive peptides and amino acids generated during gastrointestinal digestion, enhances the release of several hormones, such as CCK, PYY, GIP, GLP-1 and insulin, that lead to reduced food intake and increased satiety (Fig. 1). Insulin secretion is associated with the glucose lowering effect and with the control of food intake. The mechanism by which whey protein leads to the increased insulin secretion is currently not known and should be investigated. One possible mechanism is the production of bioactive peptides that serve as endogenous inhibitors of DPP-4 in the proximal gut, preventing the degradation of the insulinotropic incretins GLP-1 and GIP. Another mechanism may involve BCAAs, specifically leucine, which activate the mTOR signaling pathway and protein synthesis leading to elevated hormone expression and secretion and increased thermogenesis. The insulinotropic effect of whey proteins may potentially attenuate the postprandial blood

glucose excursions over the day, might improve glucose homeostasis in Type 2 diabetic patients and could possibly postpone the introduction of medical treatment. The ability to amplify insulin secretion by whey protein may be safer than the commonly used therapeutic agents. The induced satiety, increased thermogenesis and comparable magnitude of blood glucose reduction to pharmaceutical treatment support the application of whey protein in the therapeutic treatment for the management of Type 2 diabetes and obesity. Nevertheless, future studies should determine whether these beneficial effects of whey protein on food intake, subjective satiety and intake in humans are obtained from long-term whey protein daily consumption.

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