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The impact of whey protein on plasma branched-chain amino acids and glycaemic control in humans. A narrative review

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Review Article

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Abstract

Impaired glycaemic control is a major risk factor for developing type 2 diabetes (T2D), a worldwide health epidemic intrinsically linked to diet and obesity. Whey proteins (WP) are increasingly popular supplements that are a rich source of branched-chain amino acids (BCAA), essential for muscle protein synthesis and metabolic regulation. In humans, fasting plasma concentrations of BCAA are maintained around 350 μM but become chronically elevated by 10–25% in persons with T2D. Little is known about whether BCAA from WP impacts circulating BCAA concentrations and contributes to this phenomenon. This narrative review used a systematic search approach with relevant keywords to identify evidence from randomised controlled trials in normoglycaemic humans and those with insulin resistance or T2D, on the effects of WP intake on plasma BCAA and glycaemic control. This review is, to the authors' knowledge, the first to specifically examine the effects of WP intake on plasma BCAA concentrations in relation to glycaemic control. Whilst the majority of acute studies identified ($n = 6$) reported that WP consumption between 10 and 50 g significantly elevates postprandial BCAA and insulin responses (as evidenced by peak concentration and/or area under the curve), evidence from chronic studies ($n = 3$) report inconsistent findings on the impact of 9–51 g of WP/d on fasting BCAA and glycaemic control (for example, fasting glucose and insulin, insulin clearance). Findings from this literature review highlight the need for further studies that investigate the relationship between WP consumption with BCAA and glycaemic control, and to determine underlying mechanisms of action.

Introduction

Type 2 diabetes (T2D) and the dysregulation of glucose homeostasis and insulin resistance (IR) that precedes it, is a chronic and progressive metabolic disorder that is an escalating worldwide health crisis, particularly in developed countries. By 2030 the disorder is expected to affect 643 million people globally[1]. The rise is strongly associated with the parallel global obesity epidemic, the leading risk factor for T2D[2]. In the UK, 28% of adults were obese in 2021, compared with only 15% in 1993[3,4]. T2D often leads to co-morbidities including cardiovascular disease, microvascular disease (neuropathy, retinopathy, nephropathy), dyslipidaemia and hypertension[5]. Not only does this have a significant impact on the quality of life of those living with T2D and its complications[6] but also places an enormous economic burden on national health systems. In the UK, T2D cost a total of £19 billion in 2021[7].

Whey proteins (WP) have become very popular dietary supplements amongst athletes and recreational exercisers, but also amongst individuals looking to manage their body weight[8]. WP are rich in branched-chain amino acids (BCAA)[9] which are essential nutrients known for their role in muscle protein synthesis and in direct and indirect cellular signalling. However, despite their essential role, there is evidence that elevation of plasma BCAA concentrations in humans and animals increases the risk of IR and T2D[10]. Numerous studies have proposed mechanisms through which BCAA may contribute to IR. These include disturbances in how BCAA are catabolised and the impact of BCAA on metabolic pathways that regulate insulin sensitivity. Emerging research suggests specific enzymes (branched-chain ketoacid dehydrogenase (BCKDH) and branched-chain ketoacid dehydrogenase kinase (BCKDK)) have key roles in these metabolic disturbances that are linked to impaired glycaemic control[11].

Firstly, a general overview is provided of the relationship between BCAA and T2D, the underlying mechanisms that control BCAA, and the impact of dietary sources of BCAA on circulating concentrations. Secondly, the evidence on the effects of acute and chronic WP consumption on plasma BCAA concentrations and subsequent effects on glycaemic control is

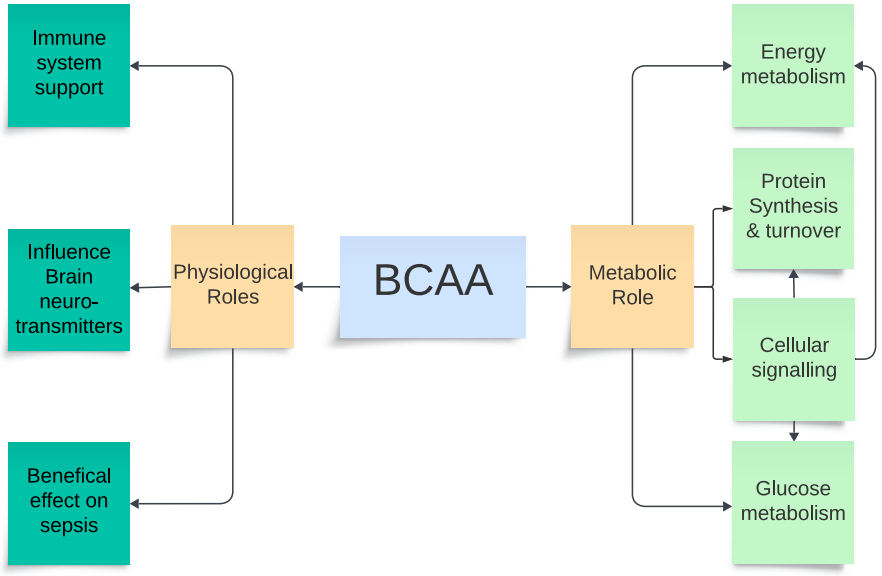

Figure 1. Emerging metabolic and physiological roles of BCAA. Figure based on the review by Monirujjaman and Ferdouse[19]. BCAA, branched-chain amino acids; T2D, type 2 diabetes.

presented and discussed. While previous reviews have explored the role of BCAA in metabolic health, none have specifically focused on the impact of WP on fasting circulating BCAA concentrations and their potential link to glycaemic control. This review addresses that gap.

BCAA and T2D

Evidence from cross-sectional and longitudinal research has shown that elevated plasma BCAA concentrations in humans are strongly associated with an increased risk of IR and T2D[12]. One meta-analysis suggests that for every one SD increase in plasma BCAA concentrations, the relative risk of developing T2D is 1·35 (95% CI: 1·25, 1·45)[13]. The BCAA are leucine, isoleucine and valine, and get their name from their hydrophobic side chains that have branched methyl groups. They are classed as essential amino acids because they cannot be synthesised by humans. They must be obtained from dietary sources and play a critical and varied role in human physiology[14]. BCAA are involved in the promotion of muscle protein synthesis[15], down-regulation of muscle protein breakdown[16], regulation of energy metabolism[17], cellular signalling[18], as well as emerging roles such as immune system support and influencing brain neurotransmitters[19]. Fig. 1 offers a schematic representation of the varied known and emerging metabolic and physiological roles of BCAA.

Circulating fasting plasma concentrations of BCAA are under the tight homeostatic control of BCAA catabolism enzymes, which respond to variations in nutrition, exercise and hormones. In the metabolically healthy, fasting plasma concentrations of BCAA are around 100 μM for leucine, 200 μM valine and 60 μM isoleucine[20]. Nutritional intake of foods high in BCAA (see Table 1) typically elevate plasma BCAA concentrations post-prandially, which then up-regulate the BCAA catabolic pathway, bringing concentrations back to baseline typically within 3–5 h of eating[21]. Exercise also increases the flux of BCAA oxidation, with concentrations decreasing during exercise as the muscle uses BCAA for fuel, returning to pre-exercise concentrations during the recovery period because of increased muscle protein turnover[22]. Fluctuations in certain hormones also influence BCAA homeostasis, with insulin, glucocorticoids, thyroid and female sex hormones known to affect key BCAA catabolic enzymes that either up-regulate or down-regulate BCAA oxidation to maintain typical concentrations[23].

It has been known since the 1960s[31] that individuals with obesity and IR or T2D have elevated fasting plasma BCAA concentrations, but there was little research interest in exploring their potential role in the aetiology of IR and T2D until the seminal work of Newgard *et al.*[32] sparked a revitalisation of interest in the topic. Newgard and colleagues identified an elevated BCAA 'signature' in obese human adults and then demonstrated in rats that a high fat diet in combination with supplementary BCAA directly contributed to the development of IR associated with obesity. Obesity related increases in human fasting plasma BCAA concentrations have been reported to range from around 10%[33] up to around 25%[34] in comparison with lean individuals.

Elevated fasting plasma BCAA are prognostic of T2D. Wang *et al.*[35] investigated 2422 normoglycaemic adults as part of a nested case–control study from the Framingham Offspring cohort. They found highly significant associations between baseline fasting plasma BCAA and future risk of T2D with those in the upper quartile of plasma BCAA concentrations (over 481·3 μM) being at least four times more likely to develop T2D during the 12-year follow up period. A systematic review of twenty-seven cross-sectional and nineteen prospective studies, which included Wang *et al.*[35], found that each SD increase in BCAA concentrations equated to a 36% increased risk of developing T2D in the future[36]. Lee *et al.*[37] examined the data of 685 adults of differing ethnicities from the Insulin Resistance Atherosclerosis Study and found that for each SD increase in plasma fasting BCAA concentrations, there was an associated two-fold increase in the odds ratio of developing T2D at the 5-year follow up in Caucasians and Hispanics but not in African Americans. The authors suggested that one explanation for the difference between ethnicities could be because BCAA concentrations are influenced by genetic factors[38], but further research was needed. In a cross-sectional cohort of sixty-nine healthy children, McCormack *et al.*[39] found that children aged 8–13 years with obesity already had elevated plasma BCAA concentrations and that baseline concentrations were moderately positively correlated (coefficient 0·44) with IR at the 18-month follow-up visit. By contrast, there have been conflicting results

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Table 1. Total branched-chain amino acid content of common supplements and foods

Food	g/100 g food	g/portion (g)
Whey protein isolate	22	6·60/30[§]
Whey protein concentrate	19	5·88/30[§]
Calcium caseinate powder	18	5·40/30[§]
Pea protein isolate	16	4·83/30[§]
Soy protein isolate	13	4·08/30[§]
Low-fat cheddar cheese	6·78	2·03/30[†]
Chicken	5·92	7·10/120[*,†]
Pork	5·21	6·25/120[*,†]
Salmon	4·76	5·71/120[*,†]
Beef	4·65	6·04/130[*,†]
Soft goat cheese	3·80	1·14/30[†]
Peanuts	3·61	0·72/20[†]
Soft cow cheese	3·00	0·90/30[†]
Almonds	2·96	0·59/20[†]
Soyabeans	2·64	2·64/100[*,‡]
Walnuts	2·53	0·50/20[†]
Egg	2·39	2·86/120[*,†]
Whole wheat bread	1·90	1·52/80[†]
Black beans	1·66	1·92/150[*,‡]
Lentils	1·65	1·98/150[*,‡]
Pecans	1·42	0·28/20[†]
Peas	1·39	1·11/80[*,‡]
Oats	0·75	0·30/40[‡]
Whole milk	0·62	1·24/200[‡]
Quinoa	0·49	0·24/50[‡]
Brown rice	0·47	0·23/50[‡]
Green beans	0·34	0·27/80[*,‡]
Sweet potatoes	0·32	0·56/175[*,‡]
Potatoes	0·26	0·46/175[*,‡]
Banana	0·18	0·14/80[‡]
Butternut squash	0·15	0·12/80[*,‡]
Mushrooms	0·14	0·11/80[*,‡]
Carrots	0·13	0·10/80[*,‡]
Apples	0·04	0·03/80[‡]

g, grams.
*Cooked portion weight
†Portion size from British Nutrition Foundation[24]
‡Portion size from British Dietetic Association[25]
§Portion size from Optimum Nutrition[26–30]

from both animal and human intervention studies that have attempted to explore whether the relationship between elevated plasma BCAA concentrations and T2D is causal or if BCAA are simply co-incidental early biomarkers of impaired insulin action. However, there is currently no consensus opinion[40]. The main hypotheses include persistent activation of the mammalian target of rapamycin (mTOR) signalling pathway and dysregulated BCAA catabolism and are outlined here.

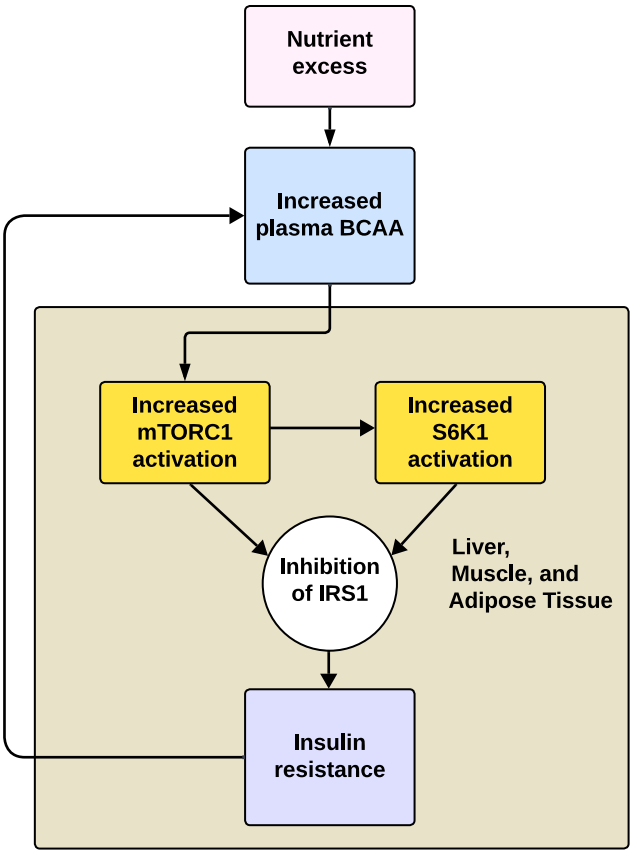

Figure 2. Proposed role of BCAA in persistent activation of mTORC1 leading to insulin resistance. Adapted from Chen and Yang[47] and Lynch and Adams[34]. BCAA, branched-chain amino acids; IRS1, insulin receptor substrate 1; mTORC1, mammalian target of rapamycin complex 1; S6K1, S6 kinase beta 1.

BCAA stimulate the mTOR signalling pathway, which coordinates regulation of metabolism and cell growth in all human tissues[41] and regulates messenger RNA (mRNA) translation which leads to an increase in muscle protein synthesis[42]. Animal studies suggest that persistent activation of the mTOR pathway by dietary BCAA induces IR by inhibiting the action of cellular insulin receptors[43,44] (Fig. 2). However, human studies have not consistently found that diets high in BCAA have this effect. Weickert *et al.*[45] reported that a diet with 25–30% of total energy from protein from low fat meat and dairy and a daily 58 g mixed whey and pea protein supplement, had only a transient effect on the expression of S6K1 (a key downstream signalling protein in the mTOR pathway)[46] in the adipose tissue of the overweight adult participants. Although IR (measured using the euglycaemic–hyperinsulinaemic clamp procedure) was found to increase at the 6-week time point, after 18 weeks of the high protein diet, the change in insulin sensitivity was no longer significant. The authors speculated this could be due to the observed adaptations to the diet in participants, which was associated with a reduction in their abdominal fat and increase in lean body mass.

Magkos *et al.*[48] examined BCAA, mTOR activity and insulin sensitivity in an obese cohort who lost body weight following gastric bypass or gastric band surgery. They found that plasma BCAA significantly decreased, whilst skeletal muscle insulin sensitivity increased. However, there was no change in the rate

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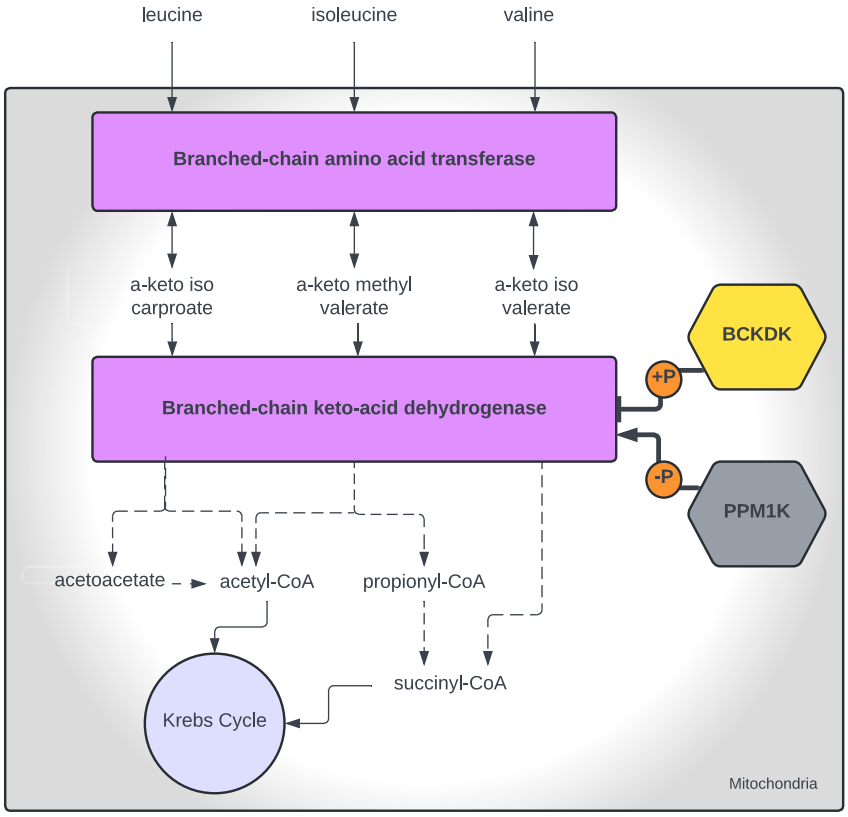

Figure 3. Overview of BCAA catabolism. BCAT and BCKDH are enzymes catalysing the first two steps of BCAA catabolism with the end products entering the Krebs cycle. BCKDH is activated via dephosphorylation by PPM1K and deactivated via phosphorylation by BCKDK. Dashed lines indicate multi-stage catabolic pathways. Adapted from Arany and Neinast[12] and Dimou *et al.*[51]. BCAA, branched-chain amino acids; BCAT, branched-chain amino acid transferase; BCKDH, branched-chain keto-acid dehydrogenase; BCKDK, branched-chain keto-acid dehydrogenase kinase; P, phosphate; PPM1K, mitochondrial protein phosphatase 1K.

of skeletal muscle mTOR phosphorylation, implying that the mTOR pathway was not the mechanism that induces IR. Smith *et al.*[49] also demonstrated in obese post-menopausal women that whilst WP ingestion (0·6 g/kg fat free mass, given during a 240-min hyperinsulinaemic–euglycaemic clamp procedure) does play a regulatory role in postprandial glucose homeostasis and IR, this was independent of the mTOR pathway. In support of these findings, Maida *et al.*[50] demonstrated that whilst manipulation of dietary BCAA did impact mTOR activation, the extent of activation (either reduced or increased) did not impact IR in murine models of obesity and T2D.

An alternative hypothesis is that high concentrations of plasma BCAA are driven by dysfunction in the BCAA catabolism pathway. Unlike other amino acids which are catabolised in the liver, BCAA escape the splanchnic territory and are catabolised mostly in skeletal muscle, but also in adipose and other tissues[14]. The first step in the BCAA catabolism pathway involves a reversible transamination, catalysed by branched-chain amino acid transferase (BCAT) which produces branched-chain keto acids (BCKA). The BCKA are then irreversibly decarboxylated by branched-chain a-ketoacid dehydrogenase (BCKDH) to produce branched-chain acyl-CoA esters. The final step of BCAA catabolism involves three separate multi-stage pathways for each BCAA, leading to the production of either acetoacetate, acetyl-CoA or succinyl-CoA[51]. An overview of BCAA catabolism is presented in Fig. 3.

She *et al.*[52] examined changes in both the BCAT and BCKDH enzymes in the tissues of obese rodents, and in tissue from obese humans before and 17 months after bariatric surgery. They found obesity-related tissue-specific changes in BCAT and BCKDH levels, both in the rodent models of obesity compared with the lean controls, and in the human tissue samples collected before and after bariatric surgery. BCAT and BCKDH were down-regulated in adipose tissue of obese rodents, but not in liver or muscle. BCAT and BCKDH were up-regulated in the human adipose tissue following bariatric surgery. The authors speculated that excess adiposity and impaired oxidation of BCAA in adipose tissue may play a previously underestimated but important role in elevating fasting BCAA concentrations. Conversely, later work by Neinast *et al.*[53] suggested that even in models of obesity, BCAA oxidation in adipose tissue only accounted for around 5% of whole-body BCAA oxidation, shedding doubt on the role of suppressed BCAA oxidation in adipose tissue as the sole cause of elevated plasma BCAA.

BCKDH is regulated by post-translational modifications, meaning its activity is controlled after the enzyme is made. BCKA dehydrogenase kinase (BCKDK) adds a phosphate group to BCKDH (phosphorylation) which inhibits its function, leading to an increase in BCAA and BCKA concentrations. By contrast, mitochondrial protein phosphatase 1K (PPM1K) removes this phosphate group (dephosphorylation) which activates BCKDH leading to a decrease in BCAA and BCKA concentrations. (Fig. 4). Several studies have shown that BCKDK is overexpressed and PPM1K underexpressed, leading to elevated BCAA and BCKA in animal models of obesity and diabetes[11,54,55]. Pharmacological interventions that target BCKDK have also provided valuable evidence by demonstrating that suppression of this enzyme leads to reduced plasma BCAA and BCKA and attenuates IR in obese mice models[55]. Bai *et al.*[56] added further evidence for the role of BCKDK/PPM1K dysregulation in elevating BCAA and inducing IR when testing high doses of the drug rosuvastatin in mice. Rosuvastatin is commonly prescribed to lower plasma/serum cholesterol but has been linked to an increased risk of developing T2D in humans. Over a 12-week period, high doses of the drug

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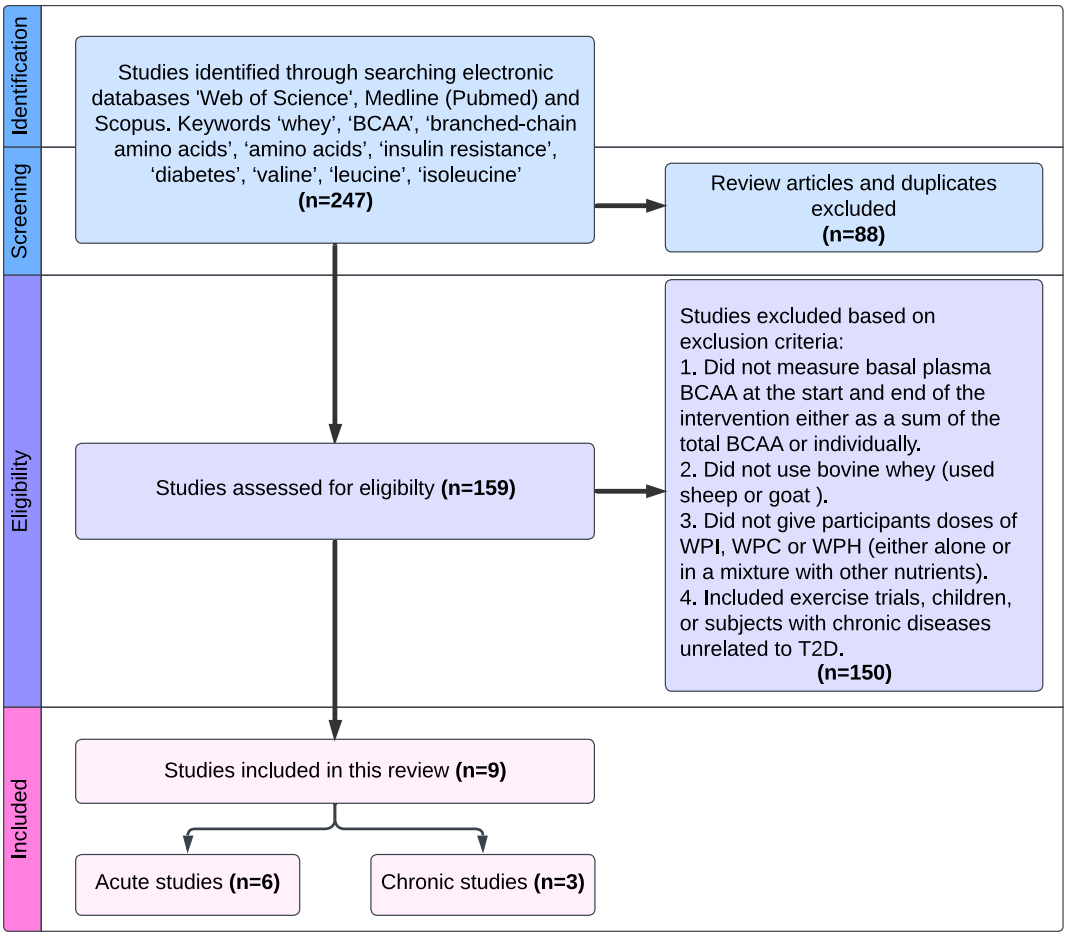

Figure 4. Flow diagram of literature search and selection. BCAA, branched-chain amino acids; T2D, type 2 diabetes; WPC, whey protein concentrate; WPH, whey protein hydrolysate; WPI, whey protein isolate.

induced a diabetic state in mice along with a 20% elevation in serum BCAA. There was up-regulation of the mRNA expression of BCKDK, and down-regulation of the expression of PPM1K in white adipose tissue and skeletal muscle, indicating that rosuvastatin affects the expression of these enzymes, which then inhibits the action of BCKDH, directly causing elevated BCAA and BCKA concentrations.

Several other mechanisms have been proposed to contribute to elevated fasting plasma BCAA. Gut microbiota in insulin-resistant individuals have been shown to have increased BCAA biosynthesis and gut BCAA transport potential, which are associated with increased plasma BCAA concentrations[57]. Certain metabolites of BCAA catabolism have been implicated in promoting insulin resistance including C3/C5 acylcarnitines[58] and 3-hydroxyisobutyrate (HIB)[59]. Brown adipose tissue has been shown to be involved in clearing BCAA and contributing to metabolic health[60]. It is beyond the scope of this review to cover all of these proposed mechanisms in detail. For further discussion of the pathophysiology and potential underlying mechanisms involved, including those mentioned here, there are several recently published comprehensive reviews on the topic[26,40,61–63].

Impact of dietary intake of BCAA

Dietary intake of BCAA varies significantly depending on the type of food consumed, as different foods have variable amounts of BCAA (Table 1 provides an overview of the BCAA content in a range of common foods and supplements). However, the impact of dietary BCAA intake on elevated plasma BCAA remains controversial. It is not clear whether specific foods high in BCAA, an overall diet high in BCAA from different sources, or the metabolic state of an individual plays the greatest role in determining fasting plasma concentrations[64]. In animal studies, She *et al.*[65] reported that plasma BCAA and BCKA, measured using metabolomics, were elevated by 45–69% in obese Zucker rats fed a standard chow diet *ad libitum* compared with the lean control rats. By contrast, Roquetto *et al.*[66] fed mice either a high-fat diet or low-fat diet with 13% of total energy intake from protein provided by WP or WPH for 16 weeks but found no elevations in plasma BCAA when compared with the casein control. In a meta-analysis of rodent studies by Solon-Biet *et al.*[67], there was a positive association between dietary BCAA intake and plasma BCAA concentration. However, the animal's existing intake of BCAA was important as to whether a dietary increase in BCAA could influence plasma BCAA. Animals on previously low BCAA diets showed the biggest increases in plasma BCAA, whereas there was little change in animals already on high BCAA diets. Interestingly, in both this meta-analysis[67] and the author's previous work[68] there was a plateau effect of dietary BCAA on circulating plasma levels in mice with a maximum plasma level of 40 μg/ml BCAA observed no matter how much further dietary intakes were increased. Whether there is a similar plateau effect in humans remains to be seen, especially since BCAA are metabolised very differently in humans compared with rodents and even other primates[69].

In humans, Woo *et al.*[70] found supplementing twelve obese prediabetic participants with 20 g of BCAA (10 g leucine, 5 g

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isoleucine, 5 g valine) a day for 4 weeks had no effect on their fasting BCAA concentrations, but did result in lower plasma glucose during an oral glucose tolerance test at the end of the study compared with baseline. However, in a 6-month intervention in elderly males with T2D, supplementation with 2·5 g leucine three times a day (7·5 g in total), increased fasting plasma leucine concentrations by 13% whilst simultaneously decreasing concentrations of isoleucine and valine by 16% and 23%, respectively, compared with the controls. However, the leucine supplementation did not change any measures of glycaemic control, which included basal fasting insulin and fasting blood glucose concentrations[71]. This study offers good evidence that dietary intake influences fasting BCAA concentrations but also suggests there may be a distinct metabolic response to supplementation with leucine alone compared with mixtures of all three BCAA.

Elshorbagy *et al.*[72] showed that thirty-six overweight participants who removed meat, poultry, eggs and dairy products (foods high in BCAA; Table 1) from their diets for 6 weeks as part of a religious fast, had a 15% decrease in fasting plasma BCAA concentrations (leucine −14%, isoleucine −12% and valine −20%) within 1 week of commencing the intervention, which was maintained until the end of the 6-week intervention. There were no changes in body weight and no changes in fasting plasma insulin or glucose concentrations. In a pilot study in six healthy participants placed on a diet where BCAA were restricted by 75% compared with the control diet, circulating plasma BCAA concentrations dropped by 50% (from 437 ± 60 to 217 ± 40 μmol/L) in just 7 d, compared with the control participants on iso-nitrogenous, iso-energetic diets. There was a marginal improvement in insulin sensitivity as measured by the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) in the BCAA-restricted group[73]. Using data from the Karlsruhe Metabolomics and Nutrition study, Merz *et al.*[64] found that an overall 'unhealthy' dietary pattern, high in saturated fat that included meat, sausages, sauces, eggs and ice cream, and low in fibre, could explain 32% of the variation in plasma BCAA of participants. The authors speculated that the high saturated fat and low fibre intakes could be responsible for the increased risk of T2D rather than the elevated BCAA. However, as a cross-sectional study they were not able to investigate any causal relationship between diet and BCAA levels. Understanding of the extent to which dietary patterns, individual foods or individual nutrients play a role in elevated BCAA and the subsequent aetiology of IR and T2D is still evolving.

Whey proteins

WP are constituents of dairy milk, comprising approximately 20% of the total protein content. WP contain all twenty amino acids[9] and are the most concentrated source of dietary BCAA available (Table 1). They comprise an array of protein sub-fractions and peptides with a remarkable range of beneficial properties, including improving markers of glycaemic control[74], improving inflammation and oxidative stress[75], as well as anti-hypertensive properties[76]. WP are extracted from the liquid whey fraction of dairy milk which is a by-product of the cheese making process. With the invention of membrane filtration technology in the 1970s it became possible to produce concentrated whey powders of up to 80 g/100 g protein content from liquid whey, known as whey protein concentrate (WPC). Further processing through micro-filtration produces whey protein isolate (WPI) which contains up to 90 g/100 g protein. WPC can be enzymatically hydrolysed to produce a partially pre-digested product known as whey protein hydrolysate (WPH). This is mainly used in infant formulas for babies who cannot tolerate standard formulas, and has antioxidant properties, making it useful as an additive in processed foods to prevent oxidative degeneration[77].

WPC and WPI are both used as sports and dietary nutritional protein supplements[78]. The market for WP in the form of supplementary protein shakes, bars and as a protein booster in many other foods has grown exponentially in the last 20 years[8]. Protein has become a very fashionable macronutrient, popularly understood to support lean muscle tissue, recovery from exercise, increase satiety and aid in fat loss or body weight maintenance[79]. Supplementary WP is now being consumed in amounts far greater than ever before[80] with the UK market predicted to grow by nearly 5% a year until 2032[81]. However, there is limited data available on specific doses and frequency of consumption in the general population in the UK.

WP and markers of IR and glycaemic control

Despite WP being the richest dietary source of BCAA, and the established link between elevated fasting plasma BCAA concentrations and increased risk of T2D, consumption of bovine dairy products, particularly WP, has been associated with a lower risk of developing T2D in large prospective cohort studies[82]. Research interest has grown in the potential role of WP as a functional food for managing or preventing T2D and associated risk factors. Human trials in both healthy individuals and those with IR or T2D have shown that WP supplementation can have a favourable effect on glycaemic control biomarkers by enhancing insulin response and lowering postprandial glucose levels[83–86]. However, a recent systematic review and meta-analysis suggests the current evidence for a beneficial effect of WP on postprandial glucose levels is 'very low to low' certainty, with further research needed[74]. Chronic studies have similarly indicated that WP may improve fasting glycaemia, reduce markers of inflammation and oxidation, and beneficially influence cardiovascular disease risk factors. A comprehensive umbrella review by Connolly *et al.*[87], which included both chronic and acute studies, supported these findings, showing WP's potential to lower HbA1c, HOMA-IR and fasting insulin, without any reported adverse effects on T2D-related risk factors.

Methods

A systematic approach was used to identify human studies that determined the effects of WP supplementation on both plasma BCAA concentrations and markers of IR and glycaemic control. An electronic search of Web of Science, Medline (PubMed) and Scopus was conducted (H.L.B.) using combinations of keywords including 'whey', 'BCAA', 'branched-chain amino acids', 'amino acids', 'insulin resistance', 'diabetes', 'valine', 'leucine', 'isoleucine'. Review articles and duplicates were excluded and the title and abstract of search results were interrogated for relevance to the topic (H.L.B.). Inclusion criteria were that studies measured basal plasma BCAA at the start and end of the intervention either as a sum of the total BCAA or individually; examined bovine whey only (sheep, goat *etc.* were excluded); doses of WPI, WPC or WPH either alone or in a mixture with other nutrients were given to participants; included participants of any age, body composition, healthy or impaired glycaemic control/T2D, and on hypo, iso or hyper energetic diets. Studies examining BCAA and glycaemic control responses to WP in the context of exercise trials, in

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children, or in subjects with chronic diseases unrelated to T2D were excluded from the present analysis.

Results and discussion

A total of 247 studies were identified. After excluding review papers and duplicates ($n = 88$), the remaining papers ($n = 159$) were examined against the inclusion and exclusion criteria. After excluding studies that did meet the criteria ($n = 150$) the remaining studies ($n = 9$) were categorised as acute ($n = 6$)[88–92] or chronic ($n = 3$)[93–95]. 'Acute' was defined as single study days that measured postprandial responses to a single or repeated dose of WP. 'Chronic' was defined as any intervention that gave a daily dose of WP over weeks or months (at least 1 week with no upper time limit) and reported long-term outcome measures. A flow diagram of study selection is presented in Fig. 4.

Acute studies

Inclusion of acute studies in this review is essential to understand the immediate metabolic responses which could contribute to long term effects of WP on plasma BCAA concentrations and markers of IR and glycaemic control, and to provide insights into the underlying mechanisms of action. Table 2 summarises data from the six acute studies identified. There is broad agreement across all six studies[88–92] that WP induces a greater increase in post-meal plasma BCAA concentrations compared with the same protein intake from other protein sources such as casein, gluten or plant-based proteins. This heightened postprandial BCAA response was associated with a greater increase in insulin secretion also observed after WP compared with the other sources of protein. However, three out of the six studies found no significant difference in postprandial plasma glucose concentrations between protein types, as might be expected to result from higher insulin concentrations. The lack of a consistent reduction in postprandial glycaemia could be due to multiple variables across the studies. These include the metabolic health of participants, meal composition, type and amount of WP, timings of feedings and timings of blood sampling. These limitations, along with others, are explored in greater detail in the 'Study limitations' section.

Chronic studies

Whilst a limited number of human intervention studies have examined the postprandial effect of WP on BCAA and markers of glycaemic control, even fewer have examined the effect of chronic WP consumption. Table 3 summarises the data from the three chronic studies identified[93–95]. Only one of the studies[93] found that chronic WPI intake resulted in an elevation in plasma fasting BCAA concentrations. In this 4-week randomised controlled trial, 158 overweight or obese men and women were divided into four groups placed on either a high (30%) or moderate (20%) protein isoenergetic diet with either 15% or 7% saturated fat content. A strength of this study was the different doses of WPI used to meet the protein targets, giving insight into a potential dose–response relationship. A dosage of 51 g/d WPI resulted in an elevation in plasma fasting BCAA concentrations, whilst a dose of 15 g WPI did not produce a significant elevation. The authors do not give details how the daily consumption was spread throughout the day or whether it was given as a single dose. The metabolomic and hormonal effects of WP differ greatly between regular small doses versus one large dose[86], so this would be useful information to have included. Although participants were divided into four diet groups with different saturated fat content, as well as different protein content, disappointingly, results for BCAA concentrations were reported as an aggregate of both high and low saturated fat groups. Therefore, it is not possible to ascertain if there was an impact of saturated fat on fasting BCAA concentrations from this key study.

The other two studies identified did not find any effect on plasma fasting BCAA concentrations following chronic daily WP supplementation, but neither used a dose as high as Chiu *et al.*[93]. Piccolo *et al.*[94] gave their participants 20 g/d WPI split into 10 g doses given at both breakfast and lunch. Byer *et al.*[95] report in their ancillary study that their participants consumed 30 g/d WPI for 18 months. However, data from the original study[96] suggest that participants were only consuming 22 g/d WPI by 6 months, and only 18 g/d by 18 months, indicating some attrition in compliance over the study duration.

Study limitations

Both the acute and chronic studies have several limitations. In the acute studies, the participant populations were limited, with only two studies using healthy participants, whilst the others included participants who were overweight or metabolically compromised. Individuals who are already less responsive to insulin may be less likely to respond to the insulinogenic effects of WP[97]. In the chronic studies, the participant populations were either obese, had metabolic syndrome or were post-menopausal women. Further research, both acute and chronic in design, is needed in younger, healthier populations who are the most likely populations to use WP supplements chronically for fitness purposes. Young healthy people are at low risk of T2D, but studying how regular, long-term, often high-dose whey protein intake affects fasting BCAA concentrations in this population is important because longitudinal studies have shown that elevations in fasting BCAA concentrations can occur many years before any signs of insulin resistance or T2D become apparent[35] and studying this group could help us understand how their long-term habits affect their future risk.

There was variability in the doses of WP across studies that complicates comparisons. The acute studies used doses ranging from 10 g to 50 g, with no agreement on what constitutes a 'high' or 'low' dose. Chronic studies also varied in the dose of WP used, from 9 to 51 g/d, as well as in duration, ranging from 4 weeks to 18 months. Importantly, two acute studies and one chronic study used different doses of WP and found a dose–response relationship, where higher doses led to more pronounced plasma BCAA response, but this was not explored across the other studies. The inconsistency in doses, as well as differences in the types of WP used (WPI, WPC or blends), which could affect rates of digestion and thus appearance of BCAA in the plasma[98], further complicates the interpretation of the results.

The acute studies varied in the composition of test meals (mixed meals or WP alone) and chronic studies varied in the broader dietary context. All studies used liquid WP shakes or soups but there is evidence that WP in different food matrix formats, such as increasingly popular protein bars, cookies or fortified yogurts, has a different postprandial impact to that given in liquid form and warrants further investigation[99]. Two chronic studies were isoenergetic, whilst one involved significant energy restriction aimed at achieving 5–10% weight loss, which likely influenced the lack of observed changes in fasting BCAA concentrations. There were also differences in the blood sampling protocols in the acute studies,

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Table 2. Acute trials of whey protein on postprandial plasma BCAA and markers of glycaemic control

Reference	Population description	Design	Test condition	Time	Outcome measures	Effect on postprandial BCAA concentrations and markers of glycaemic control
Nilsson, Holst and Bjorck (2007)[88]	$n = 12$ Healthy adults aged 20–30 years, BMI 19·5–25·7 kg/m^2	R, CO. Breakfast drinks. 1-week washout	18 g WP (type not specified) + 25 g glucose OR 3 variations of AA + 25 g glucose OR 25 g glucose (control)	120 min	BCAA, glucose, serum insulin, GIP and GLP-1	WP ↑ PP BCAA concentration Insulin AUC 60% greater WP *v.* control and glucose AUC 56% lower WP *v.* control
Tessari *et al.* (2007)[89]	$n = 12$ Older adults with T2D Average age 56·6 years Average BMI 24·3 kg/m^2	R, CO. Mixed meals. 2-week washout	0·7 g/kg BW WPI OR 0·7 g/kg BW casein OR 0·7 g/kg BW AA	180 min	AA, insulin, C-peptide, pro-insulin, glucose, GIP and GLP-1	WPI ↑ peak PP plasma BCAA 66% and insulin concentrations 40% *v.* casein PP glycaemia NS between meals
Kassis *et al.* (2018)[90]	$n = 17$ Overweight but healthy adults, 20–45 years, BMI 25–32 kg/m^2	R, CO. Breakfast drinks. 30-d washout	30 g WP (type not specified) OR 50 g WP OR 50 g casein OR 50 g Maltodextrin (control)	330 min	EE, DIT, substrate oxidation, whole body protein turnover, AA, glucose, insulin	50 g WP ↑ BCAA concentration AUC 112% *v.* 30 g WP and 60% ↑AUC *v.* 50 g casein 50 g WP ↑ insulin AUC 57% *v.* 50 g casein, but ↓ 22% *v.* 30 g WP PP glycaemia↓ for protein meals *v.* control, but NS between protein types or amounts
Pekmez *et al.* (2020)[91] PREMEAL 1	$n = 20$ adults with metabolic syndrome. Age 50·5–62·5 years, BMI 28·2–32·6 kg/m^2	R, CO. Protein drink 15 min PRIOR to standard fat rich breakfast. 1-week washout	10 g WPI OR 20 g WPI OR Water (control)	360 min	TG, ApoB-48, glucose, insulin, FFA, BCAA	10 g and 20 g WPI ↑ BCAA concentration *v.* control. 20 g and 10 g WPI ↑ insulin and ↓ glucose concentrations *v.* control with a dose–response relationship and significant difference 10 g *v.* 20 g WPI
Pekmez *et al.* (2020)[91] PREMEAL 2	$n = 16$ adults with metabolic syndrome. Age 62–68·8 years, BMI 27·5–31·7 kg/m^2	R, CO. Premeal drink or mixture 15 min or 30 min PRIOR to standard fat rich breakfast. 1-week washout	20 g WPI OR 20 g casein OR 20 g gluten	360 min	insulin, glucagon glucose, GIP, GLP-1, AA	WPI ↑ BCAA concentration AUC *v.* gluten. WPI ↑ isoleucine (but not leucine or valine) AUC *v.* casein WPI and casein ↑ peak insulin concentration *v.* gluten. WPI ↑ insulin peak *v.* casein but NS PP glycaemia NS between conditions
De Marco Castro *et al.* (2022)[92]	$n = 9$ Healthy males and females over 65 years, average BMI 25·5 kg/m^2	R, CO. Breakfast soups. 1–3-week washout	31 g WP (isolate and concentrate blend) OR 41 g plant protein (three variations)	180 min	AA, insulin, glucose	WP ↑ BCAA and insulin concentrations *v.* all other plant protein PP glycaemia NS between meals

n, number; BMI, body mass index; kg, kilograms; m^2, square metre; R, randomised; CO, crossover; g, grams; WP, whey protein; AA, amino acids; min, minutes; BCAA, branched-chain amino acids; GIP, gastric inhibitory peptide; GLP-1, glucagon-like peptide 1; PP, postprandial; AUC, area under curve; *v.*, versus; T2D, type 2 diabetes; BW, body weight; WPI, whey protein isolate; NS, no significant difference; EE, energy expenditure; DIT, dietary induced thermogenesis; TG, triacylglycerol; ApoB-48, apolipoprotein B-48; FFA, NEFA; ↑, increased; ↓, decreased.

Table 3. Chronic trials reporting effect of whey protein on fasting plasma BCAA and markers of glycaemic control

Reference	Population description	Design	Test Condition	Control/Comparison	Duration	Hypo, iso or hyper-energetic diet	Effect on fasting plasma BCAA	Effect on markers of glycaemic control
Chiu *et al.*, (2014)[93]	*n* = 158 (47M/111F). Overweight or obese adults. Average BMI 33·9 kg/m^2. Average age 38 years.	R, PL. 4 week 'run-in' diet then high protein or moderate protein diet groups further subdivided into high saturated fat or low saturated fat groups	High protein 51 g/d WPI Moderate protein 9–15 g/d WPI	15% protein (0 g WP) 15% protein (0 g WP)	4 weeks	Iso-energetic Iso-energetic	↑ *v.* control diet ↑ *v.* control diet but NS	↔ fasting glucose or insulin sensitivity ↑ plasma BCAA correlated with ↓ insulin clearance (MCRi) and ↓insulin secretion
Piccolo *et al.* (2015)[94]	*n* = 27 Obese women with metabolic syndrome. Average BMI 36·45 kg/m^2. Average age 41 years.	R, PL.	20 g/d WPI given as two 10 g doses consumed with 8 oz water 30 min before breakfast and lunch	20 g/d gelatine-based protein supplement given as two 10 g doses with 8 oz water 30 min before breakfast and lunch	8 weeks	Hypo-energetic (average participants energy reduction was ↓ 655 kcal/d)	No difference between groups	HOMA-IR ↓ after both interventions but NS
Byer *et al.* (2018)[95]	*n* = 84 Post-menopausal women Average BMI 26·3 kg/m^2 Average age 69 years	R, PL. Ancillary study to Kerstetter et al., 2015	30 g/d WPI	30 g/d maltodextrin	18 months	Iso-energetic	Not different *v.* baseline	Not different *v.* baseline, but plasma BCAA concentration positively correlated with IR after both interventions

n, number; M, male; F, female; BMI, body mass index; R, randomised; PL, parallel; g, grams; WPI, whey protein isolate; WP, whey protein; *v.*, versus; NS, no significant difference; BCAA, branched-chain amino acids; MCRi, metabolic clearance rate of insulin; oz, ounces; kcal, kilocalories; HOMA-IR, homeostasis model assessment of insulin resistance; IR, insulin resistance; ↑, increased; ↔, no change; ↓, decreased.

from timing intervals to total analysis duration, which complicate comparisons of key outcomes such as time to peak concentrations of BCAA, insulin and glucose, as well as areas under the curves.

Acute studies were conducted following overnight fasts, which is standard procedure. However, BCAA concentrations fluctuate through the day[100] and might respond differently to WP at different times of the day and to meals subsequent to breakfast. Research designs that consider circadian BCAA rhythms have more potential to reveal any plateau effect of BCAA concentrations in humans, as seen in animal studies[68]. Only the shortest chronic study showed an effect of WP on fasting plasma BCAA concentrations, which correlated with reduced insulin clearance and increased insulin secretion. This difference in outcomes might suggest an adaptive response in BCAA metabolism to prolonged dietary changes, a concept supported by Weickert *et al.*[45] who observed a temporary increase in IR before a return to baseline in their high protein diet study.

Knowledge gaps and future research

The current body of human studies, both acute and chronic, highlight knowledge gaps that need to be addressed to better understand the impact of WP consumption on plasma BCAA concentrations and markers of glycaemic control. Key areas for future investigation include the effects of WP consumption over cumulative meals throughout the day, and over longer post-prandial durations, as well as the influence of different types of WP, food matrix formats and mixed macronutrient meals versus protein alone. There is also a need to explore how these variables interact across different subject cohorts, considering factors such as age, sex, health status and disease conditions.

Looking at chronic consumption of WP, there are gaps in the knowledge of the effects on fasting plasma BCAA and markers of glycaemic control in diverse populations, including those with different dietary patterns and consumption habits. The effects of longer-term WP consumption are unclear, as are the metabolic mechanisms driving any observed changes. Studies that account for these factors are necessary to provide a clearer picture of the role of WP in both acute and chronic contexts.

Emerging research has suggested that the type of fat ingested with dairy protein has a significant impact on how quickly postprandial BCAA are cleared from plasma. Feitsma *et al.*[101] found that a milk shake containing dairy cream with milk fat globule membrane resulted in significantly lower plasma BCAA concentrations at 3 h post-meal ingestion, compared with matched macronutrient and energy meal shakes that contained only either anhydrous milk fat without milk fat globule membrane or a vegetable fat blend instead. Health-conscious consumers have historically opted for skimmed or semi-skimmed dairy products, although recent consumer research suggests this may be changing to a preference for 'full-fat' dairy[102]. WP supplement powders are typically very low fat but are often mixed with milk to make milkshakes. Understanding the full health implications of the type of milk co-ingested with the WP is clearly important and further research is needed to confirm these intriguing findings.

Another key area for future research is to investigate whether chronic WP consumption could contribute to the dysregulation of BCAA metabolism that may account for the elevated BCAA concentrations seen in obesity and T2D. Increased activity of BCKDK shows promise for providing an explanation and is also a potential target for pharmacological intervention. Studies are needed to investigate whether chronic WP intake impacts the expression and activity of BCKDK and how this affects concentration of plasma BCAA and BCAA metabolites as well as on markers of glycaemic control. Addressing these knowledge gaps will provide a clearer understanding of the impact of WP on fasting plasma BCAA concentration and markers of glycaemic control. This is essential to be able to provide evidence based nutritional guidelines on the inclusion of WP in regular daily diets.

Conclusions

With the growing popularity of WP supplements in healthy adults to support muscle gain and weight management, studies are needed to determine whether chronic intakes over the long term affect fasting BCAA concentrations and increase the risk of developing insulin resistance and T2D. Acute studies suggest that WP may offer short-term benefits by enhancing postprandial insulin responses and, in some cases, reducing postprandial glycaemia. However, the relationship between chronic WP consumption, fasting plasma BCAA concentrations and T2D risk is unclear. Future research should explore the metabolic effects of WP in different population groups, including those using WP to support muscle gain, or individuals with overweight or obesity using WP during energy restriction. Studies should include consideration of variables such as dose, frequency, and type of WP consumed.

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