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REVIEW

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The effects of whey proteins, their peptides and amino acids on vascular function

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Abstract

Cardiovascular diseases (CVD) are a significant and growing burden on global health services, and it is now accepted that impairment of vascular function represents a major preliminary step in the development of CVD. There is considerable interest in identifying both causal factors of impaired vascular function, as well as related nutritional factors that may lower the risk of developing CVD, and food-derived bioactive peptides and amino acids have emerged as one such area. Dairy foods contain two groups of proteins, whey proteins and caseins, which represent a rich source of bioactive peptides that are released during food processing and/or digestion. These peptides have a number of physiological activities including the potential to reduce blood pressure. Research, including acute and longer-term randomised controlled trials, animal models and in vitro models has demonstrated the potential impact of dairy proteins on vascular function. The purpose of this paper is to narratively review the evidence, primarily from randomised controlled trials, examining the effects of whey proteins, their peptides and amino acids on vascular function and related issues including blood pressure. In addition, it will explore the potential underlying mechanisms responsible for these effects. It concludes that there is increasing evidence that whey proteins, and notably the bioactive peptides and amino acids released during their digestion, can have beneficial effects on aspects of vascular function and thus contribute to CVD risk reduction. It also highlights a number of beneficial effects of whey proteins including those on blood pressure, arterial stiffness, nitric oxide production and inflammation.

KEYWORDS
cardiovascular diseases, hypertension, peptides, vascular endothelium, vascular function, whey proteins

INTRODUCTION

Cardiovascular diseases (CVD) including coronary heart disease (CHD), stroke and peripheral artery disease are the leading causes of death worldwide (World Health Organization, 2021). In the UK, heart and circulatory diseases still account for 27% of total deaths and represent a substantial burden on health services

(British Heart Foundation, 2021). Whilst still one of the leading causes of death, CVD-related death rates have progressively decreased in many high-income countries over the last 40 years, although the burden is still growing in low-income countries and morbidity remains high, impacting on the quality of life for many (Malik et al., 2013). Hypertension, a sustained abnormally high blood pressure, is another vascular disease and a modifiable risk factor for other related vascular issues notably stroke but also chronic kidney disease. With a prevalence of 31% and 26% in adult (>16 years) men and women in England (Health Survey for England, 2015), hypertension (defined as systolic (SBP) and diastolic blood pressure (DBP) ≥140 and ≥90 mm Hg, respectively; NHS, 2019a), is a common precursor to reduced vascular function and a loss of control of vascular reactivity and is a key risk factor for CVD. It is estimated that about 54% of strokes and 47% of CHD worldwide are the results of hypertension (Wu et al., 2015). There is also good evidence that arterial stiffness, especially of the large vessels, is an important predictor of CVD events (Cockcroft & Wilkinson, 2000). It is now clear that healthy functionality of the vasculature is a crucial modifiable risk factor for CVD, and although data are limited, cohort studies (Livingstone et al., 2013) and randomised controlled trials (Fekete et al., 2018) suggest that dietary dairy proteins, and whey proteins, in particular, are associated with reduced vascular stiffness.

This paper aims to narratively review the evidence on the effects of whey proteins, and the peptides and amino acids they contain, on vascular health, including evidence from acute and chronic human intervention studies. It will initially cover key aspects of vascular function, detail common techniques used to investigate these effects, and briefly examine some proposed underlying mechanisms for the action of whey proteins on vascular function.

VASCULAR FUNCTION

Vascular reactivity is a vital component of endothelial function, which modulates changes in blood flow when needed via alterations in vessel tone and diameter. Broadly, healthier arteries have a higher vasodilative capacity and are more reactive to external and physiological stimuli. Due to the key health-related role played by the vascular endothelium, it has been termed 'the gatekeeper of vessel health' (Cahill & Redmond, 2016).

The vascular endothelium is responsible for the production of several vasoactive agents including both relaxing and contracting factors. Vascular dysfunction occurs when there is a reduced availability or control of these compounds and functions, leading to a reduction in vascular reactivity, increased arterial stiffness and endothelial inflammation (Fekete et al., 2013). Nitric oxide (NO) is synthesised in the endothelium and is a key vasodilator, with reduced bioavailability of NO being a key factor in the development of vascular dysfunction (Schächinger & Zeiher, 2000). Blood lipids are also closely related to CVD risk. Higher circulating concentrations of low-density lipoprotein cholesterol (LDL-C) and triacylglycerol (TAG) may have a direct deleterious effect on the vascular endothelium and vascular function (Takaeko et al., 2021). Lipids, such as non-esterified fatty acids, TAG and LDL-C can all impair endothelial function, the latter, for example, inhibiting the enzyme NO synthase and thus reducing the available NO (Kim et al., 2012). One of the many functions of the vascular endothelium is to control the volume of blood vessels, influencing (along with the kidneys and other tissues) BP, with elevated BP being the most important independent risk factor for stroke and coronary artery disease, as well as renal failure and aneurysm rupture (Kjeldsen, 2018).

Globally, there is a range of classifications of hypertension. In the UK, it is defined as a peripheral BP (brachial artery) exceeding 140 mm Hg SBP and 90 mm Hg DBP (NHS, 2019a). Two main methods are used to measure peripheral BP; 'office' testing, with a single time point measurement, which is performed within a clinical setting, and 'ambulatory' testing which consists of multiple, automated tests at regular intervals over 12–24 h, with the patient or participant not confined to a clinical or research setting. Due to the reduced chance of so-called 'white coat syndrome', the ambulatory measurement is seen as having greater clinical relevance and reflects better the variability of BP throughout the day and night (NICE, 2013). It is worth noting that central aortic BP (either measured directly by invasive techniques or by means of derivation from waveforms measured at locations distal of the aorta), is more predictive of disease outcome than peripheral pressure (McEniery et al., 2014), although clearly more complex to measure, so less often used.

Vascular tissue is under constant mechanical loading from the haemodynamic forces generated by BP and these mechanical stresses can lead to morphological and biochemical changes within the endothelium. These alterations in the properties of the vasculature, including its reactivity and stiffness, in turn, lead to vascular dysfunction and the development of CVD (Baeyens et al., 2016). Techniques used to study vascular function include measures of vascular reactivity and stiffness. Flow-mediated dilatation (FMD), a technique that measures vascular reactivity in the brachial artery, has become accepted as a highly reliable method of assessment of vascular reactivity (Thijssen et al., 2019). FMD employs a cuff to occlude the brachial artery, followed by measuring the expansion of the artery after the cuff is deflated. After a period of occlusion, reactive hyperaemia increases shear stress on the endothelium, leading to a dilation

of the artery in a healthy vasculature. The extent of this dilation is usually expressed as a percentage of the baseline value and is used to gauge reactivity; it is considered the gold standard measure for assessing vascular function. Laser Doppler imaging with iontophoresis (Sandoo & Kitas, 2015) measures the reactivity of the peripheral microvasculature, the smallest blood vessels that are responsible for the transport of nutrients, water, heat and waste to and from tissues. The technique uses an electric current to move two different vasodilating compounds, sodium nitroprusside (SNP) and acetylcholine (ACh), into the top layer of the skin where they trigger dilation of the microvasculature in the skin and an increase in blood perfusion. Whilst SNP donates NO directly by breaking down in the circulation independent of the vascular endothelium, ACh works by binding to receptors on the cells of the endothelium, and its activity is dependent upon endothelial cell function. A laser is used to scan across the skin and the Doppler shift caused by laser light reflecting off moving red blood cells is then detected with imaging to identify changes in blood perfusion in response to these two locally vasoactive compounds (Turner et al., 2008).

Arterial stiffness is commonly measured by pulse wave analysis (PWA) and pulse wave velocity (PWV). PWA examines the wave form of the arterial pressure wave to calculate the augmentation index (AIx), which is a commonly used measure of arterial stiffness. The arterial pressure wave is composed of two components, an initial pressure wave from contraction of the heart and a second pressure wave that is reflected off the branching points of the major blood vessels. The second wave augments the observed systolic pressure, and two systolic peaks are seen, the first is the non-augmented wave and the second is the augmented wave. The final height of the reflected wave is in part due to the stiffness of the blood vessels the pressure wave is passing through, and AIx is defined as the ratio of the first and second systolic peaks (augmentation pressure) divided by the total pulse pressure × 100. PWV uses the speed of movement of the pressure wave caused by the ejected blood exiting the left ventricle during systole. The speed of propagation of this pressure wave is a measure of arterial stiffness, the stiffer the vessel wall, the faster the propagation of the pulse wave, and it tends to increase with age. It is worth noting that wave speed is also positively correlated with adiposity (Wildman et al., 2003) and has been shown to increase by up to 20 cm/s per year in obese young adults (Tomiyama et al., 2011). There is now good evidence that aortic PWV as a measure of arterial stiffness represents a powerful predictor of future CVD events and all-cause mortality. The meta-analysis of Vlachopoulos et al. (2010) on the association between PWV and risk of CVD events and mortality confirmed the predictive power of high aortic PWV for CVD events and which was greater in individuals with a higher background CVD risk (Sequí-Domínguez et al., 2020).

Drug therapies for hypertension, such as beta-blockers, ACE inhibitors, angiotensin-2 receptor antagonists and diuretics, whilst effective, can be associated with side effects such as fatigue and nausea (NHS, 2019b). Other strategies such as dietary and lifestyle interventions to normalise BP are of interest; the DASH diet (Dietary Approaches to Stop Hypertension) being an example of one such strategy. Specific food groups and potentially bioactive nutrients they contain are also of interest, particularly dairy foods and proteins, such as those found in whey.

WHEY PROTEINS

Dairy foods are complex and contain several nutrients hypothesised to beneficially affect vascular function, including proteins and their derived peptides and amino acids, fats, calcium, magnesium and phosphorus (Lovegrove & Hobbs, 2016). A number of studies have shown that calcium and magnesium can trigger modest, but clinically significant reductions in SBP of 1.3–4.6 mm Hg, although at doses higher than obtainable from typical dairy intakes (Kris-Etherton et al., 2009). Whey and casein proteins in dairy foods and their derived peptides and amino acids are now increasingly being studied for their ability to reduce BP and improve aspects of haemodynamics (Fekete et al., 2016b; Livingstone et al., 2013).

Whey proteins are a group of soluble proteins comprising approximately 20% of the protein content of cow's milk and are usually isolated from the liquid portion of milk during cheese making followed by filtration and drying. The whey protein fraction is made up of several proteins including β-lactoglobulin, α-lactalbumin, immunoglobulins and glycomacropeptides, which have a range of properties (Table 1). They are high-quality proteins containing all the essential and non-essential amino acids and are relatively rich in the branched-chain amino acids (BCAA) leucine, isoleucine and valine. A number of different classes of whey protein products are commercially available and include the filtrates whey protein concentrate (WPC) and whey protein isolate (WPI) and filtered, then enzymatically digested, whey protein hydrolysate (WPH). These differ in terms of nutrient profile with WPI and WPH containing the most protein in commercially available products, typically 85–95 g protein per 100 g product, and the highest concentration of BCAA in commercially available whey proteins, and indeed of all milk proteins (Almeida et al., 2016). Consumption of whey protein has, historically, been most commonly associated with bodybuilding and sports nutrition, where it is used as a supplemental protein source to enhance muscle protein synthesis. Interestingly, this application of whey

TABLE 1 Whey protein sub-fraction composition and biological activity (based on Brandelli et al., 2015; Chakrabarti & Wu, 2016; Madureira et al., 2010; Yadav et al., 2015)

Protein sub-fraction	Amount (by % weight of total protein)	Example biological roles of protein and its derived peptides
β-lactoglobulin	50%–55%	Immunocompetence Antioxidant ACE inhibitor Antimicrobial DPP IV inhibitors (anti proliferation) Opioid agonist
α-lactoalbumin	20%–25%	Antioxidant ACE inhibitors Antimicrobial Enhanced immunocompetence
Immunoglobulins	10%–15%	Improved immune function
Glycomacropeptides	10%–15%	Antithrombotic, antimicrobial
Bovine serum albumin	5%–10%	
Lactoferrin	1%–2%	Antimicrobial, anti-inflammatory
Lactoperoxidases	<1%	Antimicrobial

protein is now also being used to reduce sarcopenia in the elderly (Devries et al., 2018). Given the emerging evidence suggesting a potential role of dairy proteins in improved vascular function, whey proteins have been examined in a number of studies to determine the short- and long-term effects on the vascular system.

CHRONIC EFFECT OF WHEY PROTEINS ON VASCULAR FUNCTION

There are a small but growing number of studies investigating whey protein and its chronic (>4 weeks) impact on vascular function. Eighteen studies covering the period 1995–2021 were identified by searching Web of Science, MEDLINE, the Cochrane Library and Google Scholar, with search terms including 'whey', 'whey peptides', 'dairy protein', 'vascular' and 'BP'. Table 2 shows data from longer-term 'chronic' studies (>4 weeks) that have examined the effect of whey proteins in a number of different subject types. Table 3 provides a summary of the observed chronic effects of whey proteins on BP and vascular function from the identified studies.

The earliest study identified (Pal & Ellis, 2010) compared isoenergetic intakes of whey and casein proteins (54 g total protein/day), with a glucose control (54 g/day), over 12 weeks in 70 overweight adults. Compared to baseline, whey proteins significantly reduced peripheral SBP (from 119.3 ± 3.2 to 113.1 ± 3.02 mm Hg) and AIx by 14%. A similarly structured study conducted by our group (Fekete et al., 2016a) utilising a very similar protocol to that of Pal and Ellis (2010) compared the effects of WPI and casein proteins (2 × 28 g/day) in men and women (n = 38) with mild hypertension, with a maltodextrin control over 8 weeks. Both casein and WPI treatments decreased blood pressure, with 24-h ambulatory systolic and diastolic blood pressures being significantly lower after the whey proteins (2.0 mm Hg ±0.7 mm Hg and 2.9 mm Hg ±1.1 mm Hg SEM, respectively) and with the whey protein treatment leading to 0.59% (±0.2%) improvement in % FMD response compared with baseline, whilst the casein group saw a 0.11% (±0.2%) change. However, a more recent study in individuals who were overweight or obese (Kjølbæk et al., 2017) found no effect on BP between treatment and control groups when using a similar but lower WPC dose (equal to protein 45 g/person per day). Another notable difference with this study cohort as compared to Pal and Ellis (2010) and Fekete et al. (2016a) is that the test period came during a weight maintenance phase following an 8-week period of planned weight loss. Data collected during the weight-loss period showed the participants had lost 12.7 kg (±2.95 kg) of bodyweight, corresponding to 13.2% (±2.34% SD) of their initial bodyweight, which may have influenced BP responses during the weight maintenance phase. Small but non-significant reductions in BP have also been seen in overweight (BMI: 30.0 ± 2.8 kg/m^2 SD), middle-aged (48 ± 7.9 years SD) men and women consuming 30 g protein from WPC/day for 9 months compared with a glucose control (Weinheimer et al., 2012).

The relationship between obesity, BP and vascular stiffness is complex, and evidence strongly links both obesity and hypertension with increased vascular stiffness (Oh, 2018). Nonetheless, in slightly overweight adolescents (12–15 years, mean pre-test BMI 25.1 kg/m^2) consuming 35 g protein/day in a parallel study, as either skimmed milk, WPI or casein, over 12 weeks (Arnberg et al., 2013), the WPI treatment showed a significantly increased brachial SBP (2 ± 7.8 mm Hg SD). It is possibly important that mean baseline BP values were well within the normal range at 111.3 and 64.5 mm Hg for brachial systolic and diastolic blood pressure,

TABLE 2 Chronic trials investigating the association between whey proteins, whey proteins subfractions and peptides and vascular function and blood pressure

Reference	*n*, Cohort	Design	Duration (weeks)	Test condition (amount)	Comparison treatment or control	Primary outcome or effect
Pal and Ellis (2010)	70, (M&F) Obese, non-hypertensive	R, SB, C, PAL	12	WPI (2 × 27 g/day)	Casein protein (2 × 27 g/day)	↓SBP (wk12, −4.2%) ↓DBP (wk12, −3.3%) ↓AIx (wk12, −14%)
Petyaev et al. (2012)	40, (M&F) Pre hypertensive	R, C, DB	4	WPI (70 mg in 7 mg lycopene micelles)	WPI 70 mg, or 7 mg lycopene	↓SBP (wk4, −5.6%) ↓DBP (wk4, −5.1%) ↑FMD (wk4, 2.6%)
Arciero et al. (2014)	79, (M&F) Overweight and obese	R	16	WPC (3 × 20 g/day)	No control group	BP: No significant change
Arnberg et al. (2013)	193, (M&F) Overweight adolescents	R, C	12	WP (35 g/day)	Casein (35 g/day)	↑ aDBP (wk12, 1%) ↑ bSBP (wk12, 1.3%) cSBP (wk12, 2.2%)
Figueroa et al. (2014)	33, (F) Obese	R, C, DB	4	WP (30 g/day)	Casein (30 g/day)	↓ bSBP (−4%) ↓ aSBP (6.2%) ↓ PWV (−4.9%)
Fekete et al. (2016a)	38, (M&F) Mild hypertensive	R, C, DB, Cr	8	WPI (2 × 28 g/day)	Calcium casenate (2 × 28 g/day) or maltodextrin (2 × 27 g/day)	↓ bSBP (−3.8%) ↓ bDBP (−2.5%) ↓ mean BP ↑ FMD (0.59%)
Fluegel et al. (2010)	71, (M&F) Grouped by BP: 25 Normatensive 24 Pre-hypertensive, 4 Stage 1 Hypertensive	R, C, PAL	6	WPH (28 g)	Non hydrolysed WPC	For young pre-hypertensives: ↓ bSBP (−1.9%) ↓ bDBP (−1%) ↓ Mean arterial pressure For elevated SBP & DPB (−1.4%)
Aldrich et al. (2011)	(M&F) 27–32 kg/m^2 40–60 years		8 controlled +12 ad libitum	WP (15% calorie intake)	Protein matched diet, protein from mixed food sources	↓ SBP (−6.2%, at 5 months after the start of intervention)
Tahavorgar et al. (2015)	52, (M) Overweight and obese	R, C	12	WPI (65 g/day)	Soy protein isolate (60 g/day)	↓ bSBP (−14.5%) ↓ bDBP (−9.2%)
Kjølbæk et al. (2017)	(M&F) BMI 27.6–40.4 kg/m^2	R, DB, C	8	WP (45 g/day)	Soy protein (45 g/day), maltodextrin (48 g/day)	BP: No significant change
Lee et al. (2007)	53, Hypertensive (M&F)	R, DB, C	12	125 ml milk drink with 2.6 g/day whey peptides	125 ml milk drink with lactose	BP: No significant change
Kawase et al. (2000)	20, (M) Normotensive, high cholesterol	R, SSB, C	8	WPC 8.8g (2 × 200 ml fermented milk)	Casein 7.84 g (in milk drink)	↓ bSBS (approx. −5.5%)

(Continues)

TABLE 2 (Continued)

Reference	*n*, Cohort	Design	Duration (weeks)	Test condition (amount)	Comparison treatment or control	Primary outcome or effect
Hodgson et al. (2012)	119, (F) Hypertensive	R, DB, C	104	WPI (30 g) in a milk drink	Maltodexdrin	BP: No significant change
Vatani and Golzar (2012)	10 (M) Overweight	–	6	WPI (3 × 30 g)	Starch placebo and supplements control group	BP: No significant change
Weinheimer et al. (2012)	*n* = 112: 20 g/day *n* = 44, 40 g/day *n* = 45: 60 g/day (M&F) BMI 30.0 ± 2.8 kg/m^2 48 ± 7.9 years	R, DB, C	36	WP (0, 20, 40, 60 g)	–	BP: No significant change
Samaras et al. (2014)	19 (M&F) Ultra Marathon runners		8	WP 30.8 (after training)	Tomato juice, or a carbohydrate beverage	FMD: No significant change

Abbreviations: aDPB, aortic diastolic blood pressure; Aix, augmentation index; aSPB, aortic systolic blood pressure; bDPB, brachial diastolic blood pressure; BP, blood pressure; bPB, brachial blood pressure; bSPB, brachial systolic blood pressure; C, controlled; cDBP, central diastolic blood pressure; Cr, crossed; cSBP central systolic blood pressure; cSBP, central systolic blood pressure; DB, double-blinded;DBP, diastolic blood pressure; F, female; FMD, flow-mediated dilatation; M, male; NS, no significant result found; PWV, pulse wave velocity; R, randomised; SB, single blinded; SBP, systolic blood pressure; WP, whey protein; WPC, whey protein concentrate; WPH, whey protein hydrosalate; WPI, whey protein isolate.

respectively, with the authors speculating that whey may only have a hypotensive effect in individuals who are already hypertensive. An association of increased BMI in childhood with adult arterial stiffness has been reported previously, however it is thought to be mediated predominantly through the chronic age-related increase of BP (Liu et al., 2019).

Indeed, age is a non-modifiable risk factor for hypertension, so the effect of whey proteins on BP regulation in older individuals is of interest. Here again, the results have been mixed. Hodgson et al. (2012) studied the long-term impact of WPI in participants aged 70–80 years with hypertension using 30 g/day versus an isoenergetic carbohydrate (maltodextrin) comparison in a randomised controlled parallel trial conducted over 2 years. Mean SBP was lowered in the whey proteins treatment group by 2.3 mm Hg after 1 year and 1.6 mm Hg at 2 years, with no significant differences between groups found using an 'intention to treat analysis'. The authors speculated that the prevalence of anti-hypertensive medication and the relatively small amount of protein used in the intervention may have been limiting factors. More recently, Lefferts et al. (2020) also examined the effects of whey proteins in non-obese older adults, looking at a cognitive and vascular function in a 12-week intervention of 50 g/day WPI against an isoenergetic carbohydrate control group. At 0 and 12 weeks vascular function was assessed using carotid-femoral PWV, brachial BP and 'haemodynamic loading' score (heart rate multiplied by SBP). Although no change in cognitive function was found, there were small but significant reductions in heart rate and central haemodynamic load in the WPI group. The observed effect on PWV at 12-weeks in the whey group was relatively small (−0.5 m/s), however, the intergroup difference was 1 m/s, which, whilst still modest, is associated, as the authors note, with a 15% improvement in CVD risk.

Some studies on the effects on vascular function and hypertension have used whey proteins in conjunction with other interventions such as reduced energy diets (Aldrich et al., 2011), structured exercise (Arciero et al., 2014; Figueroa et al., 2014; Weinheimer et al., 2012) and micronutrient supplementation (Daly et al., 2014). Aldrich et al. (2011) compared energy-reduced diets designed to promote weight loss in middle-aged men and women who were overweight (*n* = 18, mean BMI 30.3 kg/m^2, age 50 years). Energy intakes were tailored to the individual (mean intake 6.7 MJ per day), however, protein intake varied across the three groups, with the two extra protein groups' diets being energy matched by reducing carbohydrate intake. The diets comprised a control diet (CD) containing 63.4 g (±0.2 g SEM) protein, and two higher protein arms with an additional 60 g/day, one of the mixed protein sources (MP), (mean daily total 124.1 g protein) and the other a WPI intervention, (mean daily total 124.2 g protein).

TABLE 3 Summary of findings of studies using chronic whey protein treatments on blood pressure and vascular function, showing the supporting references with the total number of references including blood pressure data

	Effect on blood pressure					Effect on other measures of vascular function
	↓ DPB &↓SBP	↓ DPB only	↓SBP only	↑BP	No significant effects on BP	Benefits in terms of ↓PWV and/or ↑FMD and/or ↓Aix
References	Fekete et al. (2016a), Fluegel et al. (2010), Pal and Ellis (2010), Petyaev et al. (2012), Tahavorgar et al. (2015)	Figueroa et al. (2014)	Aldrich et al. (2011), Hodgson et al. (2012), Kawase et al. (2000)	Arnberg et al. (2013)	Arciero et al. (2014), Kjølbæk et al. (2017), Lee et al. (2007), Samaras et al. (2014), Vatani and Golzar (2012), Weinheimer et al. (2012)	Fekete et al. (2016a), Figueroa et al. (2014), Pal and Ellis (2010), Petyaev et al. (2012), Samaras et al. (2014)

Abbreviations: Aix, augmentation index; BP, blood pressure; DPB, diastolic blood pressure; FMD, flow-mediated dilatation; PWV, pulse wave velocity; SBP, systolic blood pressure.

Although protein intakes were the same in the MP and WPI interventions, a 7.2 mm Hg decrease in SBP was observed in the WPI group at 5 months compared to baseline. Whilst SBP decreased in all three groups after two months only the WPI showed a significant reduction in SBP when compared to the control group.

Daily treatment of 30 g of whey proteins or casein with an isoenergetic (maltodextrin) control, in conjunction with resistance and interval training in 33 women with obesity showed the whey and casein proteins both significantly reduced brachial and aortic SBP and arterial stiffness by the end of the 4-week intervention. The whey proteins led to reductions in brachial and aortic SBP (from 131 ± 4 to 126 ± 3 and 120 ± 5 to 113 ± 4 mm Hg, respectively) and reduced arterial stiffness measured by PWV (–57 cm/s) relative to baseline (Figueroa et al., 2014). Arciero et al. (2014) compared a whey protein intake of 3 × 20 g/day over 16 weeks in three different treatment groups: whey proteins only (n = 24), whey proteins plus resistance training (n = 27) and whey proteins plus a mixed modality training programme (n = 28) in middle-aged men and women aged 46 years (±9.4 years SEM), who were overweight or obese but weight stable. Although whey proteins alone significantly reduced body fat, there was no impact on BP across all treatments, which may be related to normal BP values at baseline, although these data were not provided in the paper.

The significance of the results from studies combining dietary and exercise interventions, or dietary and structured weight-loss interventions is unclear since exercise is a known driver of improved vascular function (Green & Smith, 2018) and both weight loss and exercise are two recognised methods of BP control (Bacon et al., 2004). The studies do acknowledge the potential impact of changes in both bodyweight and physical activity with investigators advising participants to not change their levels of physical activity, monitoring activity levels and excluding participants whose physical activity levels did not fit the requirements of the study. It is worth noting that activity levels were monitored using questionnaires, such as the International Physical Activity questionnaire (Pal & Ellis, 2010). In the future, the measurement of activity may be better accomplished using direct measurement via the now commonplace wearable technologies such as smart watches.

Mainly due to their potentially lower environmental impact, there is increasing interest in plant proteins in relation to obesity development (Lin et al., 2015), hypertension, overall cardiovascular health (Alexander et al., 2017), cardiometabolic risk factors (Zhubi-Bakijaa et al., 2021) and overall and cause-specific mortality (Huang et al., 2020). The conclusions of Zhubi-Bakijaa et al. (2021) are of note. They confirm that specific amino acids have beneficial effects on risk factors for CVD such as hypertension and that proteins such as those in dairy foods, especially whey proteins, are inversely associated with hypertension and obesity and this needs recognition when replacing animal proteins with those from plants. The study of Tahavorgar et al. (2015) had indeed highlighted this. It was a double-blinded randomised study that compared a daily treatment of 54 g whey protein with 54 g soya protein isolate in men (age 30–65 years) who were overweight or obese over 12 weeks. Whilst both interventions led to a reduction in systolic and diastolic blood pressure, the effect was more pronounced in the whey proteins group, with a within-group reduction of –16.6 and –7.0 mm Hg for systolic and diastolic blood pressure, respectively, compared with –1.5 and –1.9 mm Hg in the soya protein group. However, due to the considerable differences between groups at baseline, these results may not be as large as they appear when looking at the change from baseline.

TABLE 4 Acute trials investigating the association between whey proteins, whey proteins subfractions and peptides and vascular function and blood pressure

Reference	*n*, Cohort	Design	Duration (min)	Product and amount	Results
Pal and Ellis (2011)	20, (F) Postmenopausal	R	360	WPI (45 g)	SBP & DBP: No significant change
Ballard et al. (2009)	21, (M&F) Overweight, mild hypertensive	R, DB, C, Cr	120	NOP-47 (5 g)	↑ FMD: At 30 min 4.6% at 120 min 5.1%
McDonald et al. (2019)	23, (M&F) Prediabetic	R, Cr	180	Whey (75 g)	↑ FMD (9.02%)
Pal and Ellis (2011)	20, (F) Overweight post-menopausal	R, C, Cr	480	WPI (45 g)	No significant change
Fekete et al. (2018)	30, (M&F) Mild hypertensive	R, C, DB, Cr	520	WPI (2 × 28 g/day)	↓ SBP At 300 min
Mariotti et al. (2015)	10, (M) Overweight M	R, C, Cr	360	WPI α-lactalbumin enriched WP	Casein but not whey lowered postprandial TG. No significant difference in markers of endothelial function compared to casein.
Ballard et al. (2009)	20, (M&F) Healthy	R, C, Cr	120	NOP-47 (5 g)	↑ FMD (9%) ↑ Reactive hyperaemia (29.9%)
Pal and Ellis (2011)	20, Overweight post-menopausal	R, C, Cr	360	WPI (45 g)	PWA, BP, AIx: No significant change

Abbreviations: AIx, augmentation index; BP, blood pressure; C, controlled; Cr, crossover; DB, double-blinded; DBP, diastolic blood pressure; F, female; FMD, flow-mediated dilatation; M, male; PWA, pulse wave analysis; R, randomised; SBP, systolic blood pressure; WP, whey protein; WPC, whey protein concentrate; WPH, whey protein hydrosalate; WPI, whey protein isolate.

Clearly more data are needed on the comparison between whey proteins and plant proteins, and there are a number of factors that make comparing data across the various studies above a challenge. These include the wide range of participant types, types of whey proteins used and protein dose size. The studies above include adolescent, middle-aged and elderly, non-overweight, overweight and obese participants, and normotensive, pre-hypertensive and hypertensive cohorts. The forms of whey proteins used include WPI, WPC and unspecified whey proteins, and whey proteins mixed with other dairy foods and in fermented preparations, with doses of protein used in the intervention studies ranging from 70 mg to 65 g/person per day. The techniques used to assess vascular function also vary as do the choice of comparison and controls used which includes various food proteins and carbohydrates.

Despite this high degree of heterogeneity as well as the presence of other confounding factors, such as exercise, a number of studies show a beneficial effect of whey proteins on measures of vascular health and function, with data showing both within and between treatment reductions in BP, with generally more pronounced effects seen in hypertensive participants and larger treatment doses. Whilst the evidence from some cohorts, in particular the elderly, is suggestive of an effect, other studies have failed to demonstrate an effect from whey protein treatments. Moreover, some of the larger effects have been shown in studies that combined dietary and exercise/activity interventions. In cohorts that are normotensive or have undergone recent periods of intended weight loss no reduction or non-significant reductions in BP have been shown, with one group, Arnberg et al. (2013), demonstrating an increase in SBP. Given the difficulty in comparing results due to the reasons discussed, more studies are needed in this area.

POSTPRANDIAL EFFECT OF WHEY PROTEINS ON VASCULAR FUNCTION

Many people in Western societies spend much of the day in a postprandial state (Ruge et al., 2009). In this phase, the blood concentrations of glucose and lipids are raised as food is digested and absorbed, which can increase insulin production and also lead to a transient increase in other hormones and markers of inflammation (Dimina & Mariotti, 2019). Many of these circulating factors impact the functioning of the vascular endothelium, for example, altering or inhibiting the production and reducing the bioavailability of NO, long-term reductions of which are associated with the reduced vascular function (Chen et al., 2008). Accordingly, investigating the effects of whey proteins and gauging their impact

on factors such as insulin secretion in this acute phase is of interest. It is also worth noting that in both healthy participants as well as those with impaired insulin metabolism, whey proteins stimulate the release of insulin both with and without the co-ingestion of carbohydrates (Claessens et al., 2008). The studies on postprandial effects are summarised in Table 4.

Pal and Ellis (2011) investigated the effects of whey proteins in 20 overweight or obese post-menopausal, normotensive women for 6 hours following a mixed test meal and either WPI, casein (both 45 g protein) or glucose (45 g). No difference was found in the area under the curve for SBP, DBP or AIx (measured via PWA) between groups, in the 6 h following the meal, with the authors speculating that a single dose of WPI is insufficient to observe any changes in postprandial vascular function. The authors pointed to their earlier chronic study that only found significant reductions in DBP after 12 weeks but not six and also speculated that the meal composition may have slowed stomach emptying, delaying or inactivating bioactive compounds in the whey proteins, and so attenuating the postprandial effects. Similarly, Mariotti et al. (2015) reported no significant changes in vascular function in 10 healthy overweight men in the 6 h following the ingestion of a high-fat meal (fat 70% total energy) with either α-lactalbumin enriched whey, standard WPI or casein (all 45 g protein total) against a 45 g sucrose control. A reduction in postprandial TAG and chylomicron particle concentrations over 6 h was seen in response to the casein treatment, with the authors speculating that these effects of casein may be due to a reduced rate of gastric emptying, lipolysis and/or absorption rate of fats in the meal.

In contrast, Fekete et al. (2018), using adults with a sequential two test meal design, each containing either 28 g whey protein, 28 g calcium caseinate or a carbohydrate (maltodextrin) control, the whey proteins treatment reduced SBP compared to casein (–15.2 ± 13.6 mm Hg) and the control (–23.4 ± 10.5 mm Hg) up to 5 h post-consumption. The whey proteins also improved vascular stiffness compared with the control. This randomised, controlled, double-blind, cross-over study included 30 participants (30–77 years) with mildly elevated BP (between 120/80 and 159/99 mm Hg). The significant findings in this study may have been due to the participants presenting with mild hypertension and that the interventions were given as two separate doses (breakfast and lunch).

Elevated insulin concentrations following a mixed meal may transiently impair vascular endothelial function via reduced synthesis of NO. McDonald et al. (2019) investigated the impact of dairy proteins on vascular function in 23 individuals with prediabetes in a randomised crossover study. Participants consumed 75 g of glucose alone or with 473 ml fat-free bovine milk or an isonitrogenous amount of whey protein or casein (16.5 g) with postprandial assessments made at 30-min intervals for up to 180 min. The results showed that postprandial FMD response over 180 min (assessed as the area under the curve) was impaired to the greatest extent on the glucose treatment (~–2% relative to the protein treatments) but declines in FMD were attenuated by all dairy-based treatments at 30–90 min after test meals and by both whey and casein at 120–250 min after test meals. The study indicated that whey and casein proteins may attenuate postprandial hyperglycaemia-mediated reductions in endothelial function with the authors suggesting a number of mechanisms, including delayed stomach emptying and limiting oxidative stress which in turn improves NO bioavailability to the endothelium by increasing arginine availability (Ballard et al., 2013b).

As with the chronic studies, the literature on postprandial effects shows a wide range of treatment doses, number of test meals (one or two sequential meals), as well as duration of postprandial study period (ranging from 120 to 520 min), which may limit the collective interpretation of these results and possibly those related to mechanisms of action. However, larger and multiple doses of dairy proteins appear to show the greatest effect and particularly in participants with raised BP, with results in normotensive participants being mixed or not significant.

WHEY PROTEINS AND IMPROVED VASCULAR FUNCTION: POTENTIAL MECHANISMS

Digestion or processing of milk proteins provides a rich source of bioactive peptides and physiologically active amino acids. These nutrients have a range of properties that may affect various facets of endothelial function and vascular health, including supporting the production and improving the bioavailability of NO, the reduction of inflammation and crucially, inhibition of the angiotensin-converting enzyme (ACE) a key part of the renin–angiotensin system (RAS).

Whey protein peptides and the renin–angiotensin system

The antihypertensive properties of dairy protein-derived bioactive peptides are thought to act primarily via the RAS, a major component of BP control (Figure 1). In the RAS system the hormone angiotensin I is converted to angiotensin II, a potent vasoconstrictor, by the action of ACE located on the surface of vascular endothelial cells. The evidence that peptides from milk proteins can exhibit worthwhile ACE inhibition has been known for some time as covered in reviews of FitzGerald and Meisel (2000) and FitzGerald et al.

(2004). There is also in vitro evidence from our group that peptides of the type released from dairy proteins, such as isoleucine-proline-proline, act by inhibiting the ACE enzyme (Fekete et al., 2013). The antihypertensive properties of bioactive peptides released from milk proteins have been observed both in vitro and in vivo, suggesting that some peptides may have BP-lowering properties on a par with pharmacological therapies (Alexander, 2014). Research from our own group demonstrated significantly higher ACE inhibitory activity by in vitro digested whey proteins than digested soya protein (Giromini et al., 2019; Figure 2). ACE also degrades bradykinin, a 9-amino acid residue peptide produced by the kinin–kallikrein system in a number of cell types and tissues and is a potent vasodilator. Whilst much of the focus has been on whey-derived peptides inhibiting the synthesis of angiotensin II from angiotensin I, recent evidence suggests that ACE inhibition, which reduces bradykinin breakdown, may be just as important. This allows the concentration of bradykinin to increase, in turn leading to a greater vasodilatory effect (Taddei & Bortolotto, 2016).

The impact of whey-derived peptides on the RAS system and in particular the inhibition of vasoconstriction and the promotion of vasodilation may be a key component in the long-term reduction of BP and in turn the mechanical stress on the endothelium, with an overall improvement in vascular function.

Impact on oxidative stress and NO availability

Postprandial changes in blood nutrients such as fats and carbohydrates can induce oxidative stress, which in turn can have various deleterious effects on endothelial function. NO itself is short-lived in the bloodstream and can react with the superoxide ion to form peroxynitrite, thus reducing the available NO. Additionally, reactive oxygen species can reduce the activity of the NO generating enzyme eNOS, reducing the production of NO (Chen et al., 2008). Whey proteins have a number of potential antioxidant properties, including providing antioxidant peptides and it contains relatively large quantities of cysteine (2.2 g per 100 g of amino acid), which also has antioxidant properties. Cystine (the oxidised form of cysteine) is also an important substrate for the production of the antioxidant glutathione (GSH) (Yu & Long, 2016). Whey proteins have been shown to increase circulating concentrations of plasma GSH in several human and animal studies, including randomised controlled trials (Corrochano et al., 2018). This ubiquitous tripeptide has been shown to be involved with reducing oxidative stress, and although direct evidence in humans is limited, multiple studies in animal models have explored possible mechanisms (Espinosa-Díez et al., 2018; Watanabe et al., 2013). Such studies demonstrate a link between GSH concentrations and vascular function, and in humans, reduced GSH levels are associated with deleterious changes in the vascular system and ventricular function, and cardiomyopathy (Damy et al., 2009; Watanabe et al., 2013).

The impact of whey protein-derived peptides on oxidative stress has been explored in vitro (Ballatore, et al., 2020; Contreras et al., 2011), demonstrating significant oxygen radical and hydroxyl radical absorbency capacity tested via oxygen radical absorbency capacity (ORAC) assay, and cytoprotective properties, tested using cell models. O'Keeffe et al. (2014) using a hydrolysed WPC demonstrated both increased antioxidant capacity using ORAC assay compared with undigested WPC. They also showed increased GSH activity when human umbilical vein endothelial cells were incubated with hydrolysed WPC, as well as beneficial regulation of genes associated with antioxidant activity. However, the concentrations of peptides used in the cell studies, as well as their degree of hydrolysis, may not be physiologically representative and so it is unclear how these results would translate in vivo. Ferric reducing antioxidant power is an older but relatively common in vitro method of quantifying antioxidant capacity. The hydrolysis of whey protein isolate has been shown to increase the iron(III) reducing capacity (Mohan et al., 2015), and simulated digestion of WPI using static in vitro gastrointestinal digestion (SGID) model has also demonstrated the increased antioxidant capacity of whey proteins, with peptides from α-lactalbumin showing the highest antioxidant capacity after SGID processing (Corrochano et al., 2019). Although in vitro studies may not be totally representative of the in vivo situation, they are a valuable method for exploring the potential underlying mechanisms of action of whey proteins and their digestion products.

Improved NO production

Some whey-derived peptides may enhance NO production. The commercially available peptide NOP-47, produced by in vitro enzymatic digestion of whey proteins, has been shown to increase NO production in vitro and to improve vascular function in vivo. Ballard et al. (2009) examined the effects of NOP-47 on markers of vascular function. Twenty participants (21–39 years) consumed 5 g/day for 2 weeks followed by measurement of vascular function via FMD for 2 h after ingestion of NOP-47 or placebo. There was no difference between the groups at baseline and whilst the placebo had no effect on FMD post-consumption, the FMD responses to NOP-47 were significantly improved by 8.9% at 30 min, 9.9% at 60 min and 9.0% at 90 min after ingestion. In addition, NOP-47 ingestion significantly increased hyperaemia blood flow (measured via

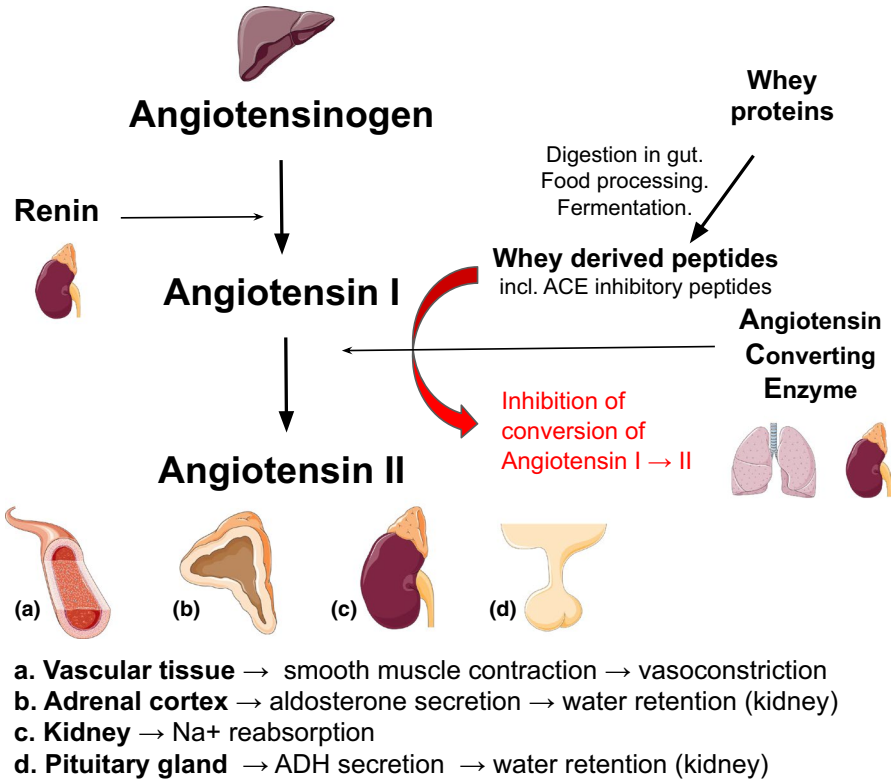

FIGURE 1 A schematic depiction of the renin–angiotensin-aldosterone system. ACE, angiotensin-converting enzyme; ADH, anti-diuretic hormone

a. Vascular tissue → smooth muscle contraction → vasoconstriction
b. Adrenal cortex → aldosterone secretion → water retention (kidney)
c. Kidney → Na+ reabsorption
d. Pituitary gland → ADH secretion → water retention (kidney)

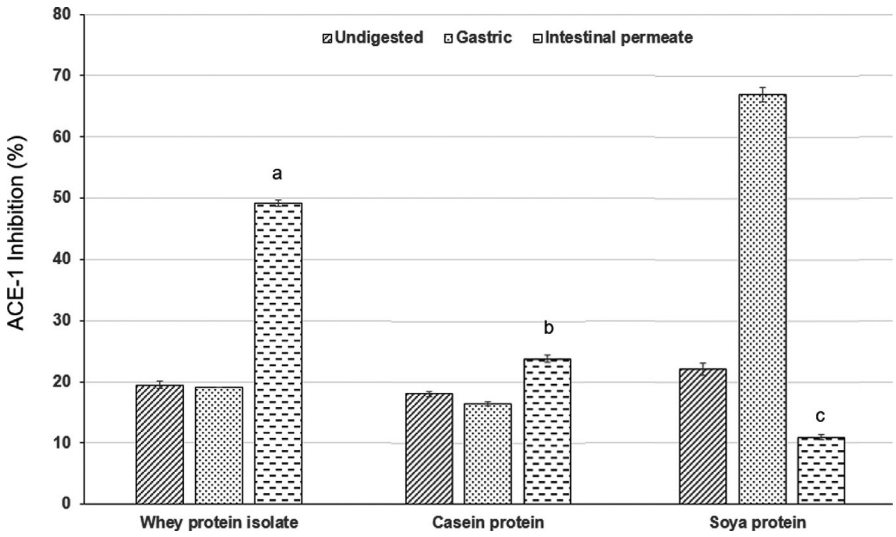

FIGURE 2 In vitro angiotensin-1 converting enzyme (ACE-1) inhibitory effect of undigested, gastric digestion and intestinal permeate of whey protein isolate, casein protein and soya protein. Data are least-square means ± SE. Different letters a–c indicate significant differences ($p < 0.05$) between intestinal permeates which represent the absorbed fraction (derived from Giromini et al., 2019)

strain gauge plethysmography) at 120 min and maintained higher plasma nitrate and nitrite concentrations than the placebo after 120 min. Ballard et al. (2013a) also showed that acute ingestion of 5 g of NOP-47 by older overweight or obese adults with an increased cardiovascular risk (age 45–65 years, BMI 25–40 kg/m^2), improved endothelium-dependent dilatation with a mechanism that was not dependent on changes in circulating vasoactive compounds including NO. It should be noted that although interesting, these data may be of limited practical use given the quantities of whey-derived peptides found in a typical diet and that the NOP-47 used was an enriched peptide preparation.

Whey proteins and some specific amino acids found in whey proteins (notably leucine, covered below) stimulate the production and activity of insulin (Adams & Broughton, 2016), and in terms of NO production and bioavailability, insulin can be seen as a double-edged sword, with insulin-mediated pathways governing both vasorelaxation and vasoconstriction. Stimulation of the ERK1/2 pathway by insulin leads to an increase in the production of endothelin, a vasoconstrictive polypeptide, but insulin signalling also stimulates the PI3K/AKT pathway, which leads to phosphorylation of eNOS and increased NO production leading to vasorelaxation (De Nigris et al.,

2015). An individual's degree of insulin sensitivity or resistance may also be a modifying factor. In people with good insulin sensitivity, the presence of insulin increases the availability of NO but in individuals with insulin resistance, the production of NO can become impaired, reducing vasodilation (Dhananjayan et al., 2016). In vitro studies showed that in insulin resistance there was impaired PI3K/AKT signalling (Janus et al., 2016) which reduced NO production, whereas the ERK1/2 pathway was unaffected. Animal studies suggest the same imbalance in insulin signalling in obese, glucose-intolerant versus lean models, leading to the suggestion that this may be a component of the reduced NO production observed in cardiometabolic disease (Symons et al., 2009).

Whey proteins derived anti-inflammatory peptides and opioids

Inflammation plays a critical role in the short and long-term development of atherosclerosis, involving acute alteration of the vasodilatory behaviour of the endothelium. It can trigger long-term modifications to the endothelium, including thickening of the vessel wall, increased permeability of the endothelium and increased expression of leucocyte adhesion molecules. This allows increased monocyte migration across the endothelial lumen, an important early stage in the formation of atherosclerotic plaque (Gerhardt & Ley, 2015). The effect of consuming 45 g/day of WPI has been compared to other dietary proteins (cod, gluten or casein) in overweight and middle-aged participants after high-fat meals, and demonstrated WPI's greater ability to reduce the markers of the inflammatory response, monocyte chemotactic protein-1 and CC chemokine ligand-5 (Holmer-Jensen et al., 2011). A number of whey protein-derived peptides have been proposed to have anti-inflammatory properties, and Swiss mouse models have demonstrated the ability of WPH, when dosed at 300 mg/kg bodyweight, to reduce markers of inflammation as measured by examining oedema levels and leucocyte activity (de Carvalho-Silva et al., 2012; Tavares et al., 2013). During digestion, α-lactalbumin and β-lactoglobulin can release the peptides α-lactorphin and β-lactorphin, respectively, and in addition to their antioxidant capacity, both have been shown to have anti-inflammatory properties, suppressing the production of the inflammatory cytokine interleukin-6 in the occluded blood vessels of male Sprague-Dawley rats (Yamaguchi & Uchida, 2007). Whilst interesting, it is important to note that far greater concentrations of peptides were used than would be obtainable through diet and that the peptides were introduced directly into the duodenum or by injection. As well as the anti-inflammatory effect, these two peptides have also been shown to have mild opioid capacity. They bind to opioid receptors, also leading to an antihypertensive effect, and this effect is naloxone sensitive. Naloxone is an opioid receptor agonist and its addition inhibits these peptide-induced antihypertensive effects, suggesting that the opioid receptor binding is responsible for some of the observed hypotensive effects (Ijäs et al., 2004; Sipola et al., 2002).

Amino acids from whey proteins

Dairy proteins are a relatively rich source of physiologically active amino acids including arginine, a precursor molecule for the synthesis of the potent vasodilator NO. The production of NO in the vascular endothelium is catalysed by endothelial NOS, using arginine and oxygen to produce NO and L-citrulline. The review of Khalaf et al. (2019) confirmed that few trials have been performed to fully assess the hypotensive effect of arginine, but they summarised the findings of an earlier meta-analysis (Dong et al., 2011). This included 11 randomised, double-blind, placebo-controlled trials involving 387 participants consuming a median dose of 9 g arginine/day for a median period of 4 weeks. The treatment reduced SBP by 5.39 mm Hg (95% CI: 2.25–8.54) and DBP by 2.66 mm Hg (95% CI: 1.54–3.77) relative to the placebo, but interestingly, no relationships between change in systolic or diastolic blood pressure and arginine dose, length of the intervention, or baseline BP were seen. The amount of arginine normally present in dairy foods, typically 188 mg in a 250 g portion of whole milk is far lower than the median of 9 g/day in the above meta-analysis.

The three BCAA leucine, isoleucine and valine, are essential amino acids found in both whey proteins and casein, with the richest source of the two being whey proteins at approximately 17 g/100 g of total protein. Administration of BCAA has been demonstrated to alter cell signalling, particularly the mechanistic target of rapamycin (mTOR) which plays a key role in both nutrient sensing and cell growth and proliferation (El Hiani et al., 2019). Circulating BCAA concentrations have been positively correlated with CVD risk including hypertension in a number of cohorts (Tobias et al., 2018; Yamaguchi et al., 2017; Yang et al., 2014). In addition, whilst BCAA intake was positively associated with hypertension in an Iranian cohort (Teymoori et al., 2017), twin studies in a UK cohort demonstrated an association between BCAA dietary intake and reduced in-twin risk of hypertension, reduced risk of insulin resistance and metabolites, such as alpha-hydroxyisovalerate, which are associated with adiposity (Jennings et al., 2016). Of the three BCAA, leucine appears to be responsible for most of the signalling properties, as administration with valine or isoleucine does not induce the same level of mTOR activity (Moberg et al., 2016; Tobias et al., 2020). The

signalling characteristics of leucine have made it of interest to those studying insulin metabolism since mTOR signalling can lead to pancreas β-cell proliferation, with the protein complex mTORC1 acting in the postprandial phase via a negative feedback loop, with increased activation of mTORC1 inhibiting insulin signalling (Yoon, 2017). However, sustained mTOR activity also leads to increased S6 kinase activity, serine phosphorylation and a decreased availability of insulin receptor substrate, leading to insulin resistance. Additionally, mTOR over-activity can eventually lead to a decreased ability of the β cells of the pancreas to release insulin (Ong et al., 2016). Another proposed mechanism may be the inhibition of endothelium-based NO synthesis by leucine stimulating mTOR, which in turn upregulates the production of glutamine, an inhibitor of NO production (Yang et al., 2015).

Leucine has been extensively studied in terms of its ability to stimulate anabolic processes such as muscle protein synthesis, but data on the effect of leucine on vascular function are very limited, with most of the available literature being either from in vitro studies or animal models, although some human observational data are available. The *PREDIMED* intervention trial cohort was set up to examine the effects of two Mediterranean diets on endpoints of cardiovascular health (Estruch et al., 2013) and a random subset of 970 individuals made up a case-cohort study (Ruiz-Canela et al., 2016). With a median on-trial duration of 4.6 years, the data highlighted a positive association between plasma leucine and isoleucine concentrations and CVD events with a significant increase in CVD risk (as defined by hazard ratio, HR) for the highest versus lowest quartile (leucine: HR 1.71, 95% CI, 1.06–2.75; isoleucine: HR 2.09, 95%CI, 1.27–3.44). It is notable that consumption of whey proteins has been shown to increase the plasma concentration of circulating BCAA from 411 nmol/ml (±26 nmol/ml SEM) to 724 nmol/ml (±58 nmol/ml SEM) in the 30 min following ingestion of 29.6 g of WPI by fasted participants (Sharp et al., 2019). However, the causal connection with CVD is unclear. In addition, numerous studies have shown that insulin resistance can lead to the accumulation of BCAA in the blood (Soleimani, 2015), with recent longitudinal data suggesting that, in some cohorts at least, the relationship may be reversed with baseline BCAA concentrations being a predictor of the incidence of type 2 diabetes (de Almeida-Pititto et al., 2021).

Given that dairy foods are not associated with increased risk of CVD or type 2 diabetes and indeed some are associated with risk reductions of both, the relevance of whey proteins as a key source of BCAA needs urgent clarification in relation to the risk of type 2 diabetes. Also as whey proteins are consumed in relatively large amounts by some sections of the population, the effect of dose needs particular examination.

CONCLUSIONS AND FUTURE RESEARCH NEEDS

It is now known that impairment of vascular function is a major contributor to CVD risk and an understanding of the impact of dietary components on vascular function, such as on NO availability, is increasingly important. Whilst the reduction of saturated fatty acid intake is a core component of public health dietary recommendations, and dairy foods are a significant source of saturated fatty acids, there is good evidence that high dairy consumption is associated with either a neutral or reduced risk of CVD and type 2 diabetes (Guo et al., 2017; Soedamah-Muthu & De Goede, 2018). Moreover, there is now increasing evidence that whey proteins, and notably the bioactive peptides and amino acids released during digestion, can have beneficial effects on aspects of vascular function and thus contribute to CVD risk reduction. This review has highlighted a number of beneficial effects of whey proteins including those on BP, vascular stiffness, NO production and inflammation.

Whilst there is an increasing number of human intervention studies examining the effects of whey proteins on aspects of vascular function, these have a high degree of heterogeneity related to treatments, exposure times and very variable measures of vascular function. Also, due to the possible changes in vascular function caused by, for example, metabolic dysregulation and age, studies in individuals from one population may have limited relevance to another. Some studies have also used diet supplements or high doses of whey proteins, which may not reflect typical dietary patterns. There are also a very limited number of studies that address the mechanistic factors involved and there is clearly a need for robust randomised, controlled human dietary intervention studies to determine in detail the impact of whey proteins, its peptides and amino acids on the multifaceted aspects of vascular function, including the emphasis on the form and dose of protein given. Dairy is a commonly consumed food group and a significant source of high-quality protein, so trials comparing the effects of whey protein with other commonly consumed, but non-dairy sources of protein on vascular function in both the acute and chronic settings are needed. Moreover, as whey proteins are an especially rich source of BCAA including leucine, future areas of study should include the impact of these amino acids on in vivo vascular function and also clarify the effect of BCAA from whey proteins on the risk of type 2 diabetes.

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CONFLICTS OF INTEREST

DP is a consultant for Innermost, XXIV Ltd.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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