REVIEW

Role of dietary proteins and peptides in cardiovascular disease

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Cardiovascular disease (CVD) is the leading cause of death for both men and women in the United States and most other countries. Therefore, a disease of such wide-ranging impact calls for the development of multiple viable strategies for prevention. Diet plays an important role in the development of the major risk factors of CVD such as low-grade systemic inflammation, hypertension, obesity, diabetes and atherosclerosis, the most significant. Thus, diet-based methods of prevention would not only be more feasible, but ultimately more cost-effective than relying on drugs to combat this condition. In recent years, peptides derived from either animal or plant sources have been found to have various bioactive properties. Nevertheless, their potential impact on inflammation and prevention of atherosclerosis has not been fully explored, particularly at the molecular level. In this review, the most current scientific information from in vitro, in vivo and clinical studies on the role of dietary proteins and peptides on CVD has been summarized and discussed.

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1 Introduction

Cardiovascular disease (CVD) is the leading cause of death in the United States [1]. Furthermore, over 80% of deaths from CVD occur in low- to middle-income countries [2]. Once a disease limited to western countries, CVD has developed into a world-wide health epidemic that transcends socio-economic barriers and equally affects both men and women. In the U.S., more than one in three Americans have CVD and approximately 2300 adults die from complications associated with CVD each day [3]. Atherosclerosis, a chronic disease that involves accumulation of lipid deposits and scar tissue in the arterial wall, is primarily responsible for clinical cases of CVD [4]. Traditionally viewed as a simple lipid disorder in the past, it is now widely recognized that inflammation is directly involved in the initiation and progression of atherosclerotic lesions.

Although the development of CVD is associated with many aspects of life-style, besides heredity, the most significant risk factors are related to the diet. In recent years, pharmacological drug use and vascular procedures aimed at treating elevated levels of cholesterol, triglycerides and hypertension have soared in conjunction with rising rates of CVD [3]. However, these late-stage methods are largely directed at reducing probability or reoccurrence of major cardiovascular events. Therefore, more upstream preventative measures focused on how the diet can positively affect cardiovascular health must be explored in order to combat such a growing epidemic.

In recent years, proteins and peptides with unique bioactive and metabolic effects have garnered significant interest due to their ability to illicit potent anti-inflammatory cellular responses. However, intercellular and intracellular interactions involving inflammation and bioactive peptides

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Abbreviations: ACE, angiotensin I-converting enzyme; **CCH,** chicken collagen hydrolysate; **CRP,** C-reactive protein; **CVD,** cardiovascular disease; **IPP,** Ile-Pro-Pro; **LF,** lactoferrin; **MCP-1,** monocyte chemoattractant protein-1; **MMP,** matrix metalloproteinase; **NF-κB,** nuclear factor-κB; **RGD,** arginine-glycine-aspartic acid; **RPI,** rice protein isolate; **SP,** sardine peptide; **TNF,** tumor necrosis factor; **VPP,** Val-Pro-Pro

found naturally in the diet have largely remained overlooked in molecular research addressing atherosclerosis. The goal of this review is to examine and discuss how dietary proteins and their respective bioactive peptides may prevent and reduce the risk of developing CVD.

2 CVD and atherosclerosis

Atherosclerosis is a persistent inflammatory disease of the arterial intima that comprises an accumulation of oxidized lipids, cholesterol and necrotic cells. The inflammatory cascade and the corresponding development of cardiovascular co-morbidities evolve from aberrant, localized arterial immune reactivity into full-fledged atherosclerotic lesions over a period of years [5]. These conditions allow circulating leukocytes to adhere to the endothelial cells lining the arterial wall and eventually penetrate into the intima. The inflamed region of the arterial wall accumulates monocytes, matures into macrophages that engulf lipid particles and finally develop into atherosclerotic lesions comprising foam cells [6]. As inflammation within this area intensifies, thrombotic and ischemic cardiac events, such as heart attacks and strokes, arise from the rupture of these lesions.

2.1 Lesion initiation and progression

Induction of atherosclerosis is initiated by a myriad of risk factors (diabetes, hypertension, overweight and obesity) and is exacerbated by environmental agonists such as smoking and physical inactivity [7]. These physiologically aggravating conditions interfere with the homeostatic balance within the endothelial cells of the arterial wall and induce atherogenesis. Under normal circumstances, endothelial cells are resistant to cellular adhesion and do not intimately interact with leukocytes within the milieu. However, inflammatory conditions upregulate the production of vascular adhesion molecule-1 on the surface of endothelial cells, attracting and binding leukocytes [8]. Highly proliferative blood monocytes accumulate around the endothelial lining by adhering to inflammatory leukocytes and secrete proinflammatory cytokines that promote chemotaxis within the intima. Further, monocyte chemoattractant protein-1 (MCP-1) becomes overexpressed in human atherosclerotic lesions and induces monocytes to penetrate into the intima [9].

2.2 Differentiation of monocytes into macrophages

Once the monocytes fully infiltrate the intima, monocyte colony stimulating factor-1 (M-CSF-1) binds to MCP-1 and promotes the differentiation of monocytes to macrophages [10]. At this point the macrophages exhibit high expressions of scavenger receptors with affinities for oxidized lipoproteins and incorporate them into the cytoplasm. As the macrophages engulf lipoproteins, the accumulation of cholesterol esters stimulates their differentiation into foam cells, which are indicative of an early atherosclerotic lesion, in in vivo and in vitro models [11, 12].

The proliferation of macrophages within the arterial intima and its subsequent progression into lipid-filled foam cells induce the chronic release of multiple proinflammatory cytokines and growth factors such as MCP-1, interleukin-1 (IL-1), IL-3, IL-6, IL-8, IL-18 and tumor necrosis factor α (TNF-α) in patients with metabolic syndrome and coronary artery disease [13, 14].

2.3 Development of complex plaque and rupture

As the atherosclerotic lesion advances toward an end-stage morphology of a vulnerable plaque, it comprises a necrotic lipid core and a fibrous cap of interstitial collagen. The necrotic core forms as a result of the failure of apoptotic macrophages to properly clear out of the site of the lesion. As a result, this propagates the release of inflammatory cytokines and eventually stimulates the release of matrix metalloproteinases (MMP-1, MMP-2, MMP-8, MMP-9, MMP-13) that degrade the fibrous cap, exposing the plaque to a risk of rupture and fatal thrombotic cardiovascular events [15]. Figure 1 depicts the components involved in the development of an atherosclerotic lesion.

2.4 Risk factors involved in CVD

As a major chronic disease, CVD is associated with a multitude of risk factors, many of which are related to the diet. The conditions that predispose individuals to the development of atherosclerosis include, besides inflammation,

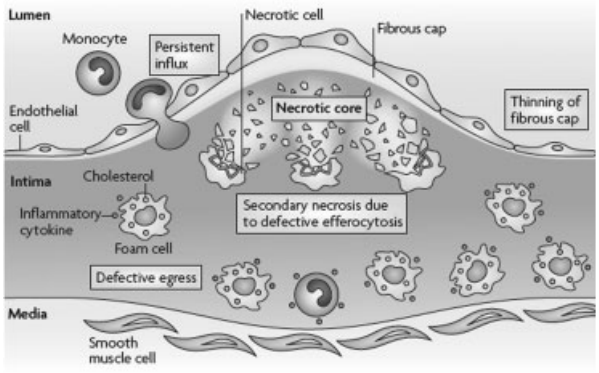

Figure 1. Schematic of an atherosclerotic lesion. Macrophage foam cells accumulate within the arterial intima and are unable to properly resolve, maintaining a persistent inflammatory state. Insufficient clearance of macrophages allows for the formation of the necrotic core, and if breached results in thrombosis, the end-point stage of an atherosclerotic lesion [8]. Reproduced with authorization from Nature Publishing Group.

hyperlipidemia, hypertension, hyperglycemia and obesity [16]. These conditions are highly affected by habitual dietary patterns, as they are influential in maintaining elevated levels of lipoproteins in the bloodstream and persistent low-grade inflammation, critical elements for the initiation of endothelial damage and atherosclerotic lesions [17]. Moreover, components of lifestyle such as inadequate physical activity and smoking are significant contributors in perpetuating the inflammatory cascade [18].

3 Addressing the problem with dietary preventative methods

In the United States, the estimated cost for treatment of CVD with drugs, surgery and revascularization procedures was over $500 billion in 2010 [3]. Therefore, CVD and its associated co-morbidities levy a significant burden not only on the medical care system but also on the long-term health of afflicted Americans. With the advent of statins and lipid-lowering drugs, major cardiovascular events such as myocardial infarctions and stroke have been reduced by over 25% [19]. Although the risk factors associated with CVD are highly correlated with the diet, there remains a heavy reliance on secondary preventative measures [20]. Therefore, this dependency on temporary pharmacological remedies and medical procedures underscores the need for alternative measures such as diet-based approaches, to enhance CVD prevention.

3.1 Physiological role of proteins and peptides

The consumption of dietary protein drives many fundamental metabolic processes and is particularly important in nutrient-based biological functions. Bioactive peptides are released from polypeptide chains through gastrointestinal and mechanical hydrolysis during food processing and fermentation. In addition to providing essential amino acids for a variety of systemic modulatory pathways, proteins and peptides may also illicit potent anti-cancer, anti-microbial, hypocholesterolemic, anti-hypertensive, anti-thrombotic and anti-inflammatory effects [21]. These biologically active peptides are derived from both plant and animal sources, with the most potential stemming from milk-based products, and legumes such as soybean and lupin. The relevant details of the studies discussed are summarized in Tables 1 and 2.

3.2 Milk proteins

Bovine milk largely comprises two major classes of protein, caseins (80%) and whey (20%). Caseins are colloidal aggregations of αs1-, αs2-, β- and κ-caseins. Whey comprises fractions of β-lactoglobulin, α-lactalbumin, lactoferrin (LF), immunoglobulins, proteose-peptone and serum albumin [22]. Milk proteins are found in dairy products and also commonly as food ingredients in concentrated, dried or liquid forms. After processing, often including fermentation, milk proteins are subjected to enzymatic hydrolysis in the gastrointestinal tract [23]. Therefore, due to exposure to these varying degrees of mechanical and biological processes, milk proteins are eventually disintegrated into smaller peptides, some of which possess bioactive properties.

Dairy products and their constituents have been extensively studied to determine their potential metabolic effects. In some studies, dairy consumption has been inversely correlated with the prevalence of overweight, hypertension, diabetes and the metabolic syndrome [24–26]. It is known that low-grade systemic inflammation is directly involved in the development and progression of CVD and comorbidities such as obesity, insulin resistance and the metabolic syndrome [27, 28]. Therefore, it is important to determine the impact of dairy on these risk factors and their relationship with markers of inflammation.

A recent large cross-sectional survey by Panagiotakos et al. [29] examined the fasting blood samples and dietary habits of 1514 men (18–87 years old) and 1528 women (18–89 years old) from the Attica region in Greece. The consumption of dairy products such as milk, cheese and yogurt were assessed and evaluated by dietitians using a validated food frequency questionnaire (EPIC-FFQ). The biochemical analyses of the blood samples found that subjects who consumed 11–14 servings of dairy products per week had 16, 5 and 12% lower levels of C-reactive protein (CRP), IL-6 and TNF-α levels, respectively, compared to those that only consumed less than eight servings ($p < 0.05$) per week. Moreover, subjects who consumed more than 14 servings per week had 29, 9 and 20% ($p < 0.01$) lower levels of CRP, IL-6 and TNF-α, respectively, after adjusting for confounders such as age, gender, smoking, physical activity, body mass and dietary habits. The inclusion criteria of this study required that participants exhibit no clinical evidence of cardiovascular or atherosclerotic disease. Therefore, no correlation between inflammatory markers and incidence of CVD were obtained. These results indicate that dairy food consumption may be associated with decreased levels of markers commonly linked with chronic low-grade inflammation and CVD risk. Whether this effect is due to milk proteins or other components within milk is unclear and has been the subject of investigation.

However, in recent years, epidemiological findings have stimulated research aimed to determine the specific constituents within milk that reduce and prevent CVD risk factors. Dairy contains calcium, folic acid, and vitamins B6 and B12, all of which may potentially confer various biological effects [30]. However, the focus has primarily been directed at the bioactive proteins and peptides found in whey and casein.

LF is a milk glycoprotein derived from whey and has been shown to have anti-inflammatory and anti-microbial

Table 1. Effect of animal-derived proteins and peptides on contributing factors of CVD

Test material	Protein/peptide	Dose	Duration	Subjects	*n*	Age (years)	Action	Reference
Dairy products (milk, cheese, yogurt)	Whole bovine milk protein	>14 servings of dairy/week	19 mo	Human, men and women	700	18–87	Immunomodulatory	[29]
Milk-ribonuclease-enriched lactoferrin tablets	Milk-ribonuclease-enriched lactoferrin	250 mg/d	6 mo	Post-menopausal women	20	45–60	Immunomodulatory	[41]
Enteric-coated lactoferrin	Lactoferrin	300 mg/d	8 wk	Human, viscerally obese men and women	13	>20	Anti-obesity effect	[42]
Dairy enriched with lactotripeptides	Ile-Pro-Pro; Val-Pro-Pro	14 mg/200 mL	8 wk	Human, hypertensive men and women	35	35–70	No effect	[46]
Casein hydrolysate tablets	Ile-Pro-Pro; Val-Pro-Pro	2.05; 1.13 mg	9 wk	Human, hypertensive men and women	12	42–53	Hypotensive	[47]
Milk protein hydrolysate capsules	Ile-Pro-Pro; Met-Ala-Pro; Leu-Pro-Pro	7.5; 6.6; 2.2 mg	4 wk	Human, hypertensive men and women	70	52–66	Hypotensive	[48]
Lactoferrin	Milk-purified lactoferrin	0.2–200 μg/mL	24 h	In vitro, endothelial cells	n/a	n/a	Immunomodulatory, anti-angiogenic	[34]
Hydrolyzed pork	Purified pork peptide	70, 210 mg/kg	120 min	In vivo, mice	120	10 wk	Antithrombotic	[56]
Chicken collagen	Chicken collagen hydrolysates	10% by feed weight	12 wk	In vivo, mice	9	5 wk	Hypocholesterolemic, hypotriglyceridemic, immunomodulatory	[57]
Sardine muscle hydrolysate	Sardine peptide	1 g/kg/d	4 wk	In vivo, rats	6	10 wk	Hypotensive	[62]

Table 2. Effect of plant-derived proteins and peptides on contributing factors of CVD

Test Material	Protein/Peptide	Dose	Duration	Subjects	*n*	Age	Action	Reference
Soy cookies	Soy protein isolate	30 g/day	2 mo	Human, hyperlipidemic men and women	25	32–64	Hypotriglyceridemic	[68]
Soy protein shakes	Soy protein concentrate	20 g/day	12 wk	Human, postmenopausal women	17	45–60	Decreased abdominal fat accumulation, immunomodulatory	[69]
Soy protein powder	Soy protein isolate	40 g/day	57 days	Human, type-2 diabetic men and women	29	>19	Hypocholesterolemic, hypotriglyceridemic	[70]
Soymilk	Whole soy protein	3 servings/day	4 wk	Human, postmenopausal women	16	40–60	No effect	[71]
Lupin-enriched protein bars	Lupin protein isolate	35 g/day	6 wk	Human, hypercholesterolemic men and women	22	21–70	Hypocholesterolemic	[90]
Lupin-enriched foods	Whole lupin protein	16–20 g/100 g food	12 mo	Human, overweight and obese men and women	46	20–71	Hypotensive, lower fasting glucose levels	[91]
Barley protein enriched bread	High-protein barley flour	30 g/day	4 wk	Human, hypercholesterolemic, men and women	23	41–69	No effect	[107]
β-conglycinin hydrolysates	KNOQKR; EITPEKNPQLR; RKQEEDEDEEQQRE	79, 27, 16 μm	48 h	In vitro, adipocytes	n/a	n/a	Hypotriglyceridemic	[84]
Lupin-enriched chow	Lupin protein isolate	200 g/kg	Until day 18 of lactation	In vivo, lactating rats	12	9 wk	Hypotriglyceridemic, hypocholesterolemic	[85]
Rice protein enriched chow	Rice protein isolate	18.4% by weight	16 wk	In vivo, mice	10	4 wk	Reduced atherosclerotic lesions, hypotriglyceridemic, antioxidant	[97]
Wheat germ	Wheat germ hydrolysate	5.86–115.20 μm	60 min	In vitro, assay	n/a	n/a	ACE inhibitory	[113]

effects in vitro [31, 32]. LF inhibits LPS-induced TNF-α, IL-1 β, IL-6 and IL-8 mRNA expression in THP-1 monocytic cells through nuclear factor-κB (NF-κB) pathways [33]. Moreover, LF inhibits the release of cytokines TNF-α, IL-1, IL-2 and cell proliferation in mixed lymphocytes. In a recent study by Yeom et al. [34], LF treatment inhibited LPS-induced expression of intercellular adhesion molecule 1, proliferation, migration and pro-inflammatory cytokines IL-1 β and IL-6 in bovine aortic endothelial cells. Puddu et al. [35] conducted an in vitro study to elucidate the immunomodulatory effect of bovine LF on human monocytes and dendritic cells. LF upregulated CD83 and cytokine/chemokine secretion in monocytes differentiated to monocyte-derived dendritic cells. Moreover, expressions of CD154, IFN-γ and IL-2 were downregulated; however, IL-6 expression was increased. On the contrary, already mature monocyte-derived dendritic cells treated with LF abolished production of IL-6 and partially inhibited toll-like receptor agonist-induced activation. The mechanism of action was suggested to be regulated through an LF receptor. Also, CD14 and its co-receptors TLR2 and TLR4 were involved in LF-induced signaling, leading to IL-6 expression. Given the immunomodulatory effects of LF in vitro, some in vivo studies have recently been conducted. A study in dogs [36] found that oral treatment with bovine LF increased neutrophil β2-integrin transcript levels, in turn leading to the upregulation of neutrophil functions. Moreover, a study performed in mice [37] found that LF supplementation reduced TNF-α and increased IL-10. LF inhibited adipocyte differentiation in MC3T3-G2/PA6 and 3T3-L1 cell lines, respectively [38, 39]. The LF receptor, LRP1, may be critically involved in its anti-adipogenic properties. Exogenously derived dietary lipids are shuttled by chylomicron remnants and absorbed into the liver primarily through LRP1. Hofmann et al. [40] found that LRP1 expression in visceral fat regulates postprandial lipid transport and glucose homeostasis in mice. Therefore, LF inhibition of LRP1 prevents lipids from being deposited in visceral fat.

A randomized controlled clinical study was conducted by Bharadwaj et al. [41] on 35 post-menopausal women (45–60 years old), in which subjects consumed 250 mg milk-ribonuclease enriched LF capsule supplements per day or a calcium placebo for 6 months. Ribonuclease, also known as angiogenin, is a protein responsible for vascularization and has been found to have reduced bone resorption and increase bone formation in post-menopausal women. The results found that milk-ribonuclease enriched LF supplementation significantly reduced pro-inflammatory cytokines IL-6 and TNF-α by 44 and 10%, respectively. In addition, levels of CRP, a marker upregulated during inflammation and atherosclerosis, were reduced by 50% compared to the placebo. Further, the important anti-inflammatory regulatory cytokine IL-10 was increased by 140%. Another randomized double-blind, placebo-controlled trial was conducted by Ono et al. [42] in men and women (ages 22–60 years old) with abdominal obesity (BMI>25 kg/m^2). In order to improve bioavailability, the subjects consumed 300 mg/day enteric-coated LF or placebo tablets for 8 wk. The enteric coating of LF prevented its degradation in the stomach by pepsin. The results indicated a significant reduction in visceral fat area ($-14.6\,cm^2$, $p = 0.009$) in the LF group compared to the placebo control. Moreover, other anthropometric measurements such as body weight, BMI and hip circumferences had statistically significant decreases compared to the placebo. Central adiposity interferes with adipokine regulation and has been associated with hyperglycemia, insulin resistance, hypertension and dyslipidemia. Therefore, these findings suggest that enteric-coated LF supplementation may help to maintain the bioavailability of LF during gastrointestinal digestion, decrease visceral fat accumulation and prevent common risk factors of CVD.

As knowledge on the bioactive components within dairy products emerged, hydrolysates and peptides from milk have garnered significant attention due to their potential antihypertensive properties. The lactotripeptides derived from milk casein, Val-Pro-Pro (VPP) and Ile-Pro-Pro (IPP), have been shown to potently reduce blood pressure by inhibiting the angiotensin I-converting enzyme (ACE) in clinical trials [43–45]. However, a randomized double-blind placebo-controlled trial by Engberink et al. [46] on 135 hypertensive Dutch men and women (ages 35–70 years old) given a daily dose of 200 mL dairy drink with 14 mg lactotripeptides for 8 wk did not significantly change systolic blood pressure ($p = 0.46$) or diastolic blood pressure ($p = 0.31$), compared with the placebo group. Conversely, a small study conducted by Nakamura et al. [47] on 12 Japanese hypertensive men and women given tablets containing a total of 1.13 mg VPP and 2.05 mg IPP for 9 wk found contradictory results. The group supplemented with VPP and IPP had a significant reduction in peripheral systolic (−21.8 mm Hg) and diastolic blood pressure (−13.6 mm Hg). In addition, a recent randomized, placebo-controlled double blind crossover study conducted by Boelsma and Kloek [48] on 70 men and women with prehypertension of stage 1 hypertension found evidence to support the antihypertensive role of lactotripeptides. The treatment groups received two daily capsules of MPH1 (7.5 mg IPP), MPH2 (6.6 mg Met-Ala-Pro, 2.3 mg Leu-Pro-Pro, 1.8 mg IPP) or cellulose placebo. The results found that MPH1 reduced systolic blood pressure by 3.8 mmHg ($p = 0.008$) and diastolic blood pressure by 2.3 mm Hg ($p = 0.0065$) compared with placebo in stage 1 hypertensive patients. Yamaguchi et al. [49], after oral administration of VPP and IPP, found ACE-inhibitory effects within the aorta and improved endothelial function by upregulating endothelial nitric oxide synthase while inhibiting activation of NF-κB. Secretion of NO by endothelial nitric oxide synthase strengthens the integrity of the vessel wall. Moreover, it was suggested that lactotripeptides inhibited degradation of bradykinin, thereby lowering blood

pressure. However, MPH2 did not have a significant effect on blood pressure.

Although several studies on milk and milk peptides have reported beneficial outcomes, some studies have found no impact on markers of inflammation related to CVD. Wennersberg et al. [50] did not observe significant differences in body weight, body composition, blood pressure, markers of inflammation (IL-6, CRP, TNF-α), endothelial function or oxidative stress in the milk versus the control group on middle-aged overweight subjects with metabolic syndrome who consumed three to five servings of dairy products daily. Moreover, van Meijl et al. [51] reported no significant differences in inflammatory markers in the milk supplemented group compared to the control. The study involved 35 (18–70 years old) overweight and obese men and women (BMI>27 kg/m^2) who consumed 500 mL of 1.5% low-fat milk and 150 g of 1.5% low-fat yogurt or a control of carbohydrate-rich products per day for 8 wk. Plasma concentrations of soluble TNF-α receptor 2 (TNFR2) increased by 227 pg/mL ($p = 0.02$) in the dairy intervention group, while there was no significant effect on IL-6, MCP-1, intracellular adhesion molecule-1 and vascular adhesion molecule-1. The results showed that the dairy intervention group did not have a significant impact on general markers of low-grade systemic inflammation indicative of CVD risk. More recently, Ivey et al. [52] reported that increased consumption of yogurt, but not of other dairy products such as milk and cheese, is associated with a lower common carotid artery intima-media thickness in a cohort of elderly women.

Although significant findings suggest the efficacy of milk protein and peptide consumption on the improvement of inflammation and CVD risk factors, the differences in results indicate the need to standardize investigations, as outcomes are highly dependent on dosage, duration, subjects' compliance and sample group.

3.3 Meat proteins

Meat proteins are derived from the skeletal muscle tissue of red meat, fish and poultry [22] and contain essential vitamins and minerals as well as significant quantities of biologically active peptides with antioxidant and antihypertensive effects [53–55]. Biologically active peptide fragments have been isolated from hydrolysates of common meats such as beef, chicken, fish and pork by enzymes derived endogenously or isolated from plants or microbes. Although the literature from the last four decades on meat-derived peptides have largely found inhibitory effects on the ACE, recent investigations have unraveled other potential benefits to cardiovascular health.

In a recent in vivo study, Shimizu et al. [56] isolated a peptide fraction from defatted pork meat by papain hydrolysis and administered it orally to mice at 70 and 210 mg/kg body weight. The antithrombotic activity of the purified papain-hydrolyzed pork peptide was assessed by calculating the total thrombosis size after laser irradiation of the carotid artery. The results indicated that the purified pork peptide significantly inhibited thrombus formation and was as effective as aspirin at 50 mg/kg body weight. Pork meat as a whole did not have anti-thrombotic effects; however, papain-hydrolyzed pork produced peptides may potentially have anti-thrombotic properties. In another study, the anti-inflammatory effect and potential preventative effect on atherosclerosis of chicken collagen hydrolysate (CCH) was recently investigated by Zhang et al. [57]. In this study, apolipoprotein E-deficient mice were fed diet supplemented with 10% CCH or a normal control diet for 12 wk. CCH comprises numerous peptides with MWs < 6,000 Da. The results indicated significant reductions in total plasma cholesterol (14.4%), total hepatic cholesterol (24.7%) and hepatic triglycerides (42.8%) in the CCH group compared with the control. Further, the concentrations of plasma proinflammatory cytokines IL-6, ICAM-1 and TNF-α were reduced by 43.4, 17.9 and 24.1%, respectively. These findings suggest that CCH supplementation may contribute in the prevention of atherosclerosis due to its anti-inflammatory effects. CCH has been found to have significant ACE-inhibitory and antihypertensive effects in spontaneously hypertensive rats [58]; also an antihypertensive effect in mildly hypertensive humans [59]. More in vivo studies are needed to determine the potential of CCH in prevention of atherosclerosis; however Zhang et al. [60] demonstrated that CCH treatment in a cardiovascular damage rat model significantly reduced BP and concentrations of ICAM-1, while increasing NO concentration. The observed effects are due to modulation of various pathways. CCH was absorbed along with other peptides through enzymatic digestion [61]. The results of this study suggest that CCH decreased lipids by regulating hepatic lipid biosynthesis, suppressing triglyceride and lowering plasma total cholesterol. CCH treatment may affect lipid metabolism by downregulating pro-inflammatory cytokines IL-6, SICAM-1 and TNF-α, important markers secreted by adipocytes.

In a study by Otani et al. [62] a sardine peptide (SP) was isolated from skeletal muscle tissue by enzymatic hydrolysis and exhibited ACE inhibitory activity. Spontaneously hypertensive rats were supplemented with 1 g/kg/day SP or 8 mg/kg/day ACE inhibitor captopril or tap water control via the drinking water for 4 wk. SP significantly reduced ACE activity in the kidneys, aorta and mesentery compared to the control. Further, SP and captopril treatment significantly inhibited blood glucose levels after glucose loading compared to the control. The results suggest that SP may have the potential in not only reducing hypertension but improving insulin resistance and preventing the development of type 2 diabetes, an important CVD risk factor. In summary, these findings illustrate the wide-ranging metabolic effects of peptides derived from animal sources. In addition to the past evidence substantiating meat peptides as effective antihypertensive compounds, they also

demonstrated potential as antithrombotic, hypoglycemic and hypolipidemic dietary therapeutics for the prevention of cardiovascular damage [60] and CVD.

3.4 Soy proteins

Soybean (*Glycine max*) comprises 35–40% protein by dry weight and the storage proteins glycinin and β-conglycinin account for 90% of the total protein content [63]. Soy foods have been consumed in Asian countries for centuries but have only recently gained substantial popularity in the U.S. and western countries due to the high publicity of its potential bioactive properties and health benefits [64]. Soy proteins are consumed in numerous products such as edamame, miso, soymilk, tempeh and tofu. Moreover, soy protein is processed as isolate and concentrates forms for the production of supplements and incorporated into a variety of processed foods and snacks in order to increase the protein content. Therefore, the food processing and fermentation techniques utilized allow for the high protein content of soybeans to be hydrolyzed into smaller bioactive peptide fragments.

In the U.S., production and consumer demand for soy products increased significantly after the Food and Drug Administration approved in 1999 the claim that 25 g of soy protein a day, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease [65]. This subsequently led to a flurry of research focusing on the impact of soy foods on animal and human cardiovascular health. The results of these studies have indicated that the consumption of soy may reduce cardiovascular risk factors such as low-density lipoprotein (LDL), cholesterol and blood triglycerides and increase high-density lipoprotein (HDL) cholesterol [66]. These studies have largely been conducted on food products containing whole soy, which also contain vitamins, minerals, isoflavones and other bioactives aside from protein. A meta-analysis conducted on the effect of soy protein supplementation found statistically significant reductions on serum lipids in studies in which high amounts of soy protein were consumed (20 to >61 g/day) [67]. In this meta-analysis, phytochemicals associated with soy protein primarily comprised phytoestrogens. Effective doses were reported from 37.5 mg aglycone units/day to 62 mg isoflavones. Therefore, more recent investigations on soy protein supplementation would shed greater light in determining the direct impact of soy protein on inflammation and CVD risk factors.

A recent study by Borodin et al. [68] assessing the impact of soy protein consumption on 30 hyperlipidemic Russian men and women who consumed 30 g protein from soy protein isolate per day or a control of skimmed milk curd protein found statistically significant decreases in total cholesterol (−6.5%, $p = 0.0099$), triglycerides (−18%, $p = 0.022$) and an increase in HDL cholesterol (+9%, $p = 0.047$). In order to determine the impact on fat metabolism and inflammation, Christie et al. [69] performed a double-blinded controlled trial on 33 post-menopausal women randomized to 20 g per day soy protein supplementation or a casein placebo for 3 months. The results showed a statistically significant reduction in total (7.5%) and subcutaneous abdominal fat (9.1%) and IL-6 (2.5%) when compared to the placebo. Due to the high prevalence of type 2 diabetes and its status as a significant risk factor in the development of CVD, Pipe et al. [70] conducted a double-blind, randomized placebo-controlled intervention with soy protein isolate to assess its impact on adults. In this study, 29 adults with type 2 diabetes consumed 40 g soy protein isolate or a placebo of milk protein isolate shakes per day for 57 days. The soy protein isolate consumption resulted in a statistically significant reduction in serum LDL cholesterol ($p = 0.04$), LDL cholesterol:HDL cholesterol ratio ($p = 0.02$) and apolipoprotein B:apolipoprotein A-I ($p = 0.05$) compared to the placebo control.

In contrast to these findings, Beavers et al. [71] did not find any statistically significant differences in plasma markers of inflammation (TNF-α, IL-1 β, IL-6, superoxide dismutatse, glutathione peroxidase and cyclooxygenase-2 in 31 post-menopausal women who consumed three servings of soymilk (18 g protein/day) for 4 wk compared to the dairy milk placebo. Although the investigators reported 80% compliance with the dietary protocol, other factors in the study design may have significantly affected the outcomes. For instance, the duration of the experiment lasted only 4 wk, which is markedly shorter than the majority of the clinical studies conducted on soy protein consumption. Furthermore, the American Heart Association reported in 2006 that based on 22 randomized trials, at least 50 g of soy protein per day was needed in order to induce benefits to cardiovascular health such as reductions in serum lipids, compared with milk and other proteins [72]. Therefore, studies focused on examining the effect of soy protein supplementation must consider adequate dosages per day to observe statistically significant changes.

In the last two decades, the strong interest in soy protein and its potential to mitigate risk factors associated with CVD led to numerous epidemiological and clinical studies. During that time, research on specific bioactive peptides within the soy protein complex also peaked and subsequently initiated efforts to elucidate the biochemical and molecular mechanisms of action. One of such peptides is lunasin, which is a 43-amino acid bioactive protein component derived from soybean that contains a unique arginine-glycine-aspartic acid (RGD) cell adhesion motif and polyaspartic acid tail responsible for its bioactive properties [73]. Among the classes of cell penetrating peptides, naturally occurring peptides derived from foods containing the unique RGD cell adhesion sequence have significant potential in the prevention of atherosclerosis and CVD. As an important mediator of cell adhesion and outside-in cellular signaling, RGD peptides also play a significant role in integrin-mediated responses such as cell proliferation,

migration and cell survival [74]. Moreover, peptides containing RGD-motifs bind to integrins with high specificity and serve as potent antagonists, leading to anti-angiogenic and anti-inflammatory effects [75–77]. As a result, RGD peptides have been synthesized as potential drug targets of integrin receptors or utilized for molecular imaging of inflammation and atherosclerotic plaques [78, 79]. However, major costs and the formidable obstacles of the drug development process make this strategy highly unfeasible. Therefore, the consumption of naturally occurring RGD peptides may potentially be instrumental in the reduction of inflammation and prevention of atherosclerosis.

In a study by de Mejia and Dia [80], lunasin inhibited pro-inflammatory cytokines IL-1 β, IL-6, cyclooxygenase-2, nitric oxidation production, inducible nitric oxide synthase expression and prostaglandin E(2) levels through suppression of NF-κB in RAW 264.7 murine macrophages. At least 10 μM of lunasin was required in order to inhibit LPS-induced inflammation in macrophages in vitro. As a result, lunasin may have a therapeutic effect on CVD risk by potentially preventing the activation of the inflammatory cascade and resolve uncontrolled inflammation in macrophages within atherosclerotic lesions. Dia et al. [81] demonstrated the bioavailability of lunasin in humans after healthy men consumed 50 g of soy protein for 5 days. At the end of day 5, the concentration of lunasin in plasma ranged from 50.2–110.6 ng/mL to 33.5–122.7 ng/mL at 30 min and 1 h, respectively. Thus, lunasin possesses promising potential in the prevention of CVD due to its anti-inflammatory effects and post-prandial biological stability. In addition to lunasin, other bioactive peptides derived from soybean have been shown to upregulate LDL receptor levels [82] and influence LDL receptor transcription [83], which may potentially contribute in the prevention of obesity and its associated co-morbidities. In a recent study by Martinez-Villaluenga et al. [84], peptides KNPQLR, EITPEKNPQLR and RKQEEDEDEEQQRE derived from soybean β-conglycinin inhibited fatty acid synthase and lipid accumulation in adipocytes, suggesting that soy protein may play a role in fatty acid metabolism and obesity. The results of these studies demonstrate the potential of soy protein and peptides to reduce fat accumulation, serum cholesterol, triglycerides and markers of inflammation. However, in light of the updated recommendation from the American Heart Association and the conclusions of these studies, higher amounts of soy protein (>50 g/day) must be consumed to illicit improvements in cardiovascular health.

3.5 Lupin protein

Lupin (*Lupinus* L.) is a legume in the same family as soybean and contains approximately 17–30% protein by weight [22]. Primarily cultivated as animal feed in the past for hundreds of years in Australia, Europe and South America, lupin has recently garnered interest in the scientific community and food industry due to its high protein content and potential bioactive components [85]. Prior studies on lupin have demonstrated hypocholesterolemic, hypotensive and hypotriglyceridemia effects in various animal models [86–89]. More recently, Bettzieche et al. [85] conducted an in vivo study on hypercholesterolemic rats fed 200 g/kg lupin protein or casein during pregnancy until day 18 of lactation. Enrichment of the diet with lupin protein reduced plasma triglyceride levels by 55% ($p < 0.05$) and significantly decreased plasma concentrations of VLDL and LDL. Moreover, HDL concentrations and hepatic LDL receptor concentrations (2.6-fold increase) were significantly higher in the lupin protein supplemented group compared to the control. Therefore, the results indicate the potential of lupin protein to modulate the regulatory pathways involved in triglyceride and cholesterol metabolism.

Due to the fact that research on lupin has only begun within the last decade, studies have primarily remained limited to animal models. However, clinical studies analyzing the effect of lupin protein consumption and its impact on CVD risk factors have recently been initiated. In a randomized, double-blind, placebo-controlled study conducted by Weisse et al. [90], 53 hypercholesterolemic men and women (ages 21–70 years old) consumed 35 g lupin protein per day or casein control for 6 wk. The results did not find significant differences in plasma concentrations of triglycerides, glucose or homocysteine between the treatment and control groups. However, lupin protein supplementation modestly reduced LDL cholesterol ($p < 0.05$) but did not affect HDL cholesterol. More recently, a randomized, controlled double-blind parallel-design trial was conducted by Belski et al. [91] to examine the role of lupin consumption on body weight and CVD risk factors. In the study, 131 overweight and obese subjects (ages 20–71 years old) were assigned to consume lupin-enriched foods or high carbohydrate control foods for 4 and 12 months. The results did not have a significant effect on total cholesterol, LDL cholesterol, triglycerides, weight loss or measures of body fat and fat-free mass when compared to the control. The lupin-enriched food group significantly lowered blood pressure at 12 months and decreased fasting insulin concentrations by 16 and 21% at 4 and 12 months, respectively. The observed marginal effect of lupin-enriched foods was not expected by the investigators and a number of factors could have contributed to the end result. During the study period, the subjects followed an ad libitum diet that incorporated assigned lupin or control foods. The lupin-enriched products of bread, biscuits and pasta were provided to the subjects on a monthly basis and the protein content ranged from 16 to 20 mg lupin protein per 100 g food. Moreover, the protein was incorporated into high-carbohydrate foods by substituting wheat flour with lupin flour, providing modest amounts of the intake per day. An additional factor that may have negatively impacted the results of this study may stem from the 29% dropout rate of the

participants and no indication of subject compliance throughout the duration of the 8-month long study. Although improvements to levels of blood pressure, cholesterol and hypertension have been reported in animal models, the potential of lupin proteins for prevention of CVD risk factors remain to be seen. In conclusion, the results indicate the need for further study, as clinical studies on lupin protein have only recently been conducted and therefore the dosages used may not be sufficient to induce the expected results. The induction of mRNA and protein levels of LDL receptor and transcriptional upregulation of CYP7A1, a key enzyme involved in the synthesis of bile acids from cholesterol, were attributed to the hypocholesterolemic effect of lupin protein [92]. Increased levels of CYP7A1 were linked to lower LDL [93]. As a major component of HDL, upregulation of mRNA expression of ApoAI may potentially be responsible for the observed significant increase of HDL cholesterol in the lupin group. Since lupin protein is classified in the same legume family as soy, there are similarities in protein composition. Conglutin-γ is a peptide derived from lupin that is associated with cholesterol-lowering effects [87].

3.6 Other plant proteins

The initial interest on soybean proteins was ignited by epidemiological studies correlating high rates of soy food consumption in Asian countries with lower incidences of CVD when compared to western countries [94]. However, the potentially protective role of rice and its protein, a staple food in Asia, on cardiovascular health has not been sufficiently investigated. Previous findings on red and black rice consumption decreased atherosclerotic plaque formation in rabbits [95]. In order to determine the component within rice exerting these atheroprotective effects, apoE$^{-/-}$ mice were fed a diet supplemented with rice protein isolate (RPI). It was found that RPI reduced the size of atherosclerotic lesions compared with the casein control [96], but the mechanism of action was not determined. In a recent study by Burris et al. [97], apoE$^{-/-}$ mice were fed RPI or casein for 16 wk. The results showed a significant reduction in the formation of atherosclerotic lesions in the aortic sinuses, plasma oxidized LDL (oxLDL), anti-oxLDL IgG levels and increased expression of mRNA levels of antioxidant enzymes involved in oxidative stress such as superoxide dismutase in the RPI group compared with the control. Multiple mechanisms of action can be attributed to these reported effects. OxLDL is implicated in the progression of CVD [98]. RPI feeding resulted in inhibition of oxLDL, possibly due to an antioxidant effect. Subsequent antioxidant capacity assays showed a significant ORAC increase in RPI-fed mice compared to the casein group. Moreover, glutathione reductase, a gene involved in regulation of GSH biosynthesis, was upregulated in the arteries of RPI-fed mice compared to casein. This result correlated with a study in which increased macrophage specific expression of glutathione reductase was reported to inhibit progression of atherosclerotic lesions in LDLR$^{-/-}$ mice [99]. Based on the findings of this study, rice protein consumption may prevent atherosclerosis by reducing oxidative stress and oxLDL. However, determining the constituents within RPI responsible for the beneficial effects to systemic cardiovascular health such as bioactive peptides would be pertinent for future studies.

Epidemiological studies on whole-grain food consumption have been highly correlated with reduced risk of coronary artery disease [100, 101], hypertension [102] and type 2 diabetes [103]. The animal and clinical studies that followed found that dietary whole grains modulate serum triglycerides [104], improve antioxidant status [105] and ameliorated vascular reactivity [106]. However, investigations to identify and characterize the therapeutic effects of bioactive proteins and peptides within grains such as barley (*Hordeum vulgare*), oat (*Avena sativa*) and wheat (*Triticum aestivum*) on CVD have not been widely studied.

In a recent randomized study by Jenkins et al. [107], 23 hypercholesterolemic men and postmenopausal women consumed bread containing 30 g barley protein or casein for 4 wk. The results found no statistically significant differences on serum LDL, CRP, measures of oxidative stress or blood pressure. The biological effects of barley have not been thoroughly investigated clinically in the past and therefore more research must be conducted to substantiate these findings. However, factors such as the short study duration (4 wk) and dosage (30 g protein) may have been confounding factors.

Oat is rich in dietary fiber and recent clinical studies have shown that the consumption of oats lowers blood pressure [108] and plasma total and LDL cholesterol [109]. The atheroprotective effects of oats have been attributed to the polysaccharide β-glucan. However, the potential of oat proteins and peptides on prevention CVD has received little attention. A recent in vitro study by Cheung et al. [110] found ACE inhibitory oat protein hydrolysates with IC_{50} values of 35–85 μg/mL after simulated gastrointestinal digestion. Wheat, another commonly consumed grain, can reduce blood pressure and glucose levels [111] but the bioactive potential of its protein has not been extensively explored. In a recent study by Yang et al. [112], five ACE inhibitory peptides with IC_{50} values as low as 5.86 μM were isolated from wheat germ with endogenous proteases. These studies identified previously unknown bioactive peptides with potential antihypertensive effects, highlighting the gap in knowledge regarding proteins and peptides in grains as common as oat and wheat.

For over two decades, the role of plant-derived proteins on CVD has been highly concentrated on soybean and therefore evidence on other naturally occurring bioactive proteins has not been extensively established. However, recent studies conducted on legumes show promising potential. In a recent meta-analysis, Bazzano et al. [113]

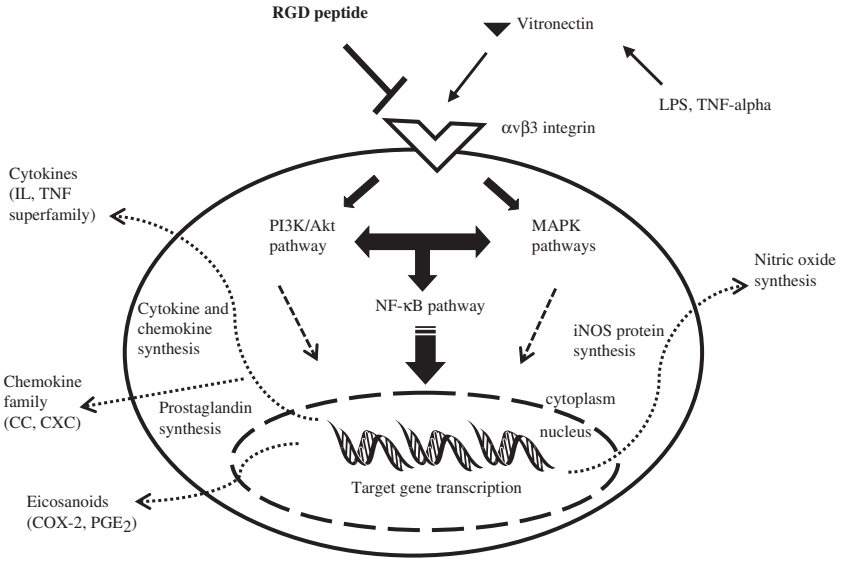

Figure 2. Modulation of multivariable pathways of inflammation within the macrophage by RGD peptides. CVD risk factors stimulate the release of integrin ligand activators, initiating the inflammatory cascade through MAPK/NF-κB dependent pathways. Adapted from [114].

examined the role of commonly consumed non-soy legumes such as beans, peas and seeds on CVD. The study found that in ten randomly controlled clinical trials, groups consuming a legume diet significantly decreased total cholesterol (−11.8% mg/dL) and LDL cholesterol (−8.0% mg/dL) compared with the control. In summary, the results underscore the wide-ranging potential of foods containing high quantities of protein on CVD prevention. Therefore, investigations must follow in order to determine the metabolic role of bioactive constituents and elucidate their respective impact physiologically.

4 Concluding remarks

The pathogenesis of CVD has now been established and important risk factors such as low-grade systemic inflammation, diabetes, hypertension and obesity are intimately responsible for its initiation, progression and deleterious endpoint. Although the advent of effective pharmacological agents has substantially reduced the rate of cardiac events, CVD remains the leading cause of death in the U.S. and most countries around the world. Since CVD is highly correlated with diet-dependent risk factors, primary preventative measures must be emphasized while the reliance on drugs and vascular surgical procedures to treat established existing diseases must be carefully controlled in order to maintain long-term health. Dietary proteins and peptides possess multiple bioactive properties and their consumption may play a significant role in promoting cardiovascular health. Figure 2 highlights the potential mechanism of action of RGD peptides, a unique class of plant-derived compounds that modulate inflammatory cascades involved in the development of atherosclerosis and CVD. Finally, Figure 3 depicts a comprehensive overview by which proteins and peptides

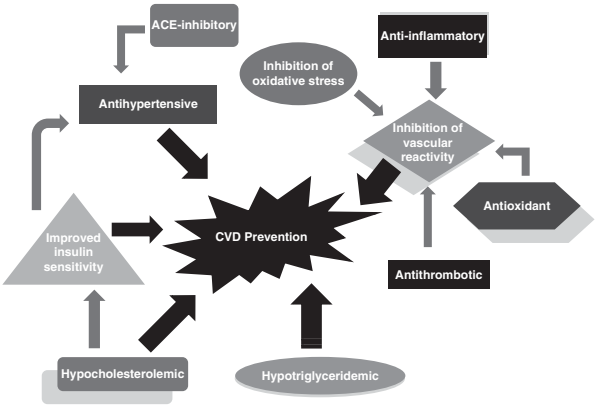

Figure 3. Regulatory pathways of CVD potentially modulated by dietary proteins and peptides.

derived from a variety of animal and plant sources may impact regulatory pathways associated with cardiovascular homeostasis. Although dietary proteins and peptides possess diverse biological characteristics and hold significant potential for the prevention of CVD, a void in the literature remains on fundamental basics such as their bioavailability after gastrointestinal digestion, their identity within protein complexes, clinical studies in which adequate doses are administered with appropriate durations and an increased focus to elucidate the molecular mechanisms of action.

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