

# The influence of the human microbiome and probiotics on cardiovascular health

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**Abbreviations:** ACE, Angiotensin converting enzyme; ASD, Autism Spectrum Disorder; BSH, Bile salt hydrolase; CLA, Conjugate linoleic acid; CVD, Cardiovascular disease; CRP, C-reactive protein; HSP, Heat shock protein; I/R, Ischemia/reperfusion; LDL-C Low density lipoprotein cholesterol; PD, Periodontal disease; TLR, Toll-like receptor; TMA, Trimethylamine; TMAO, Trimethylamine-*N*-oxide.

Cardiovascular disease (CVD) is a major cause of death worldwide. Of the many etiological factors, microorganisms constitute one. From the local impact of the gut microbiota on energy metabolism and obesity, to the distal association of periodontal disease with coronary heart disease, microbes have a significant impact on cardiovascular health. In terms of the ability to modulate or influence the microbes, probiotic applications have been considered. These are live microorganisms which when administered in adequate amounts confer a benefit on the host. While a number of reports have established the beneficial abilities of certain probiotic bacterial strains to reduce cholesterol and hypertension, recent research suggests that their use could be more widely applied. This review presents an up-to-date summary of the known associations of the microbiome with CVD, and potential applications of probiotic therapy.

## Introduction

Much has been written in recent years about the dynamic microbial communities that have an increasingly recognized impact on human health, including the cardiovascular system. The gut, skin, vagina, urinary tract and oral cavity are among several colonisation sites in which microbial communities exist in a specific equilibrium that is required for proper function and health.<sup>1</sup> As a whole, these communities in terms of their

genomes, activity, size and compositions, and surrounding ecosystems, represent the human microbiome.<sup>1–3</sup> Using high-throughput next-generation DNA sequencing of the 16 S rRNA genome, the analysis of over 4000 specimens collected from 242 adults revealed that each site has a characteristic microbiome with constituents adapted to specific niches.<sup>1,2,4</sup> The colon, for example, in North American adults appears to be dominated by 2 phyla: Firmicutes and Bacteroidetes, while other phyla, including Proteobacteria and Actinobacteria, exist in smaller proportions.<sup>5,6</sup> These bacteria perform a multitude of endogenous functions from the transformation of bile acids, breakdown of insoluble fibers, to the production of specific vitamins and cofactors,<sup>7</sup> (Figure 1, Table 1).

The effects of the microbiome on sites distant to the gut are the subject of intense investigations, particularly related to the brain, reproductive tract, mammary glands and vasculature. Only very recently has the concept of gut organisms affecting the heart been considered. The focus of the enclosed review is to examine the role of the microbiome and probiotic interventions in preventing and treating cardiovascular disease (CVD), a group of disorders of the heart and the blood vessels that supply the heart, brain, and extremities.<sup>8</sup>

## The role of the microbiome in cardiovascular health

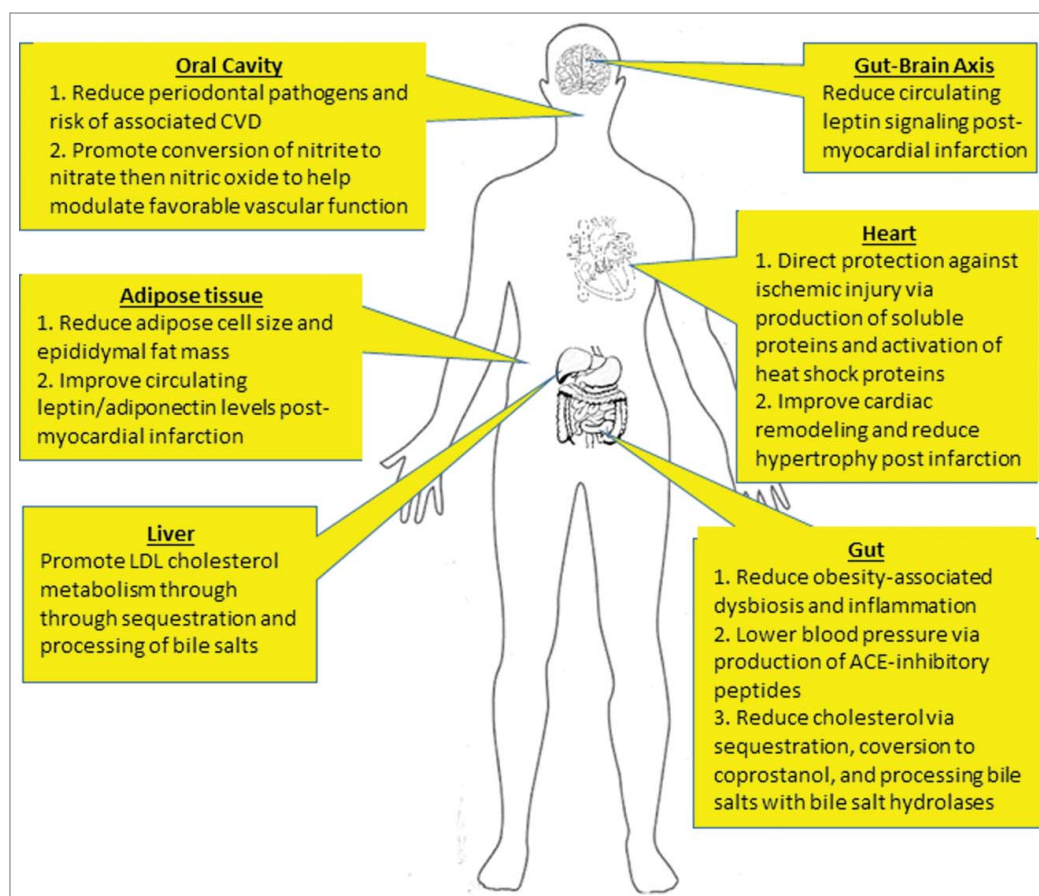
The prevalence of CVD is increasing in magnitude across the globe<sup>8</sup> and presents an immense burden to health care systems worldwide. Innovative approaches to effective prevention, intervention, and management for treating CVD are key to mitigating some of this burden. Although CVD represents a myriad of symptoms and dysfunctions, from hypertension to cardiomyopathy, there is a link between microbes and cardiovascular health.

### The oral microbiota

The teeth, tongue, cheek, attached gingiva are all surfaces among others within the oral cavity which are colonised by

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**Figure 1.** The potential sites of action of probiotics in preventing and treating CVD. Schematic diagram indicates sites throughout the body in which probiotic bacteria may confer a cardiovascular benefit on the host. Of note, many of these activities remain to be proven in humans.

and other parts of vasculature<sup>12</sup> and it is routinely carried in the oropharynx of up to 20% of children.<sup>13</sup> The ability of dental infections and other oral microbial disturbances to contribute to CVD has been closely examined over the years. In particular, periodontal disease (PD), driven by an overgrowth of anaerobic bacteria in subgingival plaque communities, has been associated with CVD through a variety of epidemiological studies and meta-analyses.<sup>14-16</sup> While these diseases share a number of risk factors (age, smoking status, oral hygiene practices) the finding of a definitive causative link between PD and specific CVD events has proven elusive.<sup>17</sup> Nevertheless consistent evidence that the presence of PD results in an increased risk of CVD.<sup>18</sup>

During PD, an overt, consistent host immune response directed against pathogenic bacteria results in chronic gingival inflammation and destruction of tissues and bones that surround and support the teeth.<sup>19</sup> Different mechanisms have been proposed to explain how PD may influence the development of CVD, with an emphasis placed on the infectious and inflammatory aspects of this oral disease. The increase in gingival bleeding during PD offers oral bacteria access to the bloodstream, where they can circulate and interact with atheromatous plaque deposits. Numerous studies have detected oral bacterial DNA in these atherosclerotic lesions.<sup>20-22</sup> These bacteria may be capable of invading and activating endothelial cells, increasing Toll-like receptor (TLR) interactions, or inducing the expression of metalloproteinases, all of which contribute to the development

distinct and complex microbial communities.<sup>9</sup> Over 500 oral bacterial species have been identified, with the healthy “core microbiome” consisting predominantly of taxa belonging to *Firmicutes*, *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, and *Fusobacteria*.<sup>10</sup> These commensals preserve homeostasis in the oral cavity by helping to produce nutrients, maintain pH, modulate saliva production, and generate inhibitory substances, all of which act to prevent colonisation and growth of exogenous or pathogenic species.<sup>11</sup>

Members of the oral microbiota have a bearing upon cardiovascular health- *Streptococcus pyogenes* being the classic example. Cross reactive antibodies for the organism affect the heart valves

in chronic gingival inflammation and destruction of tissues and bones that surround and support the teeth.<sup>19</sup> Different mechanisms have been proposed to explain how PD may influence the development of CVD, with an emphasis placed on the infectious and inflammatory aspects of this oral disease. The increase in gingival bleeding during PD offers oral bacteria access to the bloodstream, where they can circulate and interact with atheromatous plaque deposits. Numerous studies have detected oral bacterial DNA in these atherosclerotic lesions.<sup>20-22</sup> These bacteria may be capable of invading and activating endothelial cells, increasing Toll-like receptor (TLR) interactions, or inducing the expression of metalloproteinases, all of which contribute to the development

**Table 1.** Probiotic microorganisms shown to benefit cardiovascular health

| Species                                  | Strain       | Model                   | Benefit  |
|--|--------------|-------------------------|--|
| <i>Lactobacillus rhamnosus</i>           | GG           | Mouse                   | Weight reduction <sup>103</sup> , prevent ischemia in I/R injury <sup>1124</sup>                   |
|  | GR-1         | Rat                     | Attenuate heart failure, cardiac hypertrophy <sup>127</sup>  |
|  | PL60         | Mouse                   | Reduce adipose tissue mass <sup>107</sup>  |
| <i>Lactobacillus sakei</i>               | NR28         | Mouse                   | Weight reduction <sup>103</sup>  |
| <i>Lactobacillus/Bifidobacterium spp</i> | Multi-strain | Human                   | Reduce BMI in obese adults, reduce serum cholesterol <sup>105</sup>                                |
|  | Multi-strain | <i>In vitro</i> culture | Produce ACE-inhibitory peptides <sup>121,122</sup>   |
| <i>Lactobacillus plantarum</i>           | PL62 299 v   | Mouse Rat               | Reduce adipose tissue mass <sup>108</sup> Reduce severity of ischemia in I/R injury <sup>125</sup> |
| <i>Lactobacillus reuteri</i>             | NCIMB 30242  | Human                   | Lower serum cholesterol <sup>118</sup>   |

of CVD. In addition, host antibodies generated against *Porphyromonas gingivalis*, a periodontal pathogen, demonstrate cross-reactivity with human heat shock proteins (HSPs), including HSP60, commonly expressed by endothelial cells in atherosclerotic lesions.<sup>23</sup> As higher levels of anti-HSP60 antibodies correlate with morbidity and mortality from atherosclerosis,<sup>24</sup> it is possible that “auto-immunity” developed against *P. gingivalis* during PD may lead to detrimental cardiovascular outcomes.

The ability of PD to affect systemic inflammation has also garnered significant attention, given the role inflammation plays in the pathogenesis of CVD. Plasma levels of the inflammatory marker C-reactive protein (CRP) have been correlated with PD status,<sup>25,26</sup> as well as the pro-inflammatory cytokine IL-6.<sup>27</sup> These immune modulators may be either produced locally in the oral environment and subsequently dumped into systemic circulation, or arise as a result of low grade, short-lived bacteremia.<sup>28</sup> Given the high prevalence of PD in the adult population, management or prevention of this disease, in conjunction with other healthcare related interventions, may offer a way to reduce the incidence of CVD.

### The intestinal microbiota and diet

In the so-called ‘Western world’, the prevalence of heart disease coincides with other chronic diseases such as obesity, type II diabetes, and inflammation. Metabolic syndrome is largely diet-dependent and is a key preventable risk factor for CVD. It has been well documented that individuals with obesity have a gut microbiome profile distinct from those of lean individuals.<sup>5,29,30,31</sup> In diet-induced obesity, over-nutrition can alter composition of the gut microbiome, with dietary nutrients influencing the growth of certain species. Diets rich in cholesterol, saturated fats, and simple carbohydrates are associated with a gut microbiota rich in particular organisms belonging to the Firmicutes phylum.<sup>6,32</sup> In obese individuals, the decreased proportion of constituents from the Bacteroidetes phylum in comparison to Firmicutes can be normalized with a low-calorie diet-associated weight loss.<sup>6</sup> Conceivably, these obesity-associated microbiome profiles feature organisms in theory that are more adept at processing the energy-rich diets. This theory is supported by metagenomic and biochemical analyses showing that the core gut microbiome of obese individuals has an increased capacity for energy harvesting, compared to lean individuals.<sup>33</sup> Furthermore, when the gut microbes from normal mice are transplanted into germ-free recipients, there is an increase in weight and adiposity without any added food consumption.<sup>6</sup> This showed that the increased energy harvesting capacity is transmissible, although most of the microbes implicated in this outcome are based in the colon, which is distal to the small intestine where most absorption of lipids takes place. The modern Western diet, high in lipids and fructose and lacking of complex fermentable fibers, is seemingly mismatched to the capacity of our “ancestral” microbiota. This consequently results in less diversity and a shifted microbiome profile.<sup>32,34</sup> It has been suggested that a return to unprocessed food diets, consisting mainly of plant-based complex fibers, seasonally fresh raw food, fermented foods and low in red meat, can help promote the proliferation of the beneficial microbes that are considered indigenous to our gut.<sup>32</sup>

Epidemiological studies indicate that vegetarians and vegans have lower blood cholesterol and lower risk for CVD compared to omnivores.<sup>35,36</sup> The elimination of red meat from the diet is beneficial for cardiovascular health, as its consumption has long been associated with increased risk for CVD.<sup>37,38</sup> Dietary carnitine and phosphatidyl choline, predominantly from red meat, are converted to trimethylamine (TMA) by colonic microbes.<sup>39</sup> TMA is then converted to the proatherogenic metabolite trimethylamine-*N*-oxide (TMAO), which accelerates atherosclerosis in mice. The conversion of dietary carnitine to TMAO is microbe dependent, as indicated by individuals receiving oral antibiotics for a week prior to consuming red meat experienced a complete suppression of endogenous TMAO production. The same study also reports that vegetarians and vegans had significantly lower fasting baseline TMAO levels, compared to omnivores. Correspondingly, vegetarian and vegans had significantly higher abundance of *Bacteriodes* species and lower abundance of *Prevotella* species in the gut microbiome than omnivores, and a decreased risk for coronary heart disease and the traditional risk factors for CVD such as hypertension, atherosclerosis, peripheral artery disease, and stenosis. As vegetarian diet typically consists of very high portions of fermentable substrates low in carnitine, and all these components are metabolized by gut microbes, this provides strong evidence for a role of the microbiota in CVD.

In terms of prevention and treatment strategies, an understanding of the role of diet in cardiovascular health has proven extraordinarily valuable. The increased adiposity, angiogenesis, blood flow, and cardiac output associated with over-nutrition and obesity is a major risk factor for hypertension and hyperlipidemia, atherosclerosis, myocardial infarction and coronary heart disease, all of which predispose to congestive heart failure.<sup>40</sup> If modulation of the gut microbiome can interrupt this progression at any point, there is a potential to improve an individual’s cardiovascular health.

## Modulation of the human microbiome

As our understanding of the elaborate symbiotic relationship between the human microbiome and the host expands, strategies for modulating the latter have evolved. The primary modulator is antibiotics. Modern medicine has relied heavily on the prescription of antibiotics in efforts to eradicate infectious microbial pathogens. Gut infections such as *Clostridium difficile*, *Escherichia coli*, *Salmonella* spp., and *Helicobacter pylori* can be controlled with the use of antibiotics,<sup>41</sup> however not without significant detriment to the host. Antibiotics are relatively non-discriminating drugs, unable to distinguish between pathogenic and non-pathogenic bacteria at any site they encounter, thus leading to side effects such as diarrhea. The widespread use of broad spectrum antibiotics is something that should be discouraged, and alternative options are being explored.

### Application of probiotics

The consumption of fermented food products from various grains, vegetables, beans, fish, and dairy products dates back to

Neolithic times. The theorization by Metchnikoff in the early 1900s that the longevity of certain ethnic cultures is the result of consuming fermented foods is credited with reinvigorating the probiotic concept.<sup>42</sup> Today, probiotics are defined as live microorganisms which when administered in adequate amounts confer a health benefit on the host.<sup>43</sup> There is strong clinical evidence supporting probiotic treatment for relieving symptoms of bacterial vaginosis,<sup>44</sup> diarrhea,<sup>45,46</sup> and irritable bowel syndrome,<sup>47-50</sup> as well as preventing *Clostridium difficile*-associated diarrhea.<sup>51</sup> In most cases, the effects are strain specific and the precise mechanisms not well elucidated.

Probiotics can interact with the existing microbial community dynamic through competition with pathogens.<sup>52,53</sup> Various mechanisms have been studied, including production of bacteriocins, biosurfactants and simple competitive exclusion.<sup>54-56</sup> The net effect of suppressing pathogenic activity can be restoration of a 'normal' microbial community. Probiotic strains also participate in epithelial cross talk with the host immune system.<sup>57</sup> The gut epithelium is a major defense barrier against foreign pathogens and antigens. Probiotics not only improve the integrity of epithelial barriers and function of tight junctions,<sup>55,58</sup> but also can interact with toll-like receptors and transcription factors in the gut that regulate inflammatory responses.<sup>59,60</sup> Probiotic strains produce many metabolites, enzymes, co-factors, and vitamins that become active in modulating our health. For example, the fermentation of carbohydrates by the gut microbiota and probiotics results in the production of short chain fatty acids such as acetate or propionate which are used as energy in the host.<sup>7</sup> Studies also show that certain probiotic strains produce vitamin K, B2, B12, and folate,<sup>61-64</sup> all of which are utilized by the host.

There are numerous examples showing the potential for probiotics to have a positive impact on the oral cavity, such as preventing dental caries,<sup>65,66</sup> decreasing halitosis,<sup>67,68</sup> and reducing episodes of streptococcal pharyngeal infections.<sup>69</sup> Teughels et al.<sup>70</sup> recently showed that the daily usage of lozenges containing *Lactobacillus reuteri* by patients suffering from chronic periodontitis following standard dental scaling and root planing, resulted in significantly more pocket depth reduction and attachment gain in deep periodontal pockets, as well as a decrease in *P. gingivalis* levels. A similar study using *Lactobacillus salivarius* WB21-containing tablets demonstrated the ability of this probiotic to reduce the plaque index and periodontal pocket depth in subjects at high risk of PD.<sup>71</sup> It seems reasonable to now examine the potential of oral probiotics to reduce CVD risk via affecting PD.

Another area to explore is the ability of oral bacteria to convert dietary-derived inorganic nitrate (NO<sub>3</sub><sup>-</sup>) to nitrite (NO<sub>2</sub><sup>-</sup>) and other subsequent toxic compounds.<sup>72</sup> Importantly, the generation of nitric oxide (NO) through the "nitrate-nitrite-nitric oxide" pathway, of which oral bacteria play a crucial role, has been shown to increase vasodilatation, improve vascular endothelial function, and decrease blood pressure. In this process, ingested inorganic nitrate (found in high levels in leafy vegetables) is absorbed in the small intestine, where it enters the bloodstream and can be taken up and concentrated in the salivary glands. In the oral cavity, facultative anaerobic bacteria reduce some of this nitrate secreted in saliva to nitrite, which is

swallowed and can eventually be further processed to NO in the stomach, blood, or tissues.<sup>73</sup>

Human studies have shown that consumption of nitrate in the diet results in an increase in plasma nitrite levels, with corresponding decreases in systolic and diastolic blood pressures.<sup>74-76</sup> The importance of the oral microbiome in this process has been shown through the use of antibacterial mouthwashes, as the cardioprotective effects of dietary nitrate consumption are lost when oral bacterial populations are reduced.<sup>77,78</sup> Studies have shown that bacterial nitrate reduction mostly occurs at the dorsal surface of the tongue,<sup>79</sup> with several bacterial taxa identified as key players, including *Veillonella* and *Actinomyces*.<sup>80</sup> *In vitro* experiments have demonstrated the high capacity of *Actinomyces odontolyticus* to reduce nitrate without subsequent nitrite reduction.<sup>80</sup> This species could be explored as a novel probiotic designed to be taken immediately prior to, or in conjunction with a meal, in order to maximize nitrate utilization from the diet, increase NO generation, and help lower the risk of CVD.

### Probiotic influence on obesity and adiposity

Obesity is one of the primary risk factors for CVD, as the progression from overweight to obese introduces a slew of comorbidities that are detrimental to cardiovascular health.<sup>81</sup> The excessive energy intake and fat accumulation in obesity presents major risk for type II diabetes, chronic inflammation, and hyperlipidemia, all of which predispose to coronary heart disease, cardiac arrest, and heart failure. The use of probiotics to re-set the dysbiotic obese gut microbiome is one proposed approach to improving outcomes. A study performed on healthy weight mice found that there were significant changes in the gut microbiome as well as significant weight reduction in mice receiving either *Lactobacillus rhamnosus* GG or *Lactobacillus sakei* NR28 daily by oral gavage for 3 weeks.<sup>82</sup> There was no significant difference in food consumption between control and probiotic treatment groups, indicating that the significant reduction in epididymal fat mass was not due to a reduction of energy consumption. Results from this study indicate that the modulation of the gut microbiome with probiotic administration produces an anti-obesity effect that directly reduces epididymal fat mass. However, efforts to significantly alter the human gut microbiota using probiotics have shown more subtle effects<sup>83</sup> and it is expecting too much to think that a single probiotic will resolve the obesity epidemic. Nevertheless, understanding the mechanisms by which probiotics affect adipose tissue, cholesterol, satiety and other factors associated with cardiovascular health, will contribute to identifying new probiotic interventions.

The twice daily administration of a multi-strain probiotic capsule (*Streptococcus thermophilus*, *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Bifidobacterium lactis*, *Bifidobacterium longum*, and *Bifidobacterium breve*) to overweight individuals with a body mass index greater than 25 resulted in a significant reduction in weight, waist circumference, and serum cholesterol after 8 weeks.<sup>84</sup> These metabolic changes correlated to a significant increase in *Lactobacillus plantarum* populations and the overall ratio of gram negative bacteria, presumably representing the *Bacteroidetes* phylum. Conjugated linoleic acid (CLA)

produced by certain *Lactobacillus* species have shown to reduce obesity and arteriosclerosis in mice.<sup>30,85</sup> Studies investigating CLA-producing probiotic strains have demonstrated that *Lactobacillus rhamnosus* PL60 and *Lactobacillus plantarum* PL62 reduce body weight and adipose tissue mass in mice on a high fat diet in a CLA-dependent manner, without any changes in food intake.<sup>86,87</sup> Of note, while the trans-10,cis-12 isomer of CLA have been reported to reduce adiposity and increase lean mass in mice and other animals when included at <or = 1% of the diet, there remain concerns about possible deleterious effects of trans-10,cis-12 CLA on lipid profile, glucose metabolism and insulin sensitivity.<sup>88</sup> Although many studies show the potential benefits of CLS in a variety of conditions, as much dietary CLA in humans comes from dairy product consumption, debate continues over aspects of dairy foods on human health. Probiotics have also shown to directly reduce adipocyte cell size in high fat diet mice,<sup>89,90</sup> which can improve oxidative stress and the subsequent chronic inflammation that is associated with inadequate blood supply to enlarged adipocytes in obesity. Several of these results are strain-specific, highlighting the variability of mechanisms of action across the multitude of probiotic strains and species.

#### The lowering of cholesterol and hypertension by probiotics

One of the most popular and thoroughly investigated applications for probiotic therapy for CVD is the reduction of serum cholesterol. Elevated low-density lipoprotein cholesterol (LDL-C) is a major risk factor for CVD.<sup>91</sup> The accumulation of LDL-C in the blood is a precursor to hypertension, hyperlipidaemia, and causes the formation and build-up of atherosclerotic plaque in the arteries. Meta-analyses of randomized controlled clinical trials have been performed to evaluate the effect of probiotic consumption on serum LDL-C and total cholesterol levels. Pooled data from a total 485 total participants with 'high,' 'borderline high,' and 'normal' serum cholesterol levels found that probiotic consumption significantly lowered LDL-C and total cholesterol levels among all categories, compared to the control.<sup>92</sup> The cholesterol-lowering properties of probiotics are strain and species specific with several postulated mechanisms of action. Their ingestion can sequester cholesterol from the gut by incorporation into the cellular membrane.<sup>93,94</sup> They also convert cholesterol to coprostanol<sup>94</sup> which is subsequently excreted in feces, potentially reducing cholesterol absorption in the gut.

Free cholesterol, of course, is an important component for cardiomyocyte function. A study of membrane incorporation of arachidonic acid (C20:4  $\omega$ 6, AA) or docosahexaenoic acid (C22:6  $\omega$ 3, DHA) as  $\omega$ 6 or  $\omega$ 3 polyunsaturated fatty acids on cholesterol homeostasis, showed a 2.7-fold lower cholesterol biosynthesis in AA cells than the DHA cells.<sup>95</sup> The results demonstrated that AA incorporation into cardiomyocyte membranes decreased the free cholesterol turnover by markedly decreasing the endogenous cholesterol synthesis and by decreasing the ABCA1- and ABCG1-cholesterol efflux pathways, whereas DHA had the opposite effects. Yogurt lactic acid bacteria which produce high amounts of exopolysaccharide can remove free cholesterol from solution<sup>96</sup>. It is important to select the probiotic strain(s) that can lower LDL cholesterol and not adversely affect

cardiomyocyte function or increase fat deposition. This can be specific for probiotic strains, as shown in a study where administration of *B. breve* NCIMB 702258 to mice increased visceral fat mass and weight gain whereas administration of *B. breve* DPC 6330 did not.<sup>97</sup> On the other hand, *B. breve* DPC 6330 had a greater influence on the fatty acid composition of epididymal adipose tissue, with higher palmitic acid, palmitoleic acid, and DHA, while *B. breve* NCIMB 702258 had a greater effect on the fatty acid composition of the brain. These effects may be direct via the probiotic, or through modulating the gut *Clostridiaceae*, *Eubacteriaceae* or other constituents. Since AA and DHA play important roles in neurogenesis, neurotransmission, and protection against oxidative stress, and their concentrations in the brain influence cognitive processes, including learning and memory,<sup>97-99</sup> the selection and application of probiotics must be carefully conceived.

Perhaps most accepted mechanism is the processing of bile acids in the gut by Gram-positive organisms including *Lactobacillus* and *Bifidobacterium*.<sup>100,101</sup> Metabolism of cholesterol, a precursor of bile acids, is mediated through gut microbes expressing the enzyme bile salt hydrolase (BSH). Probiotics with high BSH activity promote the deconjugation of bile acids in the gut to secondary amino acid conjugates. When these secondary conjugates are excreted, cholesterol is broken down to replace the processed bile salts. Overall, this process promotes the catabolism of cholesterol, leading to reduced serum levels. There is variability in BSH phenotypes among probiotic species, indicating that the genes which encode it are likely to be horizontally acquired.<sup>102</sup> Many probiotic strains express more than one BSH homolog,<sup>103</sup> potentially helping them survive in the gut when exposed to different types of bile salts.

An important aspect of the bile acid effect is the pool size, metabolite composition and compartment concentrations which relate to the composition of the gut microbiota and how it metabolizes bile acids and impacts host metabolic processes and adiposity.<sup>104</sup> The organisms alter expression of genes controlled by the farnesoid X receptor (FXR) through bile acids, leading to differential activation by the acids and their metabolites [chenodeoxycholic acid (CDCA) > deoxycholic acid (DCA) > lithocholic acid (LCA) >> cholic acid (CA)].<sup>105</sup> Intake of 8 strain probiotic VSL#3 enhanced bile acid deconjugation and fecal excretion, albeit in mice. The mechanism was believed to be due to changes in ileal bile acid absorption, repression of the enterohepatic FXR-fibroblast growth factor 15, and increased hepatic bile acid neosynthesis.<sup>106,107</sup> Induction of such physiological effects are quite dramatic for a probiotic, and warrant further study to determine if it is due to modulation of the indigenous microbiota, or specific metabolic effects of the ingested probiotic strains.

To date, Health Canada has approved only one probiotic product with cardiovascular health claims. This product, Cardiova™, also available in the USA and Europe, contains 2 billion encapsulated *Lactobacillus reuteri* NCIMB 30242, clinically proven to lower LDL-cholesterol levels by 11.6% in hypercholesterolemic adults.<sup>108</sup>

Hypertension, closely tied to hypercholesterolemia, is a major risk factor for CVD.<sup>109</sup> About 30% of Americans are

hypertensive, doubling their risk for developing CVD. Reducing hypertension alone, using diuretics, angiotensin converting enzyme (ACE)-inhibitors and  $\beta$ -blockers is about 30% less effective than reducing hypertension by treating hypercholesterolemia in hypertensive patients. If probiotic therapy improves lipid blood chemistry, they could potentially improve hypertension and outcomes for CVD patients. A meta-analysis of 14 randomized placebo-controlled clinical trials with 702 participants show that probiotic fermented milk significantly reduced both systolic and diastolic blood pressure in pre-hypertensive and hypertensive patients.<sup>110</sup> Certain probiotic strains produce peptides with ACE-inhibitory activity through the proteolysis and fermentation of milk proteins.<sup>111,112</sup> When growth of *Lactobacillus* and *Bifidobacterium* strains was enhanced using fermentation substrates, or prebiotics (inulin, pectin, fructooligosaccharides, and mannitol), proteolytic activity and ACE inhibition was proportionally increased.<sup>112</sup>

By reducing cholesterol and hypertension, the risk for developing coronary heart disease, atherosclerosis, heart attack, and stroke is reduced by nearly half. The strong clinical evidence for the attenuation of hypercholesterolemia and hypertension with probiotic consumption provides support for use of these organisms in the treatment of CVD.

#### **The protective and therapeutic effect of probiotics against myocardial infarction and heart failure**

Until recently, probiotic applications for cardiovascular health were limited to metabolic and diet-associated processes. The aforementioned evidence for probiotic therapy for CVD pertains mostly to symptoms of CVD that are precursory to direct heart damage incurred by coronary artery disease, myocarditis, myocardial infarction, and heart failure. Now, there is emerging evidence that probiotics can provide a direct, cardioprotective effect to the heart that results in reduced ischemic injury and improved cardiac function, post-infarction. The protective role of probiotics against apoptotic injury was first investigated in intestinal cells. While exploring the mechanisms of action against inflammatory bowel disease, it was found the *L. rhamnosus* GG prevents TNF, IL-1 $\alpha$ , and IFN- $\gamma$ -induced apoptosis in mouse colon cells.<sup>113</sup> This was achieved through both activation of the anti-apoptotic Akt pathway, and inhibition of the pro-apoptotic p38 mitogen-activated protein kinase pathway. The purification of *L. rhamnosus* GG supernatant identified a novel protein, p75, to be responsible.<sup>58</sup> The effect of this protein on ischemia/reperfusion (I/R) induced heart cell injury was evaluated using a rat model. The pre-treatment of rats with the purified p75 protein isolated from *L. rhamnosus* GG 30 minutes prior to I/R surgery significantly attenuated heart tissue infarction in a dose-dependent manner. This phenotype was reportedly generated by enhanced expression of HSPs with p75 pretreatment<sup>114</sup> suggesting that proteins produced by *Lactobacillus* probiotics have a direct cardioprotective effect against ischemic injury. Further mechanistic research is required, as the isolated p75 protein, delivered in a bolus, bypassed the gastrointestinal system. Studies examining the production and kinetics of p75 from *L. rhamnosus*

GG within the gut can contribute to an understanding of the role of the microbiome in this phenotype.

A recent myocardial infarction study in rats demonstrated that the oral consumption of probiotics could be cardioprotective. Rats administered the probiotic drink marketed as “GoodBelly,” containing *Lactobacillus plantarum* 299 v, in their drinking water for up to 14 days before I/R heart surgery saw a 29% reduction in ischemia and a 23% recovery of post-ischemic mechanical, as measured by left ventricular diastolic pressure.<sup>115</sup> This cardioprotection seems to be gut microbiome-dependent, as similar administration of vancomycin generated the same phenotype. It was found that the attenuated ischemia was independent of cytokine mediation, but dependent on serum leptin reduction. There was a significant increase in serum leptin post-I/R that was attenuated with pretreatment using GoodBelly and vancomycin. Pre-administration of exogenous leptin abolished the cardioprotection. Leptin is a hormone mainly produced by adipocytes but also by cardiomyocytes, and is typically upregulated and deleterious in CVD patients. This novel finding linked communication between the gut and the heart through hormone signaling and demonstrates an ability of orally administered probiotics to protect against myocardial infarction-associated ischemic injury.

While the application of probiotic to prevent and protect against CVD and direct damage to the heart is gaining interest, the use of probiotics as a therapy for CVD after a major cardiac event may have more practical applications for 2 reasons: Firstly, many patients do not realize they are at risk of infarction and therefore would be unlikely consuming probiotics as prophylaxis; and secondly while the mortality of myocardial infarction has improved by 40% over the past decade, the prevalence of heart failure is stagnant<sup>116</sup> due to the irreversible damage to heart tissue caused by ischemia and infarction. Heart failure is a complex syndrome and in many cases is considered the final outcome of several manifestations of CVD. Myocardial infarction, coronary heart disease, hypertension, and chronic inflammation are all examples of confounding factors that instigate and perpetuate heart failure.

Heart failure is a progressive disease that is difficult to reverse. As a result, 50% of patients will die within the first 5 years of diagnosis. Novel strategies are desired for treating heart failure, to complement the current use of ACE-inhibitors and  $\beta$ -blockers. A recent animal study investigating the outcome of oral probiotic administration in rats with moderate heart failure has produced encouraging results. Following a coronary artery ligation surgery without reperfusion, rats were provided *Lactobacillus rhamnosus* GR-1 in their drinking water for *ad libitum* consumption. After 6 weeks of daily probiotic administration and sustained coronary artery ligation there was a significant attenuation of several indices of heart failure, including cardiac hypertrophy and ventricular remodeling.<sup>117</sup> Cardiac mechanical function was also maintained at normal levels whereas mechanical function significantly deteriorated in animals on placebo treatment over the course of 6 weeks. Cecum digesta microbial analysis using 16 S rRNA next-generation sequencing indicated no significant community-level changes in the microbiota. This suggests that the attenuation of heart failure by *L. rhamnosus* GR-1 may be

independent of the gut microbiome and instead due to a direct interaction of the probiotic with the heart. Studies in efforts to elucidate such mechanisms are ongoing. This seminal research is the first to report that probiotics might be an effective therapy for heart failure and other comorbidities that occur after a major cardiovascular event.

## Conclusions

Probiotics represent one of the fastest growing consumer items on the functional food and nutraceutical market today<sup>118</sup> meaning they are an affordable and accessible health product. They also have few side effects unlike chronic use of CVD medications such as  $\beta$ -blockers and ACE-inhibitors, or any pharmacological agent, which are accompanied by many adverse side effects, including cough, dizziness, hypotension, and bradycardia.<sup>119</sup>

There is a great potential to reduce the cost of CVD medication by implementing combination therapy. Patients diagnosed

with CVD spend around \$340 US per month on ACE-inhibitors or  $\beta$ -blockers alone, and around \$300 US on medication for other non-cardiovascular comorbidities such as diabetes.<sup>120</sup> For patients without access to health insurance and lacking personal financial resources, these costs stand as a strong deterrent for medication compliance. Lack of sufficient medication can exacerbate the progression of HF and eventually result in hospitalization and further economic burden. Based on the suggested retail price of commercially available probiotic products such as Good-Belly, the cost of daily consumption would be less than \$40 per month. While, significantly more research is needed to understand the role of the microbiome and probiotics in CVD, the potential exists that such studies can lead to new approaches to preventing and managing this disease.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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