Impact of probiotics, prebiotics and synbiotics on lipid metabolism in humans

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Abstract. Public health strategies for reducing the risk of coronary heart disease have focused on lowering plasma lipids, particularly cholesterol levels, with recent studies also highlighting triacylglycerol (TAG) as an important modifiable risk factor. One approach is to supplement the diet with probiotics, prebiotics or synbiotics. Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host. Putative health benefits include improved resistance to gastrointestinal infections, reduction in lipid levels and stimulation of the immune system. Prebiotics are selectively fermented dietary components that are aimed at improving host health through selective fermentation by the gut microbiota, such as bifidobacteria and lactobacilli. Animal studies have shown prebiotics to markedly reduce circulating TAG and to a lesser extent cholesterol concentrations, with favourable but inconsistent findings with respect to changes in lipid levels in human studies. Here we provide an overview of the effects, and possible mechanisms, of probiotics, prebiotics and synbiotics (combination of a probiotic and prebiotic) on circulating lipemia in humans.

Keywords: Probiotics, prebiotics, synbiotics, coronary heart disease, lipids, lactic acid bacteria, short chain fatty acids, inulin

1. Introduction

Diseases of the circulatory system account for an appreciable proportion of total morbidity and mortality in adults. Epidemiological studies examining coronary heart disease (CHD) risks in different populations have demonstrated a strong and consistent relationship between total plasma cholesterol and CHD risk [1, 2]. The positive association is largely confined to the low density lipoprotein (LDL) fraction, which transports approximately 70% of cholesterol in the bloodstream. High density lipoprotein cholesterol (HDL-C) has been shown to have a strong inverse relationship with CHD development [3]. Low levels of HDL-C are thought to reflect a compromised pathway for the excretion of cholesterol from the body (reverse cholesterol transport) and have been associated with a five-fold increase in CHD risk compared with normal values [4].

In addition to these classical risk factors, elevated levels of triacylglycerol (TAG; major fat in the blood) in both the fasted and fed (postprandial) state has been demonstrated to be associated with CHD in a number of observational studies [5]. Only more recently have three prospective cohort studies confirmed raised postprandial TAG to be an independent risk factor for CHD [6–8], and more discriminatory than fasting TAG in some population groups such as women [6, 8]. Delayed clearance of TAG from the circulation has been proposed to drive the remodelling of the lipid contents of HDL and LDL by neutral lipid exchange leading to a reduction in HDL-C concentration and an increased prevalence of small dense LDL cholesterol (LDL-C) [9]. This lipid profile has been reported in men with established CHD and those classified with the atherogenic lipoprotein phenotype and the metabolic syndrome [10, 11].

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Increasing prevalence of dyslipidaemia in the population, associated with the rising rates of obesity and type II diabetes, has generated considerable interest in dietary strategies which can reduce circulating lipid levels in both the fed and fasted state and which also offer long-term efficacy comparable with most effective drug treatments. Although low-fat, high carbohydrate diets have been proposed for the primary management of CHD, long-term compliance to such diets is difficult to maintain and there is evidence for potentially detrimental metabolic effects in some population groups, such as type II diabetics [12]. One dietary strategy which has been proposed to benefit the lipid profile involves the supplementation of the diet with probiotics, prebiotics and synbiotics (a combination of probiotics and prebiotics) which are mechanisms to improve the health of the host by supplementation and/or fortification of certain health-promoting gut bacteria. There have been a number of reviews on the impact of probiotics and prebiotics on CVD risk factors including immune function [13] and obesity [14]. This review will solely focus on the effects of probiotics, prebiotics and synbiotics on lipid metabolism in humans, and discuss potential mechanisms of action.

2. Probiotics (including fermented milk products and lactic acid bacteria)

According to the currently adopted definition by the Food and Agriculture Organisation/World Health Organisation, probiotics are defined as “live microorganisms which when administered in adequate amounts confer a health benefit on the host” [15]. The original observation of the positive role played by certain bacteria was first introduced by Russian scientist and Nobel laureate Elie Metchnikoff, who in 1907 suggested that it would be possible to modify the gut flora by replacing harmful microbes with useful microbes. However, interest in the possible lipid lowering benefits of fermented milk, and the bacteria they contained, did not occur until later in the mid-1970’s when Mann and Spoerry [16] reported an 18% fall in plasma total cholesterol after feeding four to five litres of fermented milk/day for three weeks in 24 Maasi warriors. In a small follow-up study (n = four), Mann [17] reported that four litres/day of yoghurt (microbiological activity unspecified) over a 12 day period significantly reduced (37%) serum total cholesterol. This was maintained when the intake was reduced to two litres/day, although not with one litre/day. It was suggested that this was due to a reduction in cholesterol biosynthesis possibly due to an unidentified ‘milk factor’ such as a HMG-CoA reductase inhibitor. Investigating possible candidates for the ‘milk factor’, Howard and Marks [18] fed lactose ± Ca/Mg, cheese whey or yoghurt, to volunteers over a two week period. The yoghurt, but not the lactose ± Ca/Mg or cheese whey, significantly reduced plasma total cholesterol by 5.5% (Table 1). However, this trial was confounded by the lack of dietary control which resulted in substantial changes to the volunteers’ habitual diet during the study.

Most of the early studies introduced confounding factors due to the lack of control of the subjects’ diet, in addition to being underpowered, of short duration [19] and used exceptionally high volumes of intervention foods. In addition the relatively high milk/acidified milk used as the probiotic vehicle may have impacted on the results due to the potential effects of milk on satiety, food intake and other CVD risk marker such as blood pressure. In an attempt to control for these factors, Hepner et al. [20] performed a crossover study in which 720 ml of yoghurt or milk were given each day to the subjects for a four week period. Significant reductions in plasma cholesterol were observed after the first week of both supplementation periods [20], suggesting that these changes may have been the consequence of participation in the study where conscious or even unconscious modification of the diet and other lifestyle factors is common, rather than a reflection of the bioactive properties of the intervention foods. A run-in period is often advised to counteract these effects [21].

Of the early negative studies that have been published, those of Thompson et al. [22], Massay [23] and McNamara et al. [24] incorporated a run-in period. The study performed by McNamara et al. [24] was one of the more carefully designed interventions. They investigated the effects of the ingestion of 480 ml unspecified yoghurt in a study which included a three week run-in period and four week intervention period compared to a non-fermented milk concentrate (as a control). Dietary intake and body weight remained constant and there was no change in serum total, LDL-C, HDL-C or TAG levels [24]. In another well controlled, double blind, crossover study, Kefer (500 ml/day) a fermented dairy product from the Balkans and Caucasus regions of Central Asia consumed for its potential health benefits, showed no lipid lowering properties when compared to unfermented milk [25]. From the studies mentioned above it can be concluded that there is little evidence...
<table>
<thead>
<tr>
<th>Author</th>
<th>Subjects</th>
<th>M/F</th>
<th>Study Design &amp; Intervention Duration</th>
<th>Micronutrient</th>
<th>Vehicle (volume)</th>
<th>TC</th>
<th>LDL-C (%)</th>
<th>HDL-C (%)</th>
<th>TAG (%)</th>
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</thead>
<tbody>
<tr>
<td>Mann and Spoerry [16]</td>
<td>24/0</td>
<td>SB/Parallel (2 gp)</td>
<td>3 wk</td>
<td>Unspecified</td>
<td>8.3 l</td>
<td>↓ 9.6% ***</td>
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<td>Mann [17]</td>
<td>3/1</td>
<td>Sequential 12 d</td>
<td>Unspecified</td>
<td>4 l</td>
<td>↓ 16.8% *</td>
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<tr>
<td>Howard and Marks [18]</td>
<td>10 NA</td>
<td>Parallel 3 wk</td>
<td>Unspecified</td>
<td>2 l</td>
<td>↓ 23.2% *</td>
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<td>Crossover 4 wk</td>
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<td>2 l</td>
<td>↓ 5.4% *</td>
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<td>Parallel (3 gp) 3 wk</td>
<td>Unspecified</td>
<td>2 l</td>
<td>↓ 8.7% ***</td>
<td>NA</td>
<td>↑ 8.9% ***</td>
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<td>5/16</td>
<td>Crossover 1 wk</td>
<td>Unspecified</td>
<td>550 g</td>
<td>↓ 8.7% ***</td>
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<td>58.9% ***</td>
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<tr>
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<td>0/30</td>
<td>SB/Crossover 4 wk</td>
<td>Unspecified</td>
<td>400 ml</td>
<td>↓ 11.6% *</td>
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<tr>
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<td>100 NA</td>
<td>Sequential 2 wk</td>
<td>Lactobacillus bulgaricus and Streptococcus thermophilus</td>
<td>Unspecified</td>
<td>681 g</td>
<td>↓ 11.6% *</td>
<td>NA</td>
<td>NA</td>
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<td>180</td>
<td>Crossover (2 gp) 4 wk</td>
<td>Unspecified</td>
<td>16 oz</td>
<td>↓ 11.6% *</td>
<td>NA</td>
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<tr>
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<td>DB/Crossover (2 gp) 6 wk</td>
<td>Gall Lactobacillus casei and Streptococcus thermophilus</td>
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<td>200 ml</td>
<td>↓ 11.6% *</td>
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<tr>
<td>Richelsen et al. [40]</td>
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<td>Bacteria</td>
<td>Vehicle (volume)</td>
<td>TC</td>
<td>LDL-C</td>
<td>HDL-C</td>
<td>TAG</td>
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<td>de Roos et al. [31]</td>
<td>78</td>
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<td>Lactobacillus acidophilus L-1</td>
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<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<td>DB/Parallel (2 gp) 8 wk</td>
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<td>NS</td>
<td>18.8%</td>
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<td>NA</td>
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<tr>
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<td>DB/Parallel (2 gp) 6 wk</td>
<td>Lactobacillus plantarum 299</td>
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<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
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<tr>
<td>Xiao et al. [29]</td>
<td>32/0</td>
<td>SB/Parallel 4 wk</td>
<td>Bifidobacterium lactis BL1</td>
<td>3 x 100 ml UPY</td>
<td>NS</td>
<td>15.4%</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>Rossi et al. [41]</td>
<td>44</td>
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<td>Enterococcus faecium</td>
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<td>NS</td>
<td>NS</td>
<td>310%</td>
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<td>Klein et al. [52]</td>
<td>13/13</td>
<td>Crossover (2 gp) 5 wk</td>
<td>Lactobacillus acidophilus DSM-2</td>
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<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>11.6%</td>
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<td>Sadrzadeh-Yeganeh et al. [32]</td>
<td>0/90</td>
<td>Parallel (3 gp) 6 wk</td>
<td>Bifidobacterium lactis BS-12 or yoghurt or no yoghurt (yogurt NY-FP90)</td>
<td>300 ml UPY</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>Chang et al. [50]</td>
<td>31/70</td>
<td>DB/Parallel (2 gp) 8 wk</td>
<td>Streptococcus thermophilus</td>
<td>300 ml Y</td>
<td>NS</td>
<td>7.0%</td>
<td>NS</td>
<td>NS</td>
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<tr>
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<td>50</td>
<td>DB/Parallel (2 gp) 12 wk</td>
<td>Lactobacillus salivarius</td>
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<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>Trautvetter et al. [51]</td>
<td>19/13</td>
<td>DB/Crossover (2 gp) 4 wk</td>
<td>Lactobacillus paracasei (LPC17) or Lactobacillus paracasei (LPC35) or Cap</td>
<td>100 ml UPY (soy)</td>
<td>4.0%</td>
<td>4.0%</td>
<td>Y</td>
<td>110%</td>
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<tr>
<td>Hypercholesterolaemic individuals</td>
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<td>Lactic acidophilus</td>
<td>100 ml UPY (soy)</td>
<td>4.7%</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Larkin et al. [34]</td>
<td>21/15</td>
<td>Crossover (2 gp) 5 wk</td>
<td>Lactobacillus acidophilus, Bifidobacterium lactis and Lactobacillus acidophilus DSM-2</td>
<td>100 ml UPY (soy)</td>
<td>4.7%</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>Anderson et al. [32]</td>
<td>9/20</td>
<td>SB/Parallel (2 gp) 3 wk</td>
<td>Lactobacillus acidophilus L-1 or Lactobacillus acidophilus (ATCC 8814)</td>
<td>200 ml UPY (soy)</td>
<td>2.4%</td>
<td>NA</td>
<td>3.9%</td>
<td>NA</td>
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</tr>
<tr>
<td></td>
<td>18/22</td>
<td>DB/Crossover 4 wk</td>
<td>Lactobacillus acidophilus DSM-2 or Lactobacillus acidophilus (ATCC 8814)</td>
<td>200 ml FM</td>
<td>2.9%</td>
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Table 1 (continued)

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<tr>
<th>Author</th>
<th>Subjects</th>
<th>Study Design &amp; Duration</th>
<th>Bacteria</th>
<th>Vehicle (volume)</th>
<th>TC</th>
<th>LDL-C</th>
<th>HDL-C</th>
<th>TAG</th>
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<tr>
<td>Sessions et al. [21]</td>
<td>78/76</td>
<td>DB/Crossover (2 gp) 12 wk</td>
<td>Gaio: Enterococcus faecium, Staphylococcus thermophilus</td>
<td>200 ml</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Bretzke et al. [35]</td>
<td>11/21</td>
<td>DB/Crossover (2 gp) 8 wk</td>
<td>Gaio: Enterococcus faecium, Staphylococcus thermophilus</td>
<td>200 ml</td>
<td>↓5.3% ** ↓6.2% ***</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>Simons et al. [48]</td>
<td>16/28</td>
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<td>2x</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<td>14</td>
<td>Moderate chol</td>
<td>Lactobacillus acidophilis, Bifidobacterium lactis</td>
<td>caps</td>
<td>↓5.5%</td>
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<td>NS</td>
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<tr>
<td>Karlson et al. [44]</td>
<td>150</td>
<td>DB/Parallel (2 gp) 4 wk</td>
<td>Lactobacillus plantarum</td>
<td>100 ml</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>Ejtehed et al. [28]</td>
<td>23/52</td>
<td>DB/Parallel (2 gp) 12 wk</td>
<td>Lactobacillus acidophilis, Leuconostoc lactis</td>
<td>300 g</td>
<td>↓4.5% ↓7.5%</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Jones et al. [37]</td>
<td>41/74</td>
<td>DB/Parallel (2 gp) 4 wk</td>
<td>Lactobacillus acidophilis, Leuconostoc lactis</td>
<td>250 g</td>
<td>↓4.8% ↓8.9%</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<tr>
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<td>54/73</td>
<td>DB/Parallel (2 gp) 6 wk</td>
<td>Lactobacillus acidophilis, Leuconostoc lactis</td>
<td>2 x</td>
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<td>NS</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>Fuentes et al. [36]</td>
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<td>Lactobacillus plantarum</td>
<td>Capsules</td>
<td>↓1.6%</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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</table>

Notes: F = female; M = male; WMY, whole milk yoghurt; UPY, unpasteurised yoghurt; SMY, skimmed milk yoghurt; Y, yoghurt; FM, fermented milk; NS, not significant; PY, pasteurised yoghurt, LFY, low fat yoghurt, data not available; †, transient; SB, single blind; DB, double blind; *P<0.05, **P<0.01, ***P<0.001.
that fermented milk products per se affect serum lipid parameters.

2.1. The effect of viable lactic acid bacteria on lipid parameters

Hepner et al. [20] were the first to attempt to discern whether the presence of live bacteria was important for the reported effects of yoghurt on lipid parameters. The study compared the effects of 750 ml of pasteurised and unpasteurised yoghurt, using milk as a placebo. After a 12 week intervention period all treatments significantly reduced plasma cholesterol levels, with milk resulting in a lesser reduction. Unfortunately, the nutritional and microbiological content of the products used was not reported, which severely hampers comparison with other study data. Thompson et al. [22] reported no effects of a wide range of milk based products (1 litre/day for 3 weeks) including milk supplemented with Lactobacillus acidophilus (undefined, strain code not given including milk supplemented with Lactobacillus acidophilus with Streptococcus thermophilus −1.2 × 10^7 CFU/ml). The possible importance of variation in yoghurt cultures stimulated Jaspers et al. [26] to assess the effect of 681 g/day of three strains of yoghurt fermented with Streptococcus cremoris and Streptococcus lactis −6.4 × 10^8 × 10^9 CFU/ml) and a yoghurt (fermented with Lactobacillus bulgaricus and Streptococcus thermophilus −1.2 × 10^9 CFU/ml). The results illustrate the importance of the cholesterol status of study participants on entry into the study. It is interesting to note that of the studies which investigated a variety of grouping all probiotic studies together as equal can often result in misleading conclusions.

The subject group studied also appears to be an important factor in the hypocholesterolaemic effects of lactic acid bacteria. A lack of effect was reported after obese adolescents (n = 50) consumed 200 g/day of a yoghurt with Lactobacillus salivarius Ls-SS for a 12 week period [30]. This was similar to De Roos and colleagues [31] and Sadrzadeh-Yeganeh and colleagues [32] who performed large studies (n = 78 and 90 respectively) investigating the impact of 500 ml/day of yoghurt supplemented with Lactobacillus acidophilus L-1 for a six week period in normocholesterolaemic subjects or 300 g/day of a probiotic yoghurt containing Lactobacillus acidophilus la5 and bifidobacterium lactis Bb12 compared with a conventional yoghurt or no intervention (control) for a six week period [31, 32]. It was reported that there was no significant effect on any lipid parameters determined compared with the control yoghurt, although significant total and LDL-C lowering effects were reported by Sadrzadeh-Yeganeh and colleagues compared with no yoghurt [32]. This is in contrast to the studies by Anderson et al. [33] which investigated a smaller volume (200 ml) of fermented milk (yoghurt-type) containing human Lactobacillus acidophilus L-1 or swine Lactobacillus acidophilus (ATCC 43121) in 40 hypercholesterolaemic subjects for a four week period and reported a significant reduction of 2.9% in total cholesterol compared with baseline. These data illustrate the importance of the cholesterol status of study participants on entry into the study. It is interesting to note that of the studies which investigated a variety of different probiotics in hypercholesterolaemic subjects seven out of nine (Table 1) reported a significant hypocholesterolaemic effect ranging from 2.4–13.6% reduction [27, 28, 33–38], which is in contrast to the
well controlled studies performed using normocholes-
terolemic participants.
A number of studies have tested the lipid lower-
ing effects of a particular probiotic yoghurt Gaio®
(MD Foods Aarhus, Denmark) fermented by Causid®
consisting of Enterococcus faecium (human species,
undefined, strain code not given) and two strains of
Streptococcus thermophilus with varying results. Agerbeck et al. [39] tested the effect of 200 ml (Enteroc-
coccus faecium, ~2 × 10^8 CFU/ml) per day of Gaio®
in a parallel study design, where the active yoghurt
was tested against an identical yoghurt that had been
chemically acidified with an organic acid (delta-gluco-
lactone). The intervention was for a six week period in
58 middle-aged men with moderately raised choles-
terol levels (5.0–6.5 mmol/l). They observed a 9.8% 
reduction in LDL-C levels for the live yoghurt group,
which was sustained over the intervention period
(Table 1). This was a well-controlled study, which
excluded many variables such as age, sex and body
weight. However, an unforeseen skew in the randomi-
sation resulted in significantly different baseline total
and LDL-C levels in the two groups. The fall in these
parameters observed in the active yoghurt group could
be ascribed to a regression towards the mean. Another
study performed using the same yoghurt and a similar
protocol confirmed by dietary assessment. The levels
of Enterococcus faecium in the active yoghurt were
monitored throughout the study and found to be no
lower than 1 × 10^6 CFU/ml at any time tested. Dur-
ing the two-week run-in period, both groups showed
significant reductions in plasma cholesterol levels, but
thereafter there were no further changes in either of
the groups or between the groups at any of the time
points. These data do not support previous studies dis-
cussed, but are consistent with the conclusions drawn
by Rosouw et al. [41] regarding cholesterol changes
attributed to study participation. In summary, six pub-
lished studies (Table 1) have investigated whether the
addition of Gaio® to the habitual diet can reduce plasma cholesterol. Four studies showed a small bene-
dicial short-term effect of a 6–10% reduction of LDL-C
[35, 39, 40, 42], yet the long-term effects were incon-
clusive [40] with the largest study to date failing to
show any significant effects [21]. However despite
these inconsistent reports, a meta-analysis of these
studies has concluded that both total and LDL-C was
reduced by 6% and 9% respectively compared to the
control yoghurt and provides evidence for a short-term
beneficial effect of Gaio® [42].
A similar trial of the same Gaio® product con-
taining Enterococcus faecium was conducted in 160
middle-aged men and women with moderately raised
cholesterol [21]. The study was a randomised, double-
blind, multi-centre, placebo controlled parallel study
and was the largest performed to date. Volunteers con-
sumed 200 ml/day of either the active or chemically
acidified yoghurt for a 12 week period. Stratified ran-
domisation was used to ensure that the groups were
comparable for age, sex, BMI and baseline fasting
cholesterol levels. The importance of not changing
t heir dietary habits and lifestyle during the study was
emphasised to the volunteers and adherence to the
protocol confirmed by dietary assessment. The levels
of Enterococcus faecium in the active yoghurt were
monitored throughout the study and found to be no
lower than 1 × 10^6 CFU/ml at any time tested. Dur-
ing the two-week run-in period, both groups showed
significant reductions in plasma cholesterol levels, but
thereafter there were no further changes in either of
the groups or between the groups at any of the time
points. These data do not support previous studies dis-
cussed, but are consistent with the conclusions drawn
by Rosouw et al. [41] regarding cholesterol changes
attributed to study participation. In summary, six pub-
lished studies (Table 1) have investigated whether the
addition of Gaio® to the habitual diet can reduce plasma cholesterol. Four studies showed a small bene-
dicial short-term effect of a 6–10% reduction of LDL-C
[35, 39, 40, 42], yet the long-term effects were incon-
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reduced by 6% and 9% respectively compared to the
control yoghurt and provides evidence for a short-term
beneficial effect of Gaio® [42].

The majority of the studies investigating the effect of
lactic acid bacteria and probiotics have used fermented
cow’s milk or cow’s milk yoghurt. In contrast, two stud-
ies have determined the effect of fermented soy milk
[43] and soy milk consumed with a probiotic yoghurt
[34] on lipid concentrations with conflicting results,
and one study using fermented oat milk [44]. Although
there are discrepancies in relation to the particular
components of soy that are active, there is increasing
evidence that soy consumption is related to a reduc-
tion in plasma lipids [45]. Isoflavones alone generally
do not reduce lipids [46] yet in combination with soy
proteins appear to be beneficial [45, 47]. Consequently
the impact of the addition of a probiotic to soy prod-
ucts on lipid-lowering effects of these products was
investigated. Rossi and colleagues [43] determined the impact of the addition of 200 ml/day of fermented soy milk produced with Enterococcus faecium (undefined, strain not given) and Lactobacillus acidophilus (undefined, strain not given) for a six week period in normocholesterolaemic middle-aged men. Despite containing isoflavones, soy protein and the same species as Gaio (Enterococcus faecium) this fermented soy milk did not significantly affect the total or LDL-C levels, but did result in a 10% increase in HDL-C [43]. These data are in contrast to the observations of a study which investigated the effect of soy milk and either 100 ml probiotic yoghurt (containing $10^8$ CFU/serving of each of Lactobacillus acidophilus (undefined, strain not given), Bifidobacterium bifidus (undefined, strain not given), and Lactobacillus rhamnosus GG) or yoghurt containing typical yoghurt starter cultures. It was reported that the dietary combination of soy with probiotic yoghurt resulted in a significant reduction in total cholesterol (−4.7%) and potential synergistic hypocholesterolaemic actions between soy and probiotic bacteria were suggested [34]. In another study, 100 ml of fermented oat milk containing Lactobacillus plantarum (DSM 9843, $10^8$ CFU/ml) was consumed in a double blind parallel study in 18 men with atherosclerotic plaques. Although it was reported that there was a significant increase in plasma short chain fatty acids (SCFAs) and colonic bacterial diversity, there was no significant impact on lipid concentrations compared to a control product [44].

Probiotics can also be given in the form of capsules rather than in milk or yoghurts. A study performed in 60 hypercholesterolaemic participants investigated the impact of different strains (CECT 7527, 7528 and 7520) of Lactobacillus plantarum (1.2 × $10^8$ CFU/capsule) contained within a capsule, compared with a placebo capsule containing no bacteria. A significant reduction of 13.6% in plasma total cholesterol was observed after 12 weeks. However the degree of reduction was dependent on the baseline cholesterol concentrations. Participants with high cholesterol (>6.5 mmol/l) had a greater total and LDL-C reduction compared with those with lower baseline cholesterol concentrations [36]. This impact of baseline cholesterol concentrations may have explained the lack of effect of capsules (two/day) containing Lactobacillus fermentum (2 × $10^8$ CFU/capsule) in moderate hypercholesterolaemic subjects [48], and in a study investigating a rosheip drink (400 ml/day) containing Lactobacillus plantarum 299v (5 × $10^8$ CFU/ml) in normocholesterolaemic participants [49]. Another method of probiotic delivery is that of microencapsulation, which allows determination of the impact of the probiotic, without the confounding effects of other potentially bioactive components present in the food vehicle. Two studies published by Jones and colleagues have reported significant reductions in total (4.8% and 9.1%) and LDL-C (8.9% and 11.6%) by microencapsulated Lactobacillus reuteri NCIMB 30242 in the form of yoghurt (250 g/d) or capsules (two daily) for a six and nine week period in hypercholesterolaemic subjects (n = 114 and 127) respectively [37, 38]. They proposed that the beneficial effects reported were due to an increased number of viable probiotic reaching the colon, facilitated by the microencapsulation, in addition to consuming high quantities ($10^8$ CFU) of the probiotic bacteria daily [37, 38].

From the findings available to date, it can be concluded that there is evidence to support a hypocholesterolaemic effect of a daily consumption of some lactic acid bacteria containing products, which is more apparent in those with hypercholesterolaemia. The most consistent finding is a reduction in total cholesterol, which is associated with a significant LDL-C reduction in some [35, 37–42, 50, 51], but not all studies, although some of these studies were confounded by weight reduction [50]. However only one of the studies that have measured plasma TAG (see Table 1) have reported a significant reduction (11.6 %) in this lipid level [52], with another study reporting a significant increase of 16% [22]. A current meta-analysis of randomised controlled trials investigating the effect of probiotics on plasma lipid profiles concluded that a pooled analysis of 13 trials (n = 485 participants) with high, borderline or normal cholesterol concentrations suggested mean net changes in total, LDL-C and HDL-C of −0.17, −0.13 and −0.003 mmol/l respectively and for TAG −0.04 mmol/l [53]. Therefore it is apparent that the impact of probiotic bacteria on CHD lipid risk reduction is in their potential hypocholesterolaemic effects. However results are inconsistent particularly in normocholesterolaemic groups and further suitably powered and well-controlled studies are required to determine the impact of specific probiotic strains.

2.2. Possible mechanisms of action of lactic acid bacteria

Before the possible mechanisms are considered, it is important to highlight that since viable and
biologically active micro-organisms are usually required at the target site in the host, it is essential that the probiotics not only have the characteristics that are necessary to produce the desired biological effects; but also have the required viability and are able to withstand the host’s natural barriers against ingested bacteria. The classic yoghurt starter bacteria, Streptococcus thermophilus and Lactobacillus bulgaricus are technologically effective, but they do not reach the lower intestinal tract in a viable form. Therefore, intrinsic microbiological properties, such as tolerance to gastric acid, bile and pancreatic juice are important factors when probiotic organisms are considered [54].

The mechanism of action of lactic acid bacteria and probiotics on cholesterol reduction is unclear, but there are a number of proposed possibilities which may act independently or synergistically. These include physiological actions of the end products of fermentation SCFA; cholesterol assimilation, deconjugation of bile acids and cholesterol binding to bacterial cell walls. The SCFA that are produced by the bacterial anaerobic breakdown of carbohydrate are acetate, propionate and butyrate. The physiological effects of these are discussed in detail in the Section 3.2.

It has been well documented that microbial bile acid metabolism is a peculiar effect involved in the therapeutic role of some bacteria. The deconjugation reaction is catalysed by conjugated bile acid hydrolase enzyme, which is produced exclusively by bacteria [55]. Deconjugation ability is widely found in many intestinal bacteria including genera Enterococcus, Peptostreptococcus, Bifidobacterium, Fusobacterium, Clostridium, Bacteroides and Lactobacillus [56]. This reaction liberates the amino acid moiety and the deconjugated bile acid thereby reducing cholesterol re-absorption, by increasing faecal excretion of the deconjugated bile acids. Many in vitro studies have investigated the ability of various bacteria to deconjugate a variety of different bile acids. Grill et al. [57] reported Bifidobacterium longum strains (BB536 and OC) as the most efficient bacterium among four Bifidobacterium strains (Bifidobacterium animalis; Bifidobacterium infantis; Bifidobacteria breve and Bifidobacterium thermophilum) when tested against six different bile salts. Another study reported that Lactobacillus species had varying ability to deconjugate glycocholate and taurocholate [58] with Lactobacillus fermentum KC5b reported to have the ability to deconjugate a number of bile salts in vitro [59]. Studies performed on in vitro responses are useful, but in vivo studies in animals and humans are required to determine the full contribution of bile acid deconjugation to cholesterol reduction. Intervention studies in animals and ileostomy patients have shown that oral administration of certain bacterial strains led to an increased excretion of free and secondary bile salts [29, 60, 61]. Of particular note are the studies of Jones et al. [37, 38] and Fuentes [36] in which probiotic strains were chosen for their specific bile acid deconjugation activities. As described previously Jones et al. [38] and Fuentes et al. [36] reported consumption of the probiotic Lactobacillus reuteri NCIMB 30242 and three strains of Lactobacillus plantarum (CECT 7527, CECT 7528 and CECT 7529) respectively in microencapsulated form, resulted in significant LDL-C reduction. It was proposed this was due to a reduction in lipid uptake from the gut [36–38], which was supported by the elevated plasma deconjugated bile acid concentrations which affect endogenous hepatic cholesterol metabolism [38]. It was stated by the Jones and colleagues that Lactobacillus reuteri NCIMI 30242 appeared to be superior to traditional probiotic therapy and akin to that of other cholesterol-lowering ingredients. These studies highlight the importance of probiotic selection for efficacious hypcholesterolaemic effects.

There is also some in vitro evidence to support the hypothesis that certain bacteria can assimilate (take up) cholesterol [62] It was reported that Lactobacillus acidophilus (RP32) [58], Bifidobacterium bifidum (strains BYU and RP0) [63], Lactobacillus fermentum (KC5b) [64] had the ability to assimilate cholesterol in in vitro studies, but only in the presence of bile and under anaerobic conditions. However, despite these reports, there is uncertainty whether the bacteria are assimilating cholesterol or whether the cholesterol is co-precipitating with the bile salts. Studies have been performed to address this question. Klaver and Meer [65] concluded that the removal of cholesterol from the growth medium in which Lactobacillus acidophilus (RP32) and a Bifidobacterium (MUH80) were growing was not due to assimilation, but due to bacterial bile salt deconjugase activity. The same question was addressed by Tahri et al. [66] with conflicting results, and they concluded that part of the removed cholesterol was found in the cell extracts and that cholesterol assimilation and bile acid deconjugase activity could occur simultaneously.

The mechanism of cholesterol binding to bacterial cell walls has also been suggested as a possible
3. Prebiotics

Over the past three decades, there has been increasing interest in the important nutritional role of prebiotics as functional food ingredients. This interest has been derived from animal studies which showed markedly reduced TAG and total cholesterol levels when diets containing significant amounts of a prebiotic (oligofructose (OFS)) were fed. A prebiotic is defined as 'a selectively fermented ingredient that results in specific changes, in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health' [69]. Prebiotics, most often referred to as non-digestible oligosaccharides, are extracted from natural sources (e.g. inulin and OFS) or synthesised from disaccharides (e.g. transgalacto-oligosaccharides). The most commonly studied of the prebiotics include inulin and OFS which are found in many vegetables, including onion, garlic, leek, asparagus, Jerusalem artichoke and chicory root [70]. These consist of between two and sixty fructose molecules joined by β-1 osidic linkages, which, due to the nature of this type of linkage escape digestion in the upper gastrointestinal tract and remain intact but are selectively fermented by colonic microbiota. Inulin is currently found as a food ingredient of bread, baked goods, yoghurt and ice-cream because it displays gelling and thickening properties and helps to improve the mouth feel and appearance of lower energy products [70]. In Europe, the estimated intake of inulin and OFS is between three and 12 g per day [71].

3.1. The effect of prebiotics on lipid metabolism in humans

Twenty-one studies have investigated the effects of prebiotics on fasting plasma lipids [72–92], but findings have been inconsistent (Table 2). In the eight studies with individuals with moderately raised lipids and hyperlipidaemia, one study showed a reduction in fasting total, LDL-C and TAG [90], four studies showed significant decreases in fasting total and/or LDL-C with no changes in TAG levels [85–88] whereas two studies showed a significant reduction in fasting TAG only [73, 89]. In contrast, of the three studies conducted in type II diabetics, only one study reported significant reductions in total and LDL-C [91] (Table 2). In normolipidaemic participants, four out of the ten studies have reported an effect on fasting lipid concentrations [75, 79, 81, 83] One study found reductions in total and LDL-C only (6–8%) [83], whereas the remaining three studies all showed reductions in fasting TAG levels (16–27%) [75, 79, 81]. Of these studies, one also reported a reduction in total cholesterol (8%) [75] whereas Russo et al. [81] also demonstrated a concomitant increase in HDL-C. In the study by Jackson et al. [73], follow-up blood samples were taken four weeks after completion of the inulin supplementation period by which time the concentrations of TAG had returned to baseline values, supporting the conclusion that inulin feeding may have been responsible. A meta-analysis of 15 randomised controlled trials (conducted between 1996 and 2005) has reported that inulin-type fructans can significantly decrease fasting TAG concentrations by 0.17 mmol/l [93]. These findings are in line with observed effects of inulin on lipid levels in animals in which the predominant effect is on TAG rather than cholesterol concentrations.

Data from studies in rats have shown a 40% reduction in postprandial TAG concentrations when diets containing ten per cent OFS (w/v) were fed [94]. However, very little information regarding the effect of prebiotics on postprandial lipaemia in human subjects are available, with two studies reporting a lack of effect of inulin treatment on postprandial TAG levels in middle-aged men and women with moderately raised lipids [73, 84].
Table 2
Summary of human studies to examine the effects of fructan supplementation on fasting lipid levels

<table>
<thead>
<tr>
<th>Author</th>
<th>Subjects (M/F)</th>
<th>Study design and duration</th>
<th>Fructan and dose</th>
<th>Vehicle</th>
<th>Change observed in:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TC</td>
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<tr>
<td></td>
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<td></td>
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<td>LDL-C</td>
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<td>HDL-C</td>
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<td></td>
<td></td>
<td>TAG</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal lipidaemic individuals</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Pedersen et al. [74]</td>
<td>0/66</td>
<td>DB, crossover 4 wks</td>
<td>inulin 14 g margarine breakfast</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Bologhini et al. [75]</td>
<td>1200</td>
<td>Sequential 4 wks</td>
<td>inulin 9 g cereal</td>
<td>NS</td>
<td>↓14%</td>
</tr>
<tr>
<td>Knorr et al. [76]</td>
<td>6/5</td>
<td>DB, parallel 9 wks</td>
<td>inulin 22–34 g yoghurt</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>van Dijkman et al. [77]</td>
<td>1200</td>
<td>DB, crossover 3 wks</td>
<td>inulin, OFS or GOS 15 g orange juice</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Luo et al. [78]</td>
<td>1200</td>
<td>DB, crossover 4 wks</td>
<td>OFS 20 g biscuits</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Letznher et al. [79]</td>
<td>4/5</td>
<td>DB, crossover 5 wks</td>
<td>inulin 10 g sachet</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Forcheron et al. [80]</td>
<td>6/11</td>
<td>DB, crossover 24 wks</td>
<td>inulin/OF 10 g sachet</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Russo et al. [81]</td>
<td>2200</td>
<td>DB, crossover 5 wks</td>
<td>inulin 11 g pasta</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Valevic et al. [82]</td>
<td>16/28</td>
<td>DB, crossover 10 wks</td>
<td>GOS 5.5 g sachet</td>
<td>NS</td>
<td>NA</td>
</tr>
<tr>
<td>de Lusis et al. [83]</td>
<td>4/26</td>
<td>DB, parallel 4 wks</td>
<td>inulin 3 g biscuits</td>
<td>NS</td>
<td>↓16%</td>
</tr>
<tr>
<td>Moderately hyperlipidaemic individuals</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Jackson et al. [73]</td>
<td>27/27</td>
<td>DB, parallel 8 wks</td>
<td>inulin 10 g sachet</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Giacca et al. [84]</td>
<td>20/10</td>
<td>DB, crossover 8 wks</td>
<td>OFS 5.3 g sachet</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Gentile et al. [85]</td>
<td>0/55</td>
<td>DB, parallel 16 wks</td>
<td>OFS 10 g syrup</td>
<td>NS</td>
<td>↓27%</td>
</tr>
<tr>
<td>Yen et al. [86]</td>
<td>6/40</td>
<td>DB, parallel 3 wks</td>
<td>OFS 5–10 g sachet</td>
<td>NS</td>
<td>↓7%</td>
</tr>
<tr>
<td>Hyperlipidaemic individuals</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Hideki et al. [87]</td>
<td>20/26</td>
<td>DB, parallel 5 wks</td>
<td>OFS 8 g confectionary chocolate bar, paste or sweetener</td>
<td>NS</td>
<td>↓4%</td>
</tr>
<tr>
<td>Davidson et al. [88]</td>
<td>12/13</td>
<td>DB, crossover 6 wks</td>
<td>inulin 18 g</td>
<td>NS</td>
<td>↓14%</td>
</tr>
<tr>
<td>Causey et al. [90]</td>
<td>1200</td>
<td>DB, crossover 3 wks</td>
<td>inulin 20 g low fat ice-cream</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Bakke-Moene et al. [91]</td>
<td>1200</td>
<td>DB, parallel 4 wks</td>
<td>inulin 7 g not specified</td>
<td>NS</td>
<td>↓22%</td>
</tr>
<tr>
<td>Type II diabetics</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Yamashita et al. [92]</td>
<td>8/10</td>
<td>DB, parallel 2 wks</td>
<td>OFS 8 g coffee drink or jelly</td>
<td>NS</td>
<td>↓8%</td>
</tr>
<tr>
<td>Alles et al. [72]</td>
<td>9/11</td>
<td>SB, crossover 3 wks</td>
<td>OFS 15 g not specified</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Luo et al. [92]</td>
<td>4/10</td>
<td>DB, crossover 10 wks</td>
<td>OFS 20 g liquor</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Abbreviations: DB, double blind; F, female; GOS, galacto-oligosaccharide; HDL-C, high density lipoprotein cholesterol; M, male; NA, not analysed; NS, not significant; SB, single blind; TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; OFS, oligofructose; TAG, triacylglycerol. §elderly subjects.</td>
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</table>

The marked reductions in fasting lipid levels observed in animal studies have not been consistently reproduced in human subjects. This may be explained by the amount of fructans used in the human studies which varies between 5–20 g, with a single study using 34 g (Table 2). This amount is small compared to that used in animal studies (50–200 g/day OFS of rat chow) [95] which is equivalent to a dose in humans of approximately 50–80 g of OFS/inulin per day. The fact that OFS and inulin are fibres restricts its dosage in humans to between 15–20 g per day, with intakes greater than this associated with gastrointestinal symptoms such as stomach cramps, flatulence and diarrhoea [74, 85]. However, some studies have shown tolerance of higher amounts of prebiotics by increasing the daily dose (22.8 g to 45.6 g) slowly over a number of weeks [96], and also by consuming higher doses (22–34 g) with a low-fat, high carbohydrate diet (30% energy fat, 55% carbohydrate and 15% protein) [76]. One acute postprandial study also reported the lack of gastrointestinal symptoms when 48 g of resistant starch (a fermentable carbohydrate) was split over two meals, with 24 g given with the breakfast and lunch 5 h later [97]. These studies indicate that it may be possible to introduce higher intakes of fermentable fibre into the Western-style diet in a tapered fashion thereby avoiding the reported bloating and flatulence associated with single doses of inulin and OFS given in powder form. Higher fibre intakes between 70–120 g/day have been observed in populations following traditional diets rich in fruits, vegetables and grains and also our ancestral hunter-gathers, suggesting that greater...
intakes of fermentable fibres may be possible in the diet [98]. At present, it is not known whether, at the levels used in human studies, significant effects on fasting lipid levels would also be observed in animals [99].

The types of food vehicles used to increase the amount of fructans (OFS, galacto-oligosaccharides and inulin) in the diet differ. In the case of Luo et al. [78] 100 g of biscuits were eaten every day and for Pedersen et al. [74], 40 g/day of margarine was consumed which may have contributed to the negative findings in blood lipids. In the case of Davidson et al. [88], significant changes in total and LDL-C levels were observed over six weeks with inulin in comparison with the placebo (sugar) incorporated into a number of study products including a chocolate bar, a chocolate spread and sweeteners for coffee or fruit. The percentage change in each of the lipid parameters was calculated over each of the six week treatment periods and unexpectedly, there was an increase in total cholesterol, LDL-C and TAG during the placebo phase. Non-significant falls in these variables were observed during inulin treatment and so when the net changes in the variables were calculated (change during inulin treatment minus change during control treatment) there were significant differences in total and LDL-C between the two treatments. The authors attributed the increase in total and LDL-C levels in the placebo phase to be due to the increased intake of saturated fatty acids in the chocolate products which were used as two of the vehicles in the study [88]. In later studies, the use of inulin in a powder form has enabled it to be added to many of the foods eaten in the participant’s normal diet without any need for dietary advice thus avoiding changes in body weight. Since inulin has water binding properties, in its powder form inulin could be added to orange juice, tea, coffee, yoghurt and soup [73, 79, 80, 82, 84, 86, 90].

The significant relationship between the participants initial TAG concentration and percentage change in TAG levels over the eight week study demonstrated by Jackson et al. [73] lends support to the hypothesis that the initial TAG levels could be important in determining the degree of the TAG response to inulin. The lack of response in some individuals may be as a result of them being less responsive to inulin, variations in their background diet, or non-compliance with the study protocol. Speculation as to possible reasons for variability in response would be aided by a better understanding of the mechanism of action of inulin on plasma TAG levels.

The length of the supplementation period used in the studies in Table 2 may be another factor for the inconsistent changes in TAG levels in human subjects with inulin. The studies were conducted over two to 24 weeks, with significant effects occurring as early as three to four weeks [75, 79, 83, 89, 90]. One study conducted for 24 weeks showed no effect of inulin/OFS on lipid levels in normolipidaemic participants [80], suggesting that beneficial effects observed in the short-term studies may not persist on a long-term basis. A wash out period of four weeks has been shown to be a sufficient period time for the TAG concentrations to return to baseline values [73]. This may provide an explanation for the significant findings in TAG levels in the studies of Causey et al. [89] and Brightenti et al. [75] who used three to four week sequential and crossover designs with very short wash out periods.

Whilst some of the studies, to date, support beneficial effects of inulin on plasma TAG, the findings are by no means consistent and more work is required to provide convincing evidence of the lipid lowering consequences of prebiotic ingestion.

3.2. Mechanism of lipid lowering by prebiotics

Prebiotics have been shown to be an ideal substrate for the health promoting bacteria in the colon, notably bifidobacteria and lactobacilli [100]. During the fermentation process a number of by-products are produced including gases (H2S, CO2, H2, CH4), lactate and SCFAs (acetate, butyrate and propionate). The SCFAs, acetate and propionate enter the portal blood stream where they are utilised by the liver. Acetate is converted to acetyl CoA in the liver and acts as a lipogenic substrate for de novo lipogenesis, whereas propionate has been reported to inhibit lipid synthesis [101, 102]. Butyrate, on the other hand, is taken up by the large intestinal cells (colonocytes) and has been shown to protect against tumour formation in the gut [103]. The type of SCFAs which are produced during the fermentation process is dependent on the microbiota which can be stimulated by the prebiotic. In vitro fermentation experiments have shown inulin-type fructans to increase both acetate and butyrate levels, whereas galacto-oligosaccharides and xylo-oligosaccharides increase. Synthetically produced prebiotics, for example galacto-oligosaccharides and xylo-oligosaccharides increase the production of acetate, propionate and butyrate [103–105].

The production of acetate in the intestine results in the formation of SCFAs which are transported to the liver where they are used as precursors for the synthesis of triglyceride, cholesterol and bile acids. The synthesis and secretion of bile acids depends on the rate of SCFA synthesis - the higher the rate of SCFA synthesis the greater the loss of bile acids from the body. SCFAs also inhibit the synthesis of cholesterol in the liver. The inhibition of cholesterol synthesis is dependent on the amount of SCFAs produced and their rate of transport to the liver. The type of SCFAs produced is dependent on the microbiota present in the gut and the prebiotic ingested. The type of SCFAs produced in response to prebiotic ingestion is a key factor in determining the degree of lipid lowering observed in human intervention studies.
Interestingly, Kabel et al. [104] demonstrated that these SCFA were produced at different stages of the fermentation of xylo-oligosaccharides, with the first stage (0–40 h) associated with a marked production of acetate whereas the later stage (>40 h) propionate and butyrate production predominated. These differences may reflect the type of bacteria involved in the early and later stages of the fermentation process.

Inulin and OFS have been extensively studied to determine the mechanism of action of prebiotics in animals. Early in vitro studies using isolated rat hepatocytes suggested that the hypolipidaemic action of OFS was associated with the inhibition of de novo cholesterol synthesis by the SCFA propionate following impairment of acetate utilisation by the liver for de novo lipogenesis [102]. This is in agreement with human studies in which rectal infusions of acetate and propionate resulted in propionate inhibiting the incorporation of acetate into TAGs released from the liver [106]. Fiordaliso et al. [107] demonstrated significant reductions in plasma TAGs, phospholipids and cholesterol in normolipidaemic rats fed a rat chow diet containing ten per cent (w/v) OFS. The TAG-lowering effect was demonstrated after only one week of OFS and was associated with a reduction in the secretion of very low density lipoprotein (VLDL) from the liver. The TAG-lowering effect was demonstrated after only one week of OFS and was associated with a reduction in the secretion of very low density lipoprotein (VLDL) from the liver. The TAG-lowering effect was demonstrated after only one week of OFS and was associated with a reduction in the secretion of very low density lipoprotein (VLDL) from the liver.

In summary, the mechanisms of action of prebiotics, especially inulin and OFS, have been determined largely from animal studies. Present data suggest inhibition of de novo lipogenesis as the primary mode of action of prebiotics in mediating their lipid lowering effects via down regulation of the enzymes involved. If this is the case, more modest or inconsistent effects might be expected in humans, in whom de novo lipogenesis is extremely low, or variable depending on their background diet. In animal studies, rats are fed a diet which is low in fat and high in carbohydrate and so de novo lipogenesis is an upregulated pathway in these animals for the synthesis of fatty acids. It is interesting to note that when rats are fed OFS along with a high fat diet typical of the Western-style diet, TAG levels are thought to be decreased by different mechanisms involving the enhanced clearance of TAG-rich lipoproteins. An increased secretion of glucose-dependent insulinotropic polypeptide (GIP) in OFS treated rats was observed by Kok et al. [94] and this gut hormone has been shown to enhance the activity of lipoprotein lipase, the principal enzyme involved in the clearance of TAG-rich lipoproteins following the ingestion of fat [110]. The release of GIP and GLP-1 in the intestine and colon may act as mediators of the systemic effect of prebiotics such as inulin and OFS, on blood lipid concentrations. However, further work is required to determine the metabolic pathways which are influenced by different prebiotic preparations. Their effect on gastrointestinal kinetics such as gastric emptying and its modification of the levels of TAG-rich lipoproteins (chylomicrons and VLDL) in the circulation has the same. This may provide an explanation for the variability in the lipid response observed in the human studies.
recently been proposed as a potential modulator of systemic effects [111]. Therefore, the design of future studies to investigate the effect of prebiotics in humans should consider the choice of probiotic, subject group, length of supplementation period and type of vehicle used to increase the intake of prebiotics in the diet, as these variables may influence the outcome of the study.

4. Synbiotics (including combinations of lactic acid bacteria and prebiotic fibres)

A synbiotic is defined as ‘a mixture of a prebiotic and a probiotic that beneficially affects the host by improving the survival and the implantation of live microbial dietary supplements in the gastrointestinal tract, by selectively stimulating the growth and/or by activating the metabolism of one or a limited number of health promoting bacteria’ [112]. The use of symbiotics (or other combinations of lactic acid bacteria and fibres) as functional food ingredients is a developing research area and only six human studies have been performed looking at their effects on lipid metabolism (Table 3). In normolipidaemic individuals, Schaafsma et al. [113] reported a significant decrease in total and LDL-C in men after three weeks of daily supplementation with a fermented milk product (375 ml) containing *L. acidophilus* and 2.5% FOS compared with a control milk fermented by traditional yoghurt starters (375 ml). Levels of HDL-C and TAG were unchanged, leading to an improvement in the LDL-C/HDL-C ratio. Kießling and colleagues [114] showed the consumption of a fermented milk product (containing *L. acidophilus* 145 and *B. longum* 913) with the addition of 1% OFS for six weeks to significantly increase total cholesterol (*L. gasseri* and 0.8 g inulin in hypercholesterolaemic men and women [116, 117]. Interestingly, comparable reductions in total and LDL-C were observed in these studies even though Ooi and co-workers supplemented with 8.2 g less of fructan per day. The inclusion of hyperlipidaemic participants, a longer intervention period (three vs. 12 wks) and/or a different Lactobacillus strain may have contributed to the similar findings in these two studies.

In type II diabetic women, 26% and 37% reductions in total cholesterol and TAG levels and a 35% increase in HDL-C were observed after supplementation for four weeks with a synbiotic shake containing *Lactobacillus* and *B. bifidum* and 2 g of inulin [118]. This is the only study to date which has reported a reduction in fasting TAG levels with a synbiotic product. The lower dose of inulin, relative to those presented in Table 2, suggests a potential synergistic effect of the probiotic strain and inulin on the fasting lipid profile. However, the non-significant 17% reduction in TAG in the placebo group suggests that other components in the shake (such as oatmeal and textured soy protein), in addition to the raised TAG concentrations (>1.7 mmol/l) at baseline, may have contributed to the degree of change in fasting lipid levels in these women.

The majority of research conducted so far with synbiotics has looked at their effect on the composition of the gut microbiota. In one study in healthy subjects, a *Bifidobacterium* sp. (undefined, strain not defined) fermented milk product with or without 18 g of inulin was given daily for 12 days [119]. The authors concluded that that the administration of the fermented milk product (probiotic) substantially increased the proportion of bifidobacteria in the gut, but that this increase was not enhanced by the addition of 18 g of inulin. The composition of the gut microbiota was then assessed two weeks after completing the supplementation period and it was found that subjects who received the fermented milk product and inulin maintained their bifidobacterial population in the gut compared with the subjects receiving the fermented milk product only. Although a synergistic effect on bifidobacteria in the gut was not observed with the synbiotic, these results suggest that either there was better implantation of the probiotic or a prebiotic effect on indigenous bifidobacteria [57]. In a study in elderly subjects, supplementation with a capsule containing *B. bifidum* (BB-02) and *B. lactis* (BL-01) together with an inulin-based prebiotic was shown to significantly increase total numbers of faecal bifidobacteria including *B. lactis* and *B. bifidum*, with at least one of these bifidobacteria species still detectable in the faecal samples three weeks after completion of the study [120]. The...
Table 3
Summary of human studies to examine the effects of synbiotic supplementation on fasting lipid levels

<table>
<thead>
<tr>
<th>Author</th>
<th>Subjects (M/F)</th>
<th>Study design and duration</th>
<th>Synbiotic</th>
<th>Probiotic strain</th>
<th>Change observed in</th>
<th>TC</th>
<th>LDL-C</th>
<th>HDL-C</th>
<th>TAG</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Normolipidaemic individuals</strong></td>
<td></td>
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<td></td>
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<tr>
<td>Schaafsma et al. [113]</td>
<td>30/0</td>
<td>DB, crossover 3 wks</td>
<td>fermented milk product (125 ml) with 2.5% OFS (∼9 g)</td>
<td><em>Lactobacillus</em> (DN 112.053/112.096)</td>
<td>↓4%</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td><em>L. acidophilus</em> 145</td>
<td>↓5%</td>
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<td></td>
<td><em>B. Longum</em> 913</td>
<td>NS</td>
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<tr>
<td>Greany et al. [115]</td>
<td>22/33</td>
<td>SB, parallel 7.4 wks</td>
<td>3 × capsule (containing 10^7 CFU of probiotic strain and 10–15 mg OFS per capsule)</td>
<td>*Lactobacillus DDS-1 B. Longum UADL-14</td>
<td>NS</td>
<td></td>
<td>NS</td>
<td>NS</td>
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<td><strong>Hypercholesterolaemic individuals</strong></td>
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<tr>
<td>Kießling et al. [114]</td>
<td>0/14</td>
<td>DB, crossover 6 wks</td>
<td>fermented milk product (300 g) with 1% OFS (∼3 g)</td>
<td><em>Lactobacillus</em> 145</td>
<td>↑10%</td>
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<td></td>
<td><em>B. Longum</em> 913</td>
<td>↑25%</td>
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<tr>
<td>Ooi et al. [117]</td>
<td>18/14</td>
<td>DB, parallel 12 wks</td>
<td>capsule containing probiotic with 0.8 g mulin</td>
<td><em>Lactobacillus</em> 913</td>
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<tr>
<td>Type II diabetics</td>
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<td><em>Lactobacillus</em> 913</td>
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<tr>
<td>Morotó et al. [118]</td>
<td>0/20</td>
<td>DB, parallel 4 wks</td>
<td>Shake (200 ml) with 2 g mulin</td>
<td><em>Lactobacillus</em> 913</td>
<td></td>
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</table>

Abbreviations: CFU, colony forming units; DB, double blind; F, female; GOS, galacto-oligosaccharide; M, male; NA, not analysed; NS, not significant; SB, single blind; TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; OFS, oligofructose; TAG, triacylglycerol. *HDL-C concentration was no longer significant after correction for baseline concentration.
authors commented that the changes in the numbers of *B. bifidum* were particularly evident in individuals with little or no *B. bifidum* in their faecal samples at the beginning of the study, suggesting additional benefit of synbiotic consumption in individuals with more unbalanced gut microbiota. Using 1H Nuclear Magnetic Resonance spectroscopy, Ndagijimana et al. reported that feeding healthy individuals with a synbiotic food containing *Bifidobacteria longum* (strain not defined), *Lactobacillus acidophilus* (strain not defined) and OFS (0.5 g) for 30 days significantly influenced the metabolic activity of the intestinal microbiota. In particular, an increase in specific metabolites such as the SCFA butyrate was positively correlated with the number of bifidobacteria in the faeces after supplementation and negatively correlated with the initial level [121]. Maintenance of high numbers of bifidobacteria in the gut may be beneficial in terms of healthy gut function; however, the effect of this synbiotic product on the fasting lipid profile needs to be further investigated. A lower dose of prebiotic (2.75 g) added to a lactobacillus fermented milk has been shown to significantly increase the number of bifidobacteria when fed over a seven week period in healthy human subjects [112]. If this effect was a result of the synbiotic used in this study, the use of lower doses of prebiotics that can be used in synbiotic preparations should help to reduce gastrointestinal complaints observed when a prebiotic is used alone and improve the acceptability of these types of products by the general public.

5. Conclusion

The demographic shift towards an older population in Western societies will result in a higher incidence of CHD, and a significant increase in healthcare costs [122]. As a result, there is considerable interest in dietary strategies which can reduce circulating lipid levels, but which also have comparable efficacy to commonly prescribed drug treatments. Data from animal and in vitro studies have suggested that supplementation with probiotics, prebiotics or synbiotics may be a potential strategy. Our review reports evidence that probiotics significantly reduce plasma total and LDL-C cholesterol particularly in hypercholesterolaemic groups. However, there are differential effects of bacterial species which needs further investigation in randomly controlled studies. Other lipid effects are minimal. Although prebiotics have shown marked effects on both TAG and total cholesterol levels in animal studies, a meta-analysis has reported prebiotics to have a favourable effect on fasting TAG, especially in those with hypertriglyceridaemia and type II diabetes. However, there are inconsistent findings in relation to prebiotics and further studies are warranted to provide convincing evidence on their lipid lowering properties. Only a limited number of studies have been performed with synbiotics, but initial findings with respect to changes in lipid levels are encouraging, and suggest that in combination with lactic acid bacteria, lower doses of prebiotic fibres could be used in these preparations. This may help to improve the consumer acceptability of these products by reducing the gastrointestinal complaints often associated with some high-dose prebiotic preparations.

A number of mechanisms have been proposed to explain the lipid lowering effects of both probiotics and prebiotics. Probiotics are thought to impact on cholesterol levels via production of SCFA, cholesterol assimilation or increased bile acid secretion. Fermentation of the prebiotic fibres by the gut microflora also increases the levels of SCFA, which are thought to have systemic effects on *de novo* lipogenesis. Therefore, inconsistent effects on fasting lipid levels observed with prebiotics compared with probiotics may reflect greater dependence on this pathway for modulating lipid metabolism, since *de novo* lipogenesis is extremely low in humans. Therefore, greater understanding of the mechanisms underlying the impact of probiotics, prebiotics and synbiotics on lipid metabolism, and in comparison with other conventional lipid lowering therapies in populations with raised lipid levels will inform dietary advice for populations ‘at risk’ of developing CHD.

References


K.G. Jackson and J.A. Lovegrove / Functional foods and lipid metabolism


