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Functional foods, blood lipids and coronary heart disease

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Abstract

For the past 20 years, the principal focus of public health strategies for reducing the risk of coronary heart disease (CHD) has been aimed at lowering serum cholesterol levels. However, recent findings have highlighted not only cholesterol but also triacylglycerol as a significant lipid risk factor for CHD. Dietary strategies which are able to reduce these circulating lipid levels and which are able to offer long-term efficacy comparable with effective drug treatments are currently being sought. One dietary strategy that may benefit the lipid profile involves supplementation of the diet with prebiotics, probiotics and synbiotics; this method improves the health of the host by supplementation with and/or fortification of certain health promoting bacteria present in the gastrointestinal tract, by dietary means. Probiotics (live organisms) in the form of fermented milk products have been shown to exert cholesterol lowering properties, whereas non-digestible fermentable carbohydrate prebiotics have been shown to reduce triacylglycerol levels in animal studies. However, in human studies using both prebiotics and probiotics, there have been inconsistent findings with respect to changes in lipid levels, although, on the whole, there have been favourable outcomes.

Keywords: cholesterol; fermented milk products; fructans; glucose; insulin; prebiotics; probiotics; synbiotics; triacylglycerols

Abbreviations: CHD, coronary heart disease; GIP, glucose-dependent insulinotrophic polypeptide; GLP-1, glucagon-like peptide-1; HDL, high-density lipoprotein; LDL, low-density lipoprotein; OFS, oligofructose; TAG, triacylglycerol; VLDL, very low-density lipoprotein.

1 Coronary heart disease and risk factors

Diseases of the circulatory system account for an appreciable proportion of total morbidity and mortality in adults worldwide. Coronary heart disease (CHD) accounts for around 115 000 deaths each year in the United Kingdom alone (Department of Health 1999). The rates of mortality due to CHD vary considerably throughout the world; for example, in Finland, the annual incident rate has been reported to be thirteen times higher than that reported in Japan (Keys 1980). The incidence rate is reducing in most countries, although in Eastern Europe and developing countries, CHD is still increasing in incidence.

The underlying basis for clinical cardiovascular disease is a combination of atherosclerosis and thrombosis (Mangiapane and Salter 1999). Atherosclerosis is a condition in which the arterial lining is thickened in certain locations by raised plaques which occur as a result of the excessive accumulation of modified lipids (predominantly cholesterol), and of proliferation and migration of smooth muscle cells from deeper layers within the arterial wall. Formation of an atherosclerotic plaque can occlude one or more arteries, mainly coronary and cerebral, resulting in CHD or a stroke, respectively. In addition, a superimposed thrombus or clot may further occlude the artery, with the latter being an acute event which causes a myocardial infarct (Mangiapane and Salter 1999).

CHD is a multifaceted condition which has no one single cause. Potential risk factors are being identified concurrently with progress in CHD research. The known risk factors for development of CHD are shown in Table 1. This review is mainly concerned with blood lipids and CHD risk. Epidemiological studies examining CHD risks in different populations have observed a positive correlation between fasting cholesterol levels in individuals, especially that of low-density lipoprotein (LDL) cholesterol, and the development of CHD (Keys 1970). The role of cholesterol reduction as a public health strategy in the primary prevention of CHD was unequivocally supported by the findings of the West Scotland Heart Study (Shepherd *et al.* 1995). However, in addition to plasma cholesterol (a classic risk factor) other lipid risk factors have also been identified. These include low levels of high-density lipoprotein (HDL)

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Table 1. Risk factors for the development of coronary heart disease

Unmodifiable	Gender (male)
	Increasing age
	Genetic traits (including lipid metabolism abnormalities)
	Body build
	Ethnic origin
Modifiable	Cigarette smoking
	Some hyperlipidaemias (increased plasma cholesterol and triacylglycerol)
	Low levels of high-density lipoprotein (HDL)
	Obesity
	Hypertension
	Low physical activity
	Increased thrombosis (ability to clot)
	Stress
	Alcohol consumption
Diseases	Diabetes (glucose intolerance)
Geography	Climate and season (cold weather)
	Soft drinking water

cholesterol (Gensini *et al.* 1998), an elevated concentration of triacylglycerol (TAG; a major fat in the blood) in both the fasted and fed (postprandial) states (Austin, 1991; Karpe *et al.* 1994; Wilson 1994) and insulin resistance (Reaven *et al*. 1993; Haffner *et al.* 1999).

There is a substantial, diverse, and generally consistent body of evidence linking diet and CHD. Current dietary strategies for the prevention of CHD advocate adherence to low-fat/low-saturated-fat diets (Department of Health 1994). Although there is no doubt that, under certain experimental conditions, low-fat diets do offer an effective means of reducing blood cholesterol, on a population basis they appear to be less effective. This is largely due to poor compliance attributed to the poor palatability and acceptability of such diets to the consumer. Attempts have therefore been made to identify other dietary components which can address lipid abnormalities associated with CHD. Recently, dietary intervention strategies based around probiotics and prebiotics have been used with varying degrees of success in lowering plasma cholesterol and TAG levels.

2 Effects of probiotics on blood lipids: the evidence

A probiotic has been recently redefined as 'a live microbial food ingredient that is beneficial to health' (Salminen *et al.* 1998). Since the early 1970s, research into probiotics and CHD risk has increased dramatically, although to date the data have been inconsistent and inconclusive (Table 2).

2.1 Evidence from the Maasi

Mann and Spoerry (1974) investigated the effect of an exogenous surfactant material, known to be hypercholesterolaemic, in 24 male Maasi warriors. Although a significant fall (9.8%) in serum cholesterol was reported, because of a number of confounding factors including the introduction of an exercise programme, coupled with an inability to control food intake and subject weight gain, this study is now considered simply as a curiosity and does not lend much to overall findings.

2.2 Evidence for the milk factor

As a follow-up to the Maasi trial, Mann (1977) fed a small group (n = 4) of US volunteers 4 l of yoghurt (microbiological activity unspecified) per day over a 12-day period, and reported a significant fall of 37% in serum cholesterol. When the intake of the yoghurt was reduced to 2 l per day, this hypocholesterolaemic effect was maintained, although intake of 1 l per day resulted in a return to baseline cholesterol levels. The rate of cholesterol biosynthesis was monitored by measuring specific activity of plasma digitonin-precipitated sterols, 2 h after a pulse of [14C] acetate. A 28% fall in acetate incorporation was reported 16 days after a 12 day ingestion of the high yoghurt dose (4 l per day). To help explain the fall in serum cholesterol, Mann proposed the presence of a *milk factor* such as hydroxymethyl glutarate (an inhibitor of 3 hydroxy-methyl-glutaryl CoA reductase which is an important enzyme involved with cholesterol synthesis in the liver).

Drinking milk has also been shown to be associated with reduced mortality from CHD. At least seven prospective cohort studies have investigated the effect of milk consumption on death from CHD. The body of evidence suggests that drinking milk is not detrimental and may be mildly protective against CHD (Ness *et al.* 2001). Intervention studies on milk and plasma cholesterol levels are mixed: some studies show beneficial reductions of LDL-cholesterol after three daily servings of low-fat milk (Barr *et al.* 2000; Obarzanek *et al.* 2001), while others do not (Buonopane *et al.* 1992). The general consensus is that milk consumption *per se* does not increase CHD risk, but neither does it consistently reduce lipid risk factors.

Howard and Marks (1979) investigated candidates for the milk factor and fed lactose (with or without Ca/Mg), cheese whey or yoghurt to volunteers over a 2 wk period. The yoghurt alone significantly reduced plasma cholesterol by 5.5%. However, this trial was also subject to the same problems of lack of dietary control as the Mann and Spoerry study, with substantial changes from the volunteers' habitual diet resulting in a number of confounding factors. Unfortunately, most of the early studies introduced confounding factors due to a lack of control in the subjects' diet. Hepner and colleagues (1979) performed a study which attempted to control for such factors. This investigation was a crossover study in which 720 ml of yoghurt and 750 ml milk were given to the subjects over a

Table 2. Summary of human studies to evaluate the hypocholesterolaemic properties of fermented milk products

Author	Subjects (n)	Product (vol/type)	Duration	Total cholesterol	LDL cholesterol
Mann *et al.* (1974)	24M	8.3 l lacto/yoghurt	3 weeks	-9.6% (P<0.001)	NA
Mann (1977)	3M 1F	4 l WMY	12 days	-16.8% (P<0.05)	NA
Mann (1977)	3M 2F	2l SMY	12 days	-23.2% (P<0.05)	NA
Howard *et al.* (1979)	10	3 l	3 weeks	5.5% (P<0.05)	NA
Hepner *et al.* (1979)	6M 4F	720 ml (A)	4 weeks	-5.4% (P<0.01)	NA
Hepner *et al.* (1979)	5M 3F	720 ml (B)	4 weeks	8.9% (P<0.01)	NA
Rossouw *et al.* (1979)	11M	2 l yoghurt	3 weeks	+16% (P<0.01)	+12% (P<0.001)
Thompson *et al.* (1982)	13	11 UPY	3 weeks	NS	NS
Bazzare *et al.* (1983)	5M 16F	550 g yoghurt	1 week	-8.7% NA	NA
Massay (1984)	30F	480 ml yoghurt	4 weeks	NS	NS
Jaspers *et al.* (1984)	10M	681 g yoghurt	2 weeks	-11.6% transient (P<0.05)	NS
McNamara *et al.* (1989)	18M	16 oz LFY	4 weeks	NS	NS
Agerbaek *et al.* (1995)	58M	200 ml UPY	6 weeks	-6.1% (P<0.001)	-9.8% (P<0.001)
Richelsen *et al.* (1996)	47M 43F	200 ml UPY	21 weeks	NA	-9% transient (P<0.05)
Sessions *et al.* (1997)	78M 76F	200 ml UPY	12 weeks	NS	NS
Bertolami *et al.* (1999)	11M 21F	200 ml UPY	8 weeks	-5.3% (P<0.004)	6.2% (P<0.001)
De Roos *et al.* (1999)	78	500 ml UPY	6 weeks	NS	NS
Agerholm-Larsen *et al.* (2000)	20M 50F	450 ml UPY	8 weeks	NS	-8.4% (P<0.05)

F, female; M, male; WMY, whole milk yoghurt; UPY, unpasteurised yoghurt; SMY, skimmed milk yoghurt; PY, pasteurised yoghurt; LFY, low fat yoghurt; NA, data not available; NS, not statistically significant.

Adapted from Taylor & Williams 1998

4 wk period. Significant reductions in plasma cholesterol were observed after the first week of both supplementation periods (Hepner *et al.* 1979). The observation that cholesterol levels could fall significantly after acceptance onto a study has been well documented (Rossouw *et al.* 1981; Sessions *et al.* 1997). This is probably due to a conscious or even subconscious modification of the diet by the volunteers due to awareness of dietary assessment. In an attempt to reduce this confounding factor, baseline run-in periods are essential (Sessions *et al.* 1997). Of the early negative studies that have been published, those of Thompson *et al.* (1982), Massey (1984) and McNamara *et al.* (1989) have incorporated a run-in period. The study of McNamara *et al.* was one that was more carefully controlled. They investigated effects of the ingestion of 480 ml unspecified yoghurt and reported no significant cholesterol reduction. From the evidence presented above, it can therefore be concluded that there is little evidence that milk or fermented milk products affect serum lipid parameters *per se*.

2.3 Probiotic effect on lipid parameters

Hepner *et al.* (1979) assessed whether the presence of live bacteria was important for reported effects of yoghurt on lipid parameters. The aim of the study was to compare the effects of 750 ml of pasteurised and unpasteurised yoghurt, using milk as the placebo. After a 12 wk intervention period, all treatments significantly reduced plasma cholesterol, 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.* (1982) analysed a wide range of milk-based products including milk fortified with *Lactobacillus acidophilus* (titre 1.3 x 10^7 counts/ml), buttermilk (a milk product fermented with *Streptococcus cremoris* and *Streptococcus lactis* - titre 6.4 x 10^8 counts/ml) and a yoghurt (fermented with *Lactobacillus bulgaricus* and *Streptococcus thermophilus* - titre 1.2 x 10^9 counts/ml). A 1 l supplement was offered for a 3 wk period, but no significant changes were reported in serum total cholesterol, LDL or HDL. Another study (De Roos *et al.* 1999) involving seventy-eight male and female

participants (mean cholesterol 5.4 ± 0.7 mmol/l) investigated the effect of consuming 500 ml of control yoghurt or yoghurt enriched with *Lactobacillus acidophilus* L-1 for a 6 wk period. No significant reduction in cholesterol or LDL cholesterol was reported in this study.

The possible importance of variation in yoghurt cultures stimulated Jasper *et al.* (1984) to assess the effect on plasma lipid levels of consuming 681 g per day of different samples of a non-fat unpasteurised yoghurt fermented with a 1:1 ratio of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Two yoghurt strains (CH-I and CH-II) were taken for a 14 day period each, followed by a third strain (SH-III) which was taken for 21 days. A 21 day 'washout period' was permitted between each dietary intervention period to re-establish normal serum cholesterol levels. Significant falls in serum total cholesterol and LDL cholesterol levels occurred after 1 wk with strain CH-II and 2 wk with strain SH-III. Such transient changes could be explained either by effects of volunteers commencing the study as discussed previously, or could be a true difference between the efficacious properties of different strains and indeed different types of probiotics.

A commercially available yoghurt, GAIO®, containing a specific bacterial culture, CAUSIDO® (consisting of *Enterococcus faecium* and *Streptococcus thermophilus*, and has been shown to have hypocholesterolaemic properties when tested on animals) has been studied, albeit with inconsistent findings. Agerbaek *et al.* (1995) tested the effect of GAIO® against an identical yoghurt that had been chemically fermented with an organic acid (δ-gluco-lactone). Fifty-eight middle-aged men with moderately raised cholesterol levels (5.0-6.5 mmol/l) were fed 200 ml per day of yoghurt for a 6-wk period. They observed a 9.8% reduction in LDL cholesterol levels ($P < 0.001$) for the live yoghurt group. Although this was a well-controlled study, an unforeseen skew in randomisation resulted in a significantly different baseline total and LDL cholesterol levels in the two groups. The reduction in these parameters observed in the live yoghurt group could be ascribed to a regression towards the mean. Another study performed using the same yoghurt and a similar design for a longer period (6 months) was carried out in 87 men and women aged between 50 and 70 years (Richelsen *et al.* 1996). Although after 12 wk there was a significant drop in LDL cholesterol levels in the group taking the active yoghurt, these reductions were not sustained. This was partly explained by a reduction in the titre of the yoghurt at 12 wk. A more recent study investigated the same product at 200 g per day for 8 wk with 32 patients who had mild to moderate hypercholesterolaemia. The patients were asked to follow a lipid-lowering diet for 8 wk and then were given the test or control product for two 8 wk periods. The results from this study showed a significant reduction (5.3% ($P = 0.004$) and 6.2% ($P = 0.01$), respectively) in total and LDL cholesterol after consumption of the active product. However, the authors did question whether or not an average reduction of approximately 5% for total and LDL cholesterol was clinically important (Bertolami et al. 1999). A more recent publication investigated the effects of consumption of a larger volume (450 ml per day) of GAIO® yoghurt containing the CAUSIDO® culture on seventy overweight and obese men and women for 8 wk. After adjustment for changes in body weight, a significant reduction in LDL-cholesterol (8.4%) was reported in the GAIO® group (Agerholm-Larsen *et al.* 2000a).

However, not all studies have reported a significant reduction in cholesterol after GAIO® ingestion. One study reported by Sessions *et al.* (1997) was conducted in 160 middle-aged men and women with moderately raised cholesterol. Volunteers consumed 200 ml per day of either the active (containing a minimum titre of 1×10^6 counts/ml) or chemically fermented yoghurt for a 12 wk period. Stratified randomisation was used and the study was well controlled. During the 2 wk run-in period, both groups showed significant reductions in blood cholesterol ($P < 0.05$), but thereafter there were no further changes in either group or between the groups at any of the time points.

A meta-analysis of short-term intervention studies using GAIO® was published by Agerholm-Larsen *et al.* (2000b). They concluded that consumption of the fermented yoghurt produced 4 and 5% decreases in total and LDL cholesterol respectively; however, long-term studies were required to demonstrate sustained effects on blood lipids.

2.4 Possible mechanisms of action

It is important to highlight the fact that viable and biologically active microorganisms are usually required at a specific target site in the host. It is therefore essential that the probiotics involved not only exert the characteristics that are necessary to produce any 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 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 (Huis In't Veld and Shortt 1996).

The mechanism of action of probiotics on cholesterol reduction is unclear, although a number of possible mechanisms have been proposed. These include the physiological actions of fermentation end products (short chain fatty acids), deconjugation of bile acids (which could reduce cholesterol by co-precipitation at acidic pH or by increasing excretion of bile acids, thereby increasing the amount of cholesterol required for *de novo* synthesis in the liver, or a combination of both these mechanisms), cholesterol assimilation, and cholesterol binding to bacterial cell walls. The short chain fatty acids that are produced by bacterial anaerobic breakdown of carbohydrate are acetic, propionic and butyric acids. The physiological effects of these are discussed in more detail in the prebiotic section below.

It has been well documented that microbial metabolism of bile acid is a peculiar probiotic effect involved in the therapeutic role of some bacteria. The deconjugation reaction is catalysed by a conjugated bile acid hydrolase enzyme, which is produced exclusively by bacteria. Deconjugation is widely found in many intestinal bacteria including genera such as *Enterococcus*, *Peptostreptococcus*, *Bifidobacterium*, *Fusobacterium*, *Clostridium*, *Bacteroides* and *Lactobacillus* (Hylemond 1985). This reaction liberates an amino acid moiety and a 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. For example, Grill *et al.* (1995) reported *Bifidobacterium longum* as being the most efficient bacterium when tested against six different bile salts. Another study reported that *Lactobacillus* species had varying abilities to deconjugate glycocholate and taurocholate (Gilliland and Speck 1977). Studies performed on *in vitro* responses are useful, although *in vivo* studies in animals and humans are required to more fully determine the contribution of bile acid deconjugation to cholesterol reduction. Intervention studies on animals and ileostomy patients have shown that oral administration of certain bacterial species can lead to an increased excretion of free and secondary bile salts (De Smet *et al.* 1998; Marteau *et al.* 1995).

There is also *in vitro* evidence to support the hypothesis that some bacteria can assimilate (take up) cholesterol. It has been reported that *Lactobacillus acidophilus* (Gilliland *et al.* 1985) and *Bifidobacterium bifidum* (Rasic *et al.* 1992) have the ability to assimilate cholesterol during *in vitro* studies, but only in the presence of bile salts and under anaerobic conditions. However, despite such reports, there is uncertainty about whether the bacteria are assimilating cholesterol or whether cholesterol is co-precipitating with the bile salts. Studies have been performed to address this question; for example, Klaver and Van der Meer (1993) concluded that removal of cholesterol from the medium in which *Lactobacillus acidophilus* and *Bifidobacterium* were growing was not due to assimilation, but due to bacterial bile salt deconjugase activity. The same question was addressed by Tahri *et al.* (1995) but with conflicting results: 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.

Cholesterol binding to bacterial cell walls has also been suggested as a possible mechanism for the hypocholesterolaemic effects of probiotics. Hosono and Tono-oka (1995) reported that *Lactococcus lactis* subsp. *lactis* biovar. diacetylactis R-43 had the highest binding capacity for cholesterol for a range of bacteria tested. It was speculated that differences in binding of the bacteria were due to chemical and structural properties of their cell walls, and that even non-viable cells may have the ability to bind cholesterol in the host intestinal tract.

The mechanism of action of probiotics on cholesterol reduction could include one or all of the above mechanisms, with an ability of different bacterial species to have varying effects on cholesterol lowering. Moreover, inconsistencies in the literature regarding beneficial effects of probiotics could be due to a number of different factors. The period of intervention, volume consumed and, more importantly, the bacterial titre of the product are all important considerations that may vary (Thompson *et al.* 1982; Richelsen *et al.* 1996). The baseline concentrations of cholesterol also affect study outcomes, with hypercholesterolaemic groups responding to probiotics in some (Agerbaek *et al.* 1995; Agerholm-Larsen *et al.* 2000a) but not all (Sessions *et al.* 1997; De Roos *et al.* 1999) studies. The study outcome depends also on the bacterial species used, its metabolic capabilities, and viability throughout the gastrointestinal tract (Jasper *et al.* 1984). Further research is required to more fully elucidate the relative importance of such parameters before the true beneficial effects and mechanism of action of probiotics in relation to CHD can be determined.

3 The effect of fructan-type oligosaccharide prebiotics on lipid metabolism in humans

In recent years, there has been increasing interest in the important nutritional roles of prebiotics as functional food ingredients. A prebiotic is a selectively metabolised dietary ingredient that stimulates 'beneficial' microorganisms in the lower gut. This interest has been derived from animal studies which have shown marked reductions in TAGs and, to a lesser extent, cholesterol levels when diets containing significant amounts of the prebiotic (oligofructose (OFS)) were fed. However, studies conducted in humans examining the effects of prebiotics on plasma lipids levels have generated inconsistent findings (Table 3). In individuals with raised blood lipids, three studies have shown significant decreases in fasting total and LDL cholesterol, with no significant changes in TAG levels (Yamashita *et al.* 1984; Hidaka *et al.* 1991; Davidson *et al.* 1998), whereas one recent study in type II diabetics did not reveal any change in cholesterol levels (Alles *et al.* 1999). In normolipidaemic subjects, only two studies involving supplementation with inulin have demonstrated significant changes in both fasting TAG and total cholesterol levels (Canzi *et al.* 1995; Brighenti *et al.* 1999), with another study showing a significant decrease in TAG levels only (Causey *et al.* 2000). However, Luo *et al.* (1996) and Pedersen *et al.* (1997) have recently reported no effects of OFS or inulin treatment on plasma lipid levels in young healthy subjects. In a group of middle-aged men and women, lower plasma TAG levels were observed 8 wk after inulin treatment compared to a placebo (Jackson *et al.* 1999). In this study, follow-up blood samples were taken 4 wk after completion of the supplementation period, by which time concentrations of TAG had returned to baseline values, thus supporting the conclusion that inulin feeding

Table 3. Summary of human studies to examine the effects of fructan supplementation on blood lipids

Author	Subjects	Fructan	Dose	Study design	Duration	Vehicle	Significant changes observed in:	
							Blood lipids	Glucose
Yashashati *et al*. 1984	8M and 10F Type II diabetes	OFS	8 g	DB parallel	2 weeks	packed coffee drink	↓ TC ↓ LDL-C	↓ glucose
Hidaka *et al*, 1991	37 (M and F) hyperlipidaemic	OFS	8 g	DB parallel	5 weeks	canned coffee jelly confectionery	↓ TC	NS
Canzi *et al*. 1996	12 M normolipidaemic	inulin	9 g	Sequential	4 weeks	breakfast cereal	↓ TAG ↓ TC	N/A
Luo *et al*. 1996	12 M normolipidaemic	OFS	20 g	DB crossover	4 weeks	100 g biscuits	NS	NS
Pedersen *et al*. 1997	66 F normolipidaemic	inulin	14 g	DB crossover	4 weeks	40 g margarine	NS	N/A
Davidson *et al*. 1998	21 (M and F) hyperlipidaemic	inulin	18 g	DB crossover	6 weeks	chocolate bar/paste or coffee sweetener	↓ LDL-C ↓ TC	N/A
Alles *et al*. 1999	9 M and 11 F Type II diabetes	OFS	15 g	SB crossover	3 weeks	supplement not specified	NS	NS
Brighenti *et al*. 1999	12 M normolipidaemic	inulin	9 g	Sequential	4 weeks	breakfast cereal	↓ TC ↓ TAG	NS
Jackson *et al.* 1999	54 (M and F) normolipidaemic	inulin	10 g	DB parallel	8 weeks	powder added to food and drinks	TAG	↓ insulin
Causey *et al*. 2000	12 M normolipidaemic	inulin	20 g	DB crossover	3 weeks	low-fat ice-cream	↓ TAG	NS

M, male; F, female; DB, double blind; N/A, not measured; NS, not significant; SB, single blind; TC, total cholesterol; LDL-C, LDL cholesterol; OFS, oligofructose; TAG, triacylglycerol

may have been responsible for the decrease in TAG levels.

Raised postprandial TAG concentrations have been recognised as a risk factor for CHD (Austin 1991). Data from studies in rats have shown a 40% reduction in postprandial TAG concentrations when diets containing 10% (w/v) OFS were fed (Kok *et al.* 1998). However, very little information regarding the effects of prebiotics on postprandial lipaemia in human subjects are available, although one recent study in middle aged volunteers has shown no effect of inulin treatment on postprandial TAGs (Williams 1998).

The marked reduction in fasting lipid levels, notably TAGs, which has been observed in animal studies has not been consistently reproduced in humans. The amount of fructans used in the human studies listed in Table 3 varies between 9 and 20 g; this is small compared to that used in animal trials (50-200 g OFS/kg rat chow; Roberfroid 1993) and is equivalent to a dose in humans of approximately 50-80 g OFS or inulin per day. The prebiotic nature of OFS and inulin restricts its dosage in humans of up to 15 g per day since doses greater than this cause gastrointestinal symptoms such as stomach cramps, flatulence and diarrhoea (Pedersen *et al.* 1997). It is not known whether, at the levels used in human studies, significant effects would be observed in animals (Delzenne *et al.* 1993).

The types of dietary vehicles used to increase amounts of OFS or inulin in the diet differ. In the case of Luo *et al.* (1996), 100 g of biscuits were eaten every day and for Pedersen *et al.* (1997), 40 g of margarine was consumed which may have contributed towards the negative findings in blood lipids. In the case of Davidson *et al.* (1998), significant changes in total and LDL cholesterol levels were observed over 6 wk with inulin, as compared to a placebo (sugar). The percentage change in each of the lipid parameters was calculated over each of the 6-wk treatment periods and, unexpectedly, there was an increase in total cholesterol, LDL cholesterol and TAG during the placebo phase. Non-significant falls in these variables were observed during inulin treatment; therefore, when net changes in the variables were calculated (change during inulin minus that during the control treatment), there were significant differences in total and LDL cholesterols between the two treatments. The authors attributed the increase in total and LDL cholesterol levels during the placebo phase to be related to an increased intake of saturated fatty acids in the chocolate products which were used as two of the vehicles in the study (Davidson *et al.* 1998). In later studies, the use of inulin in its powdered form enabled it to be added to many of the foods eaten in the subjects' normal diet without any need for dietary advice

thus avoiding changes in body weight. Since inulin has water binding properties, as a powder it can be added to orange juice, tea, coffee, yoghurt and soup (Jackson *et al.* 1999).

The significant relationship between subjects' initial TAG concentration and the percentage change in TAG levels over the 8 wk study demonstrated by Jackson *et al.* (1999) lends support to the hypothesis that the initial TAG levels could be important in determining the degree of response to inulin. The lack of response in some individuals may be as a result of their being less responsive to inulin, variations in their background diet or non-compliance with the study protocol. Speculation as to possible reasons for the variability in response would be aided by an improved understanding of the mechanism of action of inulin on plasma TAG levels.

The length of supplementation period used in the studies summarised in Table 3 may be another factor for the inconsistent findings in changes in TAG levels in human subjects given supplements of inulin. The studies were conducted over 2-8 wk, with significant effects occurring in only three studies conducted over 3-4 wk (Brighenti *et al.* 1999; Canzi *et al.* 1996; Causey *et al.* 2000). The lack of lipid lowering noted in the studies of Alles et al. (1999), Pedersen *et al.* (1997) and Luo *et al.* (1996) may be due to insufficient duration of supplementation in their chosen subject groups. In the study of Jackson *et al.* (1999), a trend for TAG reduction upon inulin treatment occurred sometime between 4 and 8 wk; this may reflect the period necessary for modification of the gut microflora. A 4 wk wash out seemed to be sufficient for the TAG concentrations to return to baseline values.

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

3.1 Mechanism of lipid lowering by inulin and oligofructose

Prebiotics have been shown to be an ideal substrate for health promoting bacteria in the colon, notably bifidobacteria and lactobacilli (Gibson and McCartney 1998). During the fermentation process, a number of by-products are produced, including gases (hydrogen sulphide, carbon dioxide, hydrogen and methane), lactate and short chain fatty acids (acetate, butyrate and propionate). The short chain fatty acids 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 (Wolever *et al.* 1989; Demigne *et al.* 1995). 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 (Van Loo *et al.* 1999). The type of short chain fatty acids which are produced during fermentation is dependent on the gut microflora that are stimulated by the prebiotic. Inulin, for example, has been shown to increase both acetate and butyrate levels (Van Loo *et al.* 1999).

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 hypolipaemic action of OFS was associated with an inhibition of *de novo* cholesterol synthesis by propionate, following impairment of acetate utilisation by the liver for *de novo* lipogenesis (Demigné *et al.* 1995). This is in agreement with human studies where rectal infusions of acetate and propionate resulted in the latter being able to inhibit the incorporation of acetate into TAGs released from the liver (Wolever *et al.* 1995). Fiordaliso *et al.* (1995) demonstrated significant reductions in plasma TAGs, phospholipids and cholesterol in normolipidaemic rats fed a chow diet containing 10% (w/v) OFS. The TAG-lowering effect was demonstrated after only 1 wk of OFS and was associated with a reduction in very low density lipoprotein (VLDL) secretion. TAGs and phospholipids are synthesised in the liver by esterification of fatty acids and glycerol-3-phosphate before being made available for assembly into VLDL, suggesting that the hypolipidaemic effect of OFS may be occurring in the liver. The reduction observed in cholesterol levels in the rats was only demonstrated after long term feeding (16 wk) of OFS. Recent evidence has suggested that the TAG-lowering effect of OFS occurs via a reduction in VLDL TAG secretion from the liver due to a reduction in the activity of all lipogenic enzymes (acetyl-CoA carboxylase, fatty acid synthase, malic enzyme, ATP citrate lyase and glucose-6-phosphate dehydrogenase), and, in the case of fatty acid synthase, via modification of lipogenic gene expression (Delzenne and Kok 1998; Figure 1). However, further work is required to determine the mechanisms whereby short chain fatty acids lower cholesterol levels in humans.

3.2 The effect of prebiotics on glucose and insulin levels

Very little is known about the effects of prebiotics on fasting insulin and glucose levels in humans. Of the supplementation studies that have been conducted in humans, a significant reduction in glucose was observed in subjects with type II diabetes (Yamashita *et al.* 1984) and a trend for a reduction in glucose was observed in hyperlipidaemic subjects (Hidaka *et al.* 1991) consuming OFS. However, a recent study has reported no effect of OFS on blood glucose levels in type II diabetics (Alles *et al.* 1999). A significant reduction in insulin levels was observed in healthy middle-aged subjects with inulin, although this was not accompanied by changes in plasma glucose levels (Jackson *et al.* 1999). The effect of ingestion of acute test meals containing OFS on blood glucose, insulin and C-peptide levels in healthy adults showed a

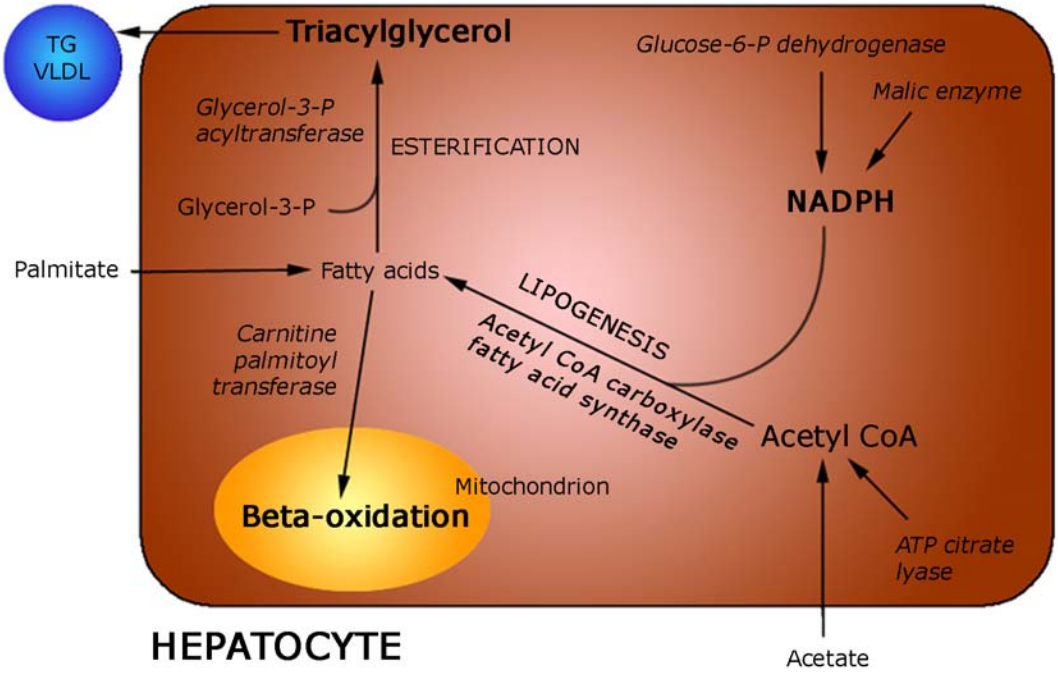

Figure 1. Hepatic fatty acid metabolism

trend for a lower glycaemic response and peak insulin levels, following the OFS enriched meals (Rumessen *et al.* 1990).

It has been suggested that the mechanism of action of prebiotics on lowering of glucose and insulin levels is associated with short chain fatty acids, especially propionate. A significant reduction in postprandial glucose concentration was observed following both acute and chronic intakes of propionate-enriched bread (Todesco *et al.* 1991). The effect of propionate intake on postprandial insulin levels was not investigated. A recent animal study has shown an attenuation of both postprandial insulin and glucose levels following 4 wk of feeding with OFS. These effects were attributed to the actions of OFS on the secretion of the gut hormones glucose dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1) (Kok *et al.* 1998). These hormones are secreted from the small intestine (GIP) and the terminal ileum and colon (GLP-1), and contribute towards the secretion of insulin following a meal in the presence of raised glucose levels (Morgan 1998).

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 that is low in fat and high in carbohydrate. Thus, *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 a different mechanism involving enhanced clearance of TAG-rich lipoproteins. An increased GIP secretion in OFS treated rats was observed by Kok *et al.* (1998) 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 fat ingestion (Knapper *et al.* 1995). The release of GIP and GLP-1 in the colon may act to mediate systemic effects of prebiotics such as inulin and OFS, on blood lipid, insulin and glucose levels. However, further work is required to determine the metabolic pathways that are influenced by prebiotics. Their effect on gastrointestinal kinetics such as gastric emptying and modification of the levels of TAG-rich lipoproteins and glucose in the circulation has recently been proposed as a potential modulator of systemic effects (Delzenne 1999). Therefore the design of future studies to investigate the effect of prebiotics in humans should consider the choice of subjects, length of supplementation period and type of vehicle used to increase the intake of prebiotics in the diet, as all of these variables may influence the outcome of the study.

4 The effects of synbiotics on coronary heart disease

The use of synbiotics as functional food ingredients is a new and developing area; very few human studies have been performed which look at their effect on risk factors for CHD. A synbiotic is a combination of both probiotic organisms and probiotic compounds. In one study, the effect of a fermented milk product with and without the addition of *Lactobacillus acidophilus* and fructooligosaccharides was examined in healthy men (Schaafsma *et al.* 1998). The design of the study was a randomised placebo controlled crossover form, in which there were two treatment periods of 3 wk, with a 1 wk washout period. The authors reported a significant reduction in total and LDL cholesterol following ingestion of the fermented milk product containing both the probiotic and prebiotic, compared to the placebo fermented milk. However, these data should be interpreted with caution since there was an increase in cholesterol levels during consumption of the placebo fermented milk product. Other research has concentrated on the composition of the gut microflora. In one study of healthy subjects, a fermented milk product containing Bifidobacterium spp. with or without 18 g of inulin was given daily for 12 days. The authors concluded that administration of the fermented milk product (probiotic) substantially increased the proportion of bifidobacteria in the gut, but that this increase was not enhanced with the addition of inulin. The composition of the gut microflora was then assessed 2 wk after completion of the supplementation period; it was found that subjects who received the fermented milk product with inulin maintained their gut bifidobacterial population compared to subjects receiving the fermented milk product only. Although a synergistic effect on the bifidobacterial population in the gut was not observed with the synbiotic, these results suggest that either there was better implantation of the probiotic and/or there was a separate prebiotic effect on bifidobacteria already present in the gut (Bouhnik *et al.* 1996). The maintenance of high numbers of bifidobacteria in the gut flora may be beneficial in terms of maintaining healthy intestinal function, however, its effect on blood lipid reduction remains to be determined. A more recent study has shown that a lower dose of prebiotic (2.75 g) added to a *Lactobacillus*-fermented milk was able to significantly increase numbers of bifidobacteria when fed over a 7 wk period in healthy human subjects (Roberfroid 1998). If this effect was a result of the synbiotic product used in the study, the use of lower doses of prebiotics in synbiotic preparations will help to reduce the gastrointestinal complaints observed with prebiotics alone and will improve the acceptability of these types of products by the general public.

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