

## Bioactive substances in milk with properties decreasing risk of cardiovascular diseases

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Milk is often seen as a potential promotor of atherosclerosis and coronary heart disease because it is a source of cholesterol and saturated fatty acids. But there are several studies indicating that milk and milk products may not affect adversely blood lipids as would be predicted from its fat content and fat composition. There are even factors in milk and milk products which may actively protect from this condition by improving several risk factors. Calcium, bioactive peptides and as yet unidentified components in whole milk may protect from hypertension, and folic acid, vitamin B<sub>6</sub> (pyridoxine) and B<sub>12</sub> (cyanocobalamin) or other unidentified components of skim milk may contribute to low homocysteine levels. Conjugated linoleic acid may have hypolipidaemic and antioxidative and thus antiatherosclerotic properties. Epidemiological studies suggest that milk and milk products fit well into a healthy eating pattern emphasizing cereals and vegetables.

### Milk: Coronary heart disease: Risk reduction

#### Introduction

The risk of coronary heart disease (CHD) incidence and mortality increases with increasing cholesterol levels (Martin *et al.* 1986, Grundy *et al.* 1988). The widely publicized 'lipid hypothesis' concluded that a high intake of cholesterol and saturated fatty acids is largely to blame for high plasma cholesterol levels and for high CHD incidence in industrialized countries.

This is one reason why milk and milk products have long had a negative image concerning atherosclerosis and CHD. Milk fat provides cholesterol and is rich in saturated fatty acids. Both components have the potential to increase plasma cholesterol levels. However, a considerable part of the saturated fatty acids in milk fat is short-chain fatty acids and the long-chain stearic acid, which do not adversely affect cholesterol levels. Saturated fatty acids with a chain length of 12–14 C atoms are reported to be hypercholesterolaemic (Grundy & Denke, 1990). Another reason for the negative image of milk is that casein (and other animal proteins) may have hypercholesterolaemic and atherogenic effects (Pfeuffer & Barth, 1990), especially reported in sensitive animal species. However, there is no hypercholesterolaemic effect of milk proteins in humans.

In more recent years additional risk factors for CHD, including plasma fibrinogen and homocysteine levels have become apparent, and the question as to what extent a high cholesterol level contributes to the overall risk as compared to other known and unidentified risk factors is more open than ever. There is some evidence against the 'lipid hypothesis'. Although there is a correlation between CHD

mortality and milk and butterfat intake, Finland and France provide important paradoxes to the lipid hypothesis (Artaud-Wild *et al.* 1993). Indeed, the discrepancy of low CHD risk despite a diet high in saturated fatty acids and cholesterol has become widely known as the 'French paradox'. There is no positive correlation between CHD risk and animal fat intake within a population (Fehily *et al.* 1993). High milk intake is reported to be associated with a decreased ischaemic heart disease risk (Shaper *et al.* 1991). These reports suggest that milk and milk products may contain antiatherogenic bioactive substances to negate the effects of saturated fatty acids and cholesterol.

This article provides a list of cardiovascular disease (CVD) risk factors and outlines how milk or milk components and milk products may modify these risk factors in a beneficial way. Such an approach was chosen because several components may be involved in amelioration of CHD or CVD.

#### Milk and lipid levels

Both high plasma cholesterol and triglyceride levels are considered risk factors for CHD (Grundy *et al.* 1998). Earlier reports showed that skim milk or yogurt consumption may decrease plasma cholesterol levels. Although whole milk intake did not lower plasma cholesterol, however, there was no increase of plasma cholesterol either (Rossouw *et al.* 1981; Massey, 1984). When adolescents consumed 2 litres of skim milk per day for three weeks, plasma cholesterol levels decreased as compared to full cream milk or yogurt (1.8 %

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fat) (Rossouw *et al.* 1981). In a more recent study in free-living adults conducted over 8 weeks, a supplement of 1 quart (0.95 litre) of skim milk decreased plasma cholesterol and triglyceride levels in hypercholesterolaemic, but not in normocholesterolaemic subjects (Buonopane *et al.* 1992). An expert panel concluded that whole milk may not affect blood lipids as would be predicted from its fat content and fat composition (Berner, 1993).

The hypocholesterolaemic effect may be even more pronounced if modified milk or processed milk products are consumed. Sharpe *et al.* (1994) found that long-term supplementation with 90 g skim milk powder per day, equivalent to about 1 litre fluid milk, from hyperimmunized cows as compared with normal skim milk powder, decreased total (-5.2 %) and LDL cholesterol (-7.9 %) levels in hypercholesterolaemic subjects. It was assumed that this intake of immunized milk changed intestinal flora, which in turn could have contributed to an increased intestinal loss of cholesterol and bile acids. The possibility that yogurt bacteria and certain strains of probiotic bacteria and thus yogurt consumption could reduce cholesterol levels has attracted much attention since Mann & Spoerry (1974) reported on the hypocholesterolaemic effect of fermented milk in Maasai. Several studies in rodents and pigs demonstrated such a hypocholesterolaemic effect (Gilliland *et al.* 1985; Grunewald, 1982; Stoll *et al.* 1991). Often *Lactobacillus acidophilus* and *Enterococcus faecium* strains were used in these studies. Because of their relative resistance towards bile and *in vitro* potency to assimilate cholesterol (Schaafsma *et al.* 1998) these organisms are the most promising candidate bacteria. In a short-term study in normocholesterolaemic subjects, daily intake of 200 ml of a yogurt-like product, fermented with *E. faecium* and *S. thermophilus*, decreased LDL cholesterol levels by 10 % (Agerbæk *et al.* 1995). However, when such a feeding regimen was maintained for a long period, the difference in LDL-cholesterol between groups disappeared after about six months, due to continuing reduction of LDL-cholesterol in the control group (Richelsen *et al.* 1996). Recently, Schaafsma *et al.* (1998), in a double-blind cross-over study, observed a hypocholesterolaemic effect of a new milk product in middle-aged healthy volunteers. This milk product was fermented by *L. acidophilus* and contained fructooligosaccharides, 1 % fat, and its fatty acid pattern was modified by the addition of soy oil.

Conjugated linoleic acid (CLA) in milk fat also has a hypolipidaemic potential, but this has so far only been demonstrated in animals. There was a hypocholesterolaemic effect in rabbits (Lee *et al.* 1994) and hamsters (Nicolosi *et al.* 1997), but not in mice (Munday *et al.* 1999), when given in concentrations up to 0.5 % (by wt.) together with a hypercholesterolaemic diet. There was a hypotriacylglycerolaemic effect in hamsters (Nicolosi *et al.* 1997) and mice (Munday *et al.* 1999), but the effect did not depend on the dose given. The study by Nicolosi *et al.* (1997) included an experimental group which was given linoleic acid, and this group showed a similar hypotriacylglycerolaemic effect like the CLA groups. The effect in rabbits (Lee *et al.* 1994) was not significant. Considering that the average CLA concentration in milk fat is around

8 mg/g (Banni *et al.* 1996) and varies considerably with season, the amount provided by milk drinks or other milk product servings is probably far less than might produce such hypocholesterolaemic effects in humans.

### Milk and atherosclerosis

Conjugated linoleic acid is a collective term designating a mixture of positional and geometric isomers of linoleic acid, all having one set of conjugated double bonds. The two double bonds in CLA are usually either at position 9 and 11 or 10 and 12. Each double bond can be in *cis*- or *trans*-configuration. Fat originating from ruminant animals contains much higher levels of CLA than fat from non-ruminants, as ruminal bacteria produce CLA from feed-derived polyunsaturated fatty acids. Therefore, milk and milk products are a rich source of CLA. CLA concentrations in dairy products typically range between 3 and 9 mg/g fat, of which the 9-*cis*,11-*trans* isomer makes up 73–93 % (Shantha *et al.* 1995; Banni *et al.* 1996). CLA content of milk fat varies with season and is highest during pasture time. Feeding regimen, processing and storage of products may increase CLA concentrations. The 9-*cis*,11-*trans* isomer is believed to be the most biologically active compound, as it is preferentially incorporated into tissues (Ha *et al.* 1990; Ip *et al.* 1991; Lee *et al.* 1994; Nicolosi *et al.* 1997).

CLA has a strong anticarcinogenic effect (Ip *et al.* 1991). It may also act as an antiatherogenic compound. Both in rabbit (Lee *et al.* 1994) and hamster experiments (Nicolosi *et al.* 1997) animals developed less aortic lesions when given up to 0.5 % (by wt.) CLA together with an atherogenic diet. However, in rabbits a significant effect of CLA was only exerted on the connective tissue in the thoracic but not the abdominal aorta, and there was no decrease in lipid deposition. The effect in hamsters was only significant if data from all treated animals (irrespective of the CLA dose given) were pooled. In mice the aortic area covered by fatty streaks was even increased with CLA feeding (Munday *et al.* 1999).

Hypolipidaemic and antioxidative effects might be responsible for any antiatherogenic effect of CLA. As outlined before, CLA feeding lowered plasma cholesterol and triglyceride levels in rabbits (Lee *et al.* 1994) and hamsters (Nicolosi *et al.* 1997) and triacylglycerol levels in mice (Munday *et al.* 1999). Ip *et al.* (1991) found less peroxides in rat mammary gland, but not in liver of CLA-fed animals, and concluded that CLA acts as an antioxidant. This is in line with *in vitro* findings (Ha *et al.* 1990) that, upon long-term (15 days) incubation at 40°C, CLA was less prone to peroxide formation than linoleic acid, and that, upon addition to linoleic acid, CLA was more protective than ascorbic acid,  $\alpha$ -tocopherol or butylated hydroxytoluene (BHT). This study also found that the protective effect disappeared with increasing doses of CLA, a phenomenon which is also seen with some other antioxidants. The alleged antioxidant properties of CLA were questioned by a more recent *in vitro* study by Berg *et al.* (1995), which showed that CLA was more prone to

oxidation than linoleic acid and was not protective for phospholipid vesicles, in contrast to  $\alpha$ -tocopherol or BHT.

Antiatherogenic effects described in some (but not all) these animal studies were demonstrated in cholesterol-sensitive and atherosclerosis-prone animals, which were given an atherogenic diet through a relatively short life span (22, 15 and 11 weeks, respectively). It is questionable as to whether the results are of major relevance to humans consuming milk and milk products throughout life.

### Milk and hypertension

A high blood pressure is defined as a systolic blood pressure above 140 mg Hg and/or a diastolic blood pressure above 90 mg Hg. Hypertension is a serious health condition. Individuals with high blood pressure are three to four times more likely to develop CHD and seven times more likely to develop a stroke.

The DASH (Dietary Approaches to Stop Hypertension) clinical intervention trial showed that a diet rich in fruits, vegetables and low-fat dairy foods reduces blood pressure both in normotensives and mildly hypertensive individuals significantly more than a fruits and vegetables diet alone (Appel *et al.* 1997). Milk non-drinkers within the Honolulu Heart Program experienced stroke twice the rate of men consuming 450 g or more milk per day (Abbott *et al.* 1996). In the study by Buonopane *et al.* (1992) a significant decrease of systolic and diastolic blood pressure by a 0.95 litre skim milk supplement was also observed in both normo- and hypercholesterolaemic participants, whereas a hypocholesterolaemic effect was only seen in hypercholesterolaemic subjects. Thus these epidemiological and intervention studies provide some indication that high intake of low fat dairy foods can reduce blood pressure, especially within the context of a diet rich in fruits and vegetables.

Calcium (Ca) in milk may contribute to reduction of blood pressure. Numerous animal and human epidemiological and intervention studies have demonstrated an inverse relationship between dietary calcium and blood pressure (Miller & Groziak, 1997; Reusser & McCarron, 1994). Not surprisingly, such a correlation was not always observed (Ascherio *et al.* 1991). As dairy foods are a rich source of calcium and the prime source of Ca in the US and other industrialized countries, one might confer this inverse correlation from Ca intake to intake of milk products. Milk contains 1200 mg Ca/litre, and bioavailability of Ca from milk is high. Possible stimulants for Ca absorption are lactose, citric acid and in particular phosphopeptides, which form complexes with Ca in the intestine (Matsui *et al.* 1997). However, it seems that blood pressure tends to decrease with increasing Ca intake only below a threshold level of 500–600 mg per day, whereas increasing Ca intake may have little impact in individuals who are already consuming adequate amounts (Miller & Groziak, 1997). Further, intake of magnesium and potassium possibly correlates inversely with blood pressure (Reusser & McCarron, 1994). Milk is a reasonable source of these minerals.

Angiotensin I-converting enzyme (ACE)-inhibitory peptides, particularly in fermented milk, may act as

hypotensive components. Such peptides containing up to ten amino acids may be released from milk proteins through the proteolytic activity of lactic acid bacteria. Casokinins derived from  $\beta$ -casein and  $\alpha_{s1}$ -casein display the highest activity. ACE catalyses both the production of the vasoconstrictor angiotensin II and the inactivation of the vasodilator bradykinin. ACE inhibitors are believed to be competitive substrates for ACE. The structure–activity relationship of ACE inhibitory peptides has not yet been established, but it appears to be strongly influenced by the C-terminal tripeptide sequence of the substrate (Meisel, 1997; Yamamoto *et al.* 1994). Milk fermented with *Lactobacillus helveticus* CP 790 (Yamamoto *et al.* 1994), sour milk fermented with *L. helveticus* and *Saccharomyces cerevisiae*, as well as the two ACE-inhibitory tripeptides, Val-Pro-Pro and Ile-Pro-Pro, isolated from the latter sour milk (Nakamura *et al.* 1995), all showed an antihypertensive effect in spontaneous hypertensive rats. Such peptides were found in the aorta of experimental animals after oral application (Masuda *et al.* 1996), which is evidence that they were indeed absorbed. There are also studies in humans showing a hypotensive effect of fermented milk (Hata *et al.* 1996; Sharpe *et al.* 1994). When Hata *et al.* (1996) gave 95 ml of the above-mentioned sour milk or control milk to elderly hypertensive patients for 8 weeks, they found that this relatively small amount of sour milk significantly decreased blood pressure. Blood pressure remained decreased for 4 weeks after cessation of the daily ingestion of sour milk, a phenomenon that was also observed with other hypotensive treatments. In another study (Sharpe *et al.* 1994) skim milk powder equivalent to 1 litre of milk from immunized cows, as compared with skim milk from control cows, lowered systolic and diastolic blood pressure along with plasma cholesterol in both normotensive and hypertensive hypercholesterolaemic subjects. As this effect cannot be due to calcium, the authors hypothesized that a small-molecular-weight fraction absorbed from the intestinal tract might have been involved. However, the components were not identified. ACE-inhibitory peptides may be released from a number of food proteins, but the antihypertensive effect in humans has not been proven for most of the peptides created by food processing.

### Milk and hyperhomocyst(e)inaemia

Homocysteine is a sulphhydryl-containing amino acid that exists in several forms. Free homocysteine constitutes only a minor fraction in plasma. Oxidation leads to homocysteine disulphides, called homocystine, and homocysteine–cysteine mixed disulphides. The collective term for all these forms, free or bound to proteins, is homocyst(e)ine, abbreviated H(e) or tHcy.

Homocysteine is an intermediate compound formed by demethylation of methionine. It is catabolized to cysteine by the pyridoxal phosphate-dependent enzymes cystathione  $\beta$ -synthase (EC 4.2.1.22) and  $\gamma$ -cystathionase (EC 4.4.1.1). It is also remethylated to methionine through folate- and vitamin B<sub>12</sub>-dependent enzymes. Folic acid and betaine serve as methyl donors, whereas vitamin B<sub>6</sub> (pyridoxine) and B<sub>12</sub> (cyanocobalamin) serve as cofactors. Their availability, together with the homocysteine-metabolizing

enzymes, are important determinants of plasma H(e) concentrations.

Hyperhomocyst(e)inaemia is considered an independent risk factor for CVD. Numerous recent studies have come to the conclusion that elevated H(e) levels are associated with increased risk of both CHD (myocardial infarction), stroke, peripheral vascular disease, and thrombosis. Moderate hyperhomocyst(e)inaemia, generally defined as plasma H(e) concentrations above 15–16  $\mu\text{mol/litre}$ , occurs in 20–40 % of patients with such vascular diseases. There is a graded and continuous correlation between plasma H(e) levels and disease risk (Duell & Malinow, 1997; Verhoef *et al.* 1998). Based on meta-analyses it has been estimated that approximately 10 % of CHD cases in the USA are attributable to this disorder (Ubbink *et al.* 1996). It has also been speculated that the French paradox, i.e. low CHD incidence despite a diet high in saturated fatty acids and cholesterol, may be explained by the high folic acid intake in this country (Parodi, 1997).

There are several plausible mechanisms by which hyperhomocyst(e)inaemia may promote atherosclerosis. These mechanisms include direct damage to the endothelium, promotion of LDL oxidation, abnormalities in platelet function and clotting factors (Duell & Malinow, 1997). Hyperhomocyst(e)inaemia may be caused by genetic defects in metabolizing enzymes. Other determinants of this condition are (male) gender, (increasing) age, smoking and impaired renal function (Verhoef *et al.* 1998). But most individuals with elevated plasma H(e) levels have sub-optimal plasma concentrations of folate, vitamin B<sub>6</sub> and B<sub>12</sub>, the strongest association being between H(e) and folate levels (Verhoef *et al.* 1998). In Framingham Heart Study participants plasma folate levels were significantly higher, and H(e) levels lower, with high intake of folate-rich food (Tucker *et al.* 1996).

However, evidence that reduction of H(e) levels decreases CVD risk has yet to be provided. It cannot yet be excluded that hyperhomocyst(e)ine is a mere marker of another metabolic disorder. The question is how much folate should be recommended in the light of a limited knowledge. It is notable that moderate folic acid supplements of 200  $\mu\text{g/d}$  were sufficient to decrease normal H(e) levels of healthy individuals by 10 % (Schorah *et al.* 1998). Folic acid is present in a wide variety of foods, especially liver, leafy vegetables, fruit, pulses, and yeast. Milk and milk products do not rank very high as a source of folic acid, but they contribute their share, particularly in countries with high milk intake. They also provide vitamin B<sub>6</sub> and B<sub>12</sub>. The data from the International Dairy Federation (1997) on intake and average concentrations of these vitamins in milk products indicate that they provide around 175, 1.6 and 26  $\mu\text{g/d}$  of the vitamins B<sub>6</sub> and B<sub>12</sub> and folic acid in Europe, and 150, 1.7 and 33  $\mu\text{g/d}$  in the USA. For vegetarians, milk and milk products are an essential source of vitamin B<sub>12</sub>. A cross-sectional study of Norwegian workers found that those with the highest intake of bread, vegetables and skim milk had the lowest H(e) levels (Oshaug *et al.* 1998). This supports again the idea that a high consumption of (low-fat) milk and milk products is part of a healthy eating pattern emphasizing cereals and vegetables.

## Conclusions

This article discusses the possibility that there are several components in milk and milk products which may have no major, but nonetheless a supportive role concerning the prevention of atherosclerosis, like folic acid, vitamin B<sub>6</sub> and B<sub>12</sub>. There are some with a probably more decisive role, like calcium and bioactive peptides. The importance of CLA is not conclusively known. Several effects concerning the hypolipidaemic and the hypotensive properties of milk were demonstrated not with defined components, but rather with whole milk products. It may well be that the effect of a particular component is enhanced by other ingredients. In this context, it is important to note that nutrients are not consumed in isolation, and that there may be physiological interactions and combined effects (Reusser & McCarron, 1994). It is certainly not justified to reject milk and milk products simply because of cholesterol and saturated fatty acids contained in them.

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