Effect of yogurt and bifidus yogurt fortified with skim milk powder, condensed whey and lactose-hydrolysed condensed whey on serum cholesterol and triacylglycerol levels in rats

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Summary. The possible hypocholesterolaemic properties of milk and fermented milk products have been investigated in groups of albino rats given a basal diet, basal diet plus cholesterol, and basal diet plus cholesterol together with whole milk or standard or bifidus yogurt. The yogurts were fortified with skim milk powder, condensed whey or lactose-hydrolysed condensed whey. After 30 d, triacylglycerols, total cholesterol, HDL-cholesterol and LDL-cholesterol were measured in serum. Whole milk and ordinary yogurt had no hypocholesterolaemic effect, but standard yogurt containing lactose-hydrolysed condensed whey and all bifidus yogurts lowered serum cholesterol. In general, yogurts changed HDL-cholesterol little, but tended to raise triacylglycerols. There was marked lowering of LDL-cholesterol in rats given either type of yogurt fortified with whey proteins. This study has demonstrated in a rat model that bifidus yogurts and yogurts fortified with whey proteins can reduce total and LDL-cholesterol, and suggests that if they have the same effect in human subjects they have potential value in cholesterol-lowering diets.

Because of the high incidence of cardiovascular disease, especially in wealthier communities, many health conscious people are adopting a low-fat diet to decrease risk by reducing blood cholesterol (particularly LDL-cholesterol) and perhaps triacylglycerols. Dairy fats are often avoided, although some studies suggest that fermented products may actually have a beneficial effect on cholesterol levels (Mann, 1977).

The use of condensed whey or lactose-hydrolysed condensed whey in yogurt mixes is potentially important in developing countries where membrane technology may be uneconomic.

In most hyperlipidaemic patients, the lipid fraction elevated is either cholesterol or triacylglycerols (Vasudevan & Sreekumari, 1995). Elevated LDL-cholesterol and triacylglycerol levels are associated with atherosclerosis, but there is an inverse relationship between HDL-cholesterol and the development of atherosclerosis (Robbins & Cotran, 1981).

The aim of the present work was to assess the potential hypocholesterolaemic properties of standard and bifidus yogurts fortified with skim milk powder, condensed whey or lactose-hydrolysed condensed whey, using albino rats as an animal model. Total cholesterol, HDL-cholesterol, LDL-cholesterol and triacylglycerol levels were measured because of their clinical significance.

MATERIALS AND METHODS

Starter culture

Lyophilized yogurt cultures containing *Streptococcus thermophilus* YH-5 and *Lactobacillus delbrueckii* subsp. *bulgaricus* YH-L (National Dairy Research Centre, Karnal 132 001, India) were separately transferred aseptically into sterilized skim milk and activated by incubating at 37 °C until coagulation. Three consecutive transfers were carried out daily in the same medium for maximum activation.

Bifidobacterium bifidum 2715 (National Collection of Food Bacteria, Institute of Food Research, Reading RG6 6BZ, UK) was transferred into sterile skim milk containing 10 g dextrose/l and 1 g yeast extract/l, and incubated anaerobically at 37 °C until coagulation. Further activation was achieved by three similar consecutive transfers in the same medium.

Routine maintenance of these cultures was effected by fortnightly transfer in sterile skim milk. Between transfers, cultures were kept at 4 °C.

Preparation of yogurts

The fermented products were prepared from fresh cows' milk (Dairy Plant, Kerala Agricultural University) standardized to 35 g fat/l. The yogurt mixes were fortified with one of three products: yogurts A_1 and B_1 with skim milk powder, yogurts A_2 and B_2 with condensed whey obtained by concentrating cottage cheese whey 8-fold using a laboratory vacuum evaporator (model Anhydro 1688; EWO, DK-1890 Copenhagen, Denmark) at 50 °C at 13·4 Pa and adjusting to 500 g total solids/l, yogurts A_3 and B_3 with lactose-hydrolysed condensed whey prepared by the action of 7·5 ml β-galactosidase (EC 3.2.1.23, 300 units/ml, Lactozym; Novo Nordisk, DK-2880 Bagsværd, Denmark) on 120 ml condensed whey at room temperature for 1 h, producing 80 g lactose/l. The lactose-hydrolysed condensed whey was heated at 80 °C for 3 min to inactivate the enzyme and added to produce a final lactose content of 40 g/l in the mix.

All mixes were heated to 60 °C, homogenized at 13·8–17·2 MPa, heated at 85 °C for 30 min and cooled to room temperature for inoculation. Yogurts A_1, A_2 and A_3 were inoculated with *Strep. thermophilus* and *Lb. bulgaricus*, each at 10 g/l; yogurts B_1, B_2 and B_3 with *Strep. thermophilus* and *Lb. bulgaricus*, each at 10 g/l, together with *Bifid. bifidum* at 100 g/l. The mixes were incubated at room temperature until a pH of 5·8 was attained, then refrigerated overnight.

Animal trial

Albino rats (54) of uniform weight (70·5 ± 0·38 g) and age (57·6 ± 0·5 d) from the University Small Animal Breeding Station were divided into nine groups each of six (three males, three females). The diets given to the groups are summarized in Table 1. Rats were caged individually, water was freely available and the experimental diets were given each morning at 100 g/kg body weight. Cholesterol (Sisco Research Laboratories, Chakala, Andheri, Bombay 400 099, India) was given at 5 g/kg body weight.

After 30 d, the rats were subjected to an overnight fast, then anaesthetized with chloroform and individual blood samples were obtained by retrobulbar puncture using heparinized capillary tubes. The blood was allowed to clot and serum was separated and stored at −25 °C until analysed.

Total cholesterol was measured by the method of Zak (1957) and HDL-cholesterol using a kit (Glaxo, Bombay 400 025, India). LDL-cholesterol was

Table 1. *Experimental groups and diets used in the trial*

Group	Diet
I	Basal†
II	Basal + cholesterol‡
III	Basal + cholesterol + yogurt A_1
IV	Basal + cholesterol + bifidus yogurt B_1
V	Basal + cholesterol + yogurt A_2
VI	Basal + cholesterol + bifidus yogurt B_2
VII	Basal + cholesterol + yogurt A_3
VIII	Basal + cholesterol + bifidus yogurt B_3
IX	Basal + cholesterol + whole milk

A_1, A_2, A_3, yogurts prepared with *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*; B_1, B_2, B_3, yogurts prepared with *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Bifidobacterium bifidum*; A_1, B_1, yogurts fortified with skim milk powder; A_2, B_2, yogurts fortified with condensed whey; A_3, B_3, yogurts fortified with lactose-hydrolysed condensed whey. Yogurts and milk were added to replace half the dry matter.

† The basal diet contained (g/l) groundnut meal 400, sesame meal 100, wheat 200, wheat bran 280, mineral mix 10, vitamin mix 10.

‡ Cholesterol was given at 5 g/kg body weight.

Table 2. *Serum triacylglycerol, total cholesterol, HDL-cholesterol and LDL-cholesterol in rats given different diets*

(Values are g/l, means ± SD for $n = 6$)

	Diet										
			Basal plus cholesterol plus								
				Yogurt							
	Basal	Basal plus cholesterol	Milk	A_1	B_1	A_2	B_2	A_3	B_3	F	SED
Total cholesterol	1·49	1·72	1·53	1·66	1·20	1·43	0·91	1·13	0·87	20·03**	0·102
Triacylglycerol	0·46	0·61	0·57	0·87	0·79	1·01	1·04	0·66	0·72	6·21**	0·112
HDL-cholesterol	0·49	0·62	0·57	0·46	0·74	0·60	0·53	0·43	0·58	5·44**	0·057
LDL-cholesterol	0·95	0·97	0·84	1·03	0·22	0·66	0·16	0·55	0·15	40·18**	0·081

**, $P < 0{\cdot}01$.

calculated as total cholesterol − (HDL-cholesterol + triacylglycerols/5). Serum triacylglycerols were measured using an Orthodiagnostic kit (Johnson and Johnson, Madras 600 018, India).

Statistical procedures

Results were analysed using one way analysis of variance, (Snedecor & Cochran, 1967) and a summary is presented in Table 2. From this it can be seen that the characteristics measured were significantly affected by the different diets given.

RESULTS

Serum total cholesterol

There was a significant increase in serum cholesterol when cholesterol was added to the basal diet. Whole milk had no hypocholesterolaemic effect. Standard yogurt A_3 and all the bifidus yogurts significantly reduced total cholesterol. Of the fortification ingredients, lactose-hydrolysed condensed whey was most effective in lowering serum cholesterol, followed by condensed whey and skim milk powder.

Serum triacylglycerols

In general, serum triacylglycerols were higher in all groups given the yogurts compared with the milk group, but this was significant only for those given A_1, A_2 and B_2.

Serum HDL-cholesterol

In most groups, the HDL-cholesterol levels were not significantly different from those for the milk-fed group. Exceptions were the group given yogurt B_1, where there was a rise, and those given yogurts A_1 and A_3, where there was a fall.

Serum LDL-cholesterol

In rats given yogurts A_2 and A_3 the LDL-cholesterol levels were lower than in rats given the milk supplement. Irrespective of the type of fortification, all the groups receiving bifidus yogurts had significantly lower serum LDL-cholesterol than those given the milk diet.

DISCUSSION

Serum cholesterol

The increase in serum cholesterol found on incorporating cholesterol in the diet is supported by the work of Eleven (1995). The lack of any hypocholesterolaemic effect of whole milk was also reported by Mann (1977), who found that consuming 2 l of whole milk daily was without benefit for hypercholesterolaemic individuals.

The lack of any difference in serum cholesterol between rats given milk and standard yogurt is consistent with the findings of Rossouw *et al.* (1981) that there was no hypocholesterolaemic effect in human subjects consuming large quantities of yogurt. However, we did find a reduction in rats given yogurt A_3, perhaps because of the increased bacterial count resulting from the easily digested form of carbohydrate present. Yogurt cultures may deconjugate bile salts (Eyssen, 1973; Beena & Prasad, 1995) and enhance excretion (Chikai *et al.* 1987), which may promote the transformation of cholesterol to bile acids and lower blood cholesterol. Rašić *et al.* (1992) reported that yogurt cultures may absorb large amounts of cholesterol. All the groups given bifidus yogurts had markedly lower serum cholesterol (cf. Homma, 1988), possibly because of a synergic effect of the standard and bifidus cultures.

The more pronounced hypocholesterolaemic effect of condensed whey and particularly lactose-hydrolysed condensed whey may also have been the result of more rapid multiplication of the starter culture and enhanced bile salt excretion.

Serum triacylglycerols

The increase in serum triacylglycerols in those rats eating yogurts may be related to a reduction in orotic acid by yogurt cultures (Ahmed *et al.* 1979), since orotic acid can depress blood triacylglycerol levels (Windmüller, 1963). Moreover, triacylglycerols are resistant to the action of the lipases of lactic acid bacteria (DeMoraes & Chandan, 1982) and may be absorbed as such. Rossouw *et al.* (1981) reported a temporary increase in serum lipids and lipoprotein in schoolboys given low-fat yogurt, and Williams & Macdonald (1982) found that female baboons given a diet high in hydrolysed lactose had increased serum triacylglycerol and reduced serum cholesterol levels. Triacylglycerol levels were unaffected by the type of fortification.

Serum HDL-cholesterol

Differences in HDL-cholesterol levels compared with the group given milk were generally small, perhaps because of the lower total cholesterol. However, we are unable to explain the lower HDL-cholesterol in rats given yogurt A_1, despite the high total cholesterol.

Serum LDL-cholesterol

Since a high blood LDL-cholesterol is associated with increased risk of atherosclerosis and cardiovascular disease, any product that lowers this level is of potential value. As 70% of total cholesterol is LDL-cholesterol (Robbins & Cotran, 1981), those processes that reduce total cholesterol – increased levels of yogurt cultures, enhancing the excretion of bile salts and so on – may also effectively lower LDL-cholesterol. Again, it is possible that the standard yogurt culture and the bifidus culture have a synergic effect.

The hypocholesterolaemic effects of these yogurts, and the advantages of whey products and bifidus cultures have been demonstrated in rats in the present study. If these effects could be confirmed in a trial with human volunteers, these products could make an effective and economic contribution to treating hypercholesterolaemia.

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