The effect of fermented and unfermented milks on serum cholesterol

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ABSTRACT Groups of 10 to 13 healthy volunteers were provided with 1 l supplements of 2% butterfat milk (2% milk), whole milk, skim milk, yogurt, buttermilk, and sweet acidophilus milk daily for a 3-wk period. Despite increases in caloric intakes on all supplements, no significant increases were found in total, low-density, and high-density lipoprotein cholesterol. A significant weight gain was seen in subjects taking yogurt, acidophilus, buttermilk, and skim milk. Weight gain was, however, most marked in the yogurt and acidophilus groups; these were the only two groups showing significant rises in triglyceride levels. These results in normal volunteers focus attention on the current practice of recommending only skim or 2% milk for hyperlipidemic individuals. Am J Clin Nutr 1982;36:1106-1111

KEY WORDS Fermented milks, blood lipids, milk fat

Introduction

Since the original studies of Mann and Spoerry (1) on Maasai tribesmen, there has been considerable interest in the ability of fermented milk products to lower the serum cholesterol. Such milk constituents as orotic acid (2), lipoproteins (3), lactose (4), calcium (5), or hydroxymethylglutaric acid (6) have been suggested as hypcholesterolemic factors. In addition, products of bacterial fermentation or the introduction of specific strains of bacteria into the gastrointestinal tract have also been held responsible (7).

The present study was undertaken to determine on a comparative basis what aspect of liquid milk products was responsible for this hypocholesterolemic action. The effect on blood lipids was assessed in healthy volunteers who took 1 l supplements of both fermented and unfermented milk products.

Methods

Sixty-eight healthy volunteers (26 male, 42 female, 22 ± 4 yr, 99.5 ± 0.74% desirable weight) took part in a 10-wk study. During the pre-study week a 3- to 4-day diet history was obtained representing their presstudy dietary intake. To test their tolerance, individuals were also asked to take a day's supplementation of the milk product which had been randomly allocated to them; the milk product was then used in the later part of the study period. At this stage one individual was reallocated to an alternative group due to inability to take the full supplement as originally assigned.

During the 9-wk study period, all individuals took a basic milk allowance of 0.25 l daily of 2% butterfat milk (2% milk). In addition to this, during the first 3 wk all subjects were provided with a supplement of 1 12% milk/day. For the middle 3 wk, only the basic allowance of 0.25 liter 2% milk was supplied, while in the last 3 wk the subjects were divided into six groups receiving either 1 l of skim milk (n = 11), 2% milk (n = 11), whole milk (n = 10), sweet acidophilus milk (n = 12), buttermilk (n = 11), or yogurt (n = 13), in addition to the basic 2% milk allowance.

Before the study two individuals routinely consumed more than 1 l of milk daily. They were, therefore, provided with 0.5 l basic allowance of 2% milk throughout the study and supplements of 1.5 l milk products.

Seven-day dietary records were kept by all subjects covering the final week of each 3-wk period. At the start of the study and at the end of each subsequent week, fasting blood samples were taken after 10- to 14-hour fasts, and the subjects' weights were recorded. At that time any change in weight in the volunteers was followed by appropriate dietary counseling.

Blood was taken into sodium EDTA tubes and stored

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on ice before centrifuging and analysis of plasma for total cholesterol and triglyceride (8). At wk 0 and at the end of wk 3, 6, and 9, plasma was also analyzed for high-density lipoprotein (HDL) from which low-density lipoprotein (LDL) cholesterol was also assessed using the equation (8):

\[
\text{total cholesterol} = (\text{HDL} + \frac{\text{TG}}{5})
\]

Serum lipids were analyzed against Lipid Research Clinic standards and the coefficients of variation between replicates for the various lipid measurements were: cholesterol, 0.94 to 1.33%; HDL cholesterol, 2.35%; and triglyceride, 2.31 to 3.09%.

Whole, skim, and 2% milk were obtained from a local dairy (Dominion Dairies Ltd). Buttermilk, yogurt, and sweet acidophilus milk were prepared by Gay Lea Foods Co from 2% milk fortified with nonfat dry milk to 13.6% total solids. Buttermilk was fermented with Streptococcus cremoris and Streptococcus lactis and yogurt with Lactobacillus bulgaricus and Streptococcus thermophilus. Sweet acidophilus milk was unfermented but inoculated with Lactobacillus acidophilus. All samples contained the same concentration of vitamin A (150 IU/100 ml) and vitamin D (36 IU/100 ml) and were free of other additives. They were analyzed for protein, ash, and total solids by standard methods (9), lactose by enzymatic technique (10), orotic acid by the method of Larson and Hegarty (11), calcium by atomic absorption spectrophotometry, cholesterol with Beckman cholesterol analyzer (12). Total bacterial counts were also carried out in each preparation. The mean analysis of the milk is shown in Table 1.

The results are expressed as means ± SEM and the significance of the change across each 3-wk supplementation period calculated using "Student's" t test for paired data. In addition, analysis of variance was undertaken separately for each of the six milk supplement groups from the last 3 wk of the study and Duncan's multiple range test was performed on the individual mean values for each variable at the three feeding intervals. Duncan's test determines significance at the 5% probability level.

Results

In general, the supplements were well tolerated with all individuals taking the full amount. The average milk intake before the study was approximately 0.5 l/day. The total milk intake during the 11 2% supplementation period, therefore, represented an intake of 0.75 l above normal. The low milk period was equivalent to 0.25 l below normal consumption levels.

Before the experiment, the majority of the group (69%) took 2% milk as their major source of liquid milk. Of the remainder, 16% took whole milk and 15% took skim milk. Sixty-three percent took yogurt regularly (390 ± 51 g/wk). Four subjects occasionally consumed buttermilk and none ever took sweet acidophilus milk.

Nutrient intake

During the study, there were significant changes in caloric intake. During the initial high 2% milk period, caloric intakes increased by 486 ± 77 cal (p < 0.001) which then fell over the low 2% milk period by 399 ± 50 cal (p < 0.001) to approximately prestudy levels (Table 2). Significant increases occurred during the final supplementation period for those taking whole milk, buttermilk, yogurt, and acidophilus milk (Table 3). Significant changes in the percentage calories from protein, fat, and carbohydrate were also observed after the supplement was added.

Plasma cholesterol

Over the first and second 3-wk periods plasma total LDL and HDL cholesterol levels remained constant (Table 2). During the last period (Fig 1), cholesterol values changed by no more than 2.5% with exception of skim milk group which fell by 8.5%, however, none of these changes reached the 5% significance level. No significant changes were seen in LDL or HDL cholesterol on any of the supplements.

<p>| TABLE 1 |
| Composition of milk and milk products |</p>
<table>
<thead>
<tr>
<th>Samples</th>
<th>Total solids</th>
<th>Fat</th>
<th>Protein</th>
<th>Lactose</th>
<th>Calcium</th>
<th>Cholesterol</th>
<th>Orotic acid</th>
<th>Viable bacterial</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>mg/100 ml</td>
<td>mg/100 ml</td>
<td>µg/ml</td>
<td>counts/ml</td>
</tr>
<tr>
<td>Skim milk</td>
<td>9.4</td>
<td>0.2</td>
<td>3.6</td>
<td>5.1</td>
<td>121</td>
<td>3</td>
<td>81.6</td>
<td></td>
</tr>
<tr>
<td>2% milk</td>
<td>11.2</td>
<td>2.0</td>
<td>3.6</td>
<td>5.0</td>
<td>120</td>
<td>5</td>
<td>76.8</td>
<td></td>
</tr>
<tr>
<td>Whole milk</td>
<td>12.4</td>
<td>3.5</td>
<td>3.5</td>
<td>4.9</td>
<td>118</td>
<td>11</td>
<td>72.0</td>
<td></td>
</tr>
<tr>
<td>Buttermilk</td>
<td>13.0</td>
<td>1.9</td>
<td>4.1</td>
<td>5.1</td>
<td>143</td>
<td>5</td>
<td>36.0</td>
<td>6.4 × 10⁴</td>
</tr>
<tr>
<td>Yogurt</td>
<td>13.2</td>
<td>1.8</td>
<td>4.1</td>
<td>5.1</td>
<td>143</td>
<td>5</td>
<td>57.6</td>
<td>1.2 × 10⁵</td>
</tr>
<tr>
<td>Sweet acidophilus</td>
<td>13.3</td>
<td>1.8</td>
<td>4.2</td>
<td>6.1</td>
<td>143</td>
<td>5</td>
<td>67.2</td>
<td>1.3 × 10⁷</td>
</tr>
</tbody>
</table>
TABLE 2
Effect of high (1.25 l) and low (0.25 l) intakes of 2% milk on plasma lipids, body weight, and caloric intakes (mean ± SEM mg/100 ml plasma or lb body weight)

<table>
<thead>
<tr>
<th></th>
<th>Wk 0</th>
<th>High milk</th>
<th></th>
<th>Low milk</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>164 ± 3.0</td>
<td>166 ± 2.8</td>
<td>160 ± 2.9</td>
<td>162 ± 3.6</td>
<td>160 ± 3.0</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>91 ± 2.7</td>
<td>89 ± 2.8</td>
<td>56 ± 1.4</td>
<td>57 ± 1.7</td>
<td>57 ± 1.7</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>57 ± 1.5</td>
<td>56 ± 1.4</td>
<td>57 ± 1.7</td>
<td>57 ± 1.7</td>
<td>57 ± 1.7</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>76 ± 3.8</td>
<td>81 ± 4.4</td>
<td>76 ± 4.1</td>
<td>77 ± 3.6</td>
<td>75 ± 3.4</td>
</tr>
<tr>
<td>Body wt</td>
<td>137.5 ± 2.9</td>
<td>138.5 ± 2.9</td>
<td>138.5 ± 2.9</td>
<td>139.1 ± 3.2</td>
<td>138.6 ± 3.5</td>
</tr>
<tr>
<td>Caloric intake</td>
<td>1993 ± 76</td>
<td>2479 ± 78</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2080 ± 84</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TABLE 3
Nutrient intakes during the supplement period

<table>
<thead>
<tr>
<th>Supplement</th>
<th>n</th>
<th>Cal</th>
<th>Protein</th>
<th>Fat</th>
<th>Carbohydrates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wk 6</td>
<td>Wk 9</td>
<td>p</td>
<td>Wk 6</td>
<td>Wk 9</td>
</tr>
<tr>
<td>2% milk</td>
<td>2087</td>
<td>2222</td>
<td>NS</td>
<td>16.0</td>
<td>19.1</td>
</tr>
<tr>
<td>Whole milk</td>
<td>2249</td>
<td>2621</td>
<td>0.02</td>
<td>13.0</td>
<td>17.0</td>
</tr>
<tr>
<td>Skim milk</td>
<td>1990</td>
<td>2139</td>
<td>NS</td>
<td>15.9</td>
<td>20.1</td>
</tr>
<tr>
<td>Yogurt</td>
<td>2060</td>
<td>2376</td>
<td>0.01</td>
<td>16.1</td>
<td>19.1</td>
</tr>
<tr>
<td>Acidophilus milk</td>
<td>2188</td>
<td>2488</td>
<td>0.01</td>
<td>15.8</td>
<td>18.2</td>
</tr>
<tr>
<td>Buttermilk</td>
<td>1900</td>
<td>2200</td>
<td>0.01</td>
<td>15.8</td>
<td>19.7</td>
</tr>
</tbody>
</table>

Serum triglyceride
No change was seen in serum triglyceride levels of the group as a whole during the initial 2% milk period or during the subsequent low milk period (Table 2). During the final period of individual milk product supplementation (Fig 1) small rises were seen in serum triglyceride on yogurt (11.3 ± 4.3 mg/100 ml, p < 0.05) and acidophilus milk (11.8 ± 5.1 mg/100 ml, p < 0.05).

Body weight
A small increase was seen in mean body weight in the group as a whole over the initial 2% milk period (0.38 ± 0.09 kg, p < 0.001) (Table 2). No significant change in weight was found during the low milk period. Over the final period all supplements (Fig 1), with the exception of whole and 2% milk, produced small but significant weight increases: skim 0.53 ± 0.58 kg (p < 0.02), acidophilus 0.69 ± 0.72 (p < 0.01), buttermilk 0.54 ± 0.65 kg (p < 0.05), yogurt 1.04 ± 0.79 kg (p < 0.001).

Relationship of nutrient intake, body weight, and lipids
Over the initial high 2% milk (wk 0 to 3) and low 2% milk periods (wk 3 to 6) where the subject group was large (n = 68) no relationship was seen between changes in caloric intake and body weight. The same lack of relationship existed between changes in these two variables, cholesterol, dietary fiber, and changes in serum lipids.

Prestudy consumption of dairy produce and blood lipids
Dividing the group into those who, before the experiment, took above (n = 18), those who took below (n = 27), and those who took equal to (n = 23) the mean liquid milk intake of 0.5 l/day did not reveal significant differences in the mean starting levels of either total, LDL, and HDL cholesterol levels or triglyceride. In addition, prestudy milk intakes did not relate to differences in the blood lipid responses to the initial 2% or subsequent low milk periods. The two subjects whose
initial milk intakes were very high and who took proportionately larger milk supplements, showed somewhat higher triglyceride levels during supplementation, other lipid and body weight responses, however, did not differ from the group as a whole.

Discussion

The dietary addition or subtraction of 1 l of 2% milk had no significant effect on serum lipids in a large group of 68 healthy individuals with normal or low blood lipids. Addition of 1 l supplements of a range of fermented and unfermented milks to smaller subgroups failed to produce any large change in lipids although in two instances (yogurt and sweet acidophilus milk) small changes were produced in serum triglyceride with significance at the 5% level. A small but insignificant rise in triglyceride was also observed by Hepner et al (7) in the yogurt-fed group.
Much interest in the possibility that fermented milk is hypocholesterolemic has been stimulated by the work of Mann and Spoerry (1) on Maasai tribesmen. In young Maasai tribesmen, 16 to 23 yr old, of comparable mean age with our own group mean, falls of 8% were seen over 3 wk when the volunteers took a mean of more than 8 l/day of fermented milk representing an estimated 3 to 4 l more than their normal daily intake. In studies of schoolboys aged 16 to 18 yr, daily supplementation with 2 l yogurt produced a nonsignificant 4% fall in serum cholesterol by the 3rd wk (13). In our own studies where only 1 l of yogurt was given daily, a nonsignificant rise of 1% was seen at the end of 3 wk of supplementation. Since these three studies have all been performed on young healthy volunteers over a 3-wk period of time the question is raised as to what extent the hypocholesterolemic action of milk is dependent on consumption of amounts in excess of 1 l/day. As in the study of Rossouw et al (13), we also found with yogurt that cholesterol levels rose at the end of the 2nd wk before falling back to base-line and that a similar pattern was obtained for whole milk. Such an effect was not seen with skim milk which fell by the end of the 1st wk.

Hepner et al (7) found that cholesterol level fell significantly by about 5% after 1 wk of supplementation of 0.75 l yogurt or 2% milk but then did not change significantly for the next 3 wk. The fall in cholesterol during the 1st wk was, however, explained by Rossouw et al (13) as attributable to spontaneous modification of dietary intake. They considered this the result of participation in an experiment and keeping a dietary record. In their own study they observed a 5 to 7% fall in cholesterol during the prestudy period which they also attributed to this result.

In the original studies of Mann and Spoerry (1), the apparently anomalous finding was that reduction in serum cholesterol was positively related to weight gain. Individuals who gained 6 lb or more lowered serum cholesterol by a mean of 28 mg/100 ml while those with a weight gain of 5 lb or less showed only an 8 mg/100 ml reduction. In our own study the mean weight gain was only 1.5 lb similar to the 2.2 lb reported by Rossouw et al (13) who found no significant reduction in serum cholesterol on yogurt.

Many factors have been put forward to account for the early observation of a hypocholesterolemic action of fermented milk. These have included bacterial metabolites of fermented milk, alteration of colonic bacterial flora or bacterial cell walls, and various milk factors such as dihydroxyethyl-glutaric acid (6), orotic acid (2), calcium, lactose (4, 5), or a lipoprotein (3).

Our studies provide no direct evidence for a hypocholesterolemic milk factor at the level of milk supplements provided here although such a factor may be present since the rise anticipated from increased fat intake. The greatest hypocholesterolemic tendency was seen with the skim milk rather than the liquid milk containing 2% butterfat or whole milk. Similarly, Rossouw et al (13) found a significant hypocholesterolemic effect with skim milk but not with whole milk. Equal effects were previously demonstrated in rats (14).

Other studies in man have indicated that calcium and lactose are not the factors responsible (4, 15).

No studies have been undertaken in man using buttermilk or sweet acidophilus milk. However, cultured buttermilk has been shown to contain a factor that inhibits cholesterol synthesis in rat liver slices (16). Also in rats sweet acidophilus milk has been shown to lower serum cholesterol level where whole milk had little or no effect (17). It has been suggested that _L acidophilus_ has a high survival rate in the gastrointestinal tract and may thus alter bile acid metabolism favoring a lowering of serum cholesterol. Our own data suggest that in man, cultured buttermilk, yogurt, and acidophilus milk given in the quantities and for the time used here do not have a noticeable effect on serum cholesterol. Since these products were prepared from milk of the same composition, it appears that bacterial metabolites from fermentation or survival of added microrganisms in the gut have only a small effect on serum cholesterol.

Perhaps the lack of appreciable elevation of blood lipids on taking milk supplements is equally surprising. Workers using laboratory animals have demonstrated a hypercholesterolemic effect of milk protein as opposed to vegetable protein (18, 19). The fat and cholesterol content of milk might also be expected to raise the serum cholesterol level. Our own findings in healthy young volun-
teers failed to show consistent elevations of serum cholesterol on supplementation with milk products in several instances despite measurable weight gain.

The present study indicates therefore that in young adults the regular consumption of large amounts of milk products is not likely to be associated in the short term with major changes in blood lipids. However, consumption of certain milk products may be preferable to consumption of eggs or carcass fat when there is concern over elevation of blood lipids.

References