Impact of buttermilk consumption on plasma lipids and surrogate markers of cholesterol homeostasis in men and women

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Abstract Background and aims: Sphingolipids (SL) are important components of the milk fat globule membrane (MFGM) found in buttermilk. While studies in animal models suggest that dietary SL may have cholesterol-lowering properties, data in human are lacking. The aim of this study was to investigate the impact of buttermilk consumption on plasma lipids and surrogate markers of cholesterol (C) homeostasis in humans.

Methods and results: Men and women (n = 34) with serum LDL-C < 5.0 mmol/L at screening (mean LDL-C = 3.8 mmol/L) were recruited in this double-blinded randomized crossover placebo controlled study. Their diets were supplemented with 45 g/d of buttermilk and with 45 g/d of a macro/micronutrient matched placebo (4 weeks each in random order). Serum lipid concentrations and surrogate markers of cholesterol homeostasis were measured post diet and compared using mixed models for repeated measures. Consumption of buttermilk led to reduction in serum cholesterol (-3.1%, P = 0.019), LDL-C (-3.1%, P = 0.057) and triacylglycerol (-10.7%, P = 0.007). Buttermilk consumption increased plasma lathosterol concentrations (+12.1%, P = 0.001), but multiple regression analysis indicated that variations in \( \beta \)-sitosterol concentrations (P = 0.002) were the only significant predictor of the LDL-C response to buttermilk consumption.

Conclusion: Buttermilk consumption may be associated with reduced cholesterol concentrations in men and women, primarily through inhibition of intestinal absorption of cholesterol.

Registration number: This trial is registered at clinicaltrials.gov as NCT01248026.

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Abbreviations: ApoB, apolipoprotein-B; BMI, body mass index; C, cholesterol; CHD, coronary heart disease; CRP, C-reactive protein; CDV, cardiovascular disease; FA, fatty acids; FFQ, food-frequency questionnaire; FSH, follicle-stimulating hormone; MFGM, milk fat globule membrane; PCSK9, protein convertase subtilisin kexin-9; SFA, saturated fatty acids; SL, sphingolipids; SM, sphingomyelin; TG, triacylglycerol.

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Introduction
The role of low-density lipoprotein cholesterol (LDL-C) in the pathogenesis of coronary heart disease (CHD) and the clinical benefit of lowering LDL-C concentrations have both been well established [1,2]. Cholesterol homeostasis and hence plasma LDL-C concentrations are maintained by a fine-tuned balance between intestinal cholesterol absorption, endogenous cholesterol synthesis and cholesterol clearance [3]. While the liver has long been recognized as a key organ regulating plasma cholesterol concentrations, the role played by the intestine in whole body cholesterol homeostasis is being increasingly recognized. There is now convincing evidence that diet-induced reduction in intestinal cholesterol absorption has a significant impact on plasma cholesterol and LDL-C concentrations. One good example of this is the introduction of phytosterols into regularly consumed foods, which consumption has been associated with significant and clinically meaningful reduction in LDL-C concentrations [4]. This is important because in low risk patients, most clinical guidelines advocate the use of non-pharmacological approaches as the first mean to lowering LDL-C concentrations [5].

Cholesterol is a highly hydrophobic molecule and for that reason, its absorption is almost entirely dependent on its solubility in bile acid micelles within the intestine [6]. Recent in vitro studies from our laboratory have shown that buttermilk, the by-product of butter manufacturing resulting from the churning of cream, inhibits cholesterol micellar solubility [7]. This phenomenon is likely due to the presence of polar lipids from the milk fat globule membrane (MFGM). Fragments of the MFGM end up in buttermilk along with most of the water-soluble cream components such as lactose, minerals and milk proteins. Most of the research so far has focused on phospholipids purified from MFGM, thereby overlooking the complex and entire MFGM mixture of bioactive proteins and polar lipids found in buttermilk. To the best of our knowledge, no study has yet documented the impact of whole buttermilk on plasma cholesterol concentrations in humans, with considerations for potential underlying mechanism. The objective of this study was to investigate the impact of buttermilk consumption on LDL-C concentration as well as on surrogate markers of cholesterol homeostasis in men and women with serum LDL-C <5.0 mmol/L. We have used plasma phytosterols concentrations as a surrogate of intestinal cholesterol absorption, plasma lathosterol concentrations as a surrogate of endogenous cholesterol synthesis, and plasma protein convertase subtilisin kenin-9 (PCSK9) concentrations as a surrogate of LDL clearance [8–10]. We hypothesized that buttermilk consumption reduces LDL-C concentrations, and that this occurs primarily through inhibited intestinal cholesterol absorption.

Methods

Population sample
Men and women were recruited in the Quebec City area via newspaper, radio and electronic news letters. Recruitment took place at the Institute of Nutraceuticals and Functional Foods (INAF) between January 2011 and April 2011. To be included in the study, participants had to be 18–65 years of age with a body mass index (BMI) ≤35 kg/m². Subjects had to have serum LDL-C concentrations below 5.0 mmol/L with a 10-years calculated Framingham risk below 10% [5]. Only participants with a stable body weight for at least 6 months prior to the study were eligible. Subjects were excluded if they had a previous history of CHD, type 2 diabetes, monogenic dyslipidemia, were using medications for hyperlipidemia or hypertension or had endocrine disorders. Among pre-menopausal women, only those with a regular menstrual cycle for the last 3 months (25–35 days) were eligible. All post-menopausal women were included, irrespective of their hormone supplementation status, as long as it remained stable throughout the study. Individuals with extreme nutritional habits such as vegetarianism, with alcohol consumption >2 drinks/day and elite athletes were not eligible. Smokers and women using contraceptive agents were not excluded from the study. Follicular-stimulating hormone (FSH) measurements were conducted if needed to confirm the postmenopausal status (FSH < 20 IU/L or FSH > 25 IU/L). Participants provided their informed consent for the study. The study protocol was approved by The Clinical Research Ethics Committees of Laval University.

Study design
The study was carried out as a double-blind, randomized, placebo controlled crossover study according to which participants were subjected to 2 consecutive treatments of 4 weeks each, in a random order. For the total duration of the study (8 weeks), participants were requested to maintain their usual diet, medication, weight, alcohol consumption, and smoking and physical activity habits, except for the 3 days preceding blood sampling, during which they were asked to remain sedentary. Vitamins and natural health product supplementation were strictly forbidden during the entire experimental period. Consumption of tea and coffee was allowed with a limit of 2 cups/day as long it remained constant throughout the study. Participants with any deviation to these recommendations were excluded from analysis.

Buttermilk and placebo formulations
The 2 test formulations, artificially chocolate flavored buttermilk and placebo, were designed to provide the equivalent of 2 servings of low fat milk (45 g/day of skim milk solids). The buttermilk powder was purchased from Westland Milk Products (Hokitika, New Zealand) and was fully characterized and formulated in ready-to-use pouches (Table 1). Each pouch contained 22.5 g of formulated buttermilk or placebo that subjects had to mix with 250 mL of water using a shaker provided by the study center. Sucralose was used to improve taste and acceptability. The placebo was formulated using dairy ingredients (calcium caseinate – DVM International, Veghel, Netherlands; whey protein isolate – Davisco Food International Inc., Eden Prairie, MN; whey protein permeate – Agropur, Longueuil, Canada; butter powder – Kerry Inc., Beloit, WI) in order to
match the macro/micronutrient composition of buttermilk with the exception of its MFGM components. This allowed us to specifically study the impact of MFGM minor proteins and phospholipids on study outcomes.

Participants were asked to consume 2 pouches every day, just before breakfast and dinner, for a total consumption of 45 g/day of buttermilk or placebo, which corresponded to 5%–10% of their daily energy intake based on a 2500 kcal/day diet. Participants received specific instruction on how to introduce the test formulations in their diet by substituting specific food items. Subjects were asked to refrain and to maintain their intake of dairy product to a maximum of 2 servings/day. Usual food intake was assessed using a validated in-house food-frequency questionnaire (FFQ) [11] along with a questionnaire specific to dairy foods on three occasions during the study: 1 – at study entry, 2 – after the first treatment and 3 – after the second treatment. The first evaluation was used as a teaching tool on how to integrate the supplements into the subject’s nutritional habits. The 2nd and 3rd evaluations were used to generate nutritional profiles during each treatment and to adjust recommendations, when necessary, for any variations that may have occurred between treatments. Data from these questionnaires were analyzed using the Nutrient Data System software based on a mix of Canadian and FDA-produced nutrient databases.

Compliance

Compliance was measured by counting the pouches returned by subjects to the research staff. A check list provided to all participants was also used for tracking unused products. Compliance ≥85% was defined as acceptable to include participants’ data into the study analyses.

Subjects had to notify the physician in charge of the clinical aspects of the study before initiating any new medication.

Risk factor assessment

Blood samples were taken at screening as well as on two consecutive days at week 4 of each treatment. For each subject and for all study outcomes described below, the average of the two post-treatment values was used. Total cholesterol (total-C), triacylglycerol (TG), and HDL-C concentrations in serum were measured using commercial reagents on a Modular P chemistry analyzer (Roche Diagnostics, Mannheim, Germany). Serum LDL-C concentrations were obtained by calculation using the Friedewald equation. Plasma apolipoprotein-B (apoB-100 and apoB-48) concentrations were measured with ELISA kits (Alerchek Inc., Springvale, ME). Plasma concentrations of C-reactive protein were measured using a commercial high sensitivity ELISA kit (BioCheck Inc., Foster City, CA).

Plasma concentrations of lathosterol, β-sitosterol and campesterol after each treatment were quantified using gas chromatography (GC) as described previously [12]. Since non-cholesterol sterols are transported in plasma by lipoproteins, their concentrations have also been expressed relative to plasma total cholesterol concentrations (102 μmol/mmol C of the same GC run) to correct for the differing number of lipoprotein acceptor particles [13]. Total plasma PCSK9 levels were measured by a commercially available ELISA kit (CircuLex Co., Ltd, Ina, Nagano, Japan).

Anthropometric measures

Anthropometric measures including waist and hip circumferences were taken at the beginning and at the end of each test diet according to standardized procedures [14].

| Table 1 Composition of the ready-to-use buttermilk and placebo pouches (22.5 g).^a |
|---------------------------------|-----------------|-----------------|
|                                | Placebo         | Buttermilk      |
|                                | Mean            | SD              | Mean            | SD              |
| Energy, kcal                   | 89.6            | ±0.5            | 88.9            | ±0.5            |
| Lactose, g                     | 11.5            | ±0.5            | 11.4            | ±0.5            |
| Total proteins, g              | 6.5             | ±0.4            | 6.4             | ±0.5            |
| Total fat, g                   | 2.0             | ±0.2            | 2.0             | ±0.2            |
| SFA, g                         | 1.15            | ±0.2            | 1.04            |
| MUFA, g                        | 0.49            | ±0.2            | 0.42            |
| PUFA, g                        | 0.07            | ±0.2            | 0.06            |
| Total phospholipids, mg        | 17.30           | ±0.5            | 93.75           |
| Phosphatidylcholine, mg        | 9.61            | ±0.4            | 28.65           |
| Phosphatidylinositol, mg       | 3.05            | ±0.4            | 11.88           |
| Phosphatidylethanolamine, mg   | 2.27            | ±0.4            | 31.91           |
| Sphingomyelin, mg              | 1.97            | ±0.4            | 11.81           |
| Phosphatidylserine, mg         | 0.40            | ±0.4            | 9.51            |
| trans-fat, g                   | 0.04            | ±0.4            | 0.05            |
| w-6, g                         | 0.04            | ±0.4            | 0.03            |
| w-3, g                         | 0.01            | ±0.4            | 0.02            |
| Ashes, g                       | 1.8             | ±0.2            | 2.0             | ±0.0            |
| Calcium, ppm                   | 6445            | ±67.0           | 8227            | ±67.5           |
| Water, g                       | 0.7             | ±0.2            | 0.7             | ±0.0            |

^a Lactose, total proteins, total fat, ashes and water values are presented as means and standard deviations (n = 3).
Statistical analysis

The study has been designed to have adequate power to test the main hypothesis that consumption of 45 g/day of buttermilk leads to significant reduction in plasma LDL-C concentrations. Sample size estimation was performed to allow the detection of a minimum of 5% reduction in plasma LDL-C concentrations. An SD of 0.39 mmol/L for the LDL-C change in response to dietary modifications has been used for sample size calculations [15]. At a mean baseline LDL-C concentration of 4.0 mmol/L, the required number of participants completing the study for optimal statistical power was n = 32 (alpha = 0.05 two-tailed, power = 90%). The anticipated dropout rate was 10%.

All data were analyzed for statistical differences using the PROC MIXED procedure for repeated measures in SAS 9.2 (SAS Institute Inc., Cary, NC). Normal distribution and homogeneity of variance were checked before further analysis. Variables with a skewed distribution were log10 transformed. The impact of buttermilk on serum lipid concentrations and surrogate markers of cholesterol homeostasis was adjusted for sex through multivariate modeling. The impact of treatment sequence was investigated using appropriate interaction terms in the MIXED model; no significant interaction was noted for any of the outcome measures. The impact of baseline values (at screening) on the response to buttermilk was also investigated using individual and interaction terms in the MIXED model. When interaction terms were significant, subgroups were created using the median values for the selected variable to present the interaction graphically. Univariate correlation analysis and multivariate stepwise regression models were used to investigate associations among outcome measures. Data are presented as means ± SDs and as percentages of change compared to the placebo unless stated otherwise. P values < 0.05 were considered statistically significant.

Results

Participants

Of the 40 subjects who met the eligibility criteria and who were recruited to start the study, 6 individuals did not complete both treatments and were excluded from further analysis (Fig. 1). Adherence to the test diets was very good, with a self-reported compliance above 97%. None of the subjects were excluded based on their lack of compliance. The characteristics at screening of subject included in the analyses are presented in Table 2. Among the 34 subjects (15 men and 19 women), body weight remained stable throughout the 8 week study period and no change was observed in hip and waist circumference measures.

Plasma lipids and lipoproteins

As shown in Table 3, buttermilk supplementation significantly reduced serum levels of total-C (−3.1%, P = 0.019) and TG (−10.7%, P = 0.007) compared with placebo. The reduction in serum LDL-C concentrations (−3.1%, P = 0.057) was borderline significant. However, there was a significant interaction between LDL-C values at screening and treatment on the LDL-C response (P for interaction = 0.028). Figure 2 illustrates the individual changes (%) in LDL-C concentrations (buttermilk vs. placebo) according to LDL-C values at screening, < or the median 3.7 mmol/L. Figure 3 illustrates how LDL-C status at screening modified the LDL-C response to buttermilk. Serum LDL-C concentrations were significantly reduced after buttermilk consumption among subjects with screening LDL-C > 3.7 mmol/L (−5.6%, P = 0.004) but not among those with screening LDL-C < 3.7 mmol/L (+0.3%, P = 0.890). Buttermilk consumption had no impact on serum HDL-C concentrations (Table 3). There was no apparent interaction between BMI (or waist circumference) and treatment on any of the study outcomes (not shown).

Cholesterol homeostasis markers

As shown in Table 3, buttermilk consumption did not significantly affect surrogate markers (mg/L) of intestinal
cholesterol absorption (i.e. campesterol −2.0% ; P = 0.527 and β-sitosterol −7.3%; P = 0.096). Figure 3 illustrates how baseline LDL-C influenced the response of plasma markers of cholesterol absorption to buttermilk consumption. The change in plasma concentrations of phytosterols (−9.3%, P = 0.009) and β-sitosterol (−11.2%, P = 0.004) were significantly reduced with buttermilk consumption compared with placebo only in subjects with high LDL-C values at screening. Buttermilk consumption compared with placebo significantly increased plasma lathosterol concentrations (−12.1%, P = 0.001), a surrogate marker of endogenous cholesterol synthesis. Baseline LDL-C did not modify the plasma lathosterol, campesterol and PCSK9 response to buttermilk (P for interaction = 0.783, 0.067 and 0.732 respectively).

Changes (%) in plasma phytosterol, β-sitosterol and PCSK9 concentrations with buttermilk vs. placebo were positively correlated with concomitant change (%) in total-C and LDL-C concentrations (Table 4). There was no such correlation between the change (%) in plasma lathosterol or campesterol concentrations and the change (%) in total-C and LDL-C levels (Table 4). In multiple stepwise regression analysis, variations (%) in β-sitosterol concentrations with buttermilk treatment were the only significant predictor of the reduction (%) in serum LDL-C (R² = 27%, P = 0.002) and total-C concentrations (R² = 42%, P < 0.001, results not shown). There was no correlation between the change in TG and the change in LDL-C with buttermilk (not shown).

**Table 3** Effect of buttermilk consumption on serum lipid concentration and surrogate markers of cholesterol homeostasis in men and women (n = 34).a

<table>
<thead>
<tr>
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<th>Placebob</th>
<th>Buttermilkb</th>
<th>Differencec</th>
<th>Pd</th>
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<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Total-C, mmol/L</td>
<td>5.92</td>
<td>±0.94</td>
<td>5.74</td>
<td>±0.81</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>3.71</td>
<td>±0.71</td>
<td>3.59</td>
<td>±0.64</td>
</tr>
<tr>
<td>Apolipoprotein B100, mg/dL</td>
<td>114.8</td>
<td>±56.5</td>
<td>117.3</td>
<td>±51.1</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.62</td>
<td>±0.47</td>
<td>1.62</td>
<td>±0.43</td>
</tr>
<tr>
<td>C/HDL-C ratio</td>
<td>3.91</td>
<td>±1.07</td>
<td>3.77</td>
<td>±0.96</td>
</tr>
<tr>
<td>Triacylglycerols, mmol/L</td>
<td>1.30</td>
<td>±0.60</td>
<td>1.16</td>
<td>±0.47</td>
</tr>
<tr>
<td>Apolipoprotein B48, mg/dL</td>
<td>6.66</td>
<td>±3.20</td>
<td>5.80</td>
<td>±2.42</td>
</tr>
<tr>
<td>C-reactive protein, mg/Le</td>
<td>1.49</td>
<td>±1.77</td>
<td>1.53</td>
<td>±1.81</td>
</tr>
<tr>
<td>Lathosterol/C, 102 mmol/mmol Ce/f</td>
<td>0.49</td>
<td>±0.17</td>
<td>0.57</td>
<td>±0.22</td>
</tr>
<tr>
<td>Phytosterols/C, 102 mmol/mmol C/6f</td>
<td>1.20</td>
<td>±0.58</td>
<td>1.18</td>
<td>±0.54</td>
</tr>
<tr>
<td>Campesterol/C, 102 mmol/mmol C</td>
<td>0.41</td>
<td>±0.20</td>
<td>0.42</td>
<td>±0.19</td>
</tr>
<tr>
<td>β-sitosterol/C, 102 mmol/mmol C/6e</td>
<td>0.79</td>
<td>±0.40</td>
<td>0.76</td>
<td>±0.36</td>
</tr>
<tr>
<td>Lathosterol, mg/L6e</td>
<td>2.89</td>
<td>±1.14</td>
<td>3.24</td>
<td>±1.38</td>
</tr>
<tr>
<td>Phytosterols, mg/L6e/f</td>
<td>7.18</td>
<td>±3.98</td>
<td>6.79</td>
<td>±3.36</td>
</tr>
<tr>
<td>Campesterol, mg/L6e</td>
<td>2.47</td>
<td>±1.38</td>
<td>2.42</td>
<td>±1.22</td>
</tr>
<tr>
<td>β-sitosterol, mg/L6e</td>
<td>4.70</td>
<td>±2.68</td>
<td>4.36</td>
<td>±2.22</td>
</tr>
<tr>
<td>PCSK9, ng/mL</td>
<td>274.2</td>
<td>±71.4</td>
<td>267.6</td>
<td>±72.9</td>
</tr>
</tbody>
</table>

a C, cholesterol; PCSK9, protein convertase subtilisin kexin-9.

b Values are means ± SD.

c Values are expressed as percentage of change compared to placebo.

d p values from the main effect of diet in the MIXED model (SAS Institut. Cary. NC). P values were obtained using the PROC MIXED procedures. The model was adjusted for sex.

e Analysis were performed on log-transformed values.

f Campesterol + β-sitosterol.

**Discussion**

This study indicates that short-term buttermilk consumption significantly reduces serum total-C and TG concentrations in men and women. The 3.1% reduction in serum LDL-C concentrations with buttermilk did not quite reach statistical significance in the entire study sample (P = 0.057), but was highly significant among subjects with higher LDL-C concentration at screening (−5.6%, P = 0.004). Plasma lathosterol concentrations were increased after buttermilk consumption but the magnitude of the change in this estimate of endogenous cholesterol synthesis did not correlate with the LDL-C response to buttermilk. Only changes (%) in surrogate markers of intestinal cholesterol absorption (plasma phytosterols and β-sitosterol) were correlated to the total-C and LDL-C response to buttermilk in multivariate analyses.

To the best of our knowledge, this is the first study to have investigated the impact of buttermilk consumption on lipid levels in humans, with considerations for potential underlying mechanism. Ohlsson et al. have investigated the acute [16] and chronic [17] impact of sphingolipids (SL)-enriched buttermilk supplementation on blood lipids in healthy human subjects and have reported no significant effect on fasting and postprandial plasma lipids concentrations. These two previous studies had smaller sample sizes and involved subjects with relatively normal LDL-C concentrations, thereby limiting the ability to observe significant effects. Furthermore, Thompson et al. [18] have studied the impact of cultured buttermilk consumption on
lipid levels in a small group of healthy subjects and reported no noticeable lipids-lowering effect. However, cultured buttermilk represents the product of cow’s milk fermentation and thus, does not possess the composition specificities of fresh buttermilk (i.e. MFGM polar lipids).

Our data have shown that the cholesterol-lowering property of buttermilk in humans appeared to be more important among individuals with higher serum LDL-C concentrations. The impact of LDL-C status on diet-induced LDL-C changes has been discussed previously [19]. The LDL-C response to buttermilk consumption (∆0.24 mmol/L for the “high” LDL-C group at screening) was close in magnitude to the cholesterol-lowering effectiveness of plant sterol therapy, generally associated with an LDL-C reduction of 0.27—0.35 mmol/L [20]. However, the effect of buttermilk may differ from the effect of plant sterol therapy by its ability to reduce serum TG levels as well. Indeed, plant sterols inhibit intestinal cholesterol absorption with no apparent effect on TG [4]. In the present study, supplementation with buttermilk reduced plasma concentrations of apoB-48 by approximately 13% but this change was not statistically significant. The absence of a correlation between the reduction in plasma TG and in LDL-C with buttermilk suggests that different mechanisms may be at play. However, the TG-lowering effect associated with buttermilk consumption and its underlying mechanisms definitely deserves further research.

Although not statistically significant when considering the whole study group, plasma phytosterols concentrations were significantly reduced in subjects with high LDL-C levels values at screening, i.e. in subjects showing a significant LDL-C reduction after buttermilk consumption. Multiple regression analyses have also shown that variations in plasma β-sitosterol concentrations were the only multivariate predictor of the buttermilk-induced changes in serum LDL-C and total-C levels. This suggests that buttermilk consumption may reduce serum cholesterol primarily through inhibition of intestinal cholesterol absorption. APOE*3Leiden mice, when fed with a Western-type diet supplemented with SL, showed significant reductions in plasma cholesterol and TG levels [21]. Supplementing the western diet with SL also impaired fatty acid (FA) absorption, probably through ionic interactions between SL and FA [21]. Authors have not discussed the possibility that SL-binding properties may also be the underlying mechanism leading to impaired cholesterol and TG absorption in the intestinal tract. SM and phosphatidylcholine, which represent respectively 25% and 35% of the bovine MFGM lipids [22] have a high binding affinity with cholesterol, thereby inhibiting intestinal cholesterol absorption in rats [23]. Furthermore, the slow rate of hydrolysis of SM in the gastrointestinal tract and its incomplete digestion may enhance its capacity to bind cholesterol throughout the small intestine [24]. This phenomenon may obstruct the hydrolysis and processing of other lipids, including micelle formation, which are essential for cholesterol absorption, thus reducing intestinal uptake [25]. In recent studies, buttermilk has been associated with a significant reduction in the micellar solubility of cholesterol in vitro, thereby providing further support to the thesis that buttermilk constituents may impair intestinal absorption of cholesterol [7,26,27].

PSCK9 plays a determinant role in cholesterol metabolism by regulating the intracellular degradation of LDL receptors [8]. Stable isotope studies in humans have shown that plasma PCSK9 is a significant correlate of the LDL fractional catabolic rate [28], and therefore can be used as a surrogate of LDL clearance [8]. Buttermilk consumption did not lead to a significant change in plasma PCSK9 concentrations compared to placebo. However, the magnitude of the variation in plasma PCSK9 with buttermilk was positively correlated with changes in serum total-C and LDL-C concentrations. Therefore, it is possible that part of the LDL-C lowering effect of buttermilk could be attributed to an increased clearance of cholesterol. However, it is also possible that the reduction in LDL-C with buttermilk in itself may have downregulated PCSK9 expression. Indeed, buttermilk consumption resulted in increased plasma lathosterol concentrations, suggestive of an up-regulated endogenous cholesterol synthesis. These data are consistent with studies in animals having shown an increased expression in genes involved in hepatic cholesterol uptake and in hepatic cholesterol synthesis in APOE*3Leiden mice supplemented with SL [21]. It is also possible that circulating products resulting from the digestion of buttermilk may directly affect cholesterol synthesis and clearance pathways. However, we propose that the apparent increase in endogenous cholesterol synthesis and possibly the apparent increase in LDL clearance associated with the
cholesterol response to buttermilk consumption may rather reflect feedback mechanisms compensating for the reduction in the body's cholesterol pool, which may be due in part to the magnitude of the buttermilk-induced inhibition of intestinal cholesterol absorption.

This study has strengths and limitations. One of its strengths resides in the fine characterization of the buttermilk and placebo fed to the subjects. This allowed us to ensure that the cholesterol and TG-lowering effects observed were attributed specifically to components of buttermilk, primarily MFGM. The repeated measurements at screening and at the end of both treatments also increased our capacity to detect very small changes in outcome measures. Cholesterol absorption, synthesis and clearance were estimated using surrogate markers and additional studies using stable isotopes should be used to confirm the findings of the present study. Indeed, although the validity of indirect markers of cholesterol homeostasis have been established [9,10,29] these markers do not provide a measure of absolute amount of cholesterol absorbed and synthesized.

In summary, the reduction in total-C and LDL-C concentrations following buttermilk consumption may be more important among subjects with high baseline LDL, and appears to be primarily attributable to inhibition of intestinal cholesterol uptake. Data from animal models suggest that the unique phospholipid content of buttermilk may be responsible for this effect. The apparent increase in estimated cholesterol synthesis with buttermilk is believed to be a compensatory feedback response, which had no incidence on serum cholesterol concentrations. Consumption of buttermilk may also affect LDL clearance directly or indirectly and this deserves further investigation. The TG-lowering effect of buttermilk is of interest and also deserves further research.

From a clinical perspective, buttermilk may be considered as a natural, well-tolerated and low cost dietary product for improving lipid profiles in low risk patients.

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