Sphingolipids in human ileostomy content after meals containing milk sphingomyelin1–3

Lena Ohlsson, Erik Hertervig, Bo AG Jönsson, Rui-Dong Duan, Lena Nyberg, Rikard Svernlov, and Åke Nilsson

ABSTRACT

Background: Sphingomyelin occurs in modest amounts in the diet, in sloughed mucosal cells, and in bile. It is digested by the mucosal enzymes alkaline sphingomyelinase and ceramidase. In humans, alkaline sphingomyelinase is also secreted in bile. The digestion of sphingomyelin is slow and incomplete, which has been linked to the inhibition of cholesterol absorption and colonic carcinogenesis.

Objective: We evaluated whether the supply of moderate amounts of milk sphingomyelin increases the exposure of the colon to sphingomyelin and its metabolites.

Design: Two experimental series were performed. In experiment A, we measured the content of sphingomyelin and ceramide in human ileostomy content by HPLC during 8 h after consumption of a test meal containing 250 mg milk sphingomyelin. In experiment B, we measured the molecular species of sphingomyelin and ceramide by HPLC–tandem mass spectrometry after doses of 50, 100, or 200 mg sphingomyelin.

Results: In experiment A, the average increase in ileostomy content of ceramide plus sphingomyelin amounted to 19% of the fed dose of sphingomyelin. In experiment B, the output of C-22:0-sphingomyelin, C-23:0-sphingomyelin, C-24:0-sphingomyelin, and sphingosine increased significantly, and palmitoyl-sphingomyelin increased proportionally less. Outputs and concentrations of palmitoyl-ceramide and sphingosine showed great individual variation, and stearoyl-sphingomyelin and stearoyl-ceramide did not increase after the meals. Although the output of long-chain sphingomyelin species increased significantly, the data indicated that >81% of all measured sphingomyelin species had been digested.

Conclusions: Humans digest and absorb most of the sphingomyelin in normal diets. The amount of sphingolipid metabolites to which the colon is exposed can, however, be influenced by realistic amounts of dietary sphingomyelin. Am J Clin Nutr 2010;91:672–8.

INTRODUCTION

Although the major dietary glycerolipids are rapidly digested and absorbed in the upper small intestine primarily by pancreatic enzymes, sphingolipids are more slowly digested by mucosal enzymes (1). Mucosal alkaline sphingomyelinase (Alk-SMase) (2), lactase (3), and ceramidase (4) sequentially catalyze the formation of ceramide, free sphingoid bases, and free fatty acids from dietary sphingomyelin and glycosphingolipids. Whereas intact sphingolipids and ceramides are poorly absorbed, sphingoid bases penetrate effectively into the mucosal cells, probably via passive permeation, and most are converted to palmitic acid, which is incorporated into chylomicron triglycerides (1, 5–7).

Rat studies indicate that the digestion of sphingomyelin is most efficient in the middle of the small intestine and that the capacity is limited (8). In other animal experiments the extended course of sphingolipid digestion has been linked to an inhibitory effect on cholesterol absorption (9) and an inhibitory effect on colon carcinogenesis in chemical carcinogen models (10, 11). Although it is known that humans express Alk-SMase (12) and intestinal ceramidase (13), sphingolipid digestion has not been characterized in humans. A specific human feature is that Alk-SMase is expressed not only in the intestine, but also in the liver, and is secreted in bile (14).

In this study we examined sphingomyelin, ceramide, and sphingosine concentrations in human ileostomy content and how they are influenced by the consumption of 50 to 250 mg milk sphingomyelins. These doses are within the range of the regular daily intake (15) or that can be attained with consumption of foods such as dairy products enriched in milk sphingolipids (16).

SUBJECTS AND METHODS

Pure milk sphingomyelin was prepared as described previously (8). In the first experimental series (experiment A), 250 mg milk sphingomyelin was sonicated in 10 mL water and mixed into 200 mL skimmed milk. In the second experimental series (experiment B), 6 subjects were given milk sphingomyelin dispersed in 200 mL of a milk-like oat drink (Oatly, Landskrona, Sweden). In experiment B, each subject was given 50, 100, or 200 mg milk sphingomyelin on 3 separate occasions ≥1 wk apart. In both experiments A and B, the fasting ileostomy

1 From the Department of Gastroenterology and Nutrition Laboratory, Biomedical Center, Lund University, Lund, Sweden; (LO, R-DD, and AN); the Departments of Gastroenterology and Nutrition (EH, RS, and AN) and Environmental and Occupational Medicine (BAGJ), Lund University Hospital, Lund, Sweden; and the Swedish Dairy Association, Lund, Sweden (LN).

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3 Address reprint requests (electronic form only) and correspondence to Å Nilsson, Department of Clinical Sciences, Gastroenterology and Nutrition, Lund University Hospital, S-22185 Lund, Sweden. E-mail: ake.nilsson@med.lu.se.

content was collected for 8 to 9 h overnight. The subjects then received the milk sphingomyelin together with a standardized breakfast containing 2 slices of bread, margarine, marmalade, plain coffee, or tea. A lunch box was provided, which contained lunch and a small morning and afternoon snack consisting of a vegetarian dish, fruit, and bread, but no milk, meat, fish, or eggs. In both experiments the overnight ileostoma output was collected just before breakfast. During the following 8 h, ileostoma output was collected in four 2-h intervals. The subjects (mean age: 38.2 y; range: 22–50 y; 4 men and 2 women in each series) had undergone a colectomy because of severe ulcerative colitis (no ileal resection) ≥6 mo before the experiment and were considered to have well-functioning ileostomies. In experiment B, however, one subject had a very irregular ileostomy flow, probably as a result of obstruction of the outlet, and was excluded. Thus, the data in experiment B are based on 5 patients. Both studies were approved by the Regional Human Ethics Committee and were in accordance with the Helsinki Declaration of 1975 as revised in 1983. All participants had given informed written consent.

Lipids were extracted with chloroform:methanol (1:1) and were subjected to HPLC analysis on a silica column (Licrosphere Si 60; 5 μm, 25 mm × 4 mm; Merck KGaA, Darmstadt, Germany) with the use of a Sedex 55 (Sedex, Sedere, France) light-scattering detector (experiment A) (17). This method provides good lipid class and individual phospholipid class separation, but does not separate individual molecular species of sphingomyelin or ceramide.

In experiment B, individual molecular species of sphingomyelin, palmitoyl-C-18-d-erythro-sphingosine (referred to as palmitoyl-sphingosine below), stearoyl-C-18-d-erythro-sphingosine (referred to as stearoyl-sphingosine below), and 18-d-erythro-sphingosine (referred to as sphingosine below) were analyzed by HPLC-tandem mass spectrometry, as described previously (18). Weighed standards of palmitoyl-sphingosine, stearoylsphingosine, sphingosine, and C-16:0- and C-18:0–C-18-d-erythrosphingosine-sphingomyelin (referred to as palmitoyl- and stearoyl-sphingomyelin below) were used to prepare the calibration curves. These standards were obtained from Avanti Polar Lipids (Alabaster, AL). For the sphingomyelin species containing C-22:0-, C-23:0-, and C-24:0 carboxylic acids and sphingosine, referred to as C-22-, C-23-, and C-24-sphingomyelin below, milk sphingomyelin containing a mixture of several molecular species of sphingomyelin was used as standard. The content of each individual C-22-sphingomyelin through C-24-sphingomyelin species in the standard sample was calculated from the total sphingomyelin mass and the reported molecular species composition of bovine milk sphingomyelin (19). Four of the sphingomyelin species measured—those containing palmitic acid, C-22:0-23:0-and C-24:0 fatty acids—and sphingosine as sphingoid base are abundant in milk sphingomyelin and were reported to account for 12.9%, 9.0%, 16.0%, and 11.6%, respectively, of total milk sphingomyelin (not including dihydro-sphingomyelin) (19). Because dihydro-sphingomyelin containing saturated sphingoid bases accounted for 20.8% and milk-sphingomyelin for 79.2% of total choline-phosphosphingolipids (19), 100 mg of the “milk-sphingomyelin” used in our experiments was expected to contain ~10.1 mg palmitoyl-, 7.1 mg C-22:0-, 12.7 mg C-23:0-, and 11.6 mg C-24:0–18-sphingomyelin. Stearoyl-sphingomyelin is a minor sphingomyelin species in milk, and 100 mg of the given material is expected to contain ~0.7 mg of this species.

Graphs were designed with Graph Pad Prism (version 5.00; Graph Pad Software, San Diego, CA) 5 software. In experiment A (Figure 1), differences in output of sphingomyelin and ceramide during overnight fasting were estimated by using paired Student’s t tests. In experiment B, statistical analyses were performed by using R version 2.9.2 (Core development team, 2009; http://www.r-project.org). Values for total output and for concentrations were compared by using one-factor analysis of variance (ANOVA) for repeated measures using R-package nlme 3.1.94. If the ANOVA was significant, multiple comparisons using the Bonferroni-Holm method were made by using the R-package multcomp. Variances were stabilized by using log transformation, and sphericity was tested by using John’s test, which is a simple function of the Greenhouse-Geisser correction factor (20); thus, we obtained the Monte Carlo critical value for the Greenhouse-Geisser under the null hypothesis by simulating variance-covariance matrices and choosing the 5000th smallest value of the Greenhouse-Geisser factor as the critical value (<0.285) under which sphericity can be said to be violated at the 5% level. If the observed Greenhouse-Geisser factor was lower than critical, the df in the ANOVA was adjusted by using the Greenhouse-Geisser procedure. The observed means for log-transformed treatments and their 95% CIs were exponentiated back to the standard scale; hence, the geometric means with 95% CIs are presented in Table 1 and Figure 2.

FIGURE 1. Mean (±SEM) output of sphingomyelin and ceramide in ileostomy content during an 8–9-h overnight fast (F) and at different time intervals after intake of a breakfast meal containing 250 mg milk sphingomyelin. **The output of both sphingomyelin and ceramide during 8 h after the meal was significantly higher than that during the 8–9-h overnight fast (paired Student’s t test): *P < 0.05 and **P < 0.01. n = 6.
RESULTS

Data from experiment A are summarized in Figure 1. When the ileostomy content was collected at 2-h intervals during 8 h after the test meal, sphingomyelin and ceramide outputs peaked between 2 and 4 h after ingestion and then declined between 4 and 8 h. The total output of both sphingomyelin ($P < 0.05$) and ceramide ($P < 0.01$) 0–8 h postprandially was greater than the fasting value. The concentrations of sphingomyelin after 2–4 h and those of ceramide after 2–4 and 4–6 h were significantly higher than in the overnight fasting samples. The average increase in sphingomyelin plus ceramide output, calculated from the difference between the output during 8 h postprandially and the fasting output overnight (8–9 h) amounted to 18.8% of the given dose of 250 mg milk sphingomyelin. Thus, even if the output of both sphingomyelin and ceramide increased, the major part of the given sphingomyelin had been digested.

### TABLE 1

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<tr>
<th>Sphingolipid</th>
<th>Sphingomyelin meal content (mg)</th>
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<td>Palmitoyl-sphingomyelin (µg)</td>
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<td>Geometric mean, fasting (95% CI)</td>
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<td>Stearoyl-sphingomyelin (µg)</td>
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<td>C-22:0-sphingomyelin (µg)</td>
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1 $n = 5$. Fasting and postprandial values obtained with different doses were compared by using one-factor ANOVA with repeated measures on logarthmic values transforming values back to geometric means (95% CIs) as described in Subjects and Methods. Differences between doses at 0 to 8 h were not statistically significant.

2–4 Significantly different from fasting: $^2P < 0.01$, $^3P < 0.05$, $^4P < 0.001$. 

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In experiment B, we asked whether sphingomyelin in doses close to what may occur in normal meals (15), i.e., 50, 100, or 200 mg, increased specific molecular species of sphingomyelin, ceramide, and sphingosine in the ileostomy content when added to a normal breakfast low in sphingolipids.

The outputs in ileostomy content of all sphingolipids measured during 8 h after the breakfast meal and during overnight fasting are compared in Table 1. The fasting ileostomy output of the different compounds ranked as follows: palmitoyl-sphingosine > palmitoyl-sphingomyelin > C-24:0-sphingomyelin > stearoyl-sphingomyelin > C-23:0-sphingomyelin > sphingosine. The relative increase after the meals differed considerably between the compounds. Although C-23:0-sphingomyelin which was low during fasting but is an abundant sphingomyelin species in milk increased significantly, no increase was observed for stearoyl-sphingomyelin, which is a minor sphingomyelin species in milk. C-22:0- and C-24:0-sphingomyelin exhibited patterns similar to that of C-23:0.
Thus, the increases compared with fasting were strongly significant for all 3 doses of the very-long-chain sphingomyelins but not for differences between doses. In case of palmitoyl-sphingomyelin, the fasting output was higher than that for the C-22:0 and C23:0 sphingomyelins, but the postprandial increase significant only for the 100- and 200-mg doses. The postprandial output of palmitoyl-sphingosine was highest of all compounds, but the individual variation was high and significantly different from fasting only with 50 and 100 mg. The sphingosine output was generally much lower than the outputs for palmitoyl-sphingosine but increased after all 3 sphingomyelin doses. No effect of sphingomyelin feeding on the output of stearoyl-sphingosine was observed.

As mentioned previously, studies by others indicated that in bovine milk sphingomyelin, 10.1% (wt:wt) is palmitoyl-, 0.7% is stearoyl-, 7.1% is C-22:0-, 12.7% is C-23:0-, and 9.2% is C-24:0-sphingomyelin (19). If it is postulated that our milk sphingomyelin has the same species composition, the recovery in ileostomy content over 8 h amounted to 6.1–11.8% for C-22:0-, 3.2–5.6% for C-23:0, 14.4–26.8% for C-24:0, and 8.0–23.1% for palmitoyl-sphingomyelin, without any relation to dose. These figures include the endogenous contribution of each species as estimated by the h0 0 values. Thus, more accurate approximations can be obtained by subtracting the fasting output. The recoveries were then 5.2–8.2% for C-22:0-, 3.0–4.6% for C-23:0, 11.7–19.2% for C-24:0-, and 4.8–9.1% for palmitoyl-sphingomyelin. Thus, the major portion of all measured sphingomyelin species had undoubtedly been digested with all 3 sphingomyelin doses.

The output of sphingolipids was compared with that of cholesterol (Table 1), which was manifold higher than the sphingolipid output. Cholesterol output did not differ significantly with sphingomyelin dose but postprandial values were higher than in the over night fasting samples which probably reflects influence of the meal on gallbladder emptying.

The concentrations of different sphingolipids after the meals in comparison with fasting values in overnight ileostoma contents are shown in Figure 2. Pooled fasting values from the 3 occasions are shown, but in the statistical evaluations the respective fasting values and postprandial values are matched. The highest concentrations of sphingomyelin species and metabolites were in most cases observed at 2 to 4 h. Because the variations in intestinal transit in this type of experiments add to other variable parameters, data are, however, presented for the 0–4- and 4–8-h intervals. The analyses showed common features as well as considerable interindividual variations. In all samples the concentration of palmitoyl-sphingomyelin was much higher than that of stearoyl-sphingomyelin. Among the C-22:0-, C-23:0-, and C-24:0-sphingomyelin species, the highest concentration was observed for C-24:0-sphingomyelin. The average concentrations of C-22:0- and C-23-sphingomyelin were lower but were still higher than the concentration of stearoyl-sphingomyelin. For the ceramides, the palmitoyl-sphingosine concentration was much higher than the stearoyl-sphingosine concentration. Also, the sphingosine concentration was much lower than the palmitoyl-sphingosine concentration. As a general feature, the interindividual variation in concentrations was higher than the intraindividual variation between the 3 occasions. For example, subject number 1 had higher palmitoyl-sphingosine and stearoyl-sphingosine concentrations than the other subjects, in both fasting and postprandial samples, at all 3 occasions, but did not have high concentrations of any of the other sphingomyelin species or of sphingosine. Subject number 2, on the other hand, had the highest palmitoyl-sphingomyelin-, C-24:0-sphingomyelin, and sphingosine concentration on all 3 occasions, but much lower concentrations of palmitoyl-sphingosine than subject number 1 (data not shown).

We compared the effect of different doses of sphingomyelin on mean concentrations during 0–8 h (data not shown) and found that the effect of all 3 sphingomyelin doses was highly significant (P < 0.001) for C-22:0-, C-23:0-, and C-24:0-sphingomyelin and sphingosine concentrations; doses of 100 and 200 mg significantly (P < 0.05) increased the palmitoyl-sphingomyelin concentration, and only the 100 mg dose increased (P < 0.05) the palmitoyl-sphingosine concentration. No effect of sphingomyelin feeding on stearoyl-sphingomyelin and stearoyl-sphingosine was seen. Differences between individual doses were, however, significant only for 200 mg compared with 50 mg for C-24:0-sphingomyelin (P < 0.05) and close (P < 0.1) to significance (P = 0.071) for 200 mg compared with 50 mg for C-23:0-sphingomyelin.

As shown in Figure 2, concentrations of all sphingolipids were generally higher at 0–4 h than after 4–8 h. For these sphingolipids, the average concentration of which increased during the whole 0–8-h interval after sphingomyelin feeding (ie, all except stearoyl-sphingomyelin and stearoyl-sphingosine), we compared the concentrations obtained with all 3 doses at 0–4 and 4–8 h and during fasting. We found that the 0–4-h concentrations were higher than the fasting values for C-23:0-sphingomyelin (P < 0.001), C-24:0-sphingomyelin, C-22:0-sphingomyelin, and sphingosine (P < 0.01) and than the 4–8-h values for C-23:0-sphingomyelin (P < 0.01) and C-24:0-sphingomyelin (P < 0.05). We therefore asked whether, for these compounds, there is a more distinct dose effect at 0–4 h, ie, when the concentration and probably the proportion of exogenous sphingolipids are highest. The concentrations of C-23:0- and C-24:0-sphingomyelin were found to be higher after 200 mg sphingomyelin (P < 0.01) than after 50 mg sphingomyelin. For C-22:0, the difference (P < 0.1) was close to significance (P = 0.066). For C-24:0-sphingomyelin the difference between the 100- and 50-mg doses (P = 0.052) and for C-23:0-sphingomyelin the difference between the 200- and 100-mg doses (P = 0.067) were also close (P < 0.1) to significance For other compounds, no significant dose response could be identified. Postprandial concentrations of palmitoyl-sphingomyelin, palmitoyl-sphingosine, and sphingosine were variable, although individual subjects reached peak concentrations much above the fasting values.

**DISCUSSION**

This article characterizes the output of sphingomyelin and sphingolipid metabolites in human ileostomy content during fasting and after consuming 50–250 mg milk sphingomyelin. After a breakfast meal with 250 mg milk sphingomyelin (experiment A), the output of sphingomyelin and ceramide increased, most at 2–4 h and less after 4–8 h. Yet, the cumulative increase in sphingomyelin plus ceramide in ileostomy content during the 8 h was only 19% of the given sphingomyelin dose, which indicated that most of the fed sphingomyelin had been digested. Earlier studies in rats showed that intact sphingomyelin and ceramide are poorly absorbed. The
ceramide after feeding 3H-dihydro-sphingomyelin was similar
ing the percentage of radiolabeled sphingomyelin recovered in feces as
sphingomyelin was also found to be limited, although the per-
SMase and ceramidase are high (1, 5). The capacity to digest
is most active in the jejunum, where the concentrations of Alk-
the rapid digestion of glycerolipids in the upper small intestine and
sequential digestion to fatty acids and sphingoid bases, which is
digestion of a 250-mg dose is indeed incomplete, although >80%
had been digested. We therefore asked, in experiment B, whether
sphingomyelin doses in the range of normal meals may increase
colon exposure to sphingolipids.

In experiment B, we fed 50, 100, and 200 mg sphingomyelin and
examined 4 of the sphingomyelin species that are abundant in milk
sphingomyelin (19), namely those containing palmitic acid, C-22:0,
C-23:0, and C-24:0 fatty acids and sphingosine as the sphingoid
base. The fifth species analyzed, ie, stearoyl-sphingomyelin, is
a minor sphingomyelin species in milk. Although there are few
human data on the molecular species composition of sphingo-
myelin in the stomach, intestinal mucosa, and bile, animal studies
have shown that sphingomyelin in the intestinal and gastric mucosa
contains a high proportion of palmitic acid linked to sphingosine in
sphingomyelin, whereas the C-22–C-24 fatty acids in sphingo-
myelin are linked to phytosphingosine (22, 23). The small amount
of sphingomyelin that is secreted in bile contains much palmitic
acid compared with tissue sphingomyelin and also relatively more
stearoyl-sphingomyelin than milk (24).

In agreement with earlier studies of mucosal and bile sphin-
gomyelin, we found high concentrations of palmitoyl species of
sphingomyelin and ceramide in fasting ileostoma samples. The
concentrations of palmitoyl sphingomyelin in relation to con-
centrations of C-22:0-, C-23:0-, and C-24:0-sphingomyelin were
higher than reported from analyses of milk sphingomyelin (19). A
major finding in the postprandial samples was that the ileostoma
output of C-22-, C-23-, and C-24:0-sphingomyelin increased
relatively more than did the output of other sphingolipids. The
palmitoyl-sphingomyelin concentration was higher than the concentra-
tion of other sphingomyelin species in both fasting and
postprandial samples and the increase after feeding milk
sphingomyelin was smaller, reflecting the greater endogenous
contribution than for the C-22:0–C-24:0 species. Stearoyl-
sphingomyelin, a minor species in milk sphingomyelin, did not
increase. Thus, the composition of molecular sphingomyelin
species changed in the direction of the milk sphingomyelin,
indicating an incomplete digestion. Yet, recoveries of individual
sphingomyelin species amounted to only 3–21% of the given
dose. The major portion of all sphingomyelin species had thus
been digested, and there was no indication that the proportion of
sphingomyelin that remained undigested increased with an in-
crease in the dose.

Analyses of the ceramides palmitoyl- and stearoyl-sphingosine
and of sphingosine showed considerable interindividual dif-
ferences. As a general feature, palmitoyl-sphingosine exhibited the
highest concentrations of all compounds examined, both in
fasting and postprandial samples. Sphingosine concentrations
were much lower, in agreement with the view that sphingosine
released by the hydrolysis of ceramide is rapidly absorbed (1),
although the output increased with sphingomyelin feeding.
Furthermore, the average concentration of palmitoyl-sphingosine
was higher than that of palmitoyl-sphingomyelin, in agreement
with rat studies, which showed that hydrolysis of ceramide
formed from exogenous sphingomyelin is less complete than
sphingomyelin hydrolysis (8). Although variable, peak post-
prandial concentrations of palmitoyl-sphingosine and sphingo-
sine always exceeded fasting concentrations in all individuals.

We do not know the reasons for the great individual variations
in both fasting and postprandial samples. Variations in intestinal
transit time, in concentrations of Alk-SMase and ceramidase, and
in the endogenous supply of sphingomyelin into the gut via
sloughed mucosal cells and bile secretion, may have contributed
to the variation. Interestingly, one subject, who had the highest
concentrations of all metabolites in the fasting state, also
exhibited early peak concentration of sphingomyelins, palmitoyl-
sphingosine, and sphingosine in ileostomy content, but not in-
creased concentrations after 6 and 8 h (data not shown). This
may indicate a rapid intestinal transit. Also, the ratios between
palmitoyl-sphingomyelin and palmitoyl-sphingosine concentra-
tions differed considerably between individuals, which suggested
that the relations between sphingomyelinase and ceramidase
activities varied. Alk-SMase and ceramidase activities are highly
influenced by bile salt/sphingomyelin and bile salt/ceramide
ratios as well as the presence of other lipids (2, 25). This may
explain why we obtained a less distinct dose response than was
expected from the rat experiments.

Earlier studies showed that dietary sphingomyelin may inhibit
cholesterol absorption, and sterols delayed the digestion of
sphingomyelin (9). In the present study, we found no significant
effect of sphingomyelin on the ileostomy output of cholesterol.
Even after feeding 200 mg sphingomyelin, the output of cholesterol
was 30-fold higher than the output of the ceramide plus sphingomy-
lin species analyzed. The degree of digestion of dietary sphingomyelin
was thus much larger than the degree of reabsorption of endogenous cholesterol. Yet, some inhibition
by sterols from the digestion of sphingomyelin, both in vivo in rats (9)
and in vitro with purified Alk-SMase (25), was observed earlier.
One may therefore ask whether the interaction between sphin-
gomyelin, ceramide, or sphingosine (26) and endogenous chole-
sterol may have contributed to the incomplete digestion of
sphingomyelin and absorption of sphingosine in humans.

Consumption of sphingolipids (0.05–0.1% wt:wt in the diet as
compared with 0.01–0.02% in a Western diet) was shown to
counteract experimental colon cancer (11, 27–29). Sphingo-
myelin has also been shown to inhibit cholesterol absorption (9)
and to lower plasma lipids in lipid- and cholesterol-fed
Leyden mice (30). This may be linked to the slow digestion
and absorption of sphingomyelin and other sphingolipids (8).
Physical interaction with sphingomyelin as well as sphingosine
action may be important (10), and ceramides, in contrast with
free sphingosine, penetrate poorly into mucosal cells (1). Fur-
thermore, both Alk-SMase and ceramidase are protease-resistant
enzymes that are, in part, released from the mucosa and trans-
ported to the colon in active form. It is thus possible that the
increase in the output of sphingomyelin and its metabolites
observed in the present study may be biologically relevant in
humans, but this remains to be established.
In conclusion, the results of the present study showed that moderate amounts of sphingomyelin are effectively digested in humans. The ileum and colon, however, are exposed to some endogenous and exogenous sphingomyelin and its metabolites, and this exposure may be influenced by the supply of moderate doses of dietary sphingomyelin. There is, however, large interindividual variation, and a distinct dose response or saturation of the capacity to digest sphingomyelin was not observed within the dose range tested. A dose of ≥250 mg sphingomyelin, which can be achieved with milk fat globule membrane–enriched dairy formulations, should be enough to increase the exposure of the colon to intact sphingomyelin, ceramide, and sphingoid bases in most individuals.

The authors’ responsibilities were as follows—R-DD, EH, ÅN, and LO: planned and organized the study, recruited the patients, and conducted the patient study; EH, RS, LO, and R-DD: stored and performed lipid extraction of samples; LN: prepared the purified milk sphingomyelin and conducted the HPLC analyses in experiment A; BAGJ: conducted the HPLC-MS/MS analyses in experiment B; and AN and LO: performed most of the work on the calculations and presentation data and coordinated the writing of the manuscript. At the time of experiment A, LN was supported by Swedish Dairy Association. None of the other authors had any potential conflicts of interest.

REFERENCES