Bile-salt–activated lipase: effect on kitten growth rate

Chi-Sun Wang, Mary E Martindale, M Margaret King, and Jordan Tang

ABSTRACT Because of the presence of bile-salt–activated lipase in cat milk, the dependence of the kitten on bile-salt–activated lipase is anticipated for milk fat absorption. To test this hypothesis, we initiated a feeding experiment comparing the growth rate of kittens fed with formula with those fed with formula and supplemented with purified human milk bile-salt–activated lipase. The results indicated that the kittens fed formula with supplemental enzyme had a growth rate twice that of kittens fed with formula alone. This study also indicated that the kitten can be utilized as an animal model in the investigation of the functional role of bile-salt–activated lipase. In this study we also performed the partial characterization of cat milk protein and fat. Am J Clin Nutr 1989;49:457-63.

KEY WORDS Human milk, cat milk, lipase, bile salt, taurocholate, infant growth

Introduction

The bile-salt–activated lipase represents the major lipolytic activity of human milk (1–3). This enzyme is inactive in the milk but is presumably activated when the milk is mixed with bile salts in the intestine. The human milk bile-salt–activated lipase has been purified (4–6) and characterized. This enzyme is functionally and structurally similar to the pancreatic carboxyl ester lipase (7, 8). Preliminary evidence suggested that this lipase may play a role in the fat digestion processes of the newborn (9). For example, the pasteurization of human milk resulted in a lowering of milk fat absorption by as much as one-third in preterm infants (9). Also, the supplementation of infant formula with human milk increased the fat absorption in low-birth-weight infants (10). Although these results imply the involvement of human milk lipase in the fat absorption processes of human infants, these data are nevertheless equivocal because these studies were not performed with the purified enzyme. Part of the difficulty in obtaining direct evidence in this area has been the lack of experimental model. Although the enzyme that can be purified is obviously insufficient to carry out nutritional experiments in human infants, there has been no animal model available for this purpose as well.

For a long time, the bile-salt–activated lipase was thought to be present only in the milk of some primates, including human and gorilla (11). However, Freed et al (12) recently described that this enzyme is also present in the milk of cats and dogs. The presence of this lipase in cat milk would suggest a possible dependence of the kitten on bile-salt–activated lipase for fat digestion. Thus, the cat may represent an effective animal model with which the nutritional role of this milk enzyme can be examined. To test these ideas we initiated feeding experiments for comparing the growth rate of kittens fed with formula with those fed with formula and supplemented with purified human bile-salt–activated lipase. We also studied the chemical composition of cat milk and performed comparative studies of the properties of human and cat milk bile-salt–activated lipase.

Experimental procedures

Materials

Unless otherwise stated, all chemicals were purchased from Sigma Chemical Co, St Louis, MO. Glycerol tri[1-C14]oleate was obtained from Amersham, Arlington Heights, IL. Lipid standards (triaclyglycerols [TG], cholesterol esters, fatty acids) were obtained from Supelco, Bellefonte, PA. The purification of human milk bile-salt–activated lipase was performed as described by Wang and Johnson (6). The antibodies against purified human milk bile-salt–activated lipase were prepared from rabbits. The peroxidase-labeled antibodies specific for rabbit γ-globulin were purchased from Bio-Rad, Richmond, CA. The 15-d postpartum whole human milk sample (n = 6) was obtained from Dr Roger Illingworth, Oregon Health Science Cen-


1 From the Oklahoma Medical Research Foundation, Oklahoma City, OK.  
2 Supported in part by NIH Grant HL23181.  
3 Address reprint requests to C-S Wang, Lipoprotein/Atherosclerosis Research Program, Oklahoma Medical Research Foundation, 825 NE 13th Street, Oklahoma City, OK 73104.  
Received December 23, 1987.  
Accepted for publication April 12, 1988.
ter. Other human milk samples (1–12 mo postpartum, n = 20) were obtained from a human milk bank (Mother's Milk Bank, Shawnee Mission Medical Center, Shawnee Mission, KS) and stored frozen at −20 °C until used. All samples were analyzed within 6 mo of storage. Sepharose 4B* was purchased from Pharmacia, Piscataway, NJ. The coupling of bile-salt–activated lipase to Sepharose 4B was performed by cyanogen bromide activation (13). The antibodies to human milk bile-salt–activated lipase were purified by affinity chromatography on an immobilized bile-salt–activated lipase Sepharose 4B column by using 3 mol NaSCN/L for the elution of the retained antibodies (13).

Collection of cat milk

Samples of cat milk were obtained from four common cats. The milk was expressed using a device similar to that described by McKenzie and Anderson (14) for milking small animals.

Bile-salt–activated lipase assay

The activity of bile-salt–activated lipase in milk (10–50 μL) was measured using glycerol tri[1-14C]oleate (trioleoylglycerol) as substrate as described previously (15). All assays were carried out in an NH4OH-HCl buffer (50 mmol/L) at pH 8.5. The substrate (trioleoylglycerol) concentration was 10 μmol/mL with a specific activity of 13.3 pBq/mol, 60 mg/mL bovine serum albumin, and 20 mmol taurocholate/L. The final volume of the assay mixture was 1 mL. After incubation at 37 °C in a shaking water bath for 1 h, the reaction was stopped by adding 4 mL of a mixture of isopropanol and 1.5 mol H2SO4/L (40:1, vol:vol). After mixing, the lipids were extracted by the further addition of 2 mL water and 5 mL hexane and shaking with a vortex. Fatty acid was extracted from 3 mL of the separated hexane layer with 1 mL of 0.1 mol KOH/L. A 0.5-mL aliquot of the aqueous KOH solution was taken for measurement of the radioactivity. One unit of activity was defined as the amount of enzyme catalyzing the release of 1 μmol of fatty acid/min at 37 °C.

Analyses of triacylglycerol molecular species

The milk TG molecular species were analyzed by a modified procedure of gas-liquid chromatography (GLC) as described by Kuksis et al (15). Fifty microliters of 25-fold diluted cat milk or 10-fold diluted human milk were mixed with 4 mL heptane: isopropanol (3:7, vol:vol) containing 50 μg of internal standard (cholesterol butyrate). After mixing, the mixture was acidified with 2.5 mL of 0.016 mol H2SO4/L and the mixture was placed on a vortex mixer for 30 s. The upper phase containing the TGs was transferred to a 3-mL conical tube and the solvent was evaporated under nitrogen. The residue was redissolved in 100 μL of n-hexane and 2-μL aliquots were used for on-column injection. Analyses were performed using a Varian 3700 gas chromatograph (Sunnyvale, CA) equipped with a series 8000 autosampler and a SpectroPhysics SP4270 integrator (Houston, TX).

Fatty acid analysis

The cat milk total fatty acid composition was analyzed by GLC. Fifteen microliter of 10-fold diluted cat milk was mixed with 15 μg C15 fatty acid internal standard. The sample was then saponified by mixing 5.0 mL alcoholic-KOH (4.7 mL 95% ethanol and 0.3 mL 33% KOH) and incubated at 60 °C for 30 min. The sample was then acidified with 0.5 mL of 5.7 mol HCl/L and extracted with hexane and methylated with boron trifluoride for the GLC analyses (17).

Protein analysis

The protein content of the milk samples was determined by a previously described procedure by using sodium dodecyl sulfate (SDS) for the solubilization of milk samples (18). Bovine serum albumin was used as protein standard. SDS-polyacrylamide gel electrophoresis was performed as described previously (19). Milk samples (50 μL) were mixed with 50 μL of a solution containing 10 g/L SDS and 8 mol urea/L in 50 mmol sodium phosphate/L with pH 8.5. The mixture was further treated by addition of 10 μL 0.05% (vol:vol) bromophenol blue, 15 μL mercaptoethanol, and 75 μL glycerol. Aliquots of 10 μL of the mixture from the milk of the cat or 25 μL of the mixture from normal human milk were applied for polyacrylamide gel electrophoresis. The gels were stained for protein with Coomasie brilliant blue. The Western-blot of cat milk bile-salt–activated lipase was performed according to the method of Towbin et al (20).

Feeding experiment

All kittens were breast fed for 48 h before initiation of the formal feeding experiment. One female and one male from each of three litters was arbitrarily selected for the control and lipase-supplemented groups. The remaining kittens were breast-fed throughout the studies. Two kittens (male) from a second litter and the other two kittens from a third litter (female) were available for the breast-fed group. The mean body weight of the kittens at the beginning of the feeding experiment was 99.5 ± 8.0 g (x ± SEM) for the lipase-supplemented group, 105.5 ± 3.3 g for the control group, and 116.0 ± 3.2 g for the breast-fed group. There was no significant difference in body weight at this time among the three groups of kittens. The kittens of the control group were fed with a mixture of Kitten Milk Replacement® (Borden Inc, New York, NY) mixed with pasteurized cow milk (3:1, vol:vol). The rationale for mixing the milk replacement with cow milk was to increase the saturated fatty acid content and thus increase the qualitative resemblance of the resulting formula fat to that of milk fat. The cow milk was obtained from a local grocery store. To simulate breast-feeding, the kittens were fed manually with the mixture hourly, 2 mL/h, for 5 d. The mixture was prepared fresh daily. The kittens fed with the supplemental lipase were fed with the same mixture with the addition of purified human milk bile-salt–activated lipase at a concentration of 0.1 mg/mL to simulate the concentration of bile-salt–activated lipase in human milk. The body weights of experimental animals were determined daily to establish the growth rates of the kittens.

Results

Effect of feeding kittens with supplemental bile-salt–activated lipase

The growth rates were compared between three groups of kittens: the breast-fed, the formula-fed, and the group fed with formula supplemented with lipase. In the latter two groups, each kitten was fed with the same amount of formula (2 mL) at hourly intervals, as described above. The mean daily weight gains of these three experimental
groups are shown in Figure 1. The kittens fed with the formula alone had a growth rate of 6.5 ± 1.0 g/d, about half of that found in kittens fed the supplemental lipase (11.4 ± 0.6 g/d). The latter had a growth rate similar to that found in the group of kittens that were breast-fed (13.3 ± 2.0 g/d). The difference between the growth rates of kittens fed by formula and formula supplemented with lipase are statistically highly significant (p < 0.01). On the other hand, the difference between the breast-fed group and the lipase-supplemented group is not significant (p > 0.25).

Studies on cat milk and its lipase

Because purified human milk bile-salt-activated lipase was able to enhance the growth of kittens fed with formula, it became interesting to establish the properties of cat milk lipase and the fat and protein contents in cat milk. The results of these studies are described below.

Bile-salt-activated lipase in cat milk

We confirmed the presence of bile-salt-activated lipase in cat milk (12) because the lipolytic activity in cat milk was observed only in the presence of bile salt (20 mmol taurocholate/L). The activity of the enzyme at different times postpartum was studied. Lipase activity tended to decrease as the lactation period increased from day 3 to days 12–18 (Table 1). However, relatively large differences in the lipolytic activities among individual animals rendered this trend statistically insignificant. Our data also show that human milk had about twice the activity found in cat milk (Table 1).

The molecular weights of the cat and human enzymes were compared by Western-blot analyses (Fig 2). The cat milk enzyme has an apparent molecular weight of 91 000 (Fig 2), which is less than the human milk enzyme (∼125 000) (1). Also, the cat lipase band appeared to be present as a doublet, which may have indicated the possible microheterogeneity of the cat enzyme. However, because the human enzyme is highly glycosylated.
(1), it is not clear whether these differences reflect the protein structures of the enzymes or whether they are due to the heterogeneity of the oligosaccharides.

The structural similarities of human and cat lipases were then studied by immunochemical experiments. Monospecific rabbit antibodies raised against human bile-salt-activated lipase partially inhibited the activity of cat milk enzyme (Fig 3). Although human milk lipase was completely inactivated by its antibodies, the cat milk lipase was inactivated to ~75% at maximum (Fig 3). Such a high degree of immunochemical cross-reactivity suggests that the amino acid sequences between the enzymes from cat and man are highly similar.

**Characterization of cat milk fat**

Because milk triacylglycerols represent the natural substrate of the lipase, we have also examined the fat distribution of cat milk TGs. Qualitatively, the cat milk had a similar distribution of TG molecular species to human milk as revealed by GLC analyses (Fig 4). Quantitatively, however, the cat milk had about a two- to three-fold higher TG content than human milk (Table 2). The total TGs in cat milk did not change as lactation progressed from day 3 to day 6 to days 12–18 (Table 1). There was a slight difference in quantitative distribution of cat milk and human TGs (Table 3). Specifically, there is a slightly higher content of TG_{54} (the number denotes the total acyl-carbon of TG) and a correspondingly lower content of TG_{42}, TG_{44}, TG_{46}, and TG_{48} in cat milk than in human milk. Our finding concerning the TG content of hu-

![Graph](image.png)

**FIG 3.** Effect of purified antibodies to human milk bile-salt-activated lipase on cat milk (●) and human milk (▲) bile-salt-activated lipase activities. The nonimmune γ-globulin fraction of rabbit serum was used as a control (○) for examining the interaction with cat milk enzyme. The stock antibody concentration was 1 mg/mL.

**TABLE 2**

<table>
<thead>
<tr>
<th>Triacylglycerol (g/L)</th>
<th>Day 6 postpartum (n = 3)</th>
<th>Days 12–18 (n = 4)</th>
<th>Human milk (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triacylglycerol</td>
<td>58 ± 5</td>
<td>71 ± 10</td>
<td>67 ± 16</td>
</tr>
<tr>
<td>Protein (g/L)</td>
<td>48 ± 3</td>
<td>45 ± 4</td>
<td>53 ± 3</td>
</tr>
</tbody>
</table>

*Mean ± SEM.
TABLE 3
Acyl-carbon number distribution of cat and human milk*

<table>
<thead>
<tr>
<th>Acyl-carbon</th>
<th>Cat milk</th>
<th>Human milk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 3 (n = 3)</td>
<td>Day 6 postpartum (n = 3)</td>
</tr>
<tr>
<td>34</td>
<td>0.1 ± 0.1</td>
<td>0.1 ± 0</td>
</tr>
<tr>
<td>36</td>
<td>0.4 ± 0.2</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>38</td>
<td>0.8 ± 0.4</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td>40</td>
<td>0.8 ± 0.3</td>
<td>0.7 ± 0.2</td>
</tr>
<tr>
<td>42</td>
<td>0.6 ± 0.3</td>
<td>0.5 ± 0.2</td>
</tr>
<tr>
<td>44</td>
<td>0.5 ± 0.2</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>46</td>
<td>2.6 ± 0.5</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td>48</td>
<td>6.3 ± 1.3</td>
<td>5.1 ± 0.4</td>
</tr>
<tr>
<td>50</td>
<td>19.5 ± 1.4</td>
<td>16.8 ± 1.1</td>
</tr>
<tr>
<td>52</td>
<td>39.5 ± 3.3</td>
<td>37.1 ± 3.5</td>
</tr>
<tr>
<td>54</td>
<td>26.0 ± 2.3</td>
<td>27.3 ± 0.7</td>
</tr>
<tr>
<td>56</td>
<td>2.8 ± 1.3</td>
<td>8.3 ± 4.3</td>
</tr>
</tbody>
</table>

* x ± SEM.
† Significantly higher than those of cat milk (p < 0.05).
‡ Significantly lower than those of cat milk (p < 0.05).

Human milk is similar to that reported by Bitman et al (21) who found a fat content of 28 g/L as measured gravimetrically from 154 milk samples. However, because our milk samples were obtained from a human milk bank, the fat content represents the average value as obtained from the milk bank under their routine protocol of collection rather than a more controlled sample collection technique as suggested by Jensen et al (22).

The similar distribution of fat species in cat and human milk triacylglycerols suggested a possible similarity in fatty acid composition of cat and human milk. The analyses of fatty acid composition (Table 4) confirmed this and also revealed minor differences. Cat milk contained higher C18:1 and C20:4, traces of C12:0, and a lower level of C14:0 fatty acids than did human milk.

Characterization of cat milk proteins

As shown in Table 2, cat milk has a higher content of triacylglycerols and also a two- to three-fold higher protein content. The higher caloric content of cat milk is probably related to the small volume of milk intake by the kitten. The SDS-polyacrylamide gel electrophoresis (Fig 5) of the two milk samples indicated that cat milk is more enriched in smaller molecular weight proteins.

Discussion

The nutritional function of milk bile-salt-activated lipases in man and a few animal species was supported only by some fairly indirect evidences (9, 10). The current study has unequivocally shown that human milk bile-salt-activated lipase has a nutritional role in a kitten model. The results indicated that, under the experimen-

tal conditions, the supplementation of human milk lipase with kitten formula significantly enhanced the rates of weight gains of the animal to a level comparable with that of the breast-fed group. These results imply that in the kitten feeding model the human milk bile-salt–activated lipase can retain its potential activity after ingestion and movement through the gastrointestinal tract. It is activated upon encountering bile salts, probably in the intestine, and carries out fat digestion activities. Because the weight gain of the group fed formula and lipase and the group fed breast milk were similar (Fig 3), the results indicate that there is sufficient nutrient intake by the former group of kittens. It is apparent that in our control experiment of the kitten formula feeding, the activity of bile-salt–activated lipase in the digestive system of the animals was low and it was the rate-limiting step of kitten weight gain. Thus, the diet with formula alone would lead to malabsorption and malabsorption. Therefore, supplementation of the formula with the enzyme increased the kitten weight gains by altering this rate-limiting step. The positive results in feeding experiments (Fig 1) further imply that during the first 2–3 wk of life the kitten must have an underdeveloped pancreatic carboxyl ester lipase activity because the activity of this enzyme is known to be very similar to that of the human milk bile-salt–activated lipase.

For practical reasons, only human enzyme can be purified in sufficient amounts for the kitten feeding experiments. However, the use of purified human milk lipase in the kitten feeding model is well justified because the enzymic properties of human and cat enzymes are very similar (12) and the structural similarity of the two enzymes was demonstrated by the high degree of immunological cross-reactivity of the two enzymes (Fig 3). These arguments, as well as those points made in the last paragraphs, predict that the physiological role of human milk bile-salt–activated lipase is also a nutritional one,

TABLE 4
Fatty acid composition of cat and human milk*

<table>
<thead>
<tr>
<th>Fatty acid†</th>
<th>Day 3 (n = 3)</th>
<th>Day 6 postpartum (n = 3)</th>
<th>Days 12–18 (n = 4)</th>
<th>Human milk (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C20:4</td>
<td>trace</td>
<td>trace</td>
<td>trace</td>
<td>4.6 ± 0.4</td>
</tr>
<tr>
<td>C18:0</td>
<td>2.0 ± 0.6</td>
<td>2.1 ± 0.3</td>
<td>1.3 ± 0.1</td>
<td>5.5 ± 0.4</td>
</tr>
<tr>
<td>C16:0</td>
<td>21.2 ± 0.5</td>
<td>21.8 ± 0.3</td>
<td>21.8 ± 0.4</td>
<td>20.4 ± 0.5</td>
</tr>
<tr>
<td>C18:1</td>
<td>5.6 ± 0.3</td>
<td>5.3 ± 0.1</td>
<td>4.3 ± 0.5</td>
<td>4.8 ± 0.2</td>
</tr>
<tr>
<td>C18:2</td>
<td>6.3 ± 0.3</td>
<td>7.0 ± 0.2</td>
<td>9.3 ± 1.0</td>
<td>8.6 ± 0.3</td>
</tr>
<tr>
<td>C18:3</td>
<td>49.0 ± 1.3</td>
<td>47.5 ± 0.6</td>
<td>45.0 ± 1.1</td>
<td>37.6 ± 0.6</td>
</tr>
<tr>
<td>C18:2</td>
<td>15.0 ± 1.1</td>
<td>15.3 ± 0.2</td>
<td>16.9 ± 1.7</td>
<td>18.5 ± 0.8</td>
</tr>
<tr>
<td>C20:4</td>
<td>0.9 ± 0.4</td>
<td>0.9 ± 0.3</td>
<td>1.5 ± 0.6</td>
<td>trace</td>
</tr>
</tbody>
</table>

* x ± SEM.
† Fatty acid nomenclature by acyl-carbon number:number of double bonds.
nately the digestion of milk fat in the human infant’s intestine. Also, during the period of life when milk is the only dietary intake, human infants may have an underdeveloped pancreatic carboxyl ester lipase activity. To the best of our knowledge, the time sequence in the development of pancreatic carboxyl ester lipase in human infants has not been established. Thus, it is not presently possible to estimate whether the intestinal carboxyl ester lipase is rate-limiting in the fat digestion and growth of human infants.

Cat milk has a high TG and protein content. This is probably related to the small volume of milk production by the cat and the requirement of a high caloric density for attaining the optimum growth of the kittens. In this respect it is interesting to note that cat milk also has a higher protein and fat content than does cow milk (23). The high protein content of cat milk is associated with the enrichment of the lower molecular-weight protein of the milk, probably for maintaining the solubility of the milk proteins. On the other hand, because of the high fat content of cat milk, the presence of bile-salt-activated lipase probably assures adequate lipolysis in the intestinal lumen of the kitten.

We gratefully acknowledge the expert technical assistance of D Downs, R Whitmer, C Brown, and HB Bass. We also thank Dr Clinton N Corder and Ms Elke Corder for their help in collecting cat milk.

### References

HUMAN AND CAT MILK LIPASE