EFFECT OF PASTEURIZATION AND OF FREEZING AND THAWING HUMAN MILK ON ITS TRIGLYCERIDE CONTENT

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ABSTRACT. Wardell, J. M., Hill, C. M. and D’Souza, S. W. (Department of Child Health, St. Mary’s Hospital, Manchester, UK). Effect of pasteurization, and freezing and thawing human milk on its triglyceride content. Acta Paediatr Scand, 70: 467, 1981.—In a study of human milk collected for banking, heating at 62.5°C for 30 min, and freezing and thawing resulted in hydrolysis of triglycerides. Freezing and thawing caused disruption of fat globules and a greater hydrolysis of triglycerides than did the heating process. There was a decrease in the percentage of the poly-unsaturated fatty acids linoleic acid (C18:2) and linolenic acid (C18:3) after freezing and thawing, and after heating, but the other fatty acids of human milk triglycerides were not affected. It is suggested that the availability of linoleic and linolenic acid in milk declines when these procedures are used in human milk banking.

KEY WORDS: Pasteurization, freezing and thawing, human milk triglycerides

Expressed breast milk is used increasingly in hospitals to feed sick, and low birth weight babies. Milk is either collected from mothers while they are in hospital, or they are instructed on how to collect the milk at home and the milk is then transported to hospital. No matter how carefully this milk is collected and stored it is often contaminated by bacteria, notably Escherichia coli, Staphylococcus aureus, and group B β-haemolytic streptococci which are harmful to babies. The presence of these bacteria has made it necessary to heat milk before it is eventually frozen and stored in a breast milk bank.

Milk stored in breast milk banks may develop a rancid flavour, and it appears that pasteurization has an adverse effect on the absorption of milk lipids, thus resulting in reduced weight gain in newborn babies (1, 2). These effects require study in more detail since pasteurization or freezing and thawing may result in some alteration in the lipid composition of milk.

It is anticipated that heating, or freezing and thawing are capable of damaging membranes surrounding milk fat globules (3). Fat globules may, therefore, undergo fragmentation and allow greater access of milk lipases to triglycerides, which account for 98% of the total lipids in human milk.

The present study was designed with a view to determining the effects of pasteurization, and of freezing and thawing on the triglyceride content of milk. We have further determined the effects of freezing and thawing on milk fat globules using nephelometry.

METHODS

Expressed breast milk was donated by mothers who had given birth at St. Mary’s Hospital, Manchester. The milk was expressed at 8.00 a.m., at the end of the first week of lactation and was used within 3 h of collection. After separating an aliquot of milk which stayed at room temperature (18–20°C) as a control, the remaining milk sample was either pasteurized or frozen and thawed.

Pasteurization. Milk was heated in a constant temperature water-bath at 62.5°C. An aliquot of milk was removed...
Acetylcholine and evaporated to dryness under a stream of nitrogen at 60°C.

Triglycerides were isolated by TLC using TLC plastic sheets coated with a layer of silica gel 60, 0.2 mm thick (Merck, Darmstadt). The plates were developed in hexane–ethyl acetate–formic acid (90%) (87.5: 12.5: 1.0 v/v) and visualized in iodine vapour (5). The triglyceride band (0.7<Rf<0.9) was eluted from silica with chloroform–methanol (9:1 v/v; 5×1.5 ml aliquots), methyl heptadecanoate (100 μl, 1 mM) added as standard for GLC, and the eluants evaporated to dryness under a stream of nitrogen at 60°C. Transmethylation of triglycerides to fatty acid methyl esters for analysis by GLC was carried out using a method described by Marinetti (6) with the following modifications: eluted triglycerides were dissolved in chloroform (1 ml) and sodium methoxide reagent (1 M, 1 ml) added. After incubating at 65°C for 30 min, HCl (5 N, 1 ml) was added and fatty acid methyl esters were extracted with petroleum spirit (redistilled, 4×1.5 ml). The extracts were blown to dryness under nitrogen at 60°C and the residues dissolved in methanol (100 μl).

GLC. A Perkin-Elmer F11 gas chromatograph interfaced to a Perkin-Elmer 159 chart recorder was used with a 2 mm internal diameter glass column packed with 20% diethylene glycol succinate on Chromosorb W (mesh size 80/100) appropriately ‘aged’. Flame ionization detection was employed with the following operating conditions: injection port temperature 185°C, column oven temperature 175°C, detector temperature 250°C, and nitrogen gas pressure 11 psi.

The constituent fatty acids of human milk triglycerides were determined from the gas-liquid chromatograms. A total peak area was calculated in each case, and the percentage of the total represented by each fatty acid was determined. By expressing the results in this way any alterations in the percentage of the triglyceride fatty acids after an experimental procedure would indicate whether there is a relatively greater loss of one fatty acid as compared with the others. Concentrations of methyl myristate, methyl palmitate, methyl stearate, and methyl oleate in the milk samples were determined by comparison of their peak areas with those given by standards under identical operating conditions.

Nephelometry. An aliquot of fresh milk was diluted (1:1000 v/v) with distilled water and its nephelometer reading determined. This was repeated after the undiluted milk was frozen (−20°C) and thawed one, two, and three times, and subsequently, after this milk was allowed to stand at room temperature for 24 h.

Statistics. The paired-comparisons t-test was used in the analysis. The free fatty acid values, or nephelometer units in each experimental milk sample were compared with that in its own control.

RESULTS

Pasteurization. Heating milk at 62.5°C resulted in partial hydrolysis of triglycerides. Fig. 1 shows that triglyceride hydrolysis had started when the temperature reached 62.5°C, and by 30 min there was a significant reduction in all four fatty acids of milk triglycerides (p<0.05).
Table 1. *Area percentages of fatty acids of human milk triglycerides in unheated, control milk, and milk which has been heated to 62.5°C*

Results are mean ± S.E. of the number of samples indicated in parentheses

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Control</th>
<th>62.5°C 'Time = 0'</th>
<th>62.5°C 'Time = 30'</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myristate</td>
<td>5.4 ± 0.69 (7)</td>
<td>5.5 ± 0.52 (7)</td>
<td>5.64 ± 0.49 (7)</td>
</tr>
<tr>
<td>Palmitate</td>
<td>33.86 ± 0.84 (7)</td>
<td>33.4 ± 1.09 (7)</td>
<td>34.6 ± 0.78 (7)</td>
</tr>
<tr>
<td>Palmitoleic</td>
<td>4.82 ± 0.31 (7)</td>
<td>4.46 ± 0.53 (7)</td>
<td>4.4 ± 0.37 (7)</td>
</tr>
<tr>
<td>Stearate</td>
<td>12.17 ± 0.53 (7)</td>
<td>12.32 ± 0.57 (7)</td>
<td>12.48 ± 0.68 (7)</td>
</tr>
<tr>
<td>Oleate</td>
<td>41.38 ± 1.38 (7)</td>
<td>41.6 ± 0.97 (7)</td>
<td>41.05 ± 0.61 (7)</td>
</tr>
<tr>
<td>Linoleate</td>
<td>3.17 ± 0.32 (7)</td>
<td>3.14 ± 0.17 (7)</td>
<td>3.01 ± 0.64 (7)</td>
</tr>
<tr>
<td>Linolenate</td>
<td>3.52 ± 0.17 (7)</td>
<td>2.72 ± 0.13 (7)**</td>
<td>2.74 ± 0.22 (7)**</td>
</tr>
</tbody>
</table>

Levels of significance (Student's t-test): Control vs. 62.5°C, time 0: **p<0.01; control vs. 62.5°C, time 30: *p<0.05.

Myristate concentration had fallen by 15%, palmitate by 8%, stearate by 6%, and oleate by 8%.

The mean (±S.E.) percentages of the constituent fatty acids of triglycerides in untreated control milk and in heated milk are shown in Table 1. There were no significant changes with the exception of linolenate which had decreased by 22% when the temperature of the milk reached 62.5°C, but there was no further change when this temperature was maintained for 30 min.

*Freezing and thawing.* Freezing at −20°C and thawing also resulted in a loss of milk triglycerides (Fig. 2). A trend in these results suggests that myristate behaved differently from the remaining triglyceride fatty acids—palmitate, stearate, and oleate. After the first freeze and thaw the concentration of myristate decreased by 14% (p<0.05) and after the second freeze and thaw by a further 18%, but there was no significant change when the procedure was repeated a third time. In contrast, there was a 20–25% decrease in the mean concentrations of palmitate, stearate, and oleate after the first freeze and thaw (p<0.05) and the concentrations remained relatively steady thereafter.

The mean percentages of fatty acids of human milk triglycerides did not show any significant change with the exception of linoleate (Table 2) which showed a 65% decrease after the second freeze and thaw. The peaks on the gas chromatograms corresponding to linolenate were not accurately measurable in this experiment.

The results of freezing milk at −196°C and thawing showed a similar trend to that observed after freezing at −20°C.

![Fig. 2. Effect of freezing (−20°C) and thawing on the concentrations of myristate, stearate, palmitate, and oleate of human milk triglycerides. Results are mean (±S.E.) of the number of samples indicated in parentheses.](image-url)
Table 2. Area percentages of fatty acids of human milk triglycerides in untreated, control milk, and in milk which has been frozen (-20°C), and thawed

Results are mean ± S.E. of the number of samples indicated in parentheses

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Control</th>
<th>Freeze &amp; thaw</th>
<th>Freeze &amp; thaw 2</th>
<th>Freeze &amp; thaw 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myristate</td>
<td>3.24±0.48 (7)</td>
<td>4.58±1.05 (7)</td>
<td>3.06±0.51 (7)</td>
<td>3.59±0.79 (7)</td>
</tr>
<tr>
<td>Palmitate</td>
<td>29.77±0.83 (7)</td>
<td>31.12±0.94 (7)</td>
<td>32.54±0.5 (7)</td>
<td>29.7±1.49 (7)</td>
</tr>
<tr>
<td>Palmitoleic</td>
<td>4.6±0.91 (7)</td>
<td>2.76±0.82 (7)</td>
<td>3.16±0.64 (7)</td>
<td>3.02±0.74 (7)</td>
</tr>
<tr>
<td>Stearate</td>
<td>13.64±1.05 (7)</td>
<td>13.72±1.15 (7)</td>
<td>15.08±1.27 (7)</td>
<td>14.55±1.2 (7)</td>
</tr>
<tr>
<td>Oleate</td>
<td>43.3±1.76 (7)</td>
<td>43.17±2.18 (7)</td>
<td>43.97±1.6 (7)</td>
<td>42.5±1.58 (7)</td>
</tr>
<tr>
<td>Linoleate</td>
<td>6.64±0.67 (7)</td>
<td>4.71±1.26 (7)</td>
<td>2.35±0.83 (7)**</td>
<td>2.60±0.63 (7)*</td>
</tr>
</tbody>
</table>

Levels of significance (Student’s t-test) of: Control vs. freeze and thaw 1: NS; control vs. freeze and thaw 2: **p<0.01; control vs. freeze and thaw 3: *p<0.002.

The above experimental results have shown that with the exception of myristate the decrease in fatty acid concentration of human milk triglycerides is less in milk heated to 62.5°C for 30 min than in milk which has been frozen and thawed once. The decrease in myristate concentration is statistically similar following both types of treatment.

**Nephelometry.** The nephelometer units of human milk compared to a standard (polystyrene latex particles) with a reading of 100 are shown in Fig. 3. After the first freeze (-20°C) and thaw there was a 12% increase (p<0.05) in milk fat globules and a further increase of 9% after the second freeze and thaw but, subsequently, after the third freeze and thaw there was a decline which seemed to continue when the milk was allowed to go rancid.

**DISCUSSION**

Milk lipids exist as microscopic globules in an emulsion which is stabilized by an interfacial surface called the ‘fat globule membrane’, composed of phospholipids and associated material (7). The fat globule of human milk is composed mainly of neutral lipids, i.e. triglycerides and their intermediary products resulting from biosynthesis or enzymic hydrolysis. In fresh milk the fat globule membrane may prevent lipases from acting on triglycerides. Serum-stimulated lipase is associated with the fat globules, but on the outer surface, while bile salt-stimulated lipase is present in the aqueous phase (8, 9). However, if these milk globules are damaged, triglycerides would be available as substrate for lipases. Such damage to milk globules could occur on heating or freezing and thawing. Indeed, it has been reported (1) that free fatty acid concentration of human milk increases after storage at -18°C.

It is apparent that in the milk specimens we studied there is much variation in the triglyceride content. Consequently, the individual fatty acid concentrations were greater in some milk specimens than in others. These differences had occurred although the milk was col-
Human milk triglycerides

lected at a similar time of day in each mother at the end of the first week of lactation. Since milk collected for banking tends to be obtained from mothers at various stages of lactation, and at different times of day a greater variability in lipid contents is to be expected. When assessing the adequacy of human milk for premature babies as much consideration should be given to its lipid content as has been given to its protein and electrolyte content.

Heating, as well as freezing and thawing, results in a relatively greater loss of polyunsaturated fatty acids from milk triglycerides than mono-unsaturated, and saturated fatty acids. The percentages of the poly-unsaturated fatty acids linoleate (C18:2) and linolenate (C18:3) were shown to decrease after freezing and thawing, and after heating milk respectively whereas the other fatty acids did not appear to be affected. Unsaturated fatty acids tend to autoxidise, and this could be a reason for the loss of linoleate and linolenate. Thus, in one set of milk samples the amounts of linolenate present were not measurable on the gas chromatograms.

The feeding of expressed breast milk which has been pasteurized, frozen, stored and then thawed could be just as artificial as the feeding of commercially available milks with modified formula. Saturated fats are not as well absorbed as unsaturated fats especially in the newborn baby, and the loss of poly-unsaturated fatty acids deprives the baby of a valuable source of calories. Moreover, the essential fatty acid linoleic acid is required to promote growth and to maintain the integrity of skin. If, therefore, milk obtained from human milk banks cannot maintain adequate growth and is lacking in its immunological protective role then its suitability in its present form for very low birth weight babies should be seriously questioned. Further research is clearly necessary into how the nutritional needs of such babies can be adequately fulfilled.

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REFERENCES


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