Lipoprotein Lipase Activity of Milk from Cows with Prolonged Subclinical Mastitis

C. DANIEL AZZARA and PAUL S. DIMICK
Department of Food Science
The Pennsylvania State University
University Park 16802

ABSTRACT

The influence of prolonged subclinical mastitis on bovine milk lipoprotein lipase activity was investigated. Nine cows with at least one quarter with prolonged subclinical mastitis and at least one nonmastitic quarter were selected in various stages of lactation. Milk from subclinical quarters had a mean somatic cell count of $5.7 \times 10^6$ cells/ml while milk from nonmastitic quarters had an average somatic cell count of $9.4 \times 10^4$ cells/ml. Quarters with a subclinical infection contained the same pathogenic organisms for a minimum of 6 wk. The average milk lipoprotein lipase activity of 108.7 units/ml milk from subclinical quarters was 27.1% higher than the average enzyme activity of 79.2 units/ml milk from nonmastitic quarters. Conditions present in the mammary gland during prolonged subclinical mastitis could lead to increased milk lipoprotein lipase activity in raw milk.

INTRODUCTION

Milk lipoprotein lipase (mLPL) (EC 3.1.1.34) is the major lipolytic enzyme in milk responsible for the lipolysis of milk fat triglycerides (17). The products of this reaction include short-chain fatty acids, which can cause the defect known as lipolyzed flavor (25). In normal bovine milk, mLPL is associated mainly with the casein fraction (13); however, the enzyme can be released from casein by the addition of heparin or 1 M sodium chloride (NaCl) (14). Milk lipoprotein lipase activity is inhibited by elevated NaCl concentrations (> .5 M), but the inhibitory effect of NaCl may depend on other components of the system (5). The source of mLPL in milk has not been determined. The enzyme is thought to arise from capillary endothelium membranes due to leakage (15) or endogenous synthesis by mammary gland secretory cells (17). Although all milk contains enough mLPL to cause significant lipolysis, very little lipolysis occurs in normal milk because the substrate is protected by the milk fat globule membrane. However, mLPL is activated under adverse handling conditions (induced lipolysis) or changes in physiological and biochemical conditions (spontaneous lipolysis) (8).

There are conflicting reports concerning the effect of mastitis on total lipolytic enzyme activity in bovine milk. Some investigators have observed higher lipolytic enzyme activity in milk from mastitic quarters (10, 21, 27), whereas others have reported lower activity (1, 11) or no significant difference in activity (23) when compared with milk from nonmastitic quarters. However, mastitis appears to increase the susceptibility of milk to lipolysis. Milk from mastitic cows has higher initial free fatty acid concentrations (27, 29) and a higher incidence of lipolysis over storage (11, 12, 21). In addition to mLPL activity, a number of other factors could influence lipolysis during infection. These factors included mLPL activator-inhibitor balance, cell lipase, proteolytic enzymes, heparin-like substances, anions, and the integrity of the milk fat globule membrane (23).

The conflicting reports concerning lipolytic enzyme activity and mastitis could be a result of the changing conditions present in the mammary gland during various degrees of infection. The purpose of this investigation was to determine the effect of prolonged subclinical mastitis on mLPL activity.

MATERIALS AND METHODS

Bovine serum albumin, heparin (159 IU/mg), phosphate-buffered saline (PBS), and Wright stain were from Sigma Chemical Co., St. Louis, MO. The RPMI 1640 with 25 mM Hepes buffer and L-glutamine, fetal bovine serum, and
gentamicin were from Gibco Laboratories, Grand Island, NY. Permoutr and xylene were from Fisher Scientific Chemical Co., Pittsburgh, PA. Liposyn was obtained from Abbott Hospital Products, North Chicago, IL, and human blood serum was donated by the Chemical Laboratory Department, Mountainview Unit of the Centre Community Hospital, State College, PA.

**Sampling Technique**

Nine lactating Holstein cows in The Pennsylvania State University dairy herd were included in this study. These cows were simultaneously involved in a separate study designed to determine the effects of backflushing on the incidence of new infections (26). The infection status of the herd was acquired from data collected during two 3-wk cycles of the backflush study. Samples were collected by standard procedures (3) for bacterial cultures and somatic cell counts. Samples for enzyme assay and leukocyte differential counts were collected aseptically into 50-ml conical centrifuge tubes and held on ice.

**Infection Status**

Milk (.01 ml) was streaked onto a quarter section of a petri dish containing trypticase soy agar (Baltimore Biological Laboratory, Baltimore, MD) with 5% washed bovine erythrocytes (24). Plates were incubated at 37°C, and growth was observed and recorded at 24 and 48 h. Bacterial growth on the media was identified by colony morphology and hemolytic and Gram-staining characteristics. Staphylococcal isolates were treated for coagulase production. Streptococcal isolates were tested on CAMP-esculin plates (3).

The remaining milk sample was used to determine somatic cell counts using the Fossomatic electronic somatic cell counter (Foss Electric, Hillerod, Denmark).

Criteria required to diagnose a quarter with prolonged subclinical mastitis were: 1) a bacterium was present during the 6 wk of the backflush study; 2) the same bacterium was isolated in a sample taken during the present study; 3) somatic cell counts in both studies were in excess of 10^6 cells/ml; and 4) the animal showed no signs of clinical infection in that the milk appeared normal and the animal had no fever, pain, or gland swelling. Milk from uninfected quarters gave negative bacterial cultures, and somatic cell counts were below 4 x 10^5 cells/ml in both studies.

**Leukocyte Differential Counts**

For differential leukocyte counts, 30 ml of milk held at 4°C were placed into a 50-ml conical centrifuge tube and diluted to 50 ml with PBS. Somatic cells were sedimented at 250 x g and the pellet was suspended in 50 ml PBS. Cells were resuspended and the pellet was resuspended in .5 ml RPMI 1640 and 25 mM Hepes buffer and L-glutamine containing 20% fetal bovine serum and .05 mg/ml gentamicin. Smears were stained with Wright stain, and slides were prepared for oil immersion by mounting a coverslip with a xylene: permount (1:1 vol/vol) mixture. Differential counts were performed on 200 cells, which were identified as lymphocytes, polymorphonuclear leukocytes (PMN), and macrophages (7).

**Milk Lipoprotein Lipase Assay**

Milk samples for enzyme assay were held on ice until 2-ml assays were conducted in triplicate within 1 h of collection. The pH stat (Radiometer, Copenhagen, Denmark) method was used to monitor changes in pH produced as a result of lipolytic activity (19). The assay system chosen for this research was based on that described by Robinson (22) and further modified by DeFeo et al. (6) to determine mLPL activity. One unit of mLPL activity was defined as the amount of enzyme that liberated 1 μmol of fatty acid/h under the conditions of the test.

**RESULTS**

Table 1 presents the somatic cell counts, leukocyte differential counts, and mLPL activities of milk from uninfected quarters and quarters with a prolonged subclinical infection. Bacterial cultures of milk samples from the nine subclinically infected quarters revealed the presence of six Staphylococcus aureus infections, two streptococcal infections other than Streptococcus agalactiae, and one coliform infection. The mean somatic cell count of milk from subclinically infected quarters was significantly (P<.05) higher than the mean somatic cell count of milk from uninfected quarters.
TABLE 1. Somatic cell counts, leukocyte differential counts, and milk lipoprotein lipase activities of milk from uninfected quarters versus quarters with a prolonged subclinical infection.

<table>
<thead>
<tr>
<th>Cow number</th>
<th>Infection</th>
<th>Cells/ml (× 10⁶)</th>
<th>Differential counts</th>
<th>Activity (units/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Macrophages (%)</td>
<td>PMN (%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>lymphocytes (%)</td>
<td></td>
</tr>
<tr>
<td>829</td>
<td>Negative</td>
<td>.20</td>
<td>60</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td><em>Staphylococcus aureus</em></td>
<td>4.70</td>
<td>39</td>
<td>52</td>
</tr>
<tr>
<td>923</td>
<td>Negative</td>
<td>.03</td>
<td>51</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td><em>Staphylococcus aureus</em></td>
<td>1.54</td>
<td>44</td>
<td>50</td>
</tr>
<tr>
<td>25</td>
<td>Negative</td>
<td>.04</td>
<td>54</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td><em>Staphylococcus aureus</em></td>
<td>9.20</td>
<td>43</td>
<td>50</td>
</tr>
<tr>
<td>934</td>
<td>Negative</td>
<td>.07</td>
<td>61</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td><em>Staphylococcus aureus</em></td>
<td>6.64</td>
<td>37</td>
<td>52</td>
</tr>
<tr>
<td>718</td>
<td>Negative</td>
<td>.06</td>
<td>55</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td><em>Staphylococcus aureus</em></td>
<td>1.30</td>
<td>38</td>
<td>52</td>
</tr>
<tr>
<td>8794</td>
<td>Negative</td>
<td>.02</td>
<td>50</td>
<td>28</td>
</tr>
</tbody>
</table>
|            | *Staphylococcus aureus*    | 5.57             | 40                  | 52                 | 89.3a
| 751        | Negative                   | .03              | 55                  | 27                 |
|            | *Streptococcus spp.*       | 1.43             | 42                  | 49                 | 90.9a |
| 153        | Negative                   | .38              | 59                  | 21                 |
|            | *Streptococcus spp.*       | 1.18             | 38                  | 51                 | 49.3a|
| 858        | Negative                   | .02              | 63                  | 22                 |
|            | *Escherichia coli*         | 19.74            | 35                  | 58                 | 104.7a |

Summary:

Uninfected quarters
Mean .094 56 25 19 79.2
Range .02-.38 50-63 14-22 35.4- 104.7

Infected quarters
Mean 5.7 39 52 9 108.7
Range 1.18- 35-44 49-58 6-11 63.2- 130.9

a,b Means from the same cow with different superscripts are significantly different (P<.05).
c,d Means from the same cow with different superscripts are significantly different (P<.01).
1 Polymorphonuclear leukocytes.
2 Means of uninfected versus infected quarters are significantly different (P<.05).

Somatic cell counts of milk from infected quarters ranged from 1.18 to 19.74 × 10⁶ cells/ml with a mean cell count of 5.7 × 10⁶ cells/ml. Milk from uninfected quarters had somatic cell counts ranging from .02 to .38 × 10⁶ cells/ml with a mean cell count of 9.4 × 10⁴ cells/ml. The high cell count of the infected quarter from cow 858 probably represented the initiation of the clinical phase of infection (9). Leukocyte differential counts performed on milk from noninfected quarters indicated that macrophages average 56% of the total cell population while PMN and lymphocytes averaged 25 and 19% of the total cell populations, respectively. Milk from quarters with prolonged subclinical mastitis averaged 39% macrophages, 52% PMN, and 9% lymphocytes. The average mLPL activity of 108.7 ± 19.24 units/ml calculated from the means of quarters with prolonged subclinical mastitis was significantly (P<.05) higher than the average mLPL activity of 79.2 ± 24.1 units/ml calculated from the means of noninfected quarters. The higher mLPL activity observed in milk from infected quarters...
quarters represented a 27.1% increase over the lipolytic activity observed in milk from uninfected quarters.

**DISCUSSION**

Conditions present in the mammary gland during a prolonged subclinical infection appear to enhance mLPL activity. Recent studies in our laboratory (4) demonstrated that macrophages isolated from bovine mammary gland secretions can secrete lipolytic enzymes. It was suggested that these enzymes could damage the milk fat globular membrane and consequently increase the availability of milk triglycerides to indigenous mLPL. Thus, higher mLPL activity observed in milk from subclinical quarters during the present study could be partially due to the increased macrophage concentrations present in these samples.

Reports of lower mLPL activity in milk from mastitic quarters (1, 11) could be related to the severity of infection. It has been suggested that permanent damage sustained by mammary tissue during a severe infection could reduce the ability of the gland to synthesize mLPL (11); however, other factors also could be responsible for lowering mLPL activity. Mastitis causes an increase in serum mLPL (1, 23) that under certain conditions may be more stable than mLPL bound to casein micelles (2). Therefore, the total mLPL activity of the system could reflect the distribution of the enzyme during various degrees of infection.

Damage to capillaries and aveolar epithelium during mastitis alters the permeability of membranes between blood and milk (16, 20, 28). Minimal damage to mammary tissue sustained during a prolonged subclinical infection could lead to a concentration of blood components in milk that activates mLPL. For example, Salih and Anderson (23) found that the addition of millimolar concentrations of sodium chloride to normal milk increased serum mLPL activity. In addition, Ostlund-Lindqvist and Iverius (18) reported that as the concentration of the activator protein, apolipoprotein C-II (apo C-II), increased from 0 to 30 μg/ml, enzyme activity increased to a maximum; however, at higher concentrations (>30 μg/ml) apoC-II acted as an inhibitor. These investigators also reported that apoC-I and apoC-III:2 enhanced mLPL activity in the presence of apoC-II and excess substrate.

In conclusion, changes that occur in the mammary gland during various degrees of infection have an influence on total mLPL activity of bovine milk. This investigation demonstrated that mLPL activity is increased in milk from quarters with prolonged subclinical mastitis.

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