Comparison of Rat Hepatic Cholesterol Biosynthesis
During Skim Milk Versus Whey Permeate Ingestion

NANCY L. KEIM, JUDITH A. MARLETT, CLYDE H. AMUNDSON, 1
and LINDA D. HAGEMANN
Department of Nutritional Sciences
University of Wisconsin
Madison 53706

ABSTRACT
Whey permeate is an ultrafiltrate of whey that is devoid of protein but contains lactose, salts, and other soluble low molecular weight compounds. These experiments compared cholesterol concentrations of blood plasma, hepatic lipids, and hepatic cholesterol biosynthesis of rats ingesting skim milk powder versus whey permeate powder. Groups of young male rats weighing 90 to 92 g were fed a casein-based diet into which skim milk powder or whey permeate powder was incorporated isocalorically. No effects of skim milk or whey permeate on plasma cholesterol concentrations were observed at any time during 5-wk of feeding. However, 3-hydroxy-3-methylglutaryl coenzyme A reductase activity was increased by either skim milk or whey permeate feeding. Hepatic cholesterol, triglyceride, and phospholipid concentrations at wk 5 were unchanged. Plasma and hepatic cholesterol responses of rats to whey permeate ingestion are similar to those that occur with skim milk consumption, and plasma and hepatic cholesterol concentrations do not reflect necessarily an increase in hepatic cholesterol biosynthesis.

INTRODUCTION
Consumption of a variety of fresh or fermented milk products along with undefined diets has been associated with lower cholesterol (CH) in plasma in healthy human subjects (15, 16, 21, 22). The initial observation was by Mann and Spoerry during a study of Maasai tribesmen; consumption of large quantities of fermented milk significantly lowered plasma CH, particularly in subjects who gained weight during the study (22). Supplementing an uncontrolled diet with yogurt produced a decline in plasma CH (15, 21); the hypocholesterolemic response was more modest with fresh milk (15, 16, 21). However, when daily nutrient intake was kept constant and skim milk (1.89 liters) incorporated on an isocaloric basis, skim milk did not lower plasma CH (17). Additional studies used rats to elucidate these hypocholesterolemic milk factors (18, 19, 20, 23, 24).

Whey permeate, obtained by the ultrafiltration of whey, is devoid of protein but contains lactose, salts, and other soluble compounds of low molecular weight similar to those in skim milk. Based on evidence that one milk factor is in the dialyzable fraction and one in the nondialyzable fraction of milk (1), the hypocholesterolemic property of whey permeate might be expected to be less than that of skim milk. The purpose of these experiments was to compare hepatic cholesterol synthesis in rats fed skim milk powder (SMP) versus whey permeate powder (WPP). Some previous studies reported significant decrease in plasma CH of rats fed skim milk although the point at which the decrease appeared varied by as much as 8 wk (19, 20, 24). In other experiments with rats, no significant hypocholesterolemia was observed with skim milk ingestion (18, 23). Because some of these unexplained differences might have occurred from unknown ingredients in the rat diet when stock diets were used, all experiments reported herein used casein-based, semipurified diets.

MATERIALS AND METHODS
All animals were 27-day-old, male Sprague-Dawley rats (Gibco Animal Resources, Madison, WI 53711). Each rat was housed individually in a facility that was lighted from 1600 to 0400 h.
For an adaptation period of 3 days, all rats received stock diet (Rat Chow, Ralston Purina Company, St. Louis, MO 63188); then experimental diets were offered when the rats were 30 days old. Body weights were monitored twice a week, food intake three times a week.

The three experimental diets, each fed to 12 rats, were 1) a casein-based control diet, 2) a casein-based diet containing 25% SMP (by wt), or 3) a casein-based diet containing 17.7% WPP (Table 1). When the SMP and WPP were incorporated into the diet, proportions of carbohydrate, protein, and fat in the diet were maintained by decreasing appropriate ingredients in the basal diet. The cholesterol content of all diets by analysis (6) was similar, and < 1.0 mg/100 mg. The WPP was incorporated into the diet to produce the same lactose content as was in the 25% SMP diet. Throughout the experiment, rats tolerated the diets well with no evidence of diarrhea.

The source of all dairy products was mixed Holstein herds. The pooled milk supply was for commercial processing in Madison WI dairy plants. All of the herds were on similar feed, and all samples were obtained and processed within 1 wk. Because a large pooled milk supply was the source, there should be no seasonal or feed variations in composition. Swiss cheese whey made from the pooled milk supply was obtained, separated, pasteurized (72°C, 15 s) and subjected to ultrafiltration in the University of Wisconsin Food Engineering pilot plant. Ultrafiltration was on a Aqua-Chem unit containing polyamide membranes with a cut-off limit of 50,000 daltons. Skim milk was prepared from raw whole milk from the pooled milk supply. This milk was separated and pasteurized (72°C, 15 s) in the University of Wisconsin Food Engineering pilot plant. Both ultrafiltered whey permeate and skim milk then were spray-dried on the University of Wisconsin tower dryer under similar conditions (12). All milk and whey processing was under the supervision and control of the authors.

Blood samples (.5 to 1.0 ml) were collected from the orbital plexus of fasting (14 to 18 h) rats under light ether anesthesia. Concentrations of CH in plasma were measured at wk 0 and after 5 wk of feeding in all 12 animals from each group; at wk 2, 3, and 4 six rats were selected randomly from each of the three diet groups for measurement of CH in blood plasma.

At the end of 5 wk of feeding, hepatic lipids, CH biosynthesis, and 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase activity were measured. Four animals from each group were fasted and killed for hepatic lipid analysis; livers were excised, weighed, and frozen for later determinations of CH (6), triglyceride (10, 11), and phospholipid (4) concentrations of total lipid extract (25).

### TABLE 1. Composition of experimental diets (%).

<table>
<thead>
<tr>
<th></th>
<th>Casein Control</th>
<th>Casein +SMP</th>
<th>Casein +WPP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein, vitamin free&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.00</td>
<td>11.80</td>
<td>22.00</td>
</tr>
<tr>
<td>Starch, Staley Pearl&lt;sup&gt;b&lt;/sup&gt;</td>
<td>48.00</td>
<td>48.00</td>
<td>45.02</td>
</tr>
<tr>
<td>Sucrose, crystalline&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.00</td>
<td>.30</td>
<td>.28</td>
</tr>
<tr>
<td>Corn oil&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.30</td>
<td>5.20</td>
<td>5.30</td>
</tr>
<tr>
<td>Salt mix&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>4.00</td>
<td>4.00</td>
<td>4.00</td>
</tr>
<tr>
<td>Vitamin mix&lt;sup&gt;d&lt;/sup&gt;</td>
<td>.50</td>
<td>.50</td>
<td>.50</td>
</tr>
<tr>
<td>Choline chloride&lt;sup&gt;a&lt;/sup&gt;</td>
<td>.20</td>
<td>.20</td>
<td>.20</td>
</tr>
<tr>
<td>Solka Flocc&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
</tr>
<tr>
<td>Skim milk powder (SMP)</td>
<td></td>
<td>25.00</td>
<td></td>
</tr>
<tr>
<td>Whey permeate powder (WPP)</td>
<td></td>
<td></td>
<td>17.70</td>
</tr>
</tbody>
</table>

<sup>a</sup>United States Biochemical Corp., Cleveland, OH 44122.

<sup>b</sup>A. E. Staley Mfg. Co., Decatur, IL 62521.

<sup>c</sup>Teklad Test Diets, Madison, WI 53711.

<sup>d</sup>As in (28).
Four other animals from each experimental group were used to study in vivo hepatic CH biosynthesis by a modification of the method of Goldfarb (12). Animals were injected intraperitoneally with 25 mCi of $^3$H$_2$O between 0900 and 1000 h, that is, in the middle of the dark cycle, placed in metabolic cages for 1 h with access to diet and water, and then sacrificed by cervical dislocation. Livers were excised quickly and frozen on dry ice. Cholesterol was isolated from lyophilized homogenates of rat liver. During the lyophilization process, care was taken to trap the water quantitatively; specific activity of the water was determined by counting 10-$\mu$l aliquots in 10 ml of Aquasol (New England Nuclear, Boston, MA 02118).

Lyophilized livers were rehydrated and saponified with 5 ml of 40% methanolic KOH for 3 h at 65°C. Separation of the nonsaponifiable lipids and precipitation of the CH by digitonin were by the method of Bernstein et al. (5). Mean recovery of CH through the digitonin precipitation procedure was 77.4 ± 2.5% (SE). The digitonin precipitate was dissolved in 3 ml of dry pyridine (3) and transferred to scintillation vials. After removal of the pyridine, radioactivity was counted (Packard Instrument Co., Inc., Downers Grove, IL 60515) with Aquasol by the AES channels ratio method used to determine the degree of quenching. Results are expressed as micromoles of $^3$H$_2$O incorporated into CH per gram liver per hour.

For determination of hepatic HMG CoA reductase [mevalonate: NADP oxidoreductase (acylating CoA) E.C.1.1.1.34] activity, four rats from each group were sacrificed at midpoint in the dark cycle by cervical dislocation. The procedure for this enzyme assay is in (13, 23). All results are mean ± SE and were compared by Duncan's new multiple range test (29).

RESULTS

Mean body weight, as well as mean daily weight gain, of the three groups of rats were comparable at wk 5 (day 33) (Table 2). Despite equivalent growth rates, mean daily food intake of the casein-control group was greater than the food intake of either experimental group. Diets tended to lose small but variable amounts of moisture during feeding, but a significant difference in food intake still existed when intakes were adjusted for these losses. We have no clear explanation for this difference in intake, although the efficiency of utilization of the control diet conceivably could be lower.

Neither SMP or WPP consumption altered mean plasma CH or triglyceride (Figure 1), or hepatic CH, triglyceride, or phospholipid concentrations (Table 3).

Hepatic HMG CoA reductase activity significantly increased in both SMP and WPP groups at wk 5 (Table 3). Although differences did not reach statistical significance, means for incorporation of $^3$H$_2$O into hepatic CH also were higher in SMP and WPP groups (Table 3). This trend towards augmented hepatic CH biosynthesis occurred in the absence of any

### TABLE 2. Effect of skim milk or whey permeate powder on rat food intake and body weight. 1

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Casein control</th>
<th>+ Skim milk powder</th>
<th>+ Whey permeate powder</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X</td>
<td>SE</td>
<td>X</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>90.2</td>
<td>1.2</td>
<td>90.8</td>
</tr>
<tr>
<td>Day 33</td>
<td>285.9</td>
<td>3.6</td>
<td>281.5</td>
</tr>
<tr>
<td>Gain/day</td>
<td>5.9</td>
<td>.1</td>
<td>5.8</td>
</tr>
<tr>
<td>Food intake (g/day)</td>
<td>21.2</td>
<td>.5ab</td>
<td>18.7</td>
</tr>
</tbody>
</table>

Numbers with same superscript differ (P<.05).

1 n = 12/group.

Journal of Dairy Science Vol. 65, No. 12, 1982
CHOLESTEROL BIOSYNTHESIS WITH SKIM MILK OR WHEY

100 80 60 50 -6 50 -3
115 (6) Plasma TG mg/dl (n)

50:3

100 80 60 50 -6 50 -3
89:5

50 Casein Control

115 (6) Plasma TG mg/dl (n)

50 Casein+SMP

45:7

50 Casein+WPP

47:5

0 15 22 29 35
day of experiment

15 6 6 8 15
number of observations

Figure 1. Failure of skim milk or whey permeate powder consumption to alter plasma cholesterol and triglyceride of rats. Plasma triglyceride (TG) is expressed as the mean ± SE. The vertical bars indicate the SE of each mean plasma cholesterol point.

Figure 1. Failure of skim milk or whey permeate powder consumption to alter plasma cholesterol and triglyceride of rats. Plasma triglyceride (TG) is expressed as the mean ± SE. The vertical bars indicate the SE of each mean plasma cholesterol point.

DISCUSSION

Effects of WPP on cholesterol metabolism are similar to those of SMP when these products are incorporated into a casein-based, semipurified diet. Further, these effects are like those when fluid skim milk was fed (23). Under our conditions plasma CH of rats fed a casein-based diet and weighing initially 90 to 92 g were not lowered by 5 wk of SMP or WPP ingestion. In a study by Kritchevsky et al. (19) a reduction in plasma CH concentration of older rats (initial wt: 179 g) was significant after 3 wk of feeding “stock” diet and fluid skim milk. Malinow and McLaughlin reported a similar decrease in plasma CH of weaning rats for Chow mixed with fluid skim milk (20). However, others did not observe a decrease in plasma CH concentrations until the 11th wk of feeding Chow plus SMP (24).

Reasons for these discrepancies might include the age of the rat at which the experimental diets were introduced. When a casein-based diet was used, plasma CH was depressed

TABLE 3. Influence of skim milk or whey permeate ingestion on hepatic lipids of rats.1

<table>
<thead>
<tr>
<th></th>
<th>Experimental group</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Casein</td>
<td>+ Skim milk powder</td>
<td>+ Whey permeate powder</td>
</tr>
<tr>
<td></td>
<td>X   SE</td>
<td>X   SE</td>
<td>X   SE</td>
</tr>
<tr>
<td>Hepatic lipids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mg/g fresh wt)</td>
<td>1.79 .27</td>
<td>1.70 .09</td>
<td>1.83 .10</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>1.79 .27</td>
<td>1.70 .09</td>
<td>1.83 .10</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>5.30 1.15</td>
<td>4.80 .40</td>
<td>4.41 .42</td>
</tr>
<tr>
<td>Phospholipid</td>
<td>22.88 3.50</td>
<td>26.42 .61</td>
<td>21.29 .91</td>
</tr>
<tr>
<td>Liver weight (g/100 g body wt)</td>
<td>3.21 .02</td>
<td>3.41 .15</td>
<td>3.40 .11</td>
</tr>
<tr>
<td>H2O Incorporation into CH (μmol/g liver per h)</td>
<td>1.76 .69</td>
<td>3.86 .68</td>
<td>3.66 .76</td>
</tr>
<tr>
<td>HMG CoA Reductase activity (nmol/mg pro per 30 min)</td>
<td>.73 .26***</td>
<td>3.00 .37***</td>
<td>2.45 .61*</td>
</tr>
</tbody>
</table>

1 n = 4; analyses after 5 wk of feeding.

*P<.05.

**P<.01.
significantly by skim milk ingestion only in rats exposed to the experimental diets within 1 wk of weaning (18, 23). In our experiment slightly older rats were used, i.e., 30 days at the start of feeding instead of 23 days, because they would tolerate the frequent bleeding better. Plasma CH concentrations of weanling rats appear to be easier to manipulate by dietary modification (14). The 9-day lapse between weaning and introduction of experimental diets may have given sufficient time for other regulatory mechanisms to become functional.

Other differences that may have produced variable results include the form of the skim milk and the composition of the basal diet. The form of skim milk, however, does not appear to be a cause of the differences. Responses of plasma CH concentrations to fluid skim milk and SMP appear comparable (23). However, ingredients in a “stock” diet, when compared with a semipurified diet containing refined cellulose and casein, may alter the response of plasma CH to SMP. Differences in kinds of vegetable protein (7) or fiber (27) appear to induce different responses in plasma CH under certain experimental conditions; sources of these ingredients could vary with the source of the rat diet. The composition of the “stock” diet used by Kritchevsky et al. (19) may have had an additive effect in promoting the hypocholesterolemic response in older rats.

Incorporation of SMP or WPP into the diet increased hepatic CH biosynthesis as measured by activity of HMG CoA reductase, which confirms (23) that SMP ingested with a casein-based diet increases HMG CoA reductase activity. Our finding that HMG CoA reductase activity increased with SMP feeding does not agree with that of Kritchevsky et al. (19), who reported a reduction in the activity of HMG CoA reductase as well as decreased activities of fatty acid synthetase and cholesterol 7α-hydroxylase.

Differences exist between our experiments and those of Kritchevsky et al. Our studies utilized younger rats, a casein-based semipurified diet, and SMP or WPP, in contrast to the older rats, “stock” diet, and fluid skim milk they used. The most likely source of the discrepancy between findings of the two laboratories may be the composition of the basal diet. At weaning, the activity of HMG CoA reductase increases dramatically, but this change can be modified by diet. In comparison to a diet of Chow, weaning onto a semi-purified, casein-based diet significantly reduces both the postweaning overshoot phenomenon, as well as adult HMG CoA reductase activity (26). Evidence of an HMG CoA reductase inhibitor could not be identified in livers of animals fed semipurified diets (27). Substitution of part of the cellulose in the semipurified diet with citrus pectin restores HMG CoA reductase activity to that during a Chow diet (27).

Hepatic CH biosynthesis increased without any concomitant increase in either hepatic or plasma CH concentrations; findings were similar when rats, either 23 or 45 days old at the start of the experiment, were fed fluid skim milk for 5 wk (23). Failure to see alterations in plasma or hepatic CH when there is a significant increase in CH biosynthesis, however, is not inconsistent. The additional CH may be excreted directly or as bile acids or stored in extrahepatic tissues. Both inhibition of HMG CoA reductase by compactin (8) and increase in activity induced by incorporation of citrus pectin into a semipurified casein diet (27) occurred without any change in plasma CH. In fact, increased sterol excretion when pectin was incorporated into the diet prompted the suggestion by these authors that CH biosynthesis increased in response to increased excretion (27).

Stimulation of cholesterol synthesis is equivalent with SMP or WPP ingestion under our experimental conditions. Whey permeate contains calcium (550 mg/100 g) and lactose (77.6 g/100 g), both of which are in SMP and influence CH metabolism in rats. Fleischman et al. (9) reported a threefold increase in fecal bile acids in rats consuming diets containing .2 and 1.2% calcium compared to a low calcium diet of .08%. Increased excretion of CH by this route might lead to increased demand for synthesis. Although lactose consumed at 40% by weight of diet decreases hepatic cholesterogenesis in rats (30), consumption of a 10% lactose diet, similar to the percent we fed, produced no change from the control in serum or hepatic CH or cecal bile acids in rats (31). The SMP diet and the WPP diet were calculated to contain 13.7% lactose and .7 to .8% calcium. Therefore, lactose might have been insufficient to alter cholesterogenesis, whereas calcium intake might contribute to increased CH synthesis through increasing excretion of sterol.
Regardless of what SMP or WPP component(s) were responsible, the enhanced cholesterogenesis with SMP and WPP ingestion occurred without alterations in hepatic lipid or plasma CH. The stimulating effect of SMP was retained in an ultrafiltrate of whey and acted in the presence of animal protein. Considering our results together with those of other laboratories, the interaction between SMP or WPP or fluid skim milk and casein-based, semipurified diet affects CH metabolism differently from when SMP or fluid skim milk are consumed along with a diet of less processed ingredients.

ACKNOWLEDGMENTS

This work was supported in part by the College of Agricultural and Life Sciences, the Graduate School, and the Food Engineering Pilot Plant, University of Wisconsin-Madison, and Hatch Project No. 5141. The authors acknowledge the able technical assistance of Cynthia Kane.

REFERENCES

