Inconsistency between glycemic and insulinemic responses to regular and fermented milk products\textsuperscript{1,3}

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ABSTRACT
Background: Foods with a low glycemic index are increasingly being acknowledged as beneficial in relation to the insulin resistance syndrome. Certain organic acids can lower the glycemic index of bread products. However, the possible effect of acids in fermented milk products on the glycemic index and on insulinemic characteristics has not been addressed. The metabolic effects of fermented milk or pickled products used as additives to mixed meals have also not been addressed.

Objectives: One objective was to characterize the glycemic and insulinemic responses after intake of regular or fermented milk products (study 1). In addition, the acute metabolic effect of fermented milk (yogurt) and pickled cucumber as supplements to a traditional breakfast based on a high–glycemic index bread was evaluated (study 2).

Design: Ten healthy volunteers were served different breakfast meals after an overnight fast. Capillary blood samples were collected before and during 2 (study 1) or 3 (study 2) h after the meal. White-wheat bread was used as a reference meal in both studies.

Results: The lactic acid in the fermented milk products did not lower the glycemic and insulinemic indexes. Despite low glycemic indexes of 15–30, all of the milk products produced high insulinemic indexes of 90–98, which were not significantly different from the insulinemic index of the reference bread. Addition of fermented milk (yogurt) and pickled cucumber to a breakfast with a high–glycemic index bread significantly lowered postprandial glycemia and insulinemia compared with the reference meal. In contrast, addition of regular milk and fresh cucumber had no favorable effect on the metabolic responses.

Conclusions: Milk products appear insulinotropic as judged from 3-fold to 6-fold higher insulinemic indexes than expected from the corresponding glycemic indexes. The presence of organic acids may counteract the insulinotropic effect of milk in mixed meals. Am J Clin Nutr 2001;74:96–100.

KEY WORDS Glycemic index, insulinemic index, milk, healthy humans, organic acids, fermentation, lactic acid, breakfast, metabolic syndrome, carbohydrates

INTRODUCTION
The glycemic index was introduced to classify carbohydrate foods according to their effect on postprandial glycemia (1). The glycemic index is defined as the incremental blood glucose area after ingestion of a test product, expressed as a percentage of the corresponding area after a carbohydrate-equivalent load of a reference product (glucose or white bread). The insulinemic index can be calculated from the corresponding incremental insulin areas.

Accumulating data now suggest that a diet with a low glycemic index improves blood glucose control, the blood lipid profile, and fibrinolytic activity (2), suggesting a therapeutic role in the treatment of diseases related to insulin resistance. Epidemiologic studies also imply that such a diet may reduce the risk of type 2 diabetes (3, 4) and myocardial infarction (5). Possibly, the lowered insulin demand generally accompanying low–glycemic index foods (6) is the key feature involved. Consequently, even a short duration of hyperinsulinemia may induce insulin resistance in healthy subjects (7).

Today, there is an international consensus regarding the nutritional relevance of the glycemic index concept. In dietary recommendations from the Food and Agriculture Organization and the World Health Organization (8), an increased consumption of low–glycemic index foods is strongly advocated. Pasta (9), legumes (10), and products based on whole cereal grains (11) are examples of commercially available low–glycemic index foods. Unfortunately, most breakfast cereals and conventional bread products belong to the group of foods that elicit high metabolic responses (12). However, it is possible to reduce the insulinemic and glycemic effects of these foods, depending on the raw materials and processes used. It is known that the use of whole cereal grains (13) and sourdough fermentation (14) in bread making produces bread products with lower glycemic indexes.

Lactic acid was shown previously to lower both the glycemic and insulinemic indexes in sourdough bread (14), and inclusion of the sodium salt of propionic acid to a whole-meal barley bread was previously reported to lower the postprandial metabolic response (15). In addition, when vinegar was served as a...

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\textsuperscript{2}Supported by grants from the Swedish Council for Forestry and Agricultural Research (project 500615/96) and the Swedish Dairy Association.

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Received June 15, 2000.
Accepted for publication October 26, 2000.
supplement to a starchy meal, both glycemia and the insulin demand were reduced (16). It can, therefore, be concluded that the presence of certain organic acids in foods, whether generated through fermentation or added, may lower postprandial glycemia and insulinemia.

In most of the Scandinavian countries, both regular and fermented milk products are important items at breakfast. In a previous study with energy-equivalent cereal-based breakfasts, the inclusion of milk did not affect the glycemic index but resulted in a significantly higher insulimemic index (17). The glycemic index of certain fermented milk products are unknown and the insulimemic indexes of milk products are extremely scarce.

Therefore, the purpose of the present study was to characterize the glycemic and insulimemic responses to milk products and to evaluate the possible influence of the lactic acid in fermented milk. Another objective was to evaluate the acute metabolic effect of fermented milk (yogurt) and pickled cucumber as supplements to a traditional breakfast based on a high–glycemic index bread.

SUBJECTS AND METHODS

Study 1
Test meals: regular and fermented milk products

Besides regular milk, 2 commercial fermented milk products were tested: långfil (ropy milk) and filmjölk. All of the milk products contained 3% fat. The regular milk and filmjölk were produced by Skånemejerier (Malmö, Sweden) and the ropy milk was produced by Arla (Gävle, Sweden). In addition, a lactose solution was included. The lactose solution was prepared from pure lactose (lactose 101394S; BDH, Poole, United Kingdom) and water before every test meal. White-wheat bread (WWB) was used as a reference meal and was prepared according to Liljeberg and Björck (18). The composition of the meals is shown in Table 1.

Chemical analysis

The contents of lactose, glucose, and galactose in the milk products were analyzed by using an enzymatic kit (Boehringer Mannheim, Mannheim, Germany). The lactose content of the regular milk was 4.9% (wt wt) and the fermented milk products contained 3.7% lactose (wt wt). The glucose and galactose contents in all 3 milk products were <0.1% (wt wt). The starch content of the WWB was analyzed according to Holm et al (19). In addition, the protein (Kjeldahl) and fat (20) contents of the WWB were determined. According to the manufacturer, the lactic acid content of the 2 fermented milk products was 0.8–0.9% (wt wt).

TABLE 1
Composition of the test meals and the white-wheat bread (WWB) reference meal in study 1

<table>
<thead>
<tr>
<th></th>
<th>WWB</th>
<th>Lactose</th>
<th>Milk</th>
<th>Fermented milk products</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>WWB</td>
</tr>
<tr>
<td>Total carbohydrate (g)</td>
<td>25</td>
<td>24</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Total protein (g)</td>
<td>4.1</td>
<td>—</td>
<td>17.3</td>
<td>19.2</td>
</tr>
<tr>
<td>Total fat (g)</td>
<td>0.8</td>
<td>—</td>
<td>15.3</td>
<td>17.4</td>
</tr>
<tr>
<td>Lactic acid (g)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>5.2</td>
</tr>
<tr>
<td>Energy (kJ)</td>
<td>525</td>
<td>408</td>
<td>1300</td>
<td>1413</td>
</tr>
<tr>
<td>(kcal)</td>
<td>125</td>
<td>97</td>
<td>309</td>
<td>336</td>
</tr>
</tbody>
</table>

Study design

Ten healthy nonsmoking volunteers (5 men and 5 women aged 28–47 y) with normal body mass indexes (23.4 ± 2.1; in kg/m²) and not receiving drug therapy participated in the study. All of the subjects drank milk regularly and did not experience any discomfort after milk consumption. The test meals, including the reference meal, contained 25 g available carbohydrate; 250 mL water was served with the WWB meal and 150 mL tea or coffee was served with each meal. The subjects were served the test meals in random order on 5 separate occasions at the same time in the morning after an overnight fast. All of the meals were consumed steadily over 12–14 min.

Finger-prick capillary blood samples were taken before the meal (0 min) and 15, 30, 45, 70, 95, and 120 min after the meal for analysis of glucose and at 0, 15, 30, 45, 95, and 120 min for analysis of insulin. Blood glucose concentrations were determined with a glucose oxidase peroxidase reagent and serum insulin concentrations were determined with an enzyme immunoassay kit (Boehringer Mannheim).

The study lasted 3 mo and all of the subjects knew that they could withdraw at any time. The study was approved by the Ethics Committee of the Faculty of Medicine at Lund University.

Study 2
Test meals: WWB with and without fermented and pickled products

The composition of the meals is described in Table 2. The 2 test meals were composed of WWB (with added butter and cheese) and

TABLE 2
Composition of the test meals and the white-wheat bread (WWB) reference meal in study 2

<table>
<thead>
<tr>
<th></th>
<th>WWB</th>
<th>WWB + norm</th>
<th>WWB + acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>WWB (g)</td>
<td>122.5</td>
<td>122.5</td>
<td>122.5</td>
</tr>
<tr>
<td>Water (g)</td>
<td>250</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Milk, 0.5% fat (g)</td>
<td>—</td>
<td>250</td>
<td>—</td>
</tr>
<tr>
<td>Yogurt, 0.5% fat (g)</td>
<td>—</td>
<td>—</td>
<td>250</td>
</tr>
<tr>
<td>Cucumber (g)</td>
<td>—</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>(fresh) (pickled)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cheese, 10% fat (g)</td>
<td>29.7</td>
<td>3.9</td>
<td>—</td>
</tr>
<tr>
<td>Butter, 80% fat (g)</td>
<td>8.0</td>
<td>9.7</td>
<td>10.2</td>
</tr>
<tr>
<td>Total carbohydrate (g)</td>
<td>50</td>
<td>63.5</td>
<td>60.8</td>
</tr>
<tr>
<td>Total protein (g)</td>
<td>17.6</td>
<td>18.2</td>
<td>17.9</td>
</tr>
<tr>
<td>Total fat (g)</td>
<td>11.1</td>
<td>11.1</td>
<td>11.1</td>
</tr>
<tr>
<td>Lactic acid (g)</td>
<td>—</td>
<td>—</td>
<td>1.3</td>
</tr>
<tr>
<td>Acetic acid (g)</td>
<td>—</td>
<td>—</td>
<td>1.6</td>
</tr>
<tr>
<td>Energy (kJ)</td>
<td>1571</td>
<td>1811</td>
<td>1760</td>
</tr>
<tr>
<td>(kcal)</td>
<td>374</td>
<td>431</td>
<td>419</td>
</tr>
</tbody>
</table>

1 WWB + norm meal includes WWB + regular milk + fresh cucumber; WWB + acid meal includes WWB + fermented milk (yogurt) + pickled cucumber.
either regular milk (Skånemejerier) and fresh cucumber (WWB + norm) or yogurt (Skånemejerier) and pickled cucumber (WWB + acid) as supplements. Both of the milk products contained 0.5% fat. The reference meal was WWB, which was balanced with butter (80% fat) and cheese (10% fat) to reach the same content of fat and protein as in the 2 test meals. The starch content of the WWB was analyzed according to Holm et al (19), and the carbohydrate content of the milk and yogurt was based on the declaration of contents by the manufacturer. In addition, the protein (Kjeldahl) and fat (20) contents of the WWB were determined. To make 0.5 kg pickled cucumber, 200 mL acetic acid (12%) and 300 mL water were used.

Study design

Ten healthy nonsmoking volunteers (3 men and 7 women aged 24–56 y) with normal body mass indexes (21.4 ± 1.9; in kg/m²) and not receiving drug therapy participated in the study. The WWB reference meal was served with 250 mL water, and 150 mL tea or coffee was served with each meal. The subjects were served the test meals in random order on 3 separate occasions at the same time in the morning after an overnight fast. All of the meals were consumed steadily over 12–14 min.

Finger-prick capillary blood samples were taken before the meal (0 min) and 30, 45, 70, 95, 120, and 180 min after the meal for analysis of glucose and at 0, 30, 45, 95, and 120 min for analysis of insulin. Blood glucose concentrations were determined with a glucose oxidase peroxidase reagent and serum insulin concentrations were determined with an enzyme immunoassay kit (Boehringer Mannheim).

The study lasted 3 mo and all of the subjects knew that they could withdraw at any time. The study was approved by the Ethics Committee of the Faculty of Medicine at Lund University.

Calculations and statistical methods

For each subject and type of test meal, the areas under the curves for glucose and insulin were calculated (GRAPHPAD PRISM, version 2.0; Graph Pad Software, San Diego). All areas below baseline were excluded from the calculations. The glycemic and insulinemic indexes were then calculated from the 95-min incremental postprandial blood glucose and insulin areas by using WWB as a reference (glycemic and insulinemic indexes = 100) (1). Statistical analyses were carried out for both indexes and for the mean glucose and insulin concentrations at each time point. Values are presented as means ± SEMs, and all statistical calculations were performed with MINITAB Statistical Software (release 12 for WINDOWS; Minitab, Inc, State College, PA). Significances were evaluated with the general linear model (analysis of variance), followed by Tukey’s multiple comparisons test. Values of P < 0.05 were considered significant.

RESULTS

Study 1: regular and fermented milk products

The blood glucose responses 15 min after the test meals with ropy milk and filmjölk were significantly lower than those after the WWB and the lactose solution, respectively (Figure 1). Between 30 and 70 min after the meal, the glucose response was lower after the lactose solution than after the WWB. From 15 to 45 min after the meal, blood glucose concentrations were significantly lower after the 2 fermented milk meals than after the lactose solution.

The glycemic indexes for all of the milk products were significantly lower than those for both the lactose solution and the WWB (Table 3). However, no differences in glycemic indexes were found between the milk products. The glycemic index was significantly lower after the lactose solution than after the WWB meal.

Forty-five minutes postprandially, the regular milk and filmjölk elicited lower insulin responses than did the WWB (Figure 2);

TABLE 3

Glycemic and insulinemic indexes for the test meals and the white-wheat bread (WWB) reference meal in study 1

<table>
<thead>
<tr>
<th>Meal</th>
<th>Glycemic index</th>
<th>Insulinemic index</th>
</tr>
</thead>
<tbody>
<tr>
<td>WWB</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Lactose</td>
<td>68 ± 8</td>
<td>50 ± 6</td>
</tr>
<tr>
<td>Regular milk</td>
<td>30 ± 4</td>
<td>90 ± 8</td>
</tr>
<tr>
<td>Fermented milk products</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Filmjölk</td>
<td>15 ± 3</td>
<td>98 ± 11</td>
</tr>
<tr>
<td>Ropy milk</td>
<td>15 ± 3</td>
<td>97 ± 13</td>
</tr>
</tbody>
</table>

\(^a\) ± SEM; \(n = 10\). Values within columns with different superscript letters are significantly different, \(P < 0.05\).
FIGURE 3. Mean (±SEM) incremental blood glucose responses (Δ) in 10 healthy subjects after ingestion of a reference white-wheat bread meal (WWB; ▲), the WWB meal plus regular milk and fresh cucumber (▲), and the WWB meal plus fermented milk (yogurt) and pickled cucumber (▼). Values with different letters are significantly different, P < 0.05.

30 and 45 min after the meal, insulin responses were significantly lower with the lactose solution than with the WWB. Insulin concentrations after röpy milk were not significantly different from concentrations after the WWB meal at all time points, except for 95 min postprandially, when insulin responses were significantly lower after the WWB meal. The insulineemic index for the milk products did not differ significantly from those for the WWB but were significantly higher than those for the lactose solution (Table 3). No differences in insulineemic indexes were found between the milk products.

Study 2: WWB with and without fermented and pickled products

The blood glucose response 30 min after the test meals was significantly lower with the WWB + acid meal than with the WWB and WWB + norm meals (Figure 3). At 45 min, the WWB + acid meal produced significantly lower glycemia than did the WWB meal. The glycemic index for the WWB + acid meal was significantly lower than that for the WWB meal (Table 4). At 45 min, the serum insulin response to the WWB + acid meal was significantly lower than the response to the WWB + norm meal (Figure 4). The insulineemic index for the WWB + acid meal was significantly lower than that for both the WWB and WWB + norm meals (Table 4).

DISCUSSION

Milk products are an important component of Scandinavian breakfasts and are generally regarded as having low glycemic indexes (21). In the present study, the low glycemic indexes of milk products were confirmed and ranged from 15 to 30. The presence of lactic acid in the fermented milk products filmjölk and röpy milk did not significantly affect glycemia, possibly because of the remarkably low incremental glycemic areas seen with all of the milk products.

In contrast with the findings for the comparison between regular and fermented milk products, there was a slight difference in glycemic index between the WWB + norm meal and the WWB + acid meal (study 2). In the WWB + acid meal, both the fermented milk (yogurt) and the pickled cucumber contained organic acids. Because the fermented milk products in study 1 did not have any significant effect on the insulin responses, it seems reasonable that the acetic acid in the pickled cucumber was mainly responsible for the insulin-lowering effect. This finding agrees with earlier findings that acetic acid lowers postprandial glycemia by reducing the gastric emptying rate (16).

The milk products and the cucumber were served in addition to the white bread; thus, the WWB + norm and WWB + acid meals contained a higher amount of carbohydrates (60–64 g) than did the WWB meal (50 g). Despite this amount, both meals with milk products produced a lower glycemic index than did the WWB meal.

Foods for which glycemic indexes have been published, in contrast with milk, are comparatively rich in carbohydrates. Most information regarding starchy foods is readily available, and for this group of products the literature indicates a linear correlation between glycemic indexes and insulineemic indexes (6), suggesting that low–glycemic index foods are also less insulin demanding. The present study with milk and meals with added milk clearly showed that this linear correlation does not apply to all low–glycemic index foods.

Obviously, fermented and nonfermented milk products give rise to insulineemic responses far exceeding what could be expected from their low glycemic indexes. In fact, insulineemic indexes for the milk products tested were not significantly different from those for the WWB meal. All of the milk products included in study 1 induced postprandial hypoglycemia after <50 min postprandially, which may be explained by the high insulin concentrations. Given the hypothesis that insulineemia is...
a modulator of insulin resistance, this is an important finding. The fact that insulinemia was greater after the milk products than after an equivalent amount of lactose indicates that some milk component in addition to lactose can stimulate insulin secretion. This finding implies that milk may in fact produce higher glycemic indexes when tested in individuals with diminished (type 2 diabetic subjects) or absent (ie, type 1 diabetic subjects) β cell function than in healthy subjects capable of responding to the insulinoactive components in milk.

It is important to note that the subjects who participated in the studies did not have any history of lactose malabsorption. Because lactase persistence varies between populations, the glycemic response to lactose in milk may vary.

The glycemic index tended to decrease and the insulinenic index tended to increase when the WWB meal was consumed with regular milk, although not significantly so. This finding supports an insulinoactive effect of milk. Interestingly, consumption of fermented milk (yogurt) and pickled cucumber significantly lowered both the glycemic and insulinenic response to the WWB meal, suggesting a favorable effect of organic acids.

In the mid-1980s, Gannon et al (22) found milk to be a potent insulin secretagogue in type 2 diabetic patients. Some years later, Schrezenmeir et al (23) reported that the postprandial glucose and insulin responses to a milk-containing breakfast did not correlate in healthy individuals. A possible explanation for an insulinoactive effect of milk involves amino acids and lipids, because it is known that these components can increase the insulin secretion or the insulin demand of a meal (24–26).

As judged from the results of the present study, the time has come to acknowledge the insulinoactive properties of milk. An interesting objective of future research is to evaluate the metabolismic effect of milk in a mixed diet and to identify the secretagogue involved.

REFERENCES