Sourdough Fermentation or Addition of Organic Acids or Corresponding Salts to Bread Improves Nutritional Properties of Starch in Healthy Humans[1,2]

HELENA G. M. LILJEBERG,[3] CLAS H. LÖNNER* AND INGER M. E. BJÖRCK

*Department of Applied Nutrition and Food Chemistry, Chemical Center, University of Lund, P.O. Box 124, S-221 00 Lund, Sweden and *Clas Lönner AB, Ideon, S-223 70 Lund, Sweden*

ABSTRACT Postprandial blood glucose and insulin responses to barley bread containing organic acids or corresponding salts were evaluated in healthy human subjects. The satiety score and the rate and extent of in vitro starch digestion were also studied. Lactic acid was generated by use of a homofermentative starter culture or added to the dough. In addition, products were baked with Ca-lactate, or with Na-propionate at two different concentrations. Consumption of the product baked with a high concentration of Na-propionate significantly lowered the postprandial blood glucose and insulin responses, and significantly prolonged the duration of satiety compared with all other breads. When subjects consumed the breads baked with sourdough, lactic acid and Na-propionate, their glucose and insulin responses were reduced compared with the wholemeal bread alone. The rate of in vitro amylolysis was reduced only by ingestion of the breads containing lactic acid, suggesting that the beneficial impact of Na-propionate on metabolic responses and satiety was related to effects other than a reduced rate of starch hydrolysis. All bread products had a similar concentration of in vitro resistant starch of 1.3–2.1 g/100 g (starch basis). It is concluded that sourdough baking and other fermentation processes may improve the nutritional features of starch. The results also demonstrate that certain salts of organic acids may have metabolic effects. J. Nutr. 125: 1503–1511, 1995.

INDEXING KEY WORDS:

- *sourdough fermentation* • *starch hydrolysis*
- *glycemic response* • *satiety* • *humans*

In recent years it has become increasingly clear that starchy foods have very different effects on postprandial blood glucose and insulin responses. Starch in legumes (Tovar et al. 1992), pasta (Granfeldt and Björck 1991) and products based on whole cereal grains (Granfeldt et al. 1994) elicits low glucose and insulin responses, whereas starch in potatoes (Collier et al. 1986) and most conventional bread products (Jenkins et al. 1988) is rapidly digested and absorbed, thus giving rise to high metabolic responses. Foods with slowly digested and absorbed carbohydrates are of special importance in the dietary management of diabetic patients (Brand et al. 1991). However, diets characterized by such foods have also been found to alter lipid and glucose metabolism in healthy subjects (Jenkins et al. 1987), as judged from reductions in total cholesterol levels and improved glucose tolerance. In fact, dietary carbohydrates that cause a rapid rise in postprandial insulin levels are extensively discussed as a risk factor for the development of metabolic diseases (Ducimetiere et al. 1980). Other advantageous effects of lente food items, registered in nondiabetic subjects, include a prolonged duration of satiety (Haber et al. 1977, Holt et al. 1992) and physical endurance during athletic performance (Thomas et al. 1991).

Because of the nutritive composition and low fat content of bread, an increased bread intake is advocated. Unfortunately, the starch in most bread products causes unfavorably high metabolic responses. Attempts to reduce the glycemic response to bread have therefore been made. Previously, we showed that cereal kernels included in bread (wheat, rye or barley) reduced the glycemic response to bread in healthy humans (Liljeberg and Björck 1994, Liljeberg et al. 1992). In fact, an inverse relationship between the ratio of barley kernels and glycemia was seen (Liljeberg and Björck 1994). The lowered metabolic response to these kernel-based products was

[1]Supported by grants from the Cerealia Foundation for Research and Development (project no. 193).

[2]The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 USC section 1734 solely to indicate this fact.

[3]To whom correspondence and reprint requests should be addressed.

0022-3166/95 $3.00
Manuscript received 29 August 1994. Initial review completed 3 October 1994. Revision accepted 1 December 1994.

closely related to a lowered enzymic susceptibility of starch, as judged from studies in vitro (Liljeberg and Björck 1994, Liljeberg et al. 1992). To provide an enzymic barrier, the cereal kernels must retain their integrity in the finished bread, and an oat kernel bread with disrupted kernels did not improve the glucose response compared with a white reference bread (Liljeberg et al. 1992).

In contrast, despite disrupted rye kernels, a commercial sourdough fermented pumpernickel bread was recently found to flatten the postprandial glucose and insulin response curves in healthy subjects (Liljeberg and Björck 1994). Improved glucose tolerance may result from the presence of organic acids that may delay gastric emptying (Hunt and Knox 1972) or inhibit amylase activity (Todesco et al. 1991) and, hence, reduce the rate of small intestinal starch uptake.

The aim of the present study was to evaluate the possible influence of acids formed during sourdough fermentation in bread on the postprandial glucose and insulin responses in healthy subjects. Lactic acid was generated by use of a homofermentative starter culture or was added to the dough. To investigate the importance of pH per se, the corresponding salt, Ca-lactate, was included in one product. In addition, two bread products were baked with different concentrations of Na-propionate. In the baking industry, propionate is commonly used to inhibit mold and bacterial growth. Also studied were the rate and extent of in vitro starch digestion. Finally, the bread products were evaluated with respect to satiety and acceptability scores.

MATERIALS AND METHODS

Bread products. White wheat flour and whole-meal barley flour were provided by Nord Mills AB (Malmö, Sweden). All bread products were made from the same basic recipe with 80% whole-meal barley flour and 20% white wheat flour. Two of the products contained lactic acid. One was baked with sourdough, generated by use of a homofermentative starter culture, which formed mainly lactic acid. The other was baked with addition of lactic acid. Two products were also baked with either the calcium salt of lactic acid, Ca-lactate, or the sodium salt of propionic acid, Na-propionate. The four bread products had the same content of organic acid or salt on a molar basis. In addition, a product with a high content of Na-propionate was included in the study. A whole-meal bread with no additives (WMB) was used as a reference product.

Recipes. To make sourdough, 3465 g water, 1540 g whole-meal barley flour and 0.75 g starter culture ($5 \cdot 10^7$ colony forming units/g flour) were mixed and stored for 20 h at 37°C. The starter culture used was a homofermentative lactic acid bacteria, *Lactobacillus plantarum* strain A1 (Clas Lönner AB, Lund, Sweden) (Spicher and Lönner 1985). The main organic acid formed during fermentation was lactic acid (**Table 1**).

The basic recipe for the whole-meal bread consisted of 3280 g water, 2960 g whole-meal barley flour, 740 g white wheat flour, 200 g yeast, 50 g NaCl, 50 g sucrose and 37 g monoglycerides. The dough was proofed for 50 min, divided into pieces of 600 g, followed by a second proofing for 20 min (38°C, 75% humidity). The bread was baked at 200°C for 30 min.

Sourdough bread was made with 4810 g sourdough starter, 1480 g whole-meal barley flour, 740 g white wheat flour and 200 g yeast. The remaining ingredients and baking procedures were the same as for the basic recipe.

To make the other four bread products, the basic recipe was used, to which was added 64 g of lactic acid (90 wt%), 95 g of Ca-lactate, 62 g of Na-propionate or 185 g of Na-propionate. The water content

TABLE 1

Composition of the bread products

Bread product	pH	S•[1]	Acid		Ethanol	Starch		Dietary fiber
			Lactic[2]	Propionic[2]		Available	Resistant	
			mmol/100 g dry wt			*g/100 g dry wt*		
Whole-meal bread (WMB)[3]	5.9	3.5	0.0	0.0	1.0	66.7	1.4	13.3
Plus sourdough	4.2	21.4	18.4	0.0	1.2	65.6	0.9	12.5
Plus lactic acid	4.0	23.1	17.9	0.0	1.1	64.0	1.1	13.5
Plus Ca-lactate	5.3	8.6	18.9	0.0	1.1	64.7	1.1	13.1
Plus Na-propionate	5.9	8.7	0.0	21.3	0.6	65.0	1.2	12.7
Plus Na-propionate, high concentration	6.0	8.5	0.0	60.8	0.3	62.6	1.1	12.0

[1] S• = acid equivalents, expressed as the amount of 0.1 mol/L NaOH consumed in mL/10 g bread (dry wt).
[2] Minor amounts, 0.04–0.08 g/100 g acetic acid (dry wt basis) formed during baking is included.
[3] WMB = whole-meal bread (whole-meal barley flour, white wheat flour, 80:20), reference product.

was adjusted accordingly when lactic acid was added. Baking procedures were the same as for the basic whole-meal bread.

Before being cut into slices, all bread products were stored at room temperature overnight. The crust was removed and three to four slices were wrapped in aluminum foil, put into plastic bags and stored at −20°C until used.

Chemical analysis. A portion from each bread (<0.8 mm) was air dried and milled (Cyclotec, Tecator, Sweden) before analysis. The bread products were analyzed for potentially available starch (Holm et al. 1986) and total dietary fiber (Asp et al. 1983). Further, total starch remnants in the fiber residues were determined enzymatically following solubilization in alkali (total in vitro resistant starch). Residual starch remnants were analyzed directly, omitting pretreatment in alkali. However, the thermostable α-amylase Termamyl was not included (Siljeström et al. 1989). The fraction that required solubilization in alkali, presumably retrograded amylose, was then calculated as the difference between total starch and residual starch remnants in the fiber residues.

The bread products were also characterized with respect to pH and acid equivalents (S°) (Lönner and Preve-Åkesson 1988). Samples for measuring the amounts of lactic acid, propionic acid and ethanol were prepared and stored as described by Lönner and Preve-Åkesson (1988). The amount of lactic acid was determined enzymatically (Boehringer Mannheim, GmbH Biochemica, Mannheim, Germany) and the amounts of acetic acid, propionic acid and ethanol were measured on a gas chromatograph (Varian 3400, Varian, Palo Alto, CA).

The composition of the bread products is shown in Table 1.

Blood glucose and insulin responses in healthy subjects. Eleven healthy volunteers (six women and five men, age 26–48 y, with normal body mass indices and without drug therapy) participated in the study. The bread products (159–161 g) were provided corresponding to 50 g of available carbohydrates and served with 8 g of butter and 20 g of cheese (17% fat, wet weight). In addition, 200 mL of water and 150 mL of coffee or tea were included in each meal. The energy content of the test meals was 1590 KJ. Values for protein and fat in the bread products were based on analysis of products with similar composition, described by Liljeberg and Björck (1994). Corresponding values for cheese and butter were obtained from food tables. The subjects were served the test products as a breakfast in random order (six separate occasions) after an overnight fast. They were asked to eat the meal over a 12–15 min period.

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

Approval of the study was given by the Ethics Committee at the University of Lund, Lund, Sweden.

In vitro starch hydrolysis. The bread products were tested in vitro to determine the rate of release of starch hydrolysis products following incubation with salivary α-amylase, pepsin and pancreatic α-amylase (Granfeldt et al. 1992). Subjects rinsed their mouths with tap water and subsequently chewed the bread products for 15 s (~15 times). They were told not to eat within 1 h before the experiment. All bread portions contained 1 g of starch and were given in a randomized order to six subjects. The products were then expectorated into a beaker containing 50 mg of pepsin (2000 FIP-U/g, Merck, Darmstadt, Germany) in 6 mL of 0.05 mol/L Na,K-phosphate buffer (containing 0.4 g/L NaCl) adjusted to pH 1.5 with 2 mol/L HCl. Finally, the subjects rinsed their mouths with 5 mL of Na,K-phosphate buffer (pH 6.9) for 60 s and expectorated the rinsing solution into the beaker.

The contents were stirred and pH adjusted to 1.5. Each sample was incubated at 37°C for 30 min with gentle mixing three times during incubation. The pH was readjusted to 6.9 with NaOH before incubation with porcine pancreatin α-amylase (A6255, Sigma Chemical, St. Louis, MO). The enzyme (110 Sigma units) was dissolved in 10 mL of buffer, and 1 mL of this solution was added to the beaker. The sample was adjusted to 30 mL with phosphate buffer, and transferred to the dialysis tubing (13 cm strips, Spectra Por no. 2, width 45 mm, molecular weight cutoff 12,000–14,000). Each bag was incubated at 37°C for 3 h in a beaker with phosphate buffer (800 mL). The beaker was placed in a stirred water bath. Every 30 min, aliquots (2 mL) from the dialysate were removed for analysis of reducing sugar content by the 3,5-dinitro salicylic acid method (Hostettler et al. 1951). A standard curve was prepared using maltose. In addition, a hydrolysis rate index was calculated as 100 times the area under the curve (0–180 min) for the product divided by the corresponding area obtained with white wheat bread chewed by the same person (Liljeberg and Björck 1994).

Satiety and acceptability scores. The satiety after the test meals was estimated numerically according to Haber et al. (1977). Assessments were done before the meal (0) and at 15, 45, 95, 120 and 180 min after the meal using a scoring system graded from −10, to represent extreme hunger, to +10, to represent extreme satiety. The area under the satiety curve was calculated above zero line.

During the satiety study, the subjects were also requested to assess the acceptability of the breads on a bipolar hedonic scale (Chapman and Wigfield 1970), in which −4 represents dislike extremely, ±0

represents neither like nor dislike and +4 represents like extremely.

Statistical methods. The results are expressed as means ± SEM, and the statistical significance of differences were assessed by the Wilcoxon matched-pair signed-ranks tests (Conover 1980). The SPSS/PC+ advanced statistics program (version 2.0, SPSS, Chicago, IL) was used. A value of $P < 0.05$ was considered significant.

RESULTS

Postprandial blood glucose and insulin responses. Consumption of the breads with added Na-propionate resulted in significantly lower blood glucose concentrations ($P < 0.05$) at the initial postprandial phase (30 min), than did consumption of the WMB (**Fig. 1** and **Table 2**). The glucose concentration measured 30 min after ingestion of the WMB plus high Na-propionate was significantly lower than with the remaining products tested. When the areas under the curves in the initial phase were calculated (0–45 min), significantly lower figures were noted as a result of consumption of the sourdough bread, and the breads with lactic acid or Na-propionate, compared with WMB (**Table 3**). However, the 0–95 and 0–120 min areas under the curves did not differ significantly. No lowering of postprandial glycemia was found as a result of ingestion of the WMB plus Ca-lactate.

More pronounced differences were obtained in postprandial insulin responses. Compared with WMB, lower insulin concentrations ($P < 0.05$) were found at 30 min when subjects consumed the sourdough bread, and the WMB plus lactic acid or Na-propionate (**Fig. 2** and **Table 4**). Also, a significantly lower insulin increment was observed following ingestion of WMB plus high concentration of Na-propionate, compared with all the remaining products. When subjects consumed the sourdough bread, and the breads with added lactic acid or Na-propionate, the 0–45 min areas under the curves were significantly distinguished from the area obtained with WMB (**Table 5**). The same differences persisted when calculating the 0–95 min areas under the curves.

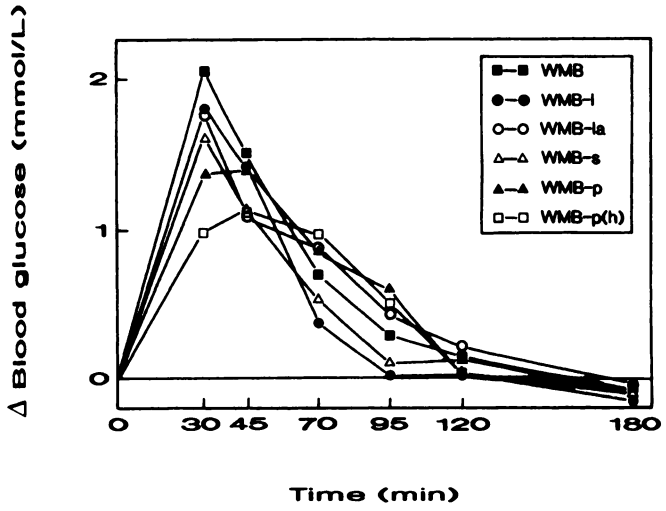

FIGURE 1 Mean incremental blood glucose responses in healthy subjects following ingestion of breakfast meals with different bread products. Values are means, $n = 11$. Error terms are given in Table 2.

Rate of in vitro starch hydrolysis. The percentage of starch hydrolyzed within 180 min (mean ± SEM) was 53.2 ± 1.0 for WMB and 48.6 ± 0.9, 46.8 ± 0.6, 58.7 ± 1.6, 55.9 ± 0.3, 55.2 ± 1.0 for the sourdough bread, WMB plus lactic acid, WMB plus Ca-lactate, WMB plus Na-propionate and WMB plus the high concentration of Na-propionate, respectively. When the hydrolysis rate index was calculated, significantly lower indices ($P < 0.05$) were found with the sourdough bread and the WMB plus lactic acid (86 and 81, respectively), compared with white wheat bread and the WMB (**Table 6**). The hydrolysis rate index obtained with the breads with added Na-propionate were not significantly distinguished from that with white wheat bread. In contrast, the indices found

TABLE 2

Incremental blood glucose responses in healthy humans after different bread meals[1]

	Glucose response					
	30 min	45 min	70 min	95 min	120 min	180 min
	Δ *mmol/L*					
Whole-meal bread	2.1 ± 0.1^{a}	1.5 ± 0.3	0.7 ± 0.2^{ab}	0.3 ± 0.1^{ab}	0.1 ± 0.1	−0.1 ± 0.1
Plus sourdough	1.6 ± 0.1^{ab}	1.1 ± 0.3	0.5 ± 0.3^{a}	0.1 ± 0.1^{a}	0.1 ± 0.1	−0.1 ± 0.1
Plus lactic acid	1.7 ± 0.2^{ab}	1.1 ± 0.2	0.9 ± 0.3^{ab}	0.4 ± 0.2^{ab}	0.2 ± 0.1	−0.1 ± 0.1
Plus Ca-lactate	1.8 ± 0.1^{a}	1.4 ± 0.2	0.4 ± 0.2^{ab}	0.0 ± 0.1^{ab}	0.0 ± 0.2	−0.2 ± 0.1
Plus Na-propionate	1.4 ± 0.2^{b}	1.4 ± 0.2	0.8 ± 0.4^{b}	0.6 ± 0.2^{b}	0.0 ± 0.1	−0.1 ± 0.1
Plus Na-propionate, high concentration	1.0 ± 0.2^{c}	1.1 ± 0.2	1.0 ± 0.3^{ab}	0.5 ± 0.2^{ab}	0.0 ± 0.1	−0.1 ± 0.1

[1]Values are means ± SEM, $n = 11$. Values not sharing the same letters are significantly different ($P < 0.05$).

TABLE 3

Blood glucose and postprandial blood glucose responses in healthy humans after different bread meals[1]

	Fasting concentration	Area under curve		
		0–45 min	0–95 min	0–120 min
	mmol/L		*mmol/L min*	
Whole-meal bread	4.4 ± 0.1[a]	57.4 ± 4.3[a]	96.9 ± 12.8	103.0 ± 13.6
Plus sourdough	4.6 ± 0.1[ab]	44.9 ± 4.3[bc]	74.1 ± 15.0	78.6 ± 15.1
Plus lactic acid	4.5 ± 0.1[a]	47.8 ± 4.6[b]	88.7 ± 12.6	97.2 ± 14.1
Plus Ca-lactate	4.6 ± 0.1[b]	51.1 ± 4.1[ab]	81.1 ± 9.6	86.1 ± 9.7
Plus Na-propionate	4.5 ± 0.1[a]	41.1 ± 4.5[bc]	87.0 ± 15.5	95.5 ± 16.2
Plus Na-propionate, high concentration	4.4 ± 0.1[ab]	30.5 ± 3.8[c]	75.9 ± 11.6	84.6 ± 12.9

[1]Values are means ± SEM, n = 11. Values not sharing the same letters are significantly different ($P < 0.05$).

with these products were significantly higher compared with WMB. Similarly, the WMB plus Ca-lactate showed a significantly higher hydrolysis rate index than white wheat bread and WMB. No significant differences in hydrolysis rate index were found between white wheat bread and WMB.

In vitro resistant starch. The contents of in vitro resistant starch were similar in all bread products (0.9–1.4 g/100 g dry weight basis), corresponding to 1.3–2.1 g/100 g starch basis (Table 1). The main indigestible fraction, or ~50–65%, consisted of a starch fraction, presumably retrograded amylose, which required solubilization in alkali to render it available for the analytical amylases.

Satiety and acceptability scores. In the early phase, 45 min after the ingested meal, the two bread products with added Na-propionate were given higher satiety scores ($P < 0.05$) than the WMB product (**Fig. 3** and **Table 7**). For the bread plus high Na-propionate, this was also true at 95 min. In addition, at 120 min, significantly higher satiety scores were registered with WMB plus Ca-lactate and WMB plus Na-propionate, compared with WMB. When calculating the satiety area under the curves (0–180 min), a significantly higher value was found with the bread baked with the high concentration of Na-propionate than with WMB (**Table 8**). The highest acceptability scores were given to the WMB, WMB plus Ca-lactate and WMB plus Na-propionate (Table 8). No significant differences ($P < 0.05$) were found between these products. The sourdough bread, WMB plus lactic acid and WMB plus high Na-propionate were liked by some, but also disliked by others, resulting in scores significantly lower than with WMB.

DISCUSSION

Results from this study indicate that consumption of bread products containing lactic acid, whether generated during fermentation or added, reduced postprandial glucose and insulin responses in healthy subjects. Also, breads baked with Na-propionate improved the same metabolic indices. The largest reduction was obtained with the bread baked with a high concentration of Na-propionate. The area under the blood glucose curve (45 min) was reduced by

TABLE 4

Incremental serum insulin responses in healthy humans after different bread meals[1]

	Insulin response			
	30 min	45 min	95 min	120 min
			Δ *nmol/L*	
Whole-meal bread	0.38 ± 0.05[a]	0.32 ± 0.07	0.11 ± 0.04[ab]	0.05 ± 0.03
Plus sourdough	0.29 ± 0.06[b]	0.24 ± 0.05	0.09 ± 0.03[a]	0.06 ± 0.02
Plus lactic acid	0.25 ± 0.03[b]	0.27 ± 0.07	0.11 ± 0.04[ab]	0.05 ± 0.02
Plus Ca-lactate	0.32 ± 0.05[ab]	0.30 ± 0.07	0.07 ± 0.02[ab]	0.05 ± 0.01
Plus Na-propionate	0.20 ± 0.04[ab]	0.22 ± 0.05	0.14 ± 0.04[b]	0.07 ± 0.02
Plus Na-propionate, high concentration	0.12 ± 0.03[c]	0.21 ± 0.05	0.11 ± 0.03[ab]	0.05 ± 0.02

[1]Values are means ± SEM, n = 11. Values not sharing the same letters are significantly different ($P < 0.05$).

TABLE 5

Fasting serum insulin and postprandial serum insulin responses in healthy humans after different bread meals[1]

	Fasting concentration	Area under curve		
		0–45 min	0–95 min	0–120 min
	nmol/L		*nmol/L min*	
Whole-meal bread	0.06 ± 0.01	10.9 ± 1.4^{a}	21.7 ± 3.7^{a}	23.7 ± 4.5
Plus sourdough	0.05 ± 0.01	8.4 ± 1.6^{bc}	16.7 ± 3.2^{bc}	18.5 ± 3.7
Plus lactic acid	0.05 ± 0.01	7.6 ± 1.2^{bc}	17.2 ± 4.1^{bc}	19.3 ± 4.8
Plus Ca-lactate	0.06 ± 0.01	9.4 ± 1.5^{ab}	18.7 ± 3.6^{ab}	20.1 ± 3.9
Plus Na-propionate	0.05 ± 0.01	6.0 ± 1.1^{bc}	15.0 ± 2.6^{bc}	17.7 ± 3.0
Plus Na-propionate, high concentration	0.05 ± 0.01	4.3 ± 1.0^{c}	12.2 ± 2.3^{c}	14.2 ± 2.4

[1]Values are means ± SEM, n = 11. Values not sharing the same letters are significantly different (P < 0.05).

~47%, compared with the reference product. This is in agreement with Todesco et al. (1991), who reported a 48% reduction of the glucose area (120 min) with a similar bread product. In the present study, the postprandial insulin areas were also evaluated. The insulin responses with WMB plus high Na-propionate were very low, and the 45- and 95-min areas were lowered by 61 and 44%, respectively.

The sourdough bread, and the breads with added lactic acid, Ca-lactate and Na-propionate were baked with the same content of organic acid or salt on a molar basis. Ingestion of the propionate bread (pH 5.9) reduced metabolic response similarly to the sourdough (pH 4.2) or lactic acid (pH 4.0) breads. The glucose areas (45 min) were reduced by 17–28%, and the effect on insulin responses was even more pronounced. Consumption of the sourdough bread, and the breads with added Na-propionate and lactic acid decreased the mean 45-min areas by 45, 23 and 30%, respectively, and the 95-min areas by 31, 23 and 21%, respectively. Although not statistically significant (P < 0.05), a similar tendency was seen with the lactate bread.

The improved glycemia, following ingestion of bread containing lactic acid, is in accordance with the low metabolic response recently reported for starch in a sourdough fermented pumpernickel bread containing mainly acetic acid (pH 4.5, S° 31.1, 29.7 mmol/100 g acetic acid on dry weight basis) (Liljeberg and Björck 1994). In addition to the effect observed with acetic acid (Liljeberg and Björck 1994) or Na-propionate (Todesco et al. 1991), a reduced glycemia has also been found in healthy subjects after consumption of fermented vegetables (Torsdottir et al. 1992) and lettuce dressed with vinegar (Brighenti and Testolin 1991).

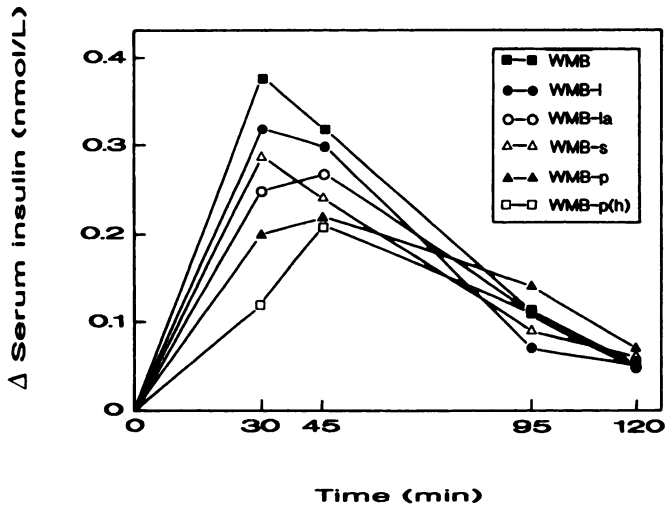

FIGURE 2 Mean incremental serum insulin responses in healthy subjects following ingestion of breakfast meals with different bread products. Values are means, n = 11. Error terms are given in Table 4.

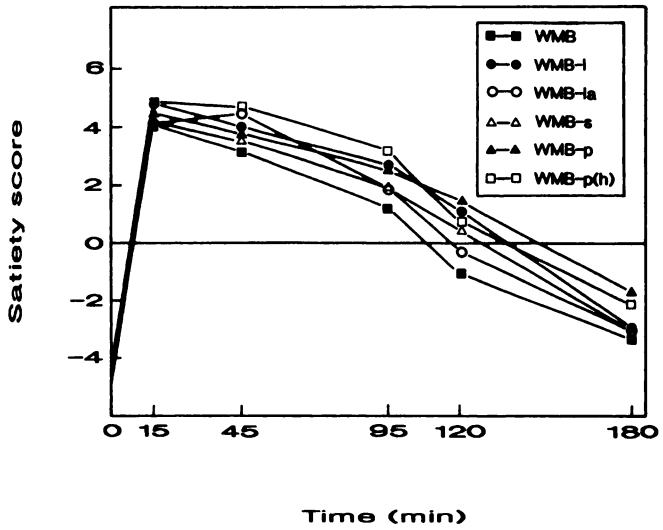

FIGURE 3 Mean satiety scores obtained in healthy subjects following ingestion of breakfast meals with different bread products. Values are means, n = 11. Error terms are given in Table 7.

TABLE 6

***Hydrolysis rate indices of the different breads*[1]**

	Hydrolysis rate index
	%
Whole-meal bread	95.5 ± 2.2[c]
Plus sourdough	85.5 ± 1.4[d]
Plus lactic acid	80.6 ± 1.3[d]
Plus Ca-lactate	113.4 ± 3.7[a]
Plus Na-propionate	101.0 ± 1.3[b]
Plus Na-propionate, high concentration	103.5 ± 2.7[b]
White wheat bread	100[bc]

[1]Values are means ± SEM, n = 11. Values not sharing the same letters are significantly different ($P < 0.05$).

According to Todesco et al. (1991), the suggested mechanism for an improved glucose tolerance by Na-propionate is an inhibition of amylolytic enzymes. Consequently, a reduced rate of starch hydrolysis by salivary amylase was noted in vitro. In contrast, as judged from in vitro results in the enzymic model used in the present study, the rate of starch hydrolysis was not affected by Na-propionate. The hydrolysis rate index values of the WMB plus Na-propionate and WMB plus high Na-propionate were found to be 101 and 104, respectively. In fact, when using the same in vitro procedure as described by Todesco et al. (1991), we failed to show any inhibition of amylase activity by Na-propionate. However, the bread products containing lactic acid (sourdough and lactic acid), significantly reduced the rate of starch hydrolysis (hydrolysis rate index 86 and 81, respectively), and the lowered hydrolysis rate index values obtained with these bread products can probably be explained by an inhibition of amylolytic enzymes.

In previous experiments, the in vitro rate of starch hydrolysis obtained with the system used in the present study was found to correlate well with blood glucose responses in healthy subjects (Granfeldt et al. 1992). Consequently, a correlation coefficient of 0.826 ($P < 0.00001$) was obtained when plotting glycemic index vs. hydrolysis rate index for an important number of cereal and legume products (glycemic index = 0.862·hydrolysis rate index + 8.198) (Granfeldt 1994). The lack of relationship between glycemic index and hydrolysis rate index in the case of the breads with added Ca-lactate or Na-propionate is thus in contrast to previous experiences with starchy products, and suggests that the cause for a reduction in glycemic and insulinemic responses with these bread products is related to a mechanism other than a reduced rate of starch digestion. When glycemic index was calculated from hydrolysis rate index in the sourdough bread and WMB plus lactic acid, predicted glycemic indices of 82 and 78, respectively, were obtained. This 20% reduction is in accordance with the lowering of the 45-min postprandial glucose areas with these products. These results suggest that the presence of lactic acid did reduce the rate of starch digestion also in the gastrointestinal tract.

The fact that no inhibitory effect, but rather an increase in hydrolysis rate index was noted with the Ca-lactate–containing product, might be related to an improved α-amylase activity in the presence of Ca^{2+} (Greenwood and Milne 1968). It is not known whether this also occurs in vivo. To our knowledge, no data are available on the potential effects of Ca-lactate on the rate of gastric emptying. However, as judged from the lack of effect of Ca-lactate on postprandial glucose or insulin responses, the overall rate of starch uptake was not affected.

The observed in vitro inhibition of enzyme activity in lactic acid–baked breads (pH 4.0–4.2) is in conflict with results reported for a sourdough fermented pumpernickel bread containing mainly acetic acid (pH 4.5). Hence, the starch in that pumpernickel bread was as rapidly digested as starch in a white wheat bread (Liljeberg and Björck 1994). However, despite the lack of effect on in vitro starch hydrolysis rate, the pumpernickel bread containing acetic acid flattened the postprandial glucose and insulin response

TABLE 7

***Satiety scores obtained in healthy humans after different bread meals*[1]**

	Satiety score					
	0 min	15 min	45 min	95 min	120 min	180 min
Whole-meal bread	–5.4 ± 0.3	4.2 ± 0.7	3.1 ± 0.6[a]	1.2 ± 0.7[a]	–1.1 ± 0.9[a]	–3.4 ± 1.1[ab]
Plus sourdough	–4.9 ± 0.4	4.2 ± 0.7	3.5 ± 0.5[ab]	1.9 ± 0.4[a]	0.4 ± 0.4[ab]	–3.1 ± 0.7[a]
Plus lactic acid	–5.2 ± 0.7	4.1 ± 0.9	4.5 ± 0.6[ab]	1.9 ± 0.9[ab]	–0.3 ± 0.8[ab]	–3.1 ± 0.9[ab]
Plus Ca-lactate	–4.6 ± 0.3	4.8 ± 0.5	4.0 ± 0.5[ab]	2.7 ± 0.7[ab]	1.1 ± 0.9[b]	–3.0 ± 0.9[ab]
Plus Na-propionate	–4.8 ± 0.5	4.5 ± 0.9	3.8 ± 0.6[b]	2.5 ± 0.6[ab]	1.4 ± 0.7[b]	–1.8 ± 0.6[b]
Plus Na-propionate, high concentration	–4.8 ± 0.4	4.9 ± 1.0	4.7 ± 0.9[b]	3.2 ± 0.8[b]	0.7 ± 1.0[ab]	–2.2 ± 1.1[ab]

[1]Values are means ± SEM, n = 11. Values not sharing the same letters are significantly different ($P < 0.05$).

TABLE 8

Satiety areas and acceptability scores for the bread products[1]

	Satiety area	Acceptability score
Whole-meal bread	316.2 ± 76.7[a]	0.8 ± 0.4[a]
Plus sourdough	317.2 ± 42.5[ab]	−1.9 ± 0.7[c]
Plus lactic acid	393.9 ± 62.5[ab]	−1.4 ± 0.7[bc]
Plus Ca-lactate	425.9 ± 75.4[ab]	0.1 ± 0.4[ab]
Plus Na-propionate	409.7 ± 72.9[ab]	−0.4 ± 0.7[abc]
Plus Na-propionate, high concentration	510.7 ± 89.8[b]	−1.2 ± 0.6[bc]

[1]Values are means ± SEM, n = 11. Values not sharing the same letters are significantly different ($P < 0.05$).

curves in healthy subjects. A further in vitro evaluation of white wheat bread products has shown that added lactic and citric acid reduced the rate of amylolysis, whereas no inhibition of amylase activity was found with added acetic or propionic acid (unpublished data). These results indicate that some acids may act as amylase inhibitors whereas others do not.

In parallel with the evaluation of metabolic responses, satiety was studied during 3 h following the test meals. All bread products containing organic acid or salt received higher satiety areas under the curves (0–180 min) than the reference product. However, this tendency only reached statistical significance in the case of a high concentration of Na-propionate. This may indicate an effect on gastric emptying. In several studies (Hunt and Knox 1969 and 1972, Hunt and Pathak 1960), it has been shown that organic acids slow gastric emptying in humans, whereas the slowing effects of most salts were found to be of minor importance (Hunt and Knox 1969, Hunt and Pathak 1960). However, the calcium and sodium salts of lactic and propionic acids, respectively, were not included. In the present study, the observations with bread products baked with Na-propionate or Ca-lactate were not consistent. Consequently, an improved glycemia was registered with the breads with added Na-propionate, whereas no effect was seen with WMB plus Ca-lactate. Propionic acid is one of the short chain fatty acids produced during fermentation of undigested carbohydrates in the colon. It has been suggested that propionic acid has some regulating functions on hepatic carbohydrate and lipid metabolism (Thorburn et al. 1993). A Na-propionate supplemented diet has also been shown to lower the hepatic total cholesterol level in rats (unpublished data). Consequently, it is likely that Na-propionate is metabolized in the liver. It is possible that consumption of Na-propionate–baked breads also may improve glycemia by influencing hepatic regulation.

The propionate bread was accepted to a higher extent than the lactic acid products. To improve palatability while still retaining effects on metabolic responses, it may be preferable to bake bread products with Na-propionate, or Na-propionate in combination with organic acid.

All bread products had a similar content of in vitro resistant starch (1.3–2.1 g/100 g starch basis). Most studies have shown equally low levels in flour-based bread (Englyst et al. 1992, Liljeberg and Björck 1994, Liljeberg et al. 1992). Recently, a high content of resistant starch was found in a commercial sourdough fermented pumpernickel bread (8.1 g/100 g starch basis) (Liljeberg and Björck 1994). The results from the present study may exclude the effect of organic acids per se on retrogradation of amylose. Probably, the high content of in vitro resistant starch was due to the long baking time at low temperature, commonly used for this type of bread.

Results from the present study show that bread products baked with lactic acid, formed during sourdough fermentation or added, improved glucose tolerance. Sourdough baking is an old, traditionally used process and its effects on dough rising, bread taste, flavor, texture and shelf-life are well established (Lönner 1988). The information in the present paper concerning improved nutritional characteristics of starch in sourdough bread is, however, new.

Favorably low glucose and insulin responses were also found with the bread products containing Na-propionate. Obviously, the metabolic effects are related to the concentration of added Na-propionate, i.e., the improved glycemia was more pronounced with high Na-propionate. The suggested mechanism for slowly absorbed starch in the presence of lactic acid is an inhibition of the amylolytic enzymes, as judged from in vitro results. However, observations with the product baked with a high concentration of Na-propionate, e.g. delayed glucose and insulin peak values, prolonged satiety and the lack of effect on in vitro starch hydrolysis rate, together suggest a mechanism related to a delay in gastric emptying. We conclude that the beneficial effects of organic acids on postprandial glycemia could motivate a renewed interest in sourdough baking and other fermentation processes. Addition of lactic acid or Na-propionate could be explored in the baking process for development of lente bread products. The metabolic effects of corresponding salts could be helpful in improving starch properties in products with less acidic characteristics.

ACKNOWLEDGMENTS

We thank Anders Drews for his invaluable assistance. The bread products were skillfully baked by Arne Bjager, Nord Mills AB (Malmö, Sweden).

LITERATURE CITED

Asp, N.-G., Johansson, C.-G., Hallmer, H. & Siljeström, M. (1983) Rapid enzymic method for assay of insoluble and soluble fiber. J. Agric. Food Chem. 31: 476–482.

Brand, J., Colagiuri, S., Crossman, S., Allen, A., Roberts, D. & Truswell, S. (1991) Low glycemic index foods improve longterm glycemic control in NIDDM. Diabetes Care 14: 95–101.

Brighenti, F. & Testolin, G. (1991) Effects of a limited dose of vinegar on blood glucose and acetate responses to a mixed meal in healthy subjects. Poster presented at the ninth international symposium on diabetes and nutrition, Diabetes and Nutrition Study Group of the EASD, June 13–15, Uppsala, Sweden.

Chapman, L. D. & Wigfield, R. (1970) Rating scales in consumer research. Food Manuf. 45: 59–62.

Collier, G. R., Wolever, T.M.S., Wong, G. S. & Josse, R. G. (1986) Prediction of glycemic response to mixed meals in noninsulin-dependent diabetic subjects. Am. J. Clin. Nutr. 44: 349–352.

Conover, W. J. (1980) Practical nonparametric statistics. John Wiley & Sons, New York, NY.

Ducimetiere, P., Eschwege, E., Papox, L., Richard, J. L., Claude, J. R. & Rosselin, G. (1980) Relationship of plasma insulin levels to the incidence of myocardial infarction and coronary heart disease mortality in a middle-aged population. Diabetologia 19: 205–210.

Englyst, H. N., Kingman, S. M. & Cummings, J. H. (1992) Classification and measurement of nutritionally important starch fraction. Eur. J. Clin. Nutr. 46 (Suppl.): S33–S50.

Granfeldt, Y. (1994) Food Factors Affecting Metabolic Responses to Cereal Products. Doctoral thesis, University of Lund, Lund, Sweden.

Granfeldt, Y. & Björck, I. (1991) Glycemic response to starch in pasta: a study of mechanism of limited enzyme availability. J. Cereal Sci. 14: 47–61.

Granfeldt, Y., Björck, I., Drews, A. & Tovar, J. (1992) An in vitro procedure based on chewing to predict metabolic response to starch in cereal and legume products. Eur. J. Clin. Nutr. 46: 649–660.

Granfeldt, Y., Liljeberg, H., Drews, A., Newman, R. & Björck, I. (1994) Glucose and insulin responses to barley products: influence of food structure and amylose-amylopectin ratio. Am. J. Clin. Nutr. 59: 1075–1082.

Greenwood, C. T. & Milne, E. A. (1968) Studies on starch-degrading enzymes. A comparison of α-amylases from different sources: their properties and action patterns. Die Stärke 20: 139–150.

Haber, G. B., Heaton, K. W., Murphy, D. & Burroughs, L. F. (1977) Depletion and disruption of dietary fibre. Effects on satiety, plasma-glucose, and serum-insulin. Lancet 1: 679–682.

Holm, J., Björck, I., Drews, A. & Asp, N.-G. (1986) A rapid method for the analysis of starch. Starch/Staerke 38: 224–226.

Holt, S., Brand, J., Soveny, C. & Hansky, J. (1992) Relationship of satiety to postprandial glycaemic, insulin and cholecystokinin responses. Appetite 18: 129–141.

Hostettler, F., Borel, E. & Devel, H. (1951) Uber die reduktion der 3,5-di-nitrosalicylsäure durch zucker. Helvet. Chim. Acta. 34: 2132–2139.

Hunt, J. N. & Knox, M. T. (1969) The slowing of gastric emptying by nine acids. J. Physiol. 201: 161–179.

Hunt, J. N. & Knox, M. T. (1972) The slowing of gastric emptying by four strong acids and three weak acids. J. Physiol. 222: 187–208.

Hunt, J. N. & Pathak, J. D. (1960) The osmotic effect of some simple molecules and ions in gastric emptying. J. Physiol. 154: 254–269.

Jenkins, D.J.A., Wolever, T.M.S., Collier, G. R., Ocana, A., Rao, A. V., Buckley, G., Lam, Y., Mager, A. & Thompson, L. U. (1987) Metabolic effects of a low-glycemic-index diet. Am. J. Clin. Nutr. 46: 968–975.

Jenkins, D.J.A., Wolever, T.M.S. & Jenkins, A. L. (1988) Starchy foods and glycemic index. Diabetes Care 11: 149–159.

Liljeberg, H. & Björck, I. (1994) Bioavailability of starch in bread products. Postprandial glucose and insulin responses in healthy subjects and in vitro resistant starch content. Eur. J. Clin. Nutr. 48: 151–163.

Liljeberg, H., Granfeldt, Y. & Björck, I. (1992) Metabolic responses to starch in bread containing intact kernels versus milled flour. Eur. J. Clin. Nutr. 46: 561–575.

Lönner, C. (1988) Starter Cultures for Rye Sour Doughs. Characteristics and Functions of Lactic Acid Bacteria. Doctoral thesis, University of Lund, Lund, Sweden.

Lönner, C. & Preve-Åkesson, K. (1988) Acidification properties of lactic acid bacteria in rye sour doughs. Food Microbiol. 5: 43–58.

Siljeström, M., Eliasson, A.-C. & Björck, I. (1989) Characterization of resistant starch from autoclaved wheat starch. Starch/Staerke 41: 147–151.

Spicher, G. & Lönner, C. (1985) Die mikroflora des sauerteiges XXI. Die in sauerteigen Schwedischer bäckereien vorkommenden Lactobacillen. Z. Lebensm. Unters. Forsch. 181: 9–13.

Thomas, D. E., Brotherhood, J. R. & Brand, J. C. (1991) Carbohydrate feeding before exercise: effect of glycemic index. Int. J. Sports Med. 12: 180–186.

Thorburn, A., Muir, J. & Proietto, J. (1993) Carbohydrate fermentation decreases hepatic glucose output in healthy subjects. Metabolism 42: 780–785.

Todesco, T., Rao, A. V., Bosello, O. & Jenkins, D.J.A. (1991) Propionate lowers blood glucose and alters lipid metabolism in healthy subjects. Am. J. Clin. Nutr. 54: 860–865.

Torsdottir, I., Blomqvist, R., Ekman, R., Hagander, B. & Sandberg, A-S. (1992) Fermented or fresh vegetables decrease the postprandial blood glucose and insulin levels in healthy persons. Scand. J. Nutr. 36: 4–7.

Tovar, J., Granfeldt, Y. & Björck, I. (1992) Effect of processing on blood glucose and insulin responses to starch in legumes. J. Agric. Food Chem. 40: 1846–1851.