

Delayed gastric emptying rate may explain improved glycaemia in healthy subjects to a starchy meal with added vinegar

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Objectives: The aim of the study was to evaluate the possible influence of acetic acid (administered as vinegar) on the postprandial glucose and insulin responses, and the potential involvement of a modified gastric emptying rate was studied by use of paracetamol as a marker.

Design: The white bread reference meal as well as the corresponding meal supplemented with vinegar had the same content of starch, protein and fat. The meals were served in the morning after an over-night fast and in random order. Capillary blood samples for analysis of glucose, insulin and paracetamol were collected postprandially.

Setting: The study was performed at the Department of Applied Nutrition and Food Chemistry, Lund University, Sweden.

Subjects: Ten healthy volunteers, seven women and three men, aged 22–51 y, with normal body mass indices were recruited.

Results: The presence of acetic acid, given as vinegar, significantly reduced the postprandial glucose (GI = 64) and insulin responses (II = 65) to a starchy meal. As judged from lowered paracetamol levels after the test meal with vinegar, the mechanism is probably a delayed gastric emptying rate.

Conclusions: Fermented foods or food products with added organic acids should preferably be included in the diet in order to reduce glycaemia and insulin demand.

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Descriptors: glucose and insulin responses; gastric emptying rate; acetic acid; vinegar; starch

Introduction

A diet characterised by foods which induce low increments in glucose and insulin is increasingly considered to play an important role in the prevention of diseases related to the metabolic syndrome. Therefore, as recently reported by Salmerón *et al* (1997a,b), the dietary glycaemic index (GI) is positively associated with a high incidence of NIDDM.

There are a number of food factors which are known to influence postprandial glycaemia by affecting the gastric emptying rate (Torsdottir *et al*, 1984) and/or the rate of digestion and absorption of carbohydrates in the small intestine (Granfeldt & Björck, 1991). Some of these factors are related to the raw material, whereas others are linked to the type and/or extent of food processing. Based on numerous studies undertaken over the past decade, it has become increasingly clear that the GI of starchy foods varies widely and may range from GI 15–130 (white bread reference). Therefore, for the following instances, starch in potatoes (Collier *et al*, 1986), conventional bread products (Jenkins *et al*, 1988a), and most breakfast cereals such as cornflakes (Jenkins *et al*, 1983), puffed rice (Brand *et al*, 1985), and rolled oat flakes or oat porridge (Granfeldt *et al*, 1995) is rapidly digested and absorbed, producing high responses of blood glucose and insulin. In contrast, pasta products (Granfeldt & Björck, 1991) and certain rice (Wolever *et al*, 1986) display GI in the lower range (GI = 45–70). The GI of legume products (Dilawari *et al*, 1981; Tovar *et al*, 1992) and intact cereal grains (Granfeldt

et al, 1994; Jenkins *et al*, 1988b) have been found to be very low, and in fact among the lowest reported for starchy foods (GI = 15–65). In a few studies it has been demonstrated that the organic acids formed during fermentation of, for instance, vegetables (Torsdottir *et al*, 1992) and bread (Liljeberg *et al*, 1995) may significantly improve the glucose tolerance to starch. Acetic acids is the major acid formed upon sourdough fermentation. Previously we observed that a commercial German sourdough fermented rye bread displayed lower GI features (GI = 68) than common flour based bread products (Liljeberg & Björck, 1994). However, the mechanism remains unclear.

The aim of the present study was to evaluate the possible influence of acetic acid (given as vinegar) on the postprandial glucose and insulin responses in healthy subjects. The potential involvement of an effect on the gastric emptying rate was studied by use of paracetamol as a marker (Liljeberg & Björck, 1996).

Materials and methods

White wheat bread (WWB)

Commercial white wheat flour was obtained from Kungsörnen AB (Järna, Sweden), and a standardised WWB was baked in a baking machine as described previously (Liljeberg & Björck, 1994). To allow indirect measurement of gastric emptying rate, the WWB was baked with the addition of paracetamol (1.0 g per test meal).

After cooling, the bread was sliced (the crust removed), wrapped in aluminium foil, put into plastic bags and stored in a freezer until utilised.

Chemical analysis

A portion of the WWB was air dried and milled (Cyclotec, Tecator, Sweden) prior to analysis. The bread product was analysed for protein (Kjeldahl), starch and fat as described previously (Liljeberg & Björck, 1996). To insure recovery of paracetamol in the WWB, the concentration was confirmed by use of an enzyme kit (Cambridge Life Sciences plc., Cambridge, England). Therefore, after baking 99% of added paracetamol was recovered. Finally, the amount of acetic acid in the vinegar was measured using gas liquid chromatography (Varian 3300, Varian, Palo Alto, CA, USA).

Blood glucose, insulin and paracetamol responses in healthy subjects

Subjects Ten healthy volunteers, seven women and three men, aged 22–51 y, with normal body mass indices ($21.3 \pm 2.0 \text{ kg/m}^2$) and without drug therapy participated in this study.

Test meals

- (1) *Reference meal*; 122 g WWB (corresponding to 50 g starch), 8 g olive oil (Filippo Berio, Lucca, Italy) and 23 g cheese (10% fat, wet wt).
- (2) *WWB + vinegar*; a vinaigrette sauce was made from 20 g white vinegar (Spice Islands, Specialty Brands Inc., San Francisco, CA, USA), 20 g water and 8 g olive oil. The WWB (122 g) was dipped in the vinaigrette sauce and eaten with cheese (23 g). In addition, 250 ml water and 150 ml coffee/tea were served with each of the two meals.

The reference meal as well as the meal supplemented with vinegar contained 50.0 g starch, 15.3 g protein and 12.0 g fat, corresponding to 1554 kJ. The subjects were served the meals in random order (two separate occasions) after an over-night fast. The tests were performed approximately one week apart and commenced at the same time in the morning. Both meals were consumed steadily over 12–14 min.

Blood analyses Finger-prick capillary samples were taken prior to 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 serum insulin level with an enzyme immunoassay kit (Boehringer Mannheim, GmgH, Mannheim, Germany). In addition, serum paracetamol was measured with an enzyme kit (Cambridge Life Sciences plc., Cambridge, England) at the following time points; prior to the meal (0) and at 15, 30, 45, 70 and 95 min after the meal.

Approval of the study was given by the Ethics Committee of the Faculty of Medicine at Lund University.

Calculations and statistical methods

The glycaemic and insulinaemic indices (GI and II, respectively) were calculated from the 95 min incremental postprandial blood glucose and insulin areas under curves, using WWB as reference (GI and II = 100, respectively). The results are expressed as means \pm s.e.m., and the significance of differences was assessed with the Wilcoxon matched-pair signed-ranking tests. The SPSS/PC+

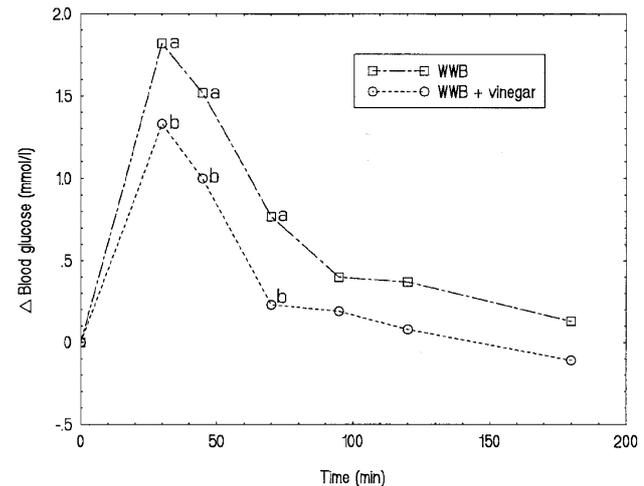


Figure 1 Mean incremental blood glucose responses in healthy subjects following ingestion of breakfast meals with white wheat bread (WWB) and WWB with added vinegar. Values with different letters are significantly different, $P < 0.05$.

advanced statistics program (version 2.0, SPSS, Chicago, IL, USA) was used. A value of $P < 0.05$ was considered significant.

Results

Postprandial blood glucose and insulin responses in healthy subjects

In the 30–70 min postprandial phase, the bread meal with added vinegar resulted in significantly lower blood glucose increments ($P < 0.05$) than did the reference meal (Figure 1), and a GI was calculated to 64.

The insulin responses were closely associated with the glucose responses (Figure 2). Compared with the reference meal, significantly lower increments ($P < 0.05$) were found at 30–45 min with the meal with included vinegar (II = 65).

Blood paracetamol responses

The postprandial blood paracetamol levels after the meal supplemented with vinegar (45–95 min) were significantly lower ($P < 0.05$) than after the reference meal, resulting

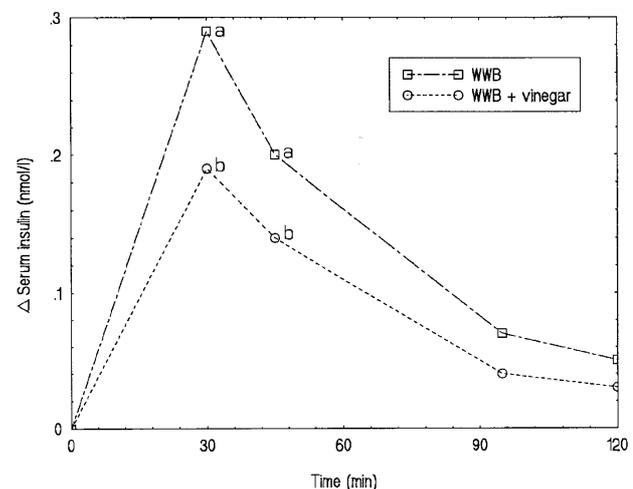


Figure 2 Mean incremental serum insulin responses in healthy subjects following ingestion of breakfast meals with white wheat bread (WWB) and WWB with added vinegar. Values with different letters are significantly different, $P < 0.05$.

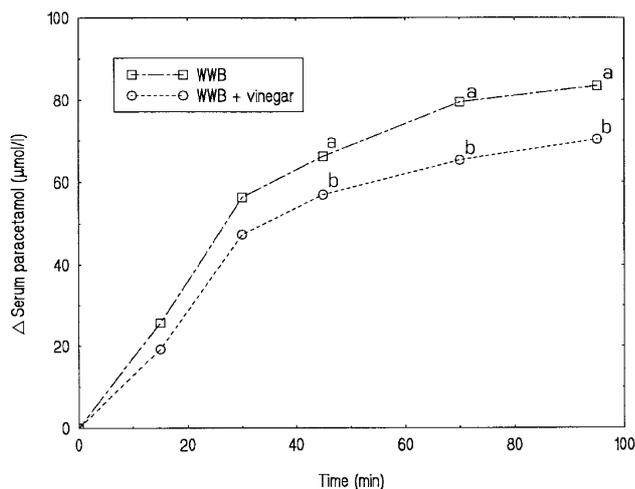


Figure 3 Mean incremental serum paracetamol responses in healthy subjects following ingestion of breakfast meals with white wheat bread (WWB) and WWB with added vinegar. Values with different letters are significantly different, $P < 0.05$.

also in a reduced total area (0–95 min) under the curve (approximately –20%) (Figure 3).

Discussion

Results from the present study show that the presence of acetic acid (18 mmol per test meal), given as vinegar, lowered the acute postprandial glucose (GI = 64) and insulin responses (II = 65) to a starchy meal. This is in accordance with Brighenti *et al* (1995), who demonstrated that the ingestion of 16 mmol of acetic acid from vinegar lowered the glucose responses in healthy subjects by 30%. The shown effect of acetic acid on the metabolic responses may confirm the results from a previous study (Liljeberg & Björck, 1994), where a commercial sourdough fermented bread with a high concentration of acetic acid (27 mmol per test meal) flattened the glucose (GI = 68) and insulin rise (II = 63). Improved glycaemia to starch following lactic acid fermentation of bread (Liljeberg *et al*, 1995) or vegetables (Torsdottir *et al*, 1992) has also been reported, suggesting beneficial effects also of other organic acids.

In this present study the influence of acetic acid on the rate of gastric emptying was evaluated. As judged from lowered postprandial blood paracetamol levels after the test meal supplemented with vinegar, in comparison with the reference meal, the mechanism appears to be a delayed gastric emptying rate. In contrast, Brighenti *et al* (1995) did not find any influence of acetic acid on the gastric emptying rate, using a method based on ultra-sound technique. In that study, the effect of vinegar in a test meal was compared with a meal where vinegar was neutralised with Na-bicarbonate, thus forming Na-acetate. No difference in gastric emptying time was noted between the two meals. However, as opposed to the meal with added vinegar, the meal with Na-acetate displayed the same high postprandial responses in glucose as a white reference bread (Brighenti *et al*, 1995).

Based on previous studies, it is possible that certain organic salts may have similar favourable metabolic effects as organic acids. Therefore, according to Todesco *et al* (1991) and studies from our laboratory (Liljeberg *et al*, 1995), the Na salt of propionic acid improved the glucose tolerance to starch. However, different mechanisms have been proposed for the lowered glucose and insulin

responses in the presence of Na-propionate. Whereas Todesco *et al* (1991), have asserted the ability of Na-propionate to reduce the rate of starch digestion, we have failed to demonstrate any inhibitory effect of the enzyme activity *in vitro* (Liljeberg *et al*, 1995). Instead a reduced rate of gastric emptying was suggested as a mechanism (Liljeberg & Björck, 1996). In contrast to Na-propionate, a hypothesis that also lactic acid added to bread may affect the gastric emptying rate was recently rejected (Liljeberg & Björck, 1996).

Conclusions

The results from this present study show that the presence of acetic acid reduced postprandial glucose and insulin responses to a bread meal in healthy subjects, and that the probable cause for this was a delayed gastric emptying rate. As judged from results obtained *in vitro*, showing that the starch in a commercial sourdough bread containing acetic acid was equally rapidly digested as in a white bread (Liljeberg & Björck, 1994), this excludes any action of acetic acid as an amylase inhibitor. Consequently, a mechanism related to the rate of gastric emptying is supported.

Taken together, the mechanisms responsible for the improved glycaemia in the presence of organic acids/salts may differ, and further work is thus necessary before drawing any conclusions regarding the effects of other acids/salt in this respect. It is concluded that fermented foods or food products with added organic acids should be included in the diet in order to reduce glycaemia and insulin demand. Such products may have a potential in diabetic diets and for individuals predisposed for metabolic diseases.

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References

- Brand JC, Nicholson PL, Thorburn AW & Truswell AS (1985): Food processing and the glycemic index. *Am. J. Clin. Nutr.* **42**, 1192–1196.
- Brighenti F, Castellani G, Benini L, Casiraghi M, Leopardi E, Crovetti R & Testolin G (1995): Effect of neutralized and native vinegar on blood glucose and acetate responses to a mixed meal in healthy subjects. *Eur. J. Clin. Nutr.* **49**, 242–247.
- Collier GR, Wolever TMS, Wong GS & Josse RG (1986): Prediction of glycemic response to mixed meals in noninsulin-dependent diabetic subjects. *Am. J. Clin. Nutr.* **44**, 349–352.
- Dilawari JB, Kamath PS, Batta RP, Mukewar S & Raghavan S (1981): Reduction of postprandial plasma glucose by Bengal gram dal (*Cicer arietinum* and *Rajmah* (*Phaseolus vulgaris*)). *Am. J. Clin. Nutr.* **34**, 2450–2453.
- 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, 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.
- Granfeldt Y, Hagander B & Björck I (1995): Metabolic responses to starch in oat and wheat products. On the importance of food structure, incomplete gelatinization or presence of viscous fibre. *Eur. J. Clin. Nutr.* **49**, 189–199.
- Jenkins DJA, Wolever TMS, Jenkins AL, Thorne MJ, Lee R, Kalmusky J, Reichert R & Wong GS (1983): The glycemic index of foods tested in diabetic patients: a new basis for carbohydrate exchange favouring the use of legume. *Diabetologia* **24**, 257–264.
- Jenkins DJA, Wolever TMS & Jenkins AL (1988a): Starchy foods and the glycemic index. *Diabetes Care* **11**, 149–159.

- Jenkins DJA, Wesson V, Wolever TMS, Jenkins AL, Kalmusky J, Guidici S, Csima A, Josse RG & Wong GS (1988b): Wholemeal versus wholegrain breads: proportion of whole or cracked grain and the glycaemic response. *Br. Med. J.* **297**, 958–960.
- 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 HGM, Lönner CH & Björck IME (1995): Sourdough fermentation or addition of organic acids or corresponding salts to bread improves nutritional properties of starch in healthy humans. *J. Nutr.* **125**, 1503–1511.
- Liljeberg HGM & Björck IME (1996): Delayed gastric emptying rate as a potential mechanism for lowered glycemia after eating sourdough bread: studies in humans and rats using test products with added organic acids or an organic salt. *Am. J. Clin. Nutr.* **56**, 389–394.
- Salmerón J, Ascherio A, Rimm E, Colditz G, Spiegelman D, Jenkins D, Stampfer M, Wing A & Willett W (1997a): Dietary fiber, glycemic load, and risk of NIDDM in men. *Diabetes Care* **20**, 545–550.
- Salmerón J, Manson J, Stampfer M, Colditz G, Wing A & Willett W (1997b): Dietary fiber, glycemic load, and risk of non-insulin-dependent diabetes mellitus in women. *JAMA* **277**, 472–477.
- Todesco T, Rao A, Bosello O & Jenkins D (1991): Propionate lowers blood glucose and alters lipid metabolism in healthy subjects. *Am. J. Clin. Nutr.* **54**, 860–865.
- Torsdottir I, Alpsten M, Andersson D, Brummer R & Andersson H (1984): Effect of different starchy foods in composite meals on gastric emptying rate and glucose metabolism. I. Comparisons between potatoes, rice and white beans. *Hum. Nutr. Clin. Nutr.* **38C**, 329–338.
- 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**, 2–7.
- Tovar J, Granfeldt Y & Björck I (1992): Effect of processing on blood glucose and insulin responses to legumes. *J. Agric. Food Chem.* **40**, 1846–1851.
- Wolever TMS, Jenkins DJA, Kalmusky J, Jenkins AL, Giordano C, Giudici S, Josse RB & Wong GS (1986): Comparison of regular parboiled rices: explanation of discrepancies between reported glycemic responses to rice. *Nutr. Res.* **6**, 349–357.