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ORIGINAL ARTICLE

Sourdough-leavened bread improves postprandial glucose and insulin plasma levels in subjects with impaired glucose tolerance

Mario Maioli · Giovanni Mario Pes · Manuela Sanna · Sara Cherchi · Mariella Dettori · Elena Manca · Giovanni Antonio Farris

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Abstract Sourdough bread has been reported to improve glucose metabolism in healthy subjects. In this study postprandial glycaemic and insulinaemic responses were evaluated in subjects with impaired glucose tolerance (IGT) who had a meal containing sourdough bread leavened with lactobacilli, in comparison to a reference meal containing bread leavened with baker yeast. Sixteen IGT subjects (age range 52–75, average BMI 29.9 ± 4.2 kg/m^2) were randomly given a meal containing sourdough bread (A) and a meal containing the reference bread (B) in two separate occasions at the beginning of the study and after 7 days. Sourdough bread was leavened for 8 h using a starter containing autochthonous *Saccharomyces cerevisiae* and several bacilli able to produce a significant amount of D-and L-lactic acid, whereas the reference bread was leavened for 2 h with commercial baker yeast containing *Saccharomyces cerevisiae*. Plasma glucose and insulin levels were measured at time 0, 30, 60, 120, and 180 min. In IGT subjects sourdough bread induced a significantly lower plasma glucose response at 30 minutes ($p = 0.048$) and a smaller incremental area under curve (AUC) Δ 0–30 and Δ 0–60 min ($p = 0.020$ and 0.018 respectively) in comparison to the bread leavened with baker yeast. Plasma insulin response to this type of bread showed lower values at 30 min ($p = 0.045$) and a smaller AUC Δ 0–30 min ($p = 0.018$). This study shows that in subjects with IGT glycaemic and insulinaemic responses after the consumption of sourdough bread are lower than after the bread leavened with baker yeast. This effect is likely due to the lactic acid produced during dough leavening as well as the reduced availability of simple carbohydrates. Thus, sourdough bread may potentially be of benefit in subjects with impaired glucose metabolism.

Keywords Sourdough bread · Impaired glucose tolerance · Lactic acid

M. Maioli (✉) · S. Cherchi · E. Manca
Institute of Internal Medicine, Metabolic Unit, University of Sassari, Viale San Pietro, 8, 07100 Sassari, Italy
e-mail: marimaio@uniss.it

G. M. Pes
Department of Biomedical Sciences, University of Sassari, Sassari, Italy
e-mail: gmpes@uniss.it

M. Sanna · M. Dettori · G. A. Farris
Department of Environmental and Agricultural Science and Biotechnology, University of Sassari, Sassari, Italy

Introduction

Until a few years ago in several areas of south Europe the Mediterranean diet was widely diffused and characterised by the prevailing consumption of low-calorie food, saturated fats, rich in fibers and carbohydrates with low glycaemic index. In particular bread represented the main component of the daily diet in such countries including Sardinia [1]. In the traditional agropastoral society of this island bread represented the base of nutrition and until approximately 30 years ago it was made nearly in all the villages of the island using "homemade" microbic starters, which were exchanged among families. Such habits have gradually diminished along with the increased tendency in nearly all the villages of the island to consume industrial made bread, while only in some rather remote areas of the island the ancient tradition of domestic breadmaking still persists. This type of traditional bread had chemical, physical and organoleptic characteristics rather different

from bread bought nowadays from bakeries where leavening is carried out with baker yeast. Sourdough bread has been reported to influence glucose metabolism in healthy subjects [2, 3]. However, so far the metabolic effect of such kind of bread has not been evaluated in subjects with impaired glucose tolerance (IGT). IGT is a condition that can be defined as "prediabetes" and is due to the combination of a resistance to the insulin action as well as to impairment of its secretion by pancreatic beta cells [4, 5]. Therefore, in this study we have evaluated in a group of subjects with IGT whether glucose and insulin response after a single ingestion of bread with characteristics similar to those of the Sardinian traditional acid-leavened bread, was lower than a reference bread similar to that currently available in industrial bakeries of the main urban areas of the island and leavened only with the common baker yeast. In IGT a reduced glucose and insulin response after consuming bread with lower GI is likely to occur, whereas in healthy subjects only insulin but not glucose modification could be seen [6]. We carried out this study using a mixed meal not only for making it more palatable but because it was suggested that the overall diet pattern, instead of single nutrients, which might have a synergistic or antagonistic effect on health [7].

Subjects and methods

Sixteen volunteers (7 women and 9 men, age range 52–75) with IGT defined according to the National Diabetes Data Group [8] were invited to participate in the study and they were selected from outpatients at the Metabolic Unit of the Department of Internal Medicine, University of Sassari. All the patients were treated with a diet and a physical activity program. The study was approved by the Ethical Committee of the Local Health Authority and a written informed consent was obtained from all the subjects prior to their inclusion in the study following the ethical standards laid down in the 1964 Declaration of Helsinki. Anthropometric measurements, routine haematologic tests, TSH, serum creatinine, and liver enzyme concentrations were collected at the beginning of the study to exclude major liver or kidney disease (Table 1). None of the patients were positive for serum markers of β-cells autoimmunity. During the 30-day period before the scheduled tests, the IGT subjects were advised to follow the usual (isocaloric) diet on the basis of a self-administered 7-day food frequency questionnaire and to abstain from strenuous physical activity on the day before the test. The 30-day period was selected to make sure that no major change of body weight occurred before the tests. The two types of bread used in this study were purposely prepared at the Dipartimento di Scienze Agrarie e Biotecnologie Alimentari of the University of Sassari [9]. The

Table 1 Baseline anthropometric and biochemical data of IGT subjects

	Values (mean ± SD)
Number	16
Age range (years)	52–75
Gender	M = 9; F = 7
BMI (kg/m^2)	29.9 ± 4.2
Smoking	Smokers = 6; non smokers = 10
Creatinine (mg/dl)	0.9 ± 0.2
Totale cholesterol (mg/dl)	194.7 ± 36.6
HDL cholesterol (mg/dl)	46.7 ± 8.8
Triglycerides (mg/dl)	99.0 ± 53.9
LDL cholesterol (mg/dl)	128.1 ± 36.7
AST (U/l)	22.4 ± 8.5
ALT (U/l)	21.1 ± 5.5
TSH (μU/ml)	1.6 ± 0.8

initial dough consisted of a mixture of 70% durum wheat semolina and 30% of cornflour, with the addition of 60% water, 1% NaCl, and 1% industrial baker yeast. After dividing this dough quantity into two exact halves, two different types of leavening agents were used: (1) the usual baker yeast available on the market, mostly consisting in *Saccharomyces cerevisiae*; (2) a sourdough starter consisting of a mixture of well-defined yeast and bacteria. This starter included an autochthonous 5.2 strain of *Saccharomyces cerevisiae*, *Lactobacillus brevis* SB3 and *Lactobacillus plantarum* SB24 [10–13], isolated from natural sourdough. This acidic starter was prepared according to preliminary bread-making experiments, which were necessary to define the optimal percentage of bacteria, to determine the proportion of ingredients, and incubation time. The dough treated with baker yeast and the one treated with acid starter were proofed for 2 and 8 h, respectively, at 30°C, 90% humidity. The choice of a fermentation time not exceeding 8 h was due to the fact that longer intervals produce a brittle and unpalatable product. The bread containing baker yeast was processed according to the same protocol (manual doughing, baking at 220°C) except for the fermentation time, which was only 2 h. During the preparation of the bread the pH of the dough in both leavening processes was measured, as well as the concentration of D-and L-lactic acid, glucose, and fructose by using commercially available spectrophotometric enzymatic kits (K-LATE, K-DATE, K-FRUGL Megazyme®) and is reported in Table 2. The production of D- and L-lactic acid during fermentation is reported in Fig. 2a, b.

IGT patients were randomly given a standard meal (total energy content about 500 kCal, 58% carbohydrates, 30% lipids and 12% proteins) consisting in: 200 ml semi-skimmed milk to ensure an optimal palatability; 10 g butter,

Table 2 Simple carbohydrates and lactic acid concentration in the tested breads

Samples	Humidity (%)	Simple carbohydrates (g/100 g)		Lactic acid (g/100 g)	
		Glucose	Fructose	D-Lactic	L-Lactic
Baking flour (70:30)	14.4	0.01	0.02	0.0002	0.013
Sourdough bread	22.9	0.06	0.26	0.224	0.148
Reference bread (baker yeast)	25.6	0.16	0.55	0.003	0.021

15 g glucose-free marmalade and 100 g bread. The latter was either sourdough bread or baker yeast bread as specified above. According to the crossover design each IGT subject had the alternative meal after 7 days. At the beginning of each test the anthropometric data and blood pressure values were registered. Blood collection was performed as follows: 15 min before the meal, at time 0, immediately before the meal; at 30, 60, 120, and 180 min after consuming all the meal. For each point, glucose, total cholesterol, HDL-cholesterol, triglycerides were determined in serum with standard enzymatic methods on a Beckman CX7 autoanalyser. LDL-cholesterol was calculated with the Friedewald formula [14]. From each sample an aliquot was drawn to determine insulin (twice for each point) with an immunoradiometric method (Insulin Irma, Immunotech). For each subject and test meal, the glucose and insulin incremental area under the curve (AUC) were calculated, excluding all areas below baseline. Environmental scanning electron microscopy (ESEM, JOEL JSM-5600LV with a magnification of 25×) was used to reveal any possible microstructural difference in bread containing baker yeast and bread containing lactic acid [15]. Values are presented as means ± SD and all statistical analyses were performed with SPSS Statistical Software (release 13 for Windows). Significance was evaluated with the Wilcoxon paired test. Values of $p < 0.05$ were considered significant.

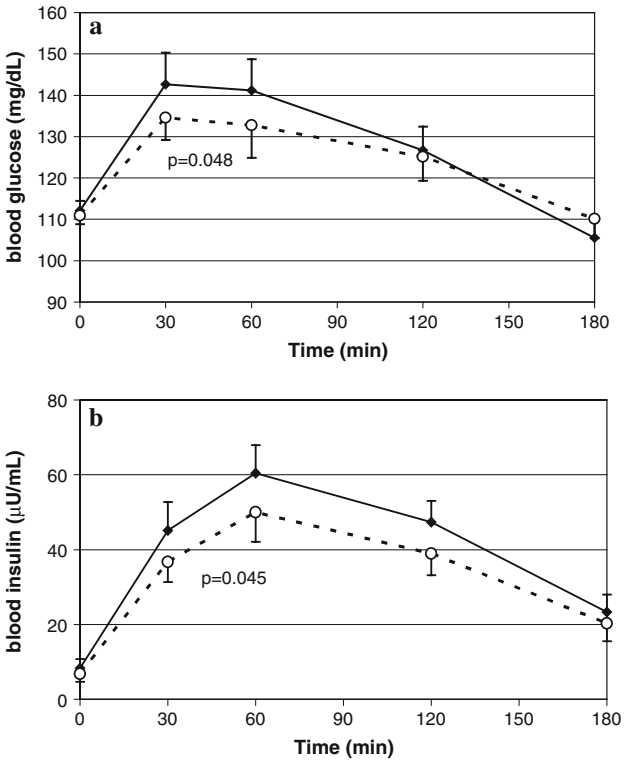

Fig. 1 Mean blood glucose (**a**) and insulin (**b**) responses to reference bread (*filled diamond*) and sourdough bread (*open circle*)

Results

In the bread leavened with baker yeast, the pH lowered from 5.78 to 5.58 after 2 h while in the sourdough bread the pH ranged between 5.63 and 5.10 in the first 2 h, eventually reducing up to 4.25 after 8 h. The average values (±SD) of blood glucose during the fasting period and after the consumption of the two kinds of bread in IGT subjects have been reported in Fig. 1. Thirty minutes after the ingestion of sourdough bread blood glucose levels resulted significantly lower (−25%, $p = 0.048$) in comparison to those obtained with the reference bread. Blood glucose AUC were significantly lower at intervals of Δ 0–30 min ($p = 0.02$) and Δ 0–60 min ($p = 0.018$) while the difference was no longer significant thereafter. As for the insulin levels, they were significantly lower ($p = 0.045$) 30 min after the ingestion of sourdough bread. Similarly, insulin AUC was significantly smaller at the interval of Δ 0–30 min ($p = 0.018$) while no significant difference was found thereafter (Fig. 2).

We did not find any significant difference in plasma cholesterol and triglycerides after consuming the two types of meals.

The carbohydrates and lactic acid concentrations in both types of bread are reported in Table 2. The ESEM analysis showed a similar microstructure in the two types of bread and no relevant difference in starch granules and porosity were observed (Fig. 3).

Discussion

In IGT subjects a mixed meal containing sourdough bread elicited a significantly lower plasma glucose and insulin levels, compared to a meal containing the reference bread. These data are interesting even if they refer to an acute

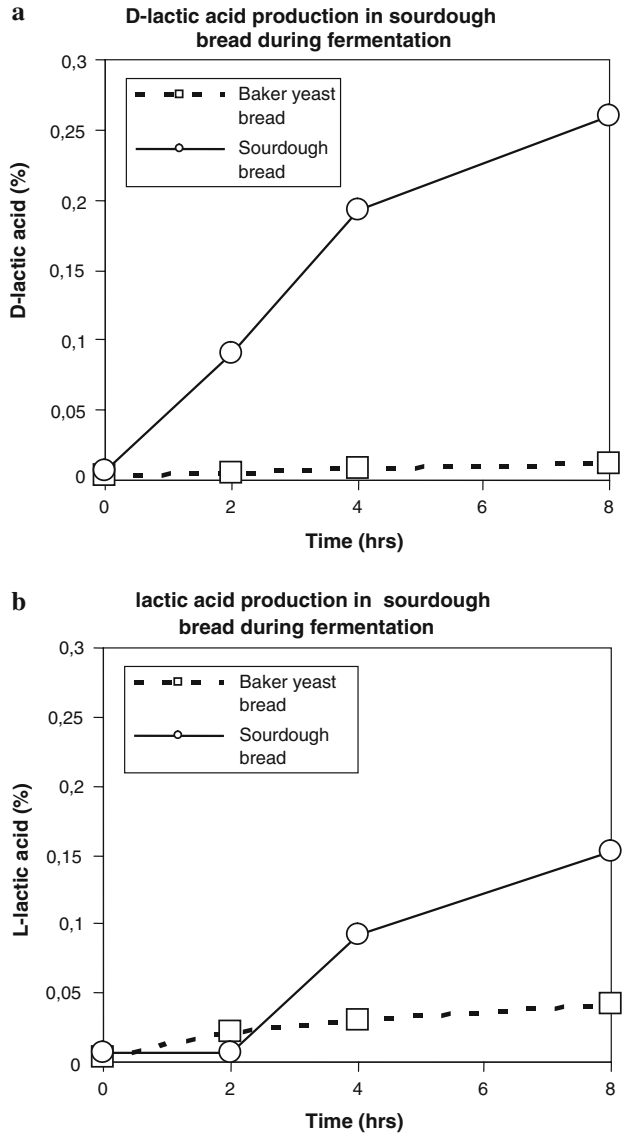

Fig. 2 D-lactic acid production in sourdough (**a**). L-lactic acid production in sourdough (**b**)

setting, as they show that an acid-leavened bread in use for centuries among the Sardinian population has clear-cut effects on glucose metabolism and, consumed together with other ingredients, shows a lower glycaemic index.

We think that the lower level of plasma glucose and insulin after the consumption of sourdough bread may be essentially attributed to two factors; an increase of the lactic acid content in the bread and a reduction of its content in simple sugars.

As far as lactic acid is concerned, the chemical analysis of dough during fermentation revealed a progressive increase of both L- and D-lactic acid concentration until the eighth hour of leavening but proofing was stopped before this time, although lactic acid production may continue for at least 30 h [16], because longer leavening times usually result in a brittle and unpalatable final product. Previous studies have already considered the effect of lactic acid in bread on the blood glucose response [17, 18]. An acutely-designed study performed by Östman et al. [19] using gruel food added with lactic acid had already pointed out that the presence of this compound is able to lower postprandial glucose levels in healthy subjects compared to the control gruel without lactic acid or gruel food in which lactic acid was added after heating. Moreover, barley bread in which lactic acid was added before baking improved glucose metabolism, which lasted for a longer time [20]. The effect of lactic acid is very important as it may affect the rate of amylolysis during the intestinal absorption of carbohydrates [19]. Furthermore, different rate of amylolysis during digestion due to ultrastructural differences between the two types of breads can be ruled out since porosity, density and distribution of gas bubbles were the same in the two types of bread. The other possible factor that could account for the observed effect is the reduced availability of simple sugars in the bread because of their transformation into lactic acid by bacteria during leavening, as demonstrated by the final values of glucose and fructose found in the tested bread after baking.

The reduction in simple carbohydrates during fermentation seems to involve mainly glucose, although even a reduction of maltose might be important, especially with longer leavening times when all monosaccharides are consumed [16].

To make the bread as similar as possible to a normal meal and to make it more palatable, milk, marmalade and butter were added. This could have contributed to the elevation of plasma glucose after the absorption of simple carbohydrates contained in milk and marmalade although the latter was poor in fibre and contained only fructose. Actually, milk was shown to have a strong insulinotropic effect, essentially due to the aminoacids of whey [21] and to produce higher than expected insulin response compared to other milk products [22]. Moreover, Frid et al. [23] attributed this effect to the release of several gastrointestinal hormones such as GIP and GLP-1. However, Nilsson et al. [24] suggested that GLP-1, which causes a delayed gastric emptying, does not increase significantly after milk ingestion. Besides, in our experiments the same amount of milk was added to both sourdough and reference meals so that the only difference in the two meals was the type of bread added. Therefore, we think that the milk added to the two types of bread could not explain the different response in glucose and insulin after eating the two types of meals in our experimental setting. Similarly, we may exclude an effect of added milk on the rate of gastric emptying on postprandial glycaemic response. Interestingly, a previous study demonstrated that despite other organic acids (e.g. the acetic acid added to the bread shortly before the meal)

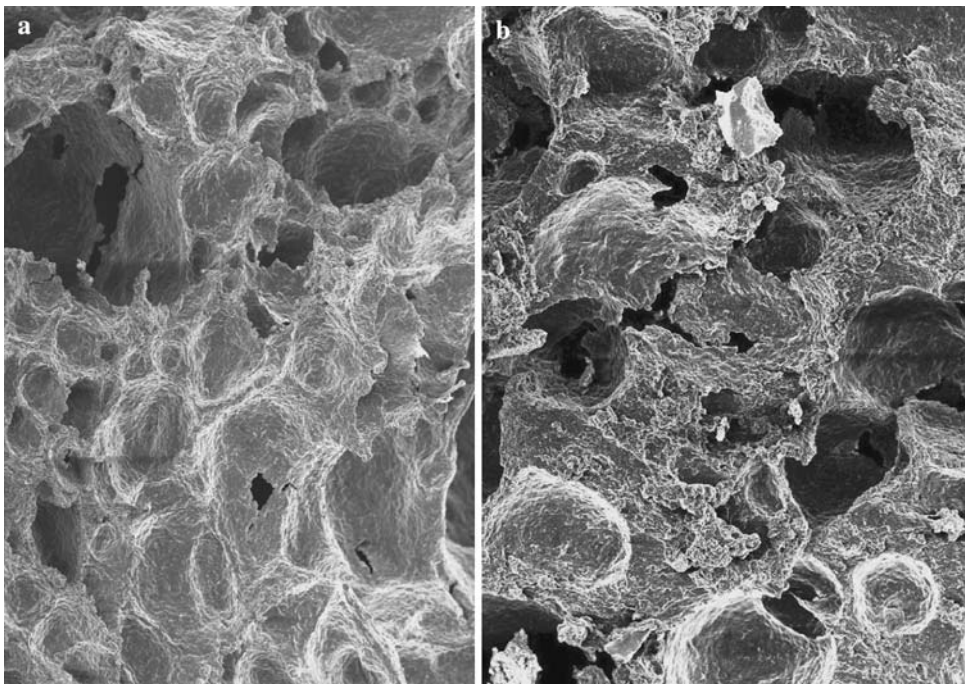

Fig. 3 Environmental scanning electron microscope analysis of sourdough bread (**a**) and baker yeast bread (**b**)

can slow down the gastric peristalsis, lactic acid failed to show the same effect, especially when added after the heating treatment [25]. Similarly, we should rule out any influence by short chain fatty acids, which are believed to be involved in gluconeogenesis [26], since the short duration of experiments did not allow the food to undergo colonic digestion where these acids are produced. Our study confirms that sourdough bread has a reduced glycaemic index compared to baker yeast bread and this is further emphasised by a reduced insulin requirement as reported previously [27]. This is of great importance as it has been reported that even short bursts of hyperglycaemia may worsen insulin resistance in diabetic patients [28].

Therefore these findings suggest that a diet program including sourdough bread may contribute to delay the evolution of IGT to diabetes. Finally, a lower postprandial hyperglycaemia elicited by this type of bread could result in fewer micro- and macro-vascular complications in patients with diabetes [29].

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