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The acute impact of the intake of four types of bread on satiety and blood concentrations of glucose, insulin, free fatty acids, triglyceride and acylated ghrelin. A randomized controlled cross-over trial

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ABSTRACT

The purpose of the present study is to compare the effects of four different breads (one commercial par-baked wheat bread, three sourdough breads prepared with commercial wheat flour, organic wheat flour, organic einkorn flour) in 16 healthy subjects. The primary outcome of this randomized cross-over trial was evaluating intra-individual changes in glycemic areas-under-the-curve (AUCs) after 50 g carbohydrate portions of each bread; secondary outcomes were changes in insulin, fatty free acids (FFA), triglyceride, acylated ghrelin and satiety AUCs. Blood samples and satiety ratings were collected every 30-min for 2-h after the consumption of each bread. The einkorn flour showed the lowest amylase activity, the commercial flour the highest; commercial bread had the highest carbohydrate content and the lowest dietary fiber content.

Glucose AUCs were significantly lower after the consumption of sourdough breads made with organic (12,754 ± 1433 mg/dL × h) and einkorn flour (12,216 ± 1210 mg/dL × h), with respect to the commercial bread (13,849 ± 2193 mg/dL × h). Insulin AUCs decreased after the consumption of all sourdough breads when compared to commercial bread. FFA and triglyceride AUCs did not differ by kind of breads. Median ghrelin AUC was significantly lower and satiety higher after the einkorn bread (3710 pg/mL × h; 3225 ± 2414, respectively) than after commercial bread consumption (4140 pg/mL × h; 1706 ± 1766, respectively), but not with other sourdough breads. In conclusion, the use of sourdough may improve the nutritional features of breads; einkorn bread induced the least disturbance in carbohydrate homeostasis and the greater satiety. If confirmed by further research, these results might have implications in the approach towards chronic dysmetabolic diseases.

1. Introduction

Bread is the staple food of Italian diet and a primary source of carbohydrates in most European countries (Cust et al., 2009). In Europe, the 2015 bread consumption was 61.7 kg/person. In Italy, the consumption of bread bought from bakeries has recently declined (−3.1%), while buying packaged bread from hypermarkets has risen (+2.3%) (Caiapic, 2016).

Postprandial glycemic and insulinemic responses, satiety and the release of hormones regulating appetite and food intake seem regulated by the types of wheat and flour employed and by the processing and preparation of bread (Rokka, Ketoja, Jarvenpaa, et al., 2013 and Burton & Lightowler, 2008). White wheat bread (*Triticum aestivum*) is by far the most consumed in the Western diet, even if recently other wheat varieties are emerging (Jones & Sheats, 2016). The flour derived from ancient grains are attracting a lot of interest (Cooper, 2015). In particular, the wheat variety Einkorn (*Triticum monococcum*) has been postulated to have different nutritional qualities and to favorably impact on the release of the gastrointestinal glucose-dependent insulinotropic polypeptide (GIP) responsible for the insulinotropic effect induced by foods (Bakhøj, Flint, Holst, & Tetens, 2003).

In Italy, as well as in Europe, the industrial production of bread is gradually replacing the traditional craft production by small bakeries.

Abbreviations: AUCs, areas-under-the-curve; BMI, body mass index; FFA, fatty free acids; GIP, gastrointestinal glucose-dependent insulinotropic polypeptide; GLP, glucagon-like peptide-1.

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The commercial bread from hypermarkets is usually obtained by completing the baking of par-baked products at the point of sale.

The use of "improvers", that is the employment of enzymes (amylase) improves the quality of the bread (Mondal & Datta, 2008 and Rosell, Haros, Escrivá, & Benedito De Barber, 2001). An amylase inhibitor (trestatin) incorporated into bread has produced significantly lower glucose and insulin responses in six individuals with normal glucose tolerance and in six patients with type 2 diabetes mellitus (Golay, Schneider, Temler, & Felber, 1991). However, the acute metabolic effects of commercial breads (by completing the baking of par-baked bread) towards sourdough breads, and those of breads obtained by commercial flour with added amylases with respect to breads obtained by biological flour without the addiction of amylases are not fully elucidated at present.

Obesity and diabetes are in epidemic growth. Prevalence of obesity increased from about 3% in 1975 to 11% in 2014 in men and from 6% to 15% in women (NCD Risk Factor Collaboration, 2016a, 2016b). Likewise, global age-standardized diabetes prevalence almost doubled from 1980 to 2014 in both genders (NCD Risk Factor Collaboration, 2016a, 2016b). Lifestyle interventions always give to unsatisfactory results (Wadden, Butryn, & Wilson, 2007 and Yoon, Kwok, & Magkidis, 2013). The use of highly satiating foods eliciting a lower glycemic and insulinemic response could be an aid in the complex approach towards the global burden of chronic dysmetabolic diseases.

The aim of the present cross-over trial was evaluating areas-under the curve (AUCs) of the blood concentrations of glucose, insulin, free fatty acids (FFA), triglycerides after the consumption of four breads (one commercial par-baked wheat bread and three sourdough breads prepared respectively with commercial wheat flour, organic wheat flour, and organic einkorn flour) in healthy individuals. The variation of satiety and of the blood concentrations of acylated ghrelin, the hunger hormone, after the intake of the four breads were evaluated too.

2. Research design and methods

2.1. Recruitment of participants

Sixteen healthy volunteers (six males and ten females) were enrolled among students and graduates attending the Department of Medical Sciences of Turin (Italy).

Inclusion criteria were: age 20–50 years, body mass index (BMI) 18–25 kg/m^2, habitual moderate exercise level and stable body weights for >1-year. Exclusion criteria were: any acute or chronic diseases, any drugs or supplementations, any alimentary restrictions, irregular eating habits or specific diets, actual smokers or quit smoking within the last 6-months, unable to give a written informed consent.

2.2. Design

This was a four-phase randomized double-blind controlled cross-over trial. Every participant consumed each of the four breads with 1-week interval among each experiment. The order of administration of each of the breads was randomized by the use of a computer-generated sequence.

2.3. Outcomes

The primary outcome was evaluating intra-individual changes in glycemic AUCs measured at 2-h after the intake of a portion of each bread containing 50 g total carbohydrates. The secondary outcomes were analyzing intra-individual changes in insulin, FFA, triglyceride, acylated ghrelin and satiety AUCs after the consumption of each bread.

2.4. Intervention

The day before each experiment, participants followed specific dietary advices (15–20% protein, 25–30% fat, 55–60% carbohydrates, 25 g dietary fiber, total energy in line with the recommended individual needs according to the Italian guidelines (SINU, 2014)) and performed moderate exercise (i.e. about 30-min brisk walking), refraining from both heavy physical activity and inactivity, and avoiding sleep deprivation. A 24-h food record was administered to evaluate the dietary intakes of the day before each experiment.

All experiments were performed in the morning at 8:30, after a 10-h fast. Participant must consume the portion of the bread within 15 min, while sitting; they were allowed to drink 250 mL of tap water during the experiment. Blood samples were collected every 30-min for 2-h after the consumption of the portion of the bread, by an indwelling venous cannula. Time 0 was at fasting; times 30, 60, 90 and 120 were referred to the time intervals in minutes after the consumption of the bread portion.

Satiety was evaluated during each experiment every 30-min by the Satiety Labeled Intensity Magnitude scale, a 10 cm line with words anchored at each end, expressing the most positive and the most negative rating to assess satiety (Cardello et al., 2005). Experiments on the same subjects were carried out with 1-week interval among experiments.

2.5. Sample size

In accordance with the previous reported reduction of about 40% of the glycemic AUC in healthy individuals after the consumption of breads with the addition of an amylase inhibitor (Golay et al., 1991), a sample size of 16 subjects was estimated to be adequate to assess a difference between the intra-subject reductions of about one standard deviation of the glycemic AUC, with a statistical power of 85% and a two sided alpha error of 0.05.

2.6. Randomization

The random sequence was computer-generated by the Unit of Clinical Epidemiology in our institution, using predefined random sequences of the four type of breads for each participant who was kept blind in each experiment about the random order of the breads.

2.7. Bread preparation

Concerning the commercial bread, the par-baked frozen dough was subjected to a defrosting period of 6 h at 4 °C and then was cooked at 200 °C for 12 min.

The sourdough breads were made with 1000 g of the specific flour (commercial wheat flour, organic wheat flour and organic einkorn flour), 700 g of water, 400 g of sourdough, and 25 g of salt. The dough was raised for 4/5 h at 28 °C and 40% of relative humidity and then baked at 230° for 35 min.

All wheat flours (commercial and organic), as well as those of the supermarket bread, were obtained by an extraction rate of 70% (in Italy, these flours are classified as "00" flours) (Pagani, Marti, & Bottega, 2014).

To make the sourdough 1000 g of flour 1000 g of water, and 50 g of starter culture ($9.5 \cdot 10^5$ colony forming units/g flour) were mixed and stored for 10 days during which every 12 h 1000 g of flour and 1000 g of water were added to refresh the mixture that was kept at 14 °C. The sourdough was made up by *Saccharomyces cerevisiae* and *Saccharomyces exiguous* in the ratio 1:250 and the domain lactic acid bacteria species were *Lactobacillus acidophilus* and *Lactobacillus casei*.