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Research report

Second meal effect on appetite and fermentation of wholegrain rye foods ☆

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ABSTRACT

Background: Wholegrain rye has been associated with decreased hunger sensations. This may be partly mediated by colonic fermentation. Sustained consumption of fermentable components is known to change the gut microflora and may increase numbers of saccharolytic bacteria. *Objective:* To investigate the effect of wholegrain rye consumption on appetite and colonic fermentation after a subsequent meal. *Methods:* In a randomized, controlled, three-arm cross-over study, twelve healthy male subjects consumed three iso-caloric evening test meals. The test meals were based on white wheat bread (WBB), wholegrain rye kernel bread (RKB), or boiled rye kernels (RK). Breath hydrogen excretion and subjective appetite sensation were measured before and at 30 min intervals for 3 h after a standardized breakfast in the subsequent morning. After the 3 h, an *ad libitum* lunch meal was served to assess energy intake. In an *in vitro* study, RKB and RK were subjected to digestion and 24 h-fermentation in order to study SCFA production and growth of selected saccharolytic bacteria. *Results:* The test meals did not differ in their effect on parameters of subjective appetite sensation the following day. *Ad libitum* energy intake at lunch was, however, reduced by 11% ($P < 0.01$) after RKB and 7% ($P < 0.05$) after RK compared with after WWB evening meal. Breath hydrogen excretion was significantly increased following RKB and RK evening meals compared with WWB ($P < 0.01$ and $P < 0.05$, respectively). Overall, RKB and RK were readily fermented *in vitro* and exhibited similar fermentation profiles, although total SCFA production was higher for RK compared with RKB ($P < 0.001$). *In vitro* fermentation of RKB and RK both increased the relative quantities of *Bifidobacterium* and decreased *Bacteroides* compared with inoculum ($P < 0.001$). The *C. coccoides* group was reduced after RKB ($P < 0.001$). *Conclusion:* Consumption of wholegrain rye products reduced subsequent *ad libitum* energy intake in young healthy men, possibly mediated by mechanisms related to colonic fermentation.

Introduction

Obesity and associated co-morbidities constitute an increasing problem worldwide (World Health Organization, 2013). Observational studies suggest a role of wholegrain in combating obesity, as wholegrain consumption has been associated with lower body weight gain (Koh-Banerjee et al., 2004; Liu et al., 2003), although this was not confirmed in a meta-analysis on the effect of wholegrain on body weight based on intervention studies. However, a small beneficial effect on body fat percentage was found (Pol et al., 2013). It is proposed that increasing wholegrain intake may enhance satiety sensation and thus may lead to a reduced energy intake and a lower body weight in the long term (Giacco, Della, Luongo, & Riccardi, 2011).

In Scandinavian countries, rye is among the most commonly consumed cereals (Frølich, Åman, & Tetens, 2013). Wholegrain rye is a rich source of fermentable dietary fibers, such as arabinoxylans, β-glucan, fructans, and resistant starch (Frølich et al., 2013; Isaksson et al., 2011), where substantial amounts of resistant starch are found in more intact food structures, such as whole and cracked kernels (Liljeberg, 2002; Nilsson, Östman, Granfeldt, & Björck, 2008a). In the colon, saccharolytic bacteria, such as *Bifidobacterium* and *Bacteroides* (Duncan, Louis, Thomson, & Flint, 2009), utilize these ferment-

Abbreviations: C. coccoides, *Clostridium* coccoide; FFQ, Food Frequency Questionnaire; PC, principal component; PCA, principal component analysis; RK, boiled rye kernels; RKB, wholegrain rye kernel bread; SCFA, short-chain fatty acids; VAS, visual analogue scales; WWB, white wheat bread.

☆ *Acknowledgement:* SI and MK designed the human study, and DS and SI collected the data. SI, LKV, and AB designed the *in vitro* digestion and fermentation experiment and SI collected the data. SI and AB analyzed resistant starch content. SI analyzed and wrote the manuscript. All authors participated in the discussion of the results and commented on the manuscript.

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able components with a resulting production of short-chain fatty acids (SCFA) and gasses, such as hydrogen, carbon-dioxide, and methane (Wong, de Souza, Kendall, Emam, & Jenkins, 2006). Regular wholegrain consumption likely leads to an increase in the abundance of saccharolytic bacteria as a high availability of fermentable substrates will promote their growth (Zhou et al., 2008).

Fermentation may be one of the mechanisms by which wholegrain consumption influences appetite, as SCFA have been reported to enhance the production of satiety-inducing hormones such as GLP-1 and PYY (Tolhurst et al., 2012; Zhou et al., 2008). However, in order for fermentation to occur, the fermentable component needs to reach the colon, hence fermentation of most cereals takes place >4 h after meal ingestion (Nilsson et al., 2008a). Therefore, effects of wholegrain fermentation on appetite are not expected to occur until the subsequent meal (Isaksson et al., 2011; Isaksson, Sundberg, Åman, Fredriksson, & Olsson, 2008).

It is difficult to assess the extent of fermentation as well as amount and type of produced SCFA. Human intervention studies often use breath hydrogen excretion as a measure of fermentation (Johansson, Nilsson, Östman, & Björck, 2013; Nilsson, Östman, Holst, & Björck, 2008b; Rosen, Östman, & Björck, 2011). However, as this does not provide information on the proportion of the different types of SCFA produced and bacteria stimulated, *in vitro* fermentation with human inoculum constitutes a method to investigate colonic fermentation in more detail (Topping & Clifton, 2001), although only comparable to a limited extent to *in vivo* situations.

In the present pilot study we investigated whether a wholegrain rye kernel bread and boiled rye kernels consumed late in the evening influenced subjective appetite sensation and breath hydrogen excretion after a standardized breakfast meal at the following morning as well as *ad libitum* energy intake at a subsequent lunch meal in healthy young subjects. The methodology of the postprandial part of the study was similar to that applied in the human intervention studies of the research center "Gut, Grain and Greens" (3G) (Ibrügger, S., Gøbel, R.J., Vestergaard, H., Licht, T.R., Frøkiær, H., Linneberg, A., et al., unpublished data). In order to study the pattern and extent of SCFA formation as well as the growth of selected saccharolytic bacteria, including the genera *Bifidobacterium*, *Bacteroides*, and the *Clostridium coccoide* (*C. coccoides*) group, an *in vitro* digestion with subsequent fermentation of the wholegrain rye products was performed.

Methods

Subjects

Twelve healthy 18–65 year-old men with normal BMI (18–25 kg/m^2) were recruited *via* advertising at university campuses at the University of Copenhagen. Exclusion criteria were known chronic diseases, regular intake of pre- or probiotics, use of dietary supplements up to one month before study start, smoking, high level of physical activity (>10 h/wk) and participation in other studies.

All participants gave written informed consent to participate in the study. The study did not require ethic approval, as no biological samples were taken. Data collection was in accordance with the Data Protection agency (2007-54-0269) and according to the Helsinki declaration. The study was carried out at the Department of Nutrition, Exercise and Sports, Faculty of Science, University of Copenhagen, Denmark.

Experimental design and procedure

The human intervention study was designed as a randomized, controlled, cross-over study. The effect of three different, isocaloric grain-based evening meals, including wholegrain rye kernel bread (RKB), boiled rye kernels (RK), or white wheat bread (WWB) as a reference, on appetite sensation and breath hydrogen excretion during the next morning was investigated. Participants were allocated to one of three different orders of test meals in a Williams design, taking first-order carry-over effects into account. This was done by simple randomization using a web-based program (http://www.randomization.com). Each meal test was completed over a two-day period and separated by at least a three-day wash-out period.

Day 1: Evening test meal

On day 1, participants were instructed not to consume any food after 17:00 and to drink 500 ml of water until arrival at the department at 21:15. During the course of the whole day, participants had to consume a diet low in dietary fibers and in order to check compliance they were asked to note down all consumed food items in a food diary. Furthermore, they were not allowed to drink alcohol and perform vigorous physical activity during the day. After arrival at the department, breath hydrogen excretion and subjective appetite sensation were measured, using visual analogue scales (VAS). The test evening meals were served at 21:30 together with 300 ml water and subsequently appetite sensation was assessed again. Participants were instructed to drink 500 ml of water till the next morning.

Day 2: Standardized breakfast and ad libitum lunch

In the morning of day 2, participants arrived in a calm mode at the department at 7:30, after an overnight fast (10 h). Upon arrival, fasting breath hydrogen and appetite sensation were measured. At the first examination, participants had their height measured to the nearest 0.5 cm by a wall-mounted stadiometer (Seca) and were weighed to the nearest 0.05 kg (Lindell Tronic 8000) in light clothing and with an empty bladder. At 8:00 a standardized breakfast meal was served consisting of white wheat bread, butter, cheese, jam, a pastry and 200 ml water (approximately 3000 kJ, 52 E% fat, 40 E% carbohydrates, 8 E% protein). Breath hydrogen excretion and appetite sensation were measured every 30 min over the following 180 min. After 90 min, 200 ml of water were served. At 11:00, an *ad libitum* lunch meal was provided in order to assess voluntary energy intake. After the meal, appetite sensation was registered again.

Composition of evening test meals

The amount of test product of the test meals was based on 50 g carbohydrate according to package labeling. Meals were isocaloric (2.5 MJ) and contained either RKB, RK or WWB together with milk (per 100 g: 190 kJ, 4.7 g carbohydrate, 1.5 g fat, 3.4 g protein), cheese (per 100 g: 1343 kJ, 0.1 g carbohydrates, 25.0 g fat, 23.5 g protein), and water (Table 1). RK were boiled for 35 min at day 1 and

Table 1
Components and macronutrient and resistant starch content of test evening meals.

	WWB meal	RKB meal	RK meal
Grain part (unprepared) (g)	–	–	83
Grain part (as eaten) (g)	111	143	147
Milk (ml)	250	250	250
Cheese (g)	66	64	65
Water (ml)	300	300	300
Energy (kJ)[a]	2475	2475	2475
Carbohydrates (g)[a]	62	62	62
Fat (g)[a]	22	21	22
Protein (g)[a]	34	32	31
Dietary fiber (g)[a]	3	11	12
Resistant starch (g)[b]	4.0	7.6	11.1

RK, boiled rye kernels; RKB, wholegrain rye kernel bread; WWB, white wheat bread.
[a] According to product labeling.
[b] Analyzed *in vitro*.