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ORIGINAL CONTRIBUTION

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Whole-grain cereal products based on a high-fibre barley or oat genotype lower post-prandial glucose and insulin responses in healthy humans

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Abstract *Background* Several factors can affect glycemic and insulinemic responses from cereal foods. Some suggested factors lowering the responses are; intact botanical structure, high amylose/ high ß-glucan cereal varieties, organic acid produced during fermentation and food processes inducing retrogradation of starch. *Aim of the study* To evaluate the impact of fermented whole grain cereal kernels with high content of amylose (40%) and/or β-glucan (4.6%) on postprandial glucose and insulin responses in healthy adults. *Methods* Thirteen healthy volunteers (4 men and 9 women) were given 25 g available carbohydrate portions of: glucose solution; tempe fermented whole-grain barley and tempe fermented whole-grain oat. Blood samples were collected directly before the meal (fasting) and 15, 30, 45, 60, 90 and 120 min after the start of the meal. The GI (glycemic index) and II (insulin index) of meals were calculated for each subject according to FAO/WHO standards. *Results* Peak glucose response was lowest after the tempe meal with high-amylose/ high-ß-glucan barley tempe while insulin response was lowest after the meal with high β-glucan oat tempe. The mean blood glucose responses for both the barley and the oat tempe meals were significantly lower than from the reference glucose load ($P < 0.0001$) during the first 60 min. The calculated GI:s for barley and oat tempe were 30 and 63, respectively. Mean serum insulin responses from barley and oat tempe were significantly lower compared with the glucose load ($P < 0.002$) during the first 60 min, and the calculated II was lower for oat tempe (21) compared with barley tempe (55). *Conclusions* The results suggest that cereal products with beneficial influence on postprandial plasma glucose and insulin responses can be tailored by fermentation and enclosure of high-amylose and/or high-β-glucan barley and oat kernels.

Key words glycemic – insulin – amylose – β-glucan – tempe fermentation

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Introduction

Whole grains are important sources of many nutrients such as dietary fibre, resistant starch, trace minerals, vitamins, and other compounds including phytoestrogens and antioxidants, of interest in disease prevention [28]. Increased intake of whole-grain foods has been related to a reduced risk of developing diabetes and heart disease [23, 24, 26]. The effect of whole grains on carbohydrate metabolism is currently being investigated from a number of scientific angles, and it has been suggested that the intact botanical structure of cereals may have a critical effect on the metabolism of insulin and glucose. Further, a preventive potential of low glycemic index/glycemic load (GI/GL) diets in relation to obesity and diabetes has been graded as 'possible' in the most recent WHO report [10]. Such a possibility was also strengthen by the data from a cross sectional study by McKeown et al. [25] demonstrating a positive relation between dietary high GI and prevalence of the metabolic syndrome.

The glycemic index (GI) was introduced to classify starchy foods according to their effect on postprandial glycaemia [19]. The GI is defined as the incremental area under the curve (AUC) for blood glucose after ingestion of a test product as a percentage of the corresponding area for a reference product (glucose or white bread). An insulinemic index (II) can be calculated from the corresponding incremental insulin AUCs. Glycemic load (GL) is the product of the amount of carbohydrate per serve and the glycemic index.

There are a number of food factors known to influence postprandial glycaemia by affecting the gastric emptying rate and/or the rate of digestion and absorption of starch in the small intestine. Some of these factors are related to the botanical composition of the raw material, while others are linked to the type and extent of food processing. Grains are composed of endosperm, germ and bran and the endosperm comprises ≈80% of the grain while germ and bran components vary. The main element of the endosperm cell walls in barley and oats is β-glucan—a polysaccharide consisting of long linear chains of glucose residues linked through both β-(1-3) and β-(1-4) linkages. β-Glucan is believed to be the active component responsible for the observed reduction of blood glucose and insulin response after a meal containing soluble fibre [5]. Factors responsible for the observed differences between foods in the postprandial glucose response also include the botanical origin, determining the amylose/amylopectin ratio. Consumption of breads containing 50–70% amylose cornstarch was found to result in lower glucose and insulin responses compared to breads made with standard cornstarch consisting of 30% amylose and 70% amylopectin [4, 11]. Another important factor is food processing, determining the extent of starch gelatinisation, particle size and the integrity of the plant cell wall. Boiled intact cereal grains such as rye, oats, barley and wheat cause low glucose and insulin responses [13, 18]. However, when the raw materials are ground into flours before boiling, the postprandial glucose and insulin responses increases significantly [14, 22, 30]. Studies have also indicated that certain acids, such as acetic, propionic and lactic acid produced during fermentation or added, have the ability to lower the postprandial blood glucose and insulin responses [21, 32]. Thus, the hypothesis of this study was that a possible means to produce healthier foods is to choose raw material with high levels of soluble fibre and amylose in combination with mild processing like e.g. fermentation. Tempe is a fermented food native of Indonesia and originally made from soybeans inoculated with a mould of the genus *Rhizopus*. In the present study, the potential of using whole kernels of a high-amylose/high-β-glucan barley and a high-β-glucan oat genotype for tempe fermentation was evaluated as a means of developing cereal products inducing low increments in glucose and insulin levels.

Subjects and methods

Raw materials

Whole barley grains (c.v. Karmosé) with high β-glucan (~6%) and high amylose content (~40%) [1] and whole oat grains (c.v. Betania) with high β-glucan content (~6%) were obtained from Svalöf Weibull AB (Svalöv, Sweden). Whole-grain barley and oat tempe were produced by Lantmännen R&D (Järna, Sweden) according to methods previously described [8]. Prior to fermentation whole kernels were soaked in 0.3% lactic acid for 10 h at 48°C. After soaking, kernels were boiled (10 min in excess water), drained and allowed to cool before inoculation with a starter culture of *Rhizopus oligosporus*. Barley and oat tempe were fermented 27 h at 32°C followed by heating in an oven (10 min, 200°C) and kept frozen (−20°C) until served.

Test meals

The human study and the preparation of the test meals were performed at the Centre for Human Studies of Foodstuffs (KPL, Uppsala, Sweden). Frozen barley and oat tempe were thawed in plastic bags

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Table 1 Nutritional content of the barley and oat tempe test meals containing 25 g available carbohydrates

	Barley tempe	Oat tempe
Portion size (g)	95	111
Water (g)	56.5	52.2
Energy (kJ)	630.0	760.9
Dietary fibre (total, g)	5.7	13.7
β-glucan total (g)	2.0	2.2
β-glucan soluble (g)	1.7	1.8
Fat (g)	1.8	5.6
Protein (g)	4.6	8.4
Ash (g)	0.9	1.2

(15 min, 50°C) prior to preparation of the test meals. Each portion was heated in an oven (7.5 min, 200°C) and served with 200 ml of water.

A measure of 'available carbohydrates' in barley and oat tempe was obtained as total carbohydrate by difference, minus dietary fibre, analysed using AOAC methods [2]. Based on this estimate in accordance with the recommended procedure for GI determination [9], portion sizes of 95 g barley tempe and 111 g oat tempe, were calculated to provide 25 g 'available carbohydrates' (Table 1). As a reference a glucose solution was used. The glucose solution was prepared from pure glucose (Apoteket AB Production & Laboratories, Sweden) and water. Water-free glucose (25 g) was dissolved in 200 ml water and stored in refrigerator until use (within 3 days).

Subjects

Healthy adult, men and women aged 20–75 years were recruited from the Uppsala region by newspaper advertisements. Before entering the study the subjects blood glucose, Hb, ASAT, ALAT, creatinin and TSH status were assessed by standard laboratory tests performed at Uppsala University Hospital, Akademiska sjukhuset, Uppsala, Sweden. The subjects had to meet the following criteria: (1) absence of any disease (2) Age 20–75; (3) body mass index ≥20 to ≤31 kg/m^2; (4) fasting serum glucose ≤6.0 mmol/l; (5) Hb ≥120 g/l for women and ≥130 g/l for men; (6) signed informed consent.

In all, 24 persons were deemed eligible to participate in a screening visit based on their response to a brief medical history interview. Of these, 16 met the trial eligibility criteria after the screening visit. Of this group, 13 subjects (9 women, 4 men) participated in the study. Physical and biochemical characteristics of the participants are shown in Table 2. The experimental protocol was approved by the Ethical Committee of the Medical Faculty at Uppsala University.

Experimental design

Glycaemic and insulinaemic responses were determined for barley and oat tempe using glucose solution as reference. The volunteers were randomly assigned to start with one of the two different tempe products. The subject randomisation order was made according to 'block scaling': each week three subjects consumed barley or oat tempe while the rest of the subjects ingested glucose solution. The subjects were served the test meals on two separate occasions and the glucose solution on three separate occasions, at the same time in the morning after an overnight fast. The meals were eaten within 15 min. Blood samples were taken before the meal and at 15, 30, 45, 60, 90 and 120 min for analysis of glucose. Blood glucose concentrations were determined directly in finger-prick capillary blood collected from the subjects by the glucose-dehydrogenase-based reaction using HemoCue blood glucose photometer (HemoCue AB). Serum insulin concentrations were measured with an electrochemical luminescence immunoassay (Modular E170, RocheA/G) in venous blood.

Calculations and statistical analyses

For each subject and test meal, the glucose and insulin areas under the curves were calculated. All areas be-

Table 2 Characteristics of the subjects at the time of entry into the study

	Age	BMI (kg/m^2)	HB[a] (g/l)	ALAT[b] (μkat/l)	ASAT[c] (μkat/l)	Glucose[d] (mmol/l)	Creatinin[e] (μmol/l)	TSH[f] (μU/l)
n	13	13	12	13	13	13	12	13
Mean ± SD	56 ± 13.2	24.4 ± 2.6	139.8 ± 10.36	0.32 ± 0.10	0.33 ± 0.12	4.7 ± 0.37	89.1 ± 12.42	1.47 ± 0.61
Median	60	24.6	137	0.3	0.3	4.7	90.5	1.17
Range	27–72	21.4–30.1	125–159	0.22–0.60	0.20–0.61	4.3–5.6	72–116	0.71–3.04

[a]Hemoglobin (whole blood)
[b]Alanin-amino transferase (serum)
[c]Aspartat-amino transferase (serum)
[d]Fasting glucose (plasma)
[e]Creatinin (plasma)
[f]Thyreoid stimulating hormone (serum)

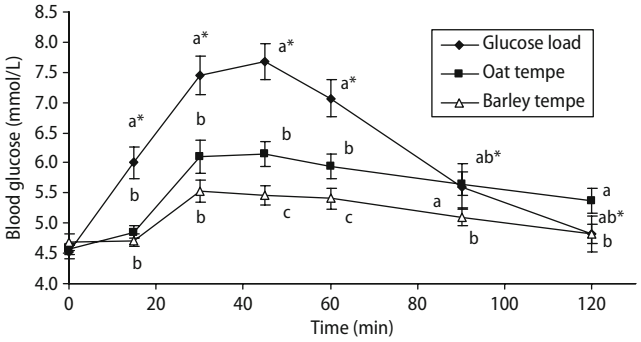

Fig. 1 Blood glucose responses of barley tempe, oat tempe and glucose load. Values are mean ± SEM; (n = 13). For each time point assigned different superscripts, blood glucose values were significantly different ($P < 0.01$). *The values of the glucose load are means of three ingestions

low baseline were excluded from the calculations. The GI was calculated from the 0–120 min incremental glucose area using glucose solution as a reference. The II was calculated in a similar way from the 0–120 min insulin response curves. GI was calculated as $(AUC_{sample}/AUC_{standard}) \times 100$ as described in the FAO/WHO report 'Carbohydrates in human nutrition' [9]. The AUCs were evaluated statistically using t-test: paired two samples for means, with each test subject being his or her own control. The mean blood glucose and serum insulin responses at each time point were statistically evaluated also using t test: paired two samples for means. Differences resulting in P-values below 0.05 were considered significant.

Results

The mean blood glucose responses for both barley and oat tempe meals were lower than for the reference glucose load (Fig. 1). The blood glucose levels after

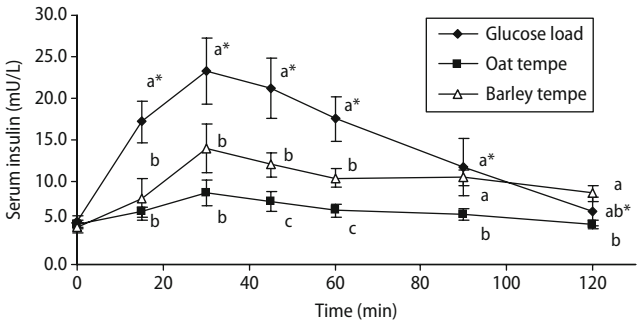

Fig. 2 Serum insulin responses of barley tempe, oat tempe and glucose load. Values are mean ± SEM; n = 9 (oat) or n = 10 (barley, glucose). For each time point assigned different superscripts, serum insulin values were significantly different ($P < 0.05$). *The values of the glucose load are means of three ingestions

Table 3 Blood glucose and serum insulin responses (AUC) and the glycemic and insulin indexes of the two tempe meals and the glucose load

	Blood glucose		Serum insulin	
	AUC	GI	AUC	II
Glucose load	204 ± 18[a]	100	1,212 ± 154[b]	100
Oat tempe	125 ± 13[a]	63 ± 6	222 ± 533	21 ± 6
Barley tempe	57 ± 9[a]	30 ± 5	669 ± 1,102	55 ± 4

Values are means for all subjects ± SEM
[a]n = 13
[b]n = 10
[c]n = 9

barley and oat tempe meals were significantly lower than after the glucose load ($P < 0.0001$) at 15, 30, 45 and 60 min, and the two tempe meals exerted significantly different responses ($P < 0.002$) at 45, 60, 90 and 120 min. At 45 and 60 min the responses from barley tempe, oat tempe and glucose load were all significantly different ($P < 0.002$) from each other. In addition, both barley and oat tempe curves showed no obvious peaks, while the glucose load curve peaked at 45 min.

Serum insulin concentrations are shown in Fig. 2. The serum insulin levels of barley and oat tempe were significantly lower ($P < 0.002$) at 15, 30, 45, and 60 min than after the glucose load. The two tempe meals had significantly different responses ($P < 0.02$) at 45, 60 and 90 min with the lowest insulin response observed after consumption of the oat tempe meal. At 45 and 60 min, serum insulin responses from the two meals and the glucose load collections were significantly different ($P < 0.02$) from each other.

The GI, calculations were based on data from 13 subjects while the II calculations were based on 10 subjects for barley tempe and nine subjects for the oat tempe. All data from three subjects were excluded because of haemolysis and from one subject due to difficulties to collect blood.

The glucose AUCs (Table 3) of the barley tempe and oat tempe were significantly lower than the AUC for the reference glucose load (Ref/Oat $P < 0.0001$, Ref/Barley $P < 0.0001$, Barley/Oat $P < 0.0001$). The insulin AUCs of both the barley and oat tempe differed significantly from that of the glucose reference (Ref/Oat $P < 0.0002$, Ref/Barley $P < 0.0001$, Barley/Oat $P < 0.004$). The calculated GI:s for the barley and oat tempe were 30 ± 5 and 63 ± 6, respectively, while the calculated II was lower for oat tempe (21 ± 6) compared to barley tempe (55 ± 4).

Discussion

The present study shows that consumption of fermented whole-grain barley or oat with high amylose

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and/or β-glucan content can improve the glucose and insulin responses of healthy subjects. Glucose and insulin levels after consumption of barley tempe and oat tempe were significantly lower than those after the glucose load, and the glucose areas under the curve (AUCs) from 45 to 120 min was significantly lower for the high-amylose barley tempe compared to the oat tempe (Fig. 1). Improvement in glycemic response after foods containing high-amylose starch or resistant starch has also been reported in previous studies [3, 11]. Meals containing high-amylose cornstarch (70–75% amylose) have been shown to reduce postprandial glucose and insulin responses. Hydrolysis of amylose has been suggested to result in fewer glucose molecules being liberated at once than during hydrolysis of the highly branched amylopectin chains [15]. According to Behall and Hallfrisch [3], the amylose content of the starch needs to be >50% to significantly reduce plasma glucose and insulin.

However, high-amylose starches are also thought to be less digestible than standard starches containing lower amounts of amylose due to the presence or development of resistant starch. In a recent study by Behall et al. [5], significant reduction of glucose and insulin responses was observed after the consumption of meals with different levels of resistant starch from high-amylose cornstarch. In addition, the reduction in glycemic response was enhanced by combining resistant starch and β-glucan. Behall et al. [5] concluded that soluble fibre appears to have a greater effect on postprandial insulin response while glucose reduction was greater after intake of resistant starch from high-amylose corn starch. These observations are in agreement with our findings; where the $AUC_{insulin}$ after consumption of the high-β-glucan oat tempe meal was lower than the $AUC_{insulin}$ from high-amylose barley tempe meal (Fig. 2). Furthermore, the glucose response was significantly lower from the high-amylose barley tempe meal compared to the high-β-glucan oat tempe meal. In the study by Behall et al. [5] the β-glucan intake averaged 0.3, 0.9 or 3.7 g in three different meals. In the present study, the total β-glucan content was 2.0 g (barley tempe) and 2.2 g (oat tempe). Hence, one would expect the insulin response from barley tempe to be as low as from oat tempe due to almost similar β-glucan content. However, Granfeldt et al. [12] reported that, in healthy subjects, less insulin is needed for the control of postprandial glucose excursions after the consumption of oat products than after barley products. In their study on rolled oats and barley, a high glucose and insulin response from thick barley flakes was observed, in contrast to the corresponding oat flakes which had a lower insulin index [12]. This was also indicated in a report by Juntunen et al. [20], who studied postprandial glucose and insulin responses to grain products in healthy subjects. Their data suggested that the form of food and botanical structure rather than the amount of fibre or type of cereal determines the postprandial insulin response. However, Heaton et al. [16] compared particle size of wheat, maize and oat meals and the consequential effects on glucose and insulin responses. They found that oat-based meals evoked smaller glucose and insulin responses than wheat- or maize-based meals and that particle size of wheat and maize, but not oats, influenced the digestion rate. With oats the in vitro digestion rate was found to increase as the particle size decreased, however, in vivo the plasma glucose and insulin responses were not significantly different between three oat-based meals of different particle size. It was suggested that this difference between wheat, maize and oats might be explained by the viscous properties of the soluble β-glucans in oats, limiting the digestion or absorption in vivo. Numerous other studies have reported inverse relationships between β-glucan content and glucose and/or insulin responses [17, 29, 31] and the suggested mechanisms include viscosity of the soluble fibres resulting in delayed or reduced carbohydrate absorption from the gut.

Evidence from in vitro studies suggests that dietary fibre can alter the activity of pancreatic amylase [7]. The inhibitory effects of fibre on pancreatic enzyme activities have been attributed to various factors including pH changes, ion-exchange properties, enzyme inhibitors and adsorption. Rather than a chemical enzyme-fibre interaction, the presence of fibre, through its particulate or viscous nature, has also been suggested to impede enzyme-substrate interactions [9]. Further, the presence of fibre in a form that restricts starch gelatinization or access of the hydrolytic enzymes to starch can slow the rate of starch digestion. In addition, resistance of starch to pancreatic hydrolysis may result from the presence of intact cell walls, which survive processing and cooking and insulates starch in a manner that partly obstructs digestion and absorption. The factors responsible for the lowered insulin response after consumption of oat tempe compared to barley tempe are not completely evident. However, the structures of the continuous matrix and starch granules probably differ between barley and oats and could in part explain the lower insulinemic response from oat tempe.

It has also been observed that bread containing lactic acid produced during sourdough fermentation or added directly, has the ability to lower the postprandial glucose and insulin responses in humans [32]. Thus, the preparation of cereal tempe from whole kernels soaked in lactic acid is probably an important part of the observed effects on postprandial

plasma glucose and insulin responses in the present study. Furthermore, grains contain components like phytic acid, lectins, phenolic compounds, amylase inhibitors and saponins. These compounds have been found to lower one or more of the following substances in plasma: glucose, insulin, cholesterol and triacylglycerol [6, 27].

Fibre-rich, whole-grain foods may indeed have many overlapping physiologic effects and, the physical form and high fibre content of whole-grain tempe as well as presence of organic acids, appear to work synergistically to affect digestion and absorption of carbohydrates. Therefore, the metabolic benefits of using whole grains, fermentation and cereal genotypes with elevated contents of amylose or β-glucans are evident. Also, the tailoring of whole-grain products offer a particular challenge since palatable and effective high-fibre products could play an important part in increasing whole-grain intakes and reducing the risk for development of illnesses such as type II diabetes and cardiovascular disease.

In conclusion, the results suggest that tempe fermentation of whole-grain barley and oats is a possible means to obtain healthy foods with low GI and II.

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