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Short Communication

Wheat-fibre-induced changes of postprandial peptide YY and ghrelin responses are not associated with acute alterations of satiety

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Weight gain and risk of type 2 diabetes are inversely associated with a high intake of insoluble cereal fibres. Because nutrient-induced changes of 'satiety hormones' from the gut may play a role in this process, we evaluated the effects of purified insoluble fibres on postprandial responses of plasma peptide YY (PYY), serum ghrelin and satiety as secondary outcome measures of a study investigating effects of cereal fibres on parameters of glucose metabolism. Fourteen healthy women were studied on six occasions in a randomized, single-blind, controlled crossover design. After 24 h run-in periods and 10 h overnight fasts, subjects ingested isoenergetic and macronutrient matched portions of control white bread or fibre-enriched bread (wheat-fibre or oat-fibre) at 08.15 hours. Gut hormones and hunger scores were measured for 300 min. Basal PYY and ghrelin concentrations were not different between the test meals ($P>0{\cdot}15$). Postprandial responses of PYY and ghrelin were blunted after the intake of wheat-fibre (total area under the curve (AUC) PYY, 177·9 (SEM 8·1) (pmol/l) min; $P=0{\cdot}016$; ghrelin 51·0 (SEM 2·5) (pmol/l) min; $P=0{\cdot}003$), but not after oat-fibre (PYY 226·7 (SEM 25·7) (pmol/l) min; $P>0{\cdot}15$; ghrelin 46·2 (SEM 1·6) (pmol/l) min; $P=0{\cdot}127$), compared to control (PYY 247·5 (SEM 25·6) (pmol/l) min; ghrelin 42·5 (SEM 1·3) (pmol/l) min). Postprandial hunger scores were unaffected by the different test meals ($P>0{\cdot}15$). Thus, oat- and wheat-fibre consumption result in different postprandial responses of PYY and ghrelin, but interestingly do not differ in satiety effects.

Insoluble cereal fibre: Satiety: Peptide YY: Ghrelin

Nutrient-induced changes of biomarkers involved in the neuroendocrine regulation of satiety may contribute to the consistently shown beneficial effects of diets high in insoluble cereal fibres (Jenkins *et al.* 2000). Peptide YY (PYY) and ghrelin are gut-derived hormones commonly linked to the regulation of food intake (Small & Bloom, 2004). To date, potential effects of insoluble cereal fibres on PYY and ghrelin responses, and their relation to hunger and satiety ratings, have not been reported. Enteroendocrine L-cells postprandially release PYY[1-36] into the circulation, in response to fat-, carbohydrate- and protein-rich meals, but not after an equal volume of water (Pedersen-Bjergaard *et al.* 1996). About 40–50 % of the thirty-six-amino acid PYY is enzymatically cleaved by dipeptidyl peptidase-IV to produce PYY[3-36], which is considered to have potent anorexigenic properties. Ghrelin is an orexigenic hormone primarily produced by oxyntic X/A-like cells in the stomach. Circulating ghrelin is suppressed by food intake, with gastric distension not being a sufficient trigger (Shiiya *et al.* 2002). Exogenous administration of ghrelin potently stimulates food intake (Small & Bloom, 2004), and preprandial increases of ghrelin correlate with hunger scores in human subjects initiating meals voluntarily (Cummings *et al.* 2004). However, it is not clear whether physiological postprandial ghrelin suppression plays a role in the acute regulation of satiety in man (Erdmann *et al.* 2004). Insoluble cereal fibres are non-viscous and have only small effects on macronutrient absorption from the gut or gastric emptying (Jenkins *et al.* 2000). Unrefined whole grains, fruit and vegetables, which are

Abbreviations: AUC, area under the curve; PYY, peptide YY.

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frequently related to fibre consumption, are highly complex substances containing various other biologically active substances. Thus, the aim of the present study was to investigate effects of purified insoluble cereal fibres on postprandial PYY and ghrelin responses and their relation to satiety ratings, assuming that the predominant insoluble fraction of cereal fibres is involved in their beneficial actions.

Experimental methods

The Ethics Committee of Potsdam University, Germany approved the study. Postprandial hunger, satiety and gut hormone responses were secondary outcome measures of an already published study, aimed at assessing effects of insoluble dietary fibres on parameters of glucose metabolism. Subjects, study design and the composition of the test meals have been described in detail (Weickert *et al.* 2005).

Study design

This was a randomized, controlled, single-blind, within-subject crossover study. Fourteen healthy women were invited to the metabolic unit after 10 h overnight fasts on six occasions. Subjects consumed three isoenergetic portions (at 08.15, 13.15 and 21.45 hours) of low-fibre white bread (control) or fibre-enriched bread (10·5 g wheat-fibre or 10·6 g oat-fibre per portion). All test breads were consumed within a 15 min period, together with 250 ml tap water. All subjects were invited the following day to perform a second meal test with control. All subjects received all three treatments (wheat-fibre followed by control; oat-fibre followed by control; control followed by control) during this crossover study. Fibre enrichment was within the recommended range of daily fibre intake. Blood samples for the measurement of PYY and ghrelin as well as satiety ratings were evaluated for 300 min (from 08.15 hours). Subjects recorded their diet for 24 h before the first study day and ate identically before each of the following study days to control for the background diet. Between study days there was a washout period of at least 7 d.

Test meals

Test breads were produced and analysed in one batch by the Institute for Cereal Processing (IGV, Potsdam-Rehbruecke, Germany). The oat-fibres (VITACEL OF101) and wheat-fibres (VITACEL WF101) and their analysis were provided by Rettenmaier & Söhne (Rosenberg, Germany). The macronutrient contents of the test breads only marginally differed and have been previously described in detail, as well as the processing of the fibre products (Weickert *et al.* 2005). The wheat-fibre- and the oat-fibre-enriched test meals could not be distinguished by taste, by smell or by visual appearance.

Hormone assays

Immunoreactive total serum ghrelin was measured by a commercially available RIA (Phoenix Pharmaceuticals, Mountain View, CA, USA), using ^{125}I-labelled bioactive ghrelin as a tracer, and a polyclonal antibody raised in rabbits against the C-terminal end of human ghrelin. Intra- and inter-assay CV were 5·3 and 13·6 %. RIA of plasma PYY was performed as previously described (Naslund *et al.* 1999), using antiserum code no. 84122II. The PYY assay reacts equally well with PYY[1-36] and PYY[3-36]. The detection limit of the assay was below 1 pmol/l. Intra-assay CV was below 5 %.

Satiety and hunger scores

Hunger and satiety ratings were evaluated using an equilateral seven-point rating scale anchored at −3 (extremely hungry) and +3 (extremely full) with a mid-point at 0 (neutral) (Holt *et al.* 1995). Subjects did not discuss or compare their hunger ratings with each other and did not refer to their previous ratings when marking the scale. For graphical presentation, the results obtained from the seven-point rating scale were converted to a scale from 6 (extremely hungry) to 0 (extremely full). Corresponding hunger scores at the fasting state were set as the baseline, and hunger ratings at 60, 120, 180, 240 and 300 min were expressed relative to the baseline.

Statistical analysis

Results are presented as means and their standard errors. Repeated measures ANOVA with treatment and time as within-subject factors and Huynh-Feldt ϵ as correction factor was used do determine significant main effects and interactions. Potential confounders like the use of oral contraceptives or a history of smoking were tested and excluded from the model when $P > 0{\cdot}15$. When there were significant interactions or a main effect of time, mean contrasts according to Bonferroni inequalities were used to analyse significance at specific time-points. Areas under the curves (AUC) were compared using two-tailed Student's *t* test for paired samples. Percentage changes of PYY and ghrelin responses were examined by using Pearson correlation coefficients. Statistical significance was defined as $P < 0{\cdot}05$. Calculations were performed using SPSS version 12.0 (SPSS Inc., Chicago, IL, USA).

Results

Relative postprandial plasma PYY, serum ghrelin and hunger responses are presented in Fig. 1. Because of missing values for two subjects, plasma PYY was calculated for twelve subjects. Baseline concentrations of PYY and ghrelin showed no significant differences (PYY control 13·3 (SEM 1·8), wheat-fibre 16·7 (SEM 1·9), oat-fibre 15·4 (SEM 2·5) pmol/l, $P > 0{\cdot}15$; ghrelin control 113·9 (SEM 13·4), wheat-fibre 103·7 (SEM 10·9), oat-fibre 118·0 (SEM 14·8) pmol/l, $P > 0{\cdot}15$), as well as baseline hunger ratings after the 10 h overnight fasts ($P > 0{\cdot}15$). Peak PYY concentration markedly increased both after the consumption of oat-fibre (+72·2 (SEM 23·6) %, $P = 0{\cdot}011$) and control (+73·6 (SEM 25·2) %, ($P = 0{\cdot}014$)). This was associated with a significant decrease in minimal ghrelin concentrations (oat-fibre −26·7 (SEM 2·5) %, $P < 0{\cdot}001$; control −31·4 (SEM 2·4) %, $P < 0{\cdot}001$). Relative postprandial responses after the consumption of wheat-fibre were attenuated for PYY (+28·1 (SEM 9·9) %, $P = 0{\cdot}016$) and ghrelin (−18·9 (SEM 3·2) %, $P < 0{\cdot}001$), and significantly different from control ($P = 0{\cdot}041$ for PYY; $P = 0{\cdot}002$ for ghrelin). Postprandial PYY and ghrelin responses between fibre-enriched test meals were significantly different after 60 min (wheat-fibre *v.* oat-fibre, PYY $P = 0{\cdot}023$, ghrelin $P = 0{\cdot}048$). Percentage changes of PYY and ghrelin responses

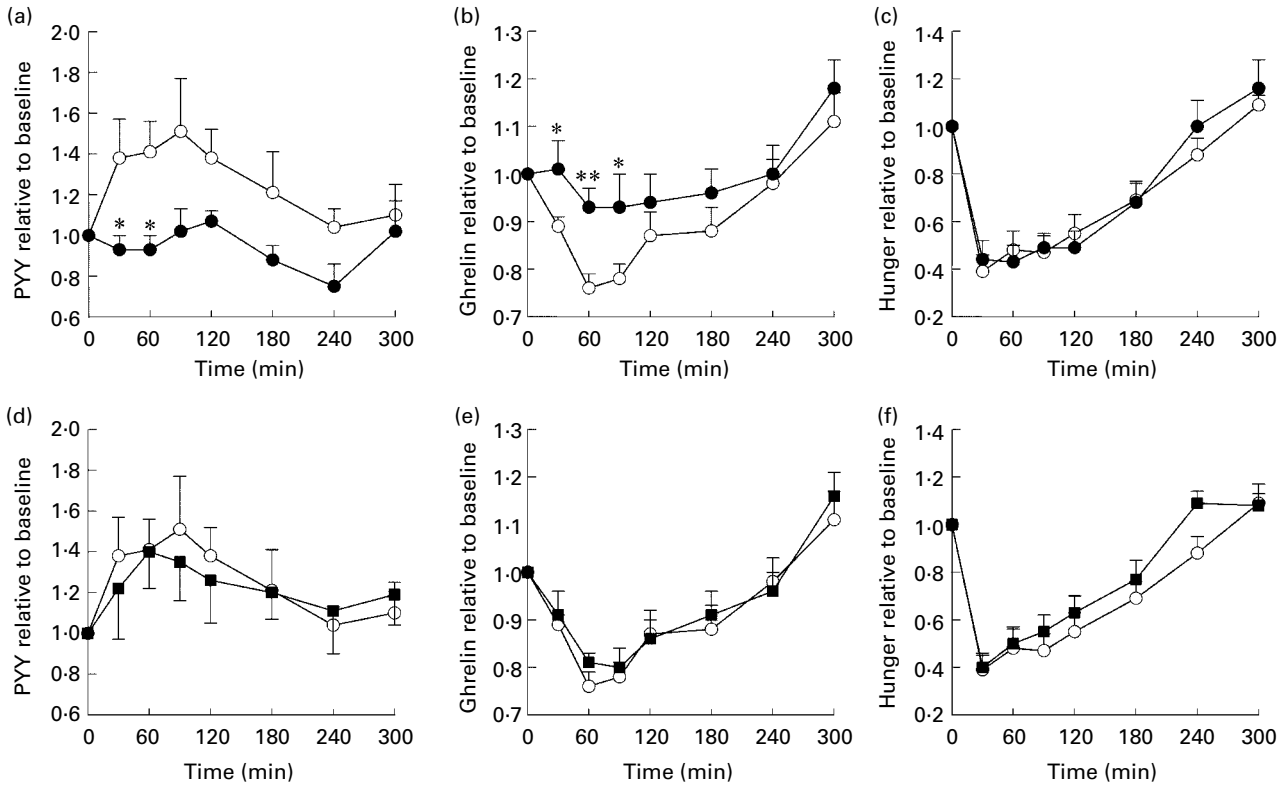

Fig. 1. Effects of fibre intake on acute plasma peptide YY (PYY) (a, d), serum ghrelin (b, e) and hunger responses (c, f). For details of procedures, see p. 796. Values (means with their standard errors depicted by vertical bars) are percentage plasma PYY (*n* 12), serum ghrelin (*n* 14) and hunger responses (*n* 14) after consumption of control white bread (○), or fibre-enriched breads (wheat-fibre (a–c, ●), oat-fibre (d–f, ■)). Lower values for hunger scores (c, f) indicate reduced hunger sensations. Bonferroni correction factor was used to analyse significance at specific time-points. Mean values were significantly different from those of the control group: **P*< 0·05; ***P*< 0·01.

on all study days showed a weak, but statistically significant negative correlation (r −0·13, P=0·002). PYY measured as $AUC_{180\,min}$ was reduced after wheat-fibre (177·9 (SEM 8·1) (pmol/l) min, P=0·016), but not after oat-fibre (226·7 (SEM 25·7) (pmol/l) min, P=0·597), compared to control (247·5 (SEM 25·6) (pmol/l) min). Repeated measures ANOVA showed an effect of fibre consumption on the time courses of PYY only after wheat-fibre (P=0·029), but not after oat-fibre (P=0·329), compared to control. Ghrelin calculated as $AUC_{180\,min}$ was increased after wheat-fibre (51·0 (SEM 2·5) (pmol/l) min, P=0·003), but not after oat-fibre (46·2 (SEM 1·6) (pmol/l) min, P=0·127), compared to control (42·5 (SEM 1·3) (pmol/l) min). Repeated measures ANOVA showed no significant effect of fibre consumption on the time courses of ghrelin (wheat-fibre P=0·128; oat-fibre P=0·729), compared to control. Hunger scores were significantly suppressed within 30 min after ingestion of all test meals (P< 0·001), and no differences were observed between the fibre-enriched meals and control, assessed by fibre-by-time interaction and measured as AUC (P> 0·15). Baseline and postprandial PYY and ghrelin responses upon the ingestion of control white bread in the second meal test on day 2 showed no significant differences (P> 0·15). There was, however, a small but statistically significant difference between the prior consumption of three portions of wheat fibre and control, which was mainly caused by increased satiety after the consumption of control rather than by reduced satiety after the consumption of wheat fibre the previous day (data not shown).

Discussion

In the present study the effects of purified insoluble dietary fibres on postprandial responses of PYY, ghrelin and satiety ratings were investigated. Two new findings are presented: (1) the consumption of isoenergetic and macronutrient matched test meals enriched with insoluble dietary wheat-fibre, but not oat-fibre, led to significantly blunted postprandial responses of PYY and ghrelin concentrations, compared to control; (2) this was, however, not associated with any alterations of postprandial hunger sensations. The anorexigenic activity of PYY[3-36] in rodents has recently been critically discussed, and published reports remain controversial (Halatchev & Cone, 2005). PYY is commonly thought to be released in proportion to the energy content of a test meal (Adrian *et al.* 1985). However, in the present study test meals were isoenergetic and contents of protein, fat and carbohydrates were virtually identical. Thus, other yet unknown mechanisms seem to be involved in the regulation of postprandial PYY secretion. The exact signals mediating meal-related ghrelin suppression are not known (Overduin *et al.* 2005). There is increasing evidence that macronutrients differently influence postprandial ghrelin concentrations (Erdmann *et al.* 2004), with carbohydrates being the most potent agent to suppress ghrelin in man. Insulin and glucose have been discussed as regulators of postprandial ghrelin suppression with conflicting results. We and others have shown that parenteral administration of insulin in supraphysiological doses or for

prolonged periods reduces circulating serum ghrelin (Mohlig *et al.* 2002; Flanagan *et al.* 2003). However, in physiological doses mimicking postprandial concentrations, parenteral administration of insulin or glucose leaves ghrelin unaffected (Caixas *et al.* 2002; Schaller *et al.* 2003). In addition, differences in postprandial ghrelin responses between wheat-fibre and oat-fibre are unexplained by time courses of insulin or glucose in the present study (Weickert *et al.* 2005). Because the wheat-fibre- and the oat-fibre-enriched breads could not be distinguished by taste, by smell or by visual appearance, it is also unlikely that the observed differences in postprandial ghrelin responses in the present study were elicited by vagal mechanisms involved in the cephalic phase of food intake (Arosio *et al.* 2004). Reduced satiety upon intake of a second meal test 24 h after wheat-fibre consumption, as observed in the current study, is difficult to interpret. Intravenous PYY[3-36] infusions for 90 min have been shown to decrease food intake for a 12 h post-infusion period, with plasma ghrelin levels only being altered for about 150 min (Batterham *et al.* 2003). It has also been shown that ghrelin infusions only have transient effects on hunger sensations (Akamizu *et al.* 2004). However, in the present study gut hormone concentrations were only measured after the first of the three test meals provided per day, which was 24 h before the second meal test. Even when assuming that PYY and ghrelin responses were comparable after the test meals ingested later in the day, the lack of an acute postprandial effect on satiety remains unexplained. A limitation of the present study is that no information about acylated octanoyl-ghrelin, generally thought to be the biologically active form, is available. However, to date effects of cereal fibres on total ghrelin responses have not been reported, and the lack of any association of postprandial total ghrelin with satiety ratings after isoenergetic and macronutrient matched test meals is a novel finding. Particularly, the hemicellulose contents of various plant fibres are highly complex and may be involved in the observed differences between PYY and ghrelin responses after wheat- and oat-fibre consumption.

In conclusion, we show that insoluble wheat-fibre consumption significantly affected postprandial PYY and ghrelin responses, both compared to the consumption of insoluble oat-fibre or control. This was, however, not associated with altered postprandial satiety ratings, indicating that physiological postprandial alterations of plasma PYY and serum ghrelin are unlikely to play a major role in the acute regulation of satiety in man.

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