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Impact of cereal fibre on glucose-regulating factors

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Abstract *Aims/hypothesis:* Insoluble dietary fibre intake is associated, by unknown mechanisms, with a reduced risk of type 2 diabetes. We investigated whether a short-term dietary intervention with purified insoluble fibres influences acute and delayed responses of glucose, insulin, glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1. *Methods:* Fourteen healthy women with NGT were studied for 300 min on six to eight occasions. Subjects consumed three matched portions of control (C) or fibre-enriched bread (10.4–10.6 g/portion; wheat fibre [WF], oat fibre [OF], and, in a substudy [n=9], resistant starch [RS]) followed by control (C-C, C-WF, C-OF, C-RS) on subsequent days. *Results:* Fibre enrichment accelerated the early insulin response (fibre×time interaction p=0.026 for WF, p<0.001 for OF, p=0.126 for RS; time of maximal concentration [T_{max}], C 57.9±5.9, WF 49.3±2.5 [p=0.086], OF 46.1±2.9 [p=0.026], RS 46.7±5.8 min [p=0.029]). It was also associated with an earlier postprandial GIP response after OF (T_{max}, C 83.6±7.2, WF 70.7±6.0 [p=0.054], OF 64.3±6.9 [p=0.022], RS 60.0±5.0 [p>0.15]). Increased fibre intake for 24 h was further associated with a reduced postprandial glucose response on the following day subsequent to ingestion of a control meal ($AUC_{C\text{-}C}$ 4,140±401, $AUC_{C\text{-}WF}$ 2,850±331 [p=0.007], $AUC_{C\text{-}OF}$ 2,830±277 [p=0.011]), with no difference in maximal concentration and T_{max} of glucose responses. No differences in insulin responses were observed 24 h after the fibre-enriched diets compared with control (p>0.15). Colonic fermentation was increased only on study days C-OF (p=0.017) and C-RS (p=0.016). *Conclusions/interpretation:* The consumption of highly purified insoluble dietary fibres accelerated the acute GIP and insulin response and was further associated with enhanced postprandial carbohydrate handling the following day upon ingestion of a control meal.

Keywords Colonic fermentation · Diabetes · Early insulin response · Glucose-dependent insulinotropic polypeptide · Glucagon-like peptide · Insoluble dietary fibre

Abbreviations C: control · C_{max}: individual concentration maximum · WF: wheat fibre · OF: oat fibre · RS: resistant starch · H_2: hydrogen · GLP-1: glucagon-like-peptide 1 · GIP: glucose-dependent insulinotropic polypeptide · T_{max}: time of maximal concentration

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Introduction

Type 2 diabetes is preceded by insulin resistance. It affects more than 150 million people worldwide and the number affected is increasing. It is assumed that metabolic changes caused by the Western diet might be involved. Observational studies have shown that diets rich in insoluble cereal fibres are associated with a reduced risk of diabetes and cardiovascular disease [1–9]. Interestingly, the diabetes

death rate in England decreased by approximately 55% between 1941 and 1954–1957, when low-fibre white flour was replaced by the wartime high-fibre national flour [10]. Acute effects of dietary fibres have been studied extensively, the focus of interest being soluble, viscous fibres, which can delay gastric emptying [11] and the absorption of macronutrients from the gut [12], influencing lipid metabolism, postprandial glycaemic responses to carbohydrate-rich meals [13] and insulin sensitivity in rat muscles [14]. However, in population studies soluble fibres are not associated with a decreased risk of type 2 diabetes or cardiovascular disease [3, 4, 7, 15].

Postprandial insulin secretion is stimulated by the increase in glucose and the secretion of the incretin hormones glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1) (reviewed in [16]). Few studies have investigated the effects of dietary fibre on postprandial GIP and GLP-1 responses. Soluble dietary fibres seem to reduce GIP and to augment GLP-1 responses [17–19], probably because of reduced carbohydrate absorption. Oligofructose has been shown to increase serum GIP in rats [20]. Even less is known about the effects of insoluble dietary fibres on postprandial incretin responses. In a study of pigs, GIP was not reduced after the intake of cellulose (but was reduced after viscous guar gum) [21]. Importantly, most published studies of postprandial incretin responses do not report the concentrations of the biologically active hormones [16].

Non-viscous cereal fibres have only small effects on macronutrient absorption [12] and their beneficial effects lack an obvious mechanism. Influences of isolated insoluble fibres on biologically active incretin responses have not yet been investigated. Using stable isotope techniques, it has been shown that insoluble fibre increases the rate of glucose disappearance [22], whereas glucose absorption is not influenced. Fermentation processes in the colon with the production of short-chain fatty acids have been discussed as possible mechanisms of positive effects on hepatic glucose metabolism [23, 24]. In some studies the contents of both soluble and insoluble fibres were increased in the intervention group [25, 26], or not all potential confounding factors (e.g. the protein contents of the test foods) were controlled [22]. Interpretation of the results after interventions with unrefined whole grains is difficult because of the presence of other active ingredients, such as minerals, phyto-oestrogens and antioxidants [27]. Bran products, which showed improved glycaemic control in some [28, 29] but not all [30] longer-term intervention studies, contain starch, protein and lipids in addition to dietary fibres.

Therefore, the aim of this study was to investigate the acute and delayed effects of identically processed purified insoluble dietary fibres on postprandial glucose, insulin and incretin responses. In a subanalysis, the effects of highly fermentable resistant starch (RS) were investigated to clarify whether increased colonic fermentation would influence the results.

Subjects and methods

Subjects

The Ethics Committee of the University of Potsdam, Germany, approved the study. Written informed consent was obtained prior to the study. Sixteen healthy women with NGT and no menstrual cycle irregularities participated.

Subjects recorded their diet for 24 h before the first study day and were asked to eat identically prior to each of the following study days to control for background diet. On the study days at 8.15 and 13.15 hours subjects consumed only the test breads; the third portion (consumed at 21.45 hours) was ingested together with the same filling on each study day. Dietary compliance was assessed before each study day. If the background diet was not identical, subjects were not studied that day. Subjects were instructed to refrain from physical exercise for 3 days before each of the study days. Three of the subjects had a history of smoking. Fourteen of the subjects were included in the final analysis. One subject had to be excluded because of dietary noncompliance, another one because of pregnancy. Nine of the subjects participated in a substudy to investigate the effects of RS. Seven subjects were taking oral contraceptives throughout the study; no further medication was taken. Characterisation of the subjects, including an OGTT, was performed on an additional study day (Table 1). Trained personnel performed anthropometric measurements. Body weight was determined on each study day. Lean body mass was measured by air displacement plethysmograghy (Life Measurement, Concord, CA, USA).

Study design

The study had a randomised, single-blind, within-subject crossover design (Fig. 1). Subjects attended the metabolic unit after 10-h overnight fasts on six to eight occasions for 300 min. Subjects remained in an upright sitting position throughout the experiments, with significant physical activity only every 60 min for taking breath samples. On

Table 1 Baseline characteristics of the study group

	Mean	SD
Age (years)	23.6	1.69
BMI (kg/m^2)	21.3	1.51
Waist-to-hip ratio	0.74	0.03
Lean body mass (%)	75.8	6.18
Plasma glucose (mmol/l)	4.83	0.41
Serum insulin (mU/l)	5.56	2.17
HOMA-IR	1.10	0.49
Cholesterol (mmol/l)	4.53	0.71
Triglycerides (mmol/l)	0.97	0.30
NEFA (mmol/l)	0.56	0.18

HOMA-IR, homeostasis model assessment for insulin resistance (calculated as [fasting insulin (mU/l) × fasting glucose (mmol/l)/22.5])

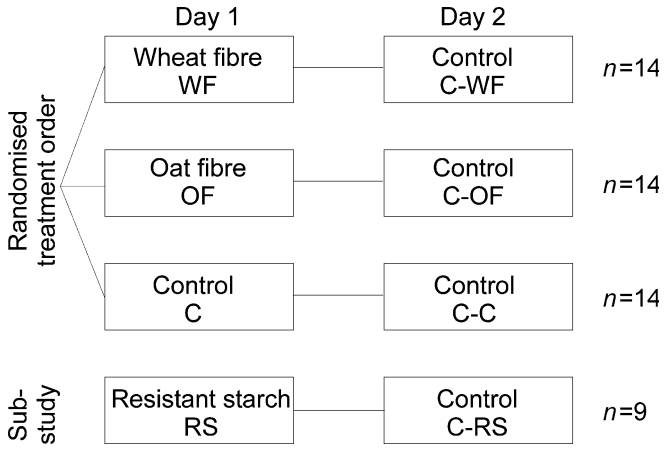

Fig. 1 Study design and labels. White bread without fibre supplement is labelled as control C, or control C-WF, C-OF, C-RS and C-C when preceded by three portions of bread enriched with wheat fibre (WF), oat fibre (OF) or resistant starch (RS) or by control bread (C) on day 1

arrival, an indwelling venous catheter was inserted into the forearm vein for blood sampling. After basal blood and breath samples had been taken, subjects consumed three isoenergetic portions (at 08.15, 13.15 and 21.45 hours) of low-fibre control white bread (C) or fibre-enriched bread (10.5 g wheat-fibre [WF], 10.6 g oat-fibre [OF]; and in a substudy 10.4 g RS [RS] per portion) with 250 ml tap water. The first two portions of the test breads were consumed in the metabolic unit and the third was ingested at home. On subsequent days after 10-h overnight fasts, subjects consumed a portion of bread C. Sampling conditions were identical to the preceding days. Between each set of study days (days C and C-C, WF and C-WF, OF and C-OF, RS and C-RS) there were washout periods of at least 7 days.

Test meals

The test breads were produced and analysed in one batch by the Institute for Cereal Processing (IGV, Potsdam-Rehbruecke, Germany). The characteristics of the test breads are presented in Table 2. The commercially available wheat (Vitacel WF101) and oat (Vitacel HF101) fibres and their analysis (Table 3) were provided by Rettenmaier and Soehne, Rosenberg, Germany. WF is extracted from the straw of the wheat plant. Processing steps include pre-grinding, mechanical removing of fines and foreign material, aqueous extraction of carbohydrates, lignin and disintegration of cellulose and hemicelluloses (with sodium hydroxide, >100°C, overpressure; hydrogen peroxide is used to reduce microbial count), filtration and washing in order to separate undesired components, drying, dry grinding and sieving. This results in a product with a particle size of 50 μm. OF is extracted from oat hulls (70°C, 1 bar) using otherwise identical processing steps. Notably, most of the soluble fibre content, including β-glucans, starch, proteins and lipids, is removed in preparation of these products. RS (Hi-maize 1043) was provided by National Starch and Chemical, Manchester, UK and was baked and stored under identical conditions.

Table 2 Macronutrient composition of the test breads (per portion)

	None (control bread, C)	Wheat fibre (bread WF)	Oat fibre (bread OF)	Resistant starch (bread RS)
Portion (g) per 50 g of available carbohydrates	103	131	133	123
Energy (kcal)	240	241	240	241
Available carbohydrates (g)	50	50	50	50
Fat (g)	0.82	0.85	0.84	0.96
Fibre content (g)	2.9	13.4	13.5	13.3
Total minerals (g)	1.74	1.87	1.80	2.13
Magnesium (mg/100 g)	20.1	17.4	17.5	13.9
Protein (g)	7.2	7.3	7.3	7.2

Analytical procedures

Blood samples were taken basally and 15, 30, 45, 60, 75, 90, 105, 120, 150, 180, 210, 240, 270 and 300 min postprandially for the measurement of glucose, insulin and C-peptide, half-hourly for NEFA, GIP and GLP-1, and at 0 and 300 min for butyrate and magnesium. Blood samples were analysed in random order to exclude systemic bias due to interassay variation. Control specimens were analysed simultaneously on each plate. Capillary blood glucose concentrations from a hyperaemic ear lobe were determined with the glucose oxidase method (glucose photometer; Hemocue, Ängelholm, Sweden). Serum insulin was measured using an insulin ELISA (Mercodia, Uppsala, Sweden) with a negligible cross-reaction with C-peptide (<0.01%) and sensitivity <6 pmol/l. Intra- and interassay CVs were 4%. Serum C-peptide was measured by a C-peptide ELISA (Mercodia); cross-reaction was <0.1% with insulin and 100% with proinsulin. Intra- and interassay CVs were 5% and the sensitivity limit <14.9 pmol/l. Plasma NEFA were

Table 3 Composition of the cereal fibres: content per 100 g

	Wheat fibre	Oat fibre
Total fibre content (%)	97	96
Insoluble fibre (% of total)	94.5	93
Soluble fibre (% of total)	2.5	3.0
β-Glucan	0.2	0.2
Cellulose (%)	74	70
Hemicellulose (%)	26	25
Lignin (%)	<0.5	3–5
Protein (%)	0.4	0.25
Fat (%)	0.2	0.1
Particle size (μm)	50	50

quantified using a calorimetric assay (NEFA C, Wako, Neuss, Germany) performed on a Cobas-Mira analyser (Roche, Mannheim, Germany). Interassay CV was 4.7%.

Butyric acid was quantified by a modified version of a method described previously [31] and analysed by gas chromatography (HP 5890 II GC; Hewlett-Packard, Böblingen, Germany) using a capillary column (HP-20M; Hewlett-Packard, Palo Alto, CA, USA) and a flame-ionisation detector.

GIP and GLP-1 concentrations in plasma were measured after extraction of plasma with 70% ethanol. For the GIP radioimmunoassay [32], we used the C-terminally directed antiserum R-65, which cross-reacts with human GIP but not with GIP 8000. Human GIP and 125-I-GIP (70 MBq/nmol) were used for standards and tracer. Blood samples for measurement of GLP-1 were drawn into chilled tubes containing EDTA and aprotinin (Trasylol; 500 KIU/ml; Bayer, Leverkusen, Germany) and kept on ice. Plasma concentrations of GLP-1 were measured by comparison with standards of synthetic GLP-17-36 amide using an antiserum (code no. 89390) specific for the amidated C-terminus of GLP-1 [33]. This assay accurately reflects the rate of secretion of GLP-1, measuring both intact GLP-1 and the primary metabolite, GLP-1 9–36 amide [34]. Sensitivity was <1 pmol/l, intra-assay CV was <6% at 20 pmol/l, and recovery of standard was approximately 100%.

Intestinal transit time

The intestinal transit time was estimated by a non-invasive method [35], which uses self-recorded data on stool form, defaecation frequency and the interdefaecatory time interval. Because of missing data, gut transit time was calculated for 12 subjects (eight subjects in the substudy).

Breath analysis

Breath analysis was undertaken basally and hourly for 300 min. All subjects were capable of producing breath hydrogen. Air breath samples were collected and measured using a breath hydrogen analyser (Quintron Model-12i-Microlyzer; Quintron Instruments, Milwaukee, WI, USA).

Statistical analysis

The characteristics of the subjects are presented as means± SD and all other results as means±SEM. Time-courses of glucose, insulin, GIP and GLP-1 were compared by calculating the incremental AUC using the trapezoidal method. Hydrogen (H_2) and NEFA responses are expressed as total AUC. Repeated measures ANOVA with treatment and time as within-subject factors and Huynh–Feldt epsilon correction was used do determine significant main effects and interactions. Potential confounders, such as the use of oral contraceptives and a history of smoking, were tested and excluded from the model when $p>0.150$. 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. When there were significant differences in basal values (plasma glucose on day 2), postprandial responses were calculated relative to the baseline. Individual concentration maximum (C_{max}), individual time of maximal concentration (T_{max}), AUCs, butyrate and magnesium values were compared using two-tailed Student's *t*-test for paired samples. Partial intraclass correlations (r_{ic}) were calculated between insulin as the dependent variable and GIP, GLP-1 and glucose as independent variables and with simultaneous adjustment for time, bread, GIP, GLP-1 and glucose. Control values for RS are given separately (n=9). Statistical significance was defined as $p<0.05$. Calculations were performed using SPSS version 12.0 (SPSS, Chicago, IL, USA).

Results

Acute effects measured after the consumption of the first portion of the test breads, day 1

Postprandial insulin and glucose responses, day 1

Time courses of the acute insulin and glucose responses on day 1 are presented in Fig. 2. The insulin response to WF and OF was earlier than that to control C, and a trend was observed after RS (fibre × time interaction, WF p=0.026, OF $p<0.001$, RS p=0.126). After OF, the earlier postprandial insulin response exceeded that of C at 30 and 45 min ($insulin_{30\ min}$, C 21.7±3.2, OF 31.6±4.2 [p=0.014]; $insulin_{45\ min}$, C 29.8±3.3, OF 35.2±3.3 mU/l [p=0.047]) and was reduced at 105 min and 210 min ($insulin_{105\ min}$, C 20.0±2.9, OF 13.4±1.6 mU/l [p=0.017]; $insulin_{210\ min}$, C 8.6±1.2, OF 6.1±0.8 mU/l [p=0.029]). After WF, a trend to an earlier postprandial insulin response at 45 min was observed ($insulin_{45\ min}$, C 29.8±3.3, WF 34.2±2.8; p=0.106), and insulin was reduced after 75 min ($insulin_{75\ min}$, C 25.6±3.5, WF 19.6±2.4; p=0.031) and in trend after 90 min ($insulin_{90\ min}$, C 23.9±4.1, WF 16.4±2.3 mU/l; p=0.072). Insulin response after RS tended to be earlier at 30 min ($insulin_{30\ min}$, C 25.2±4.1, RS 34.9± 6.1 mU/l; p=0.064). Individual time of maximal concentration (T_{max}) was earlier after OF and RS and a trend was observed after WF (T_{max}, C 57.9±0.1, WF 49.3±0.0 [p=0.086], OF 46.1±0.0 [p=0.026], RS 46.7±0.1 min [p=0.029]). Insulin $AUC_{180\ min}$ was unchanged after WF (2,193±212), OF (2,331±265) and RS (2,695±411) compared with C (2,451±312) ($p>0.15$; control for RS, 2,780± 399). Early insulin response measured as $AUC_{45\ min}$ was significantly increased after OF (697±93; p=0.027) but not after WF (567±51) or RS (740±142) ($p>0.15$; control for RS, 560±92) compared with control (502±73). Later postprandial insulin response, measured as $AUC_{45–180\ min}$, tended to be lower after RS (1,955±315; p=0.096; control

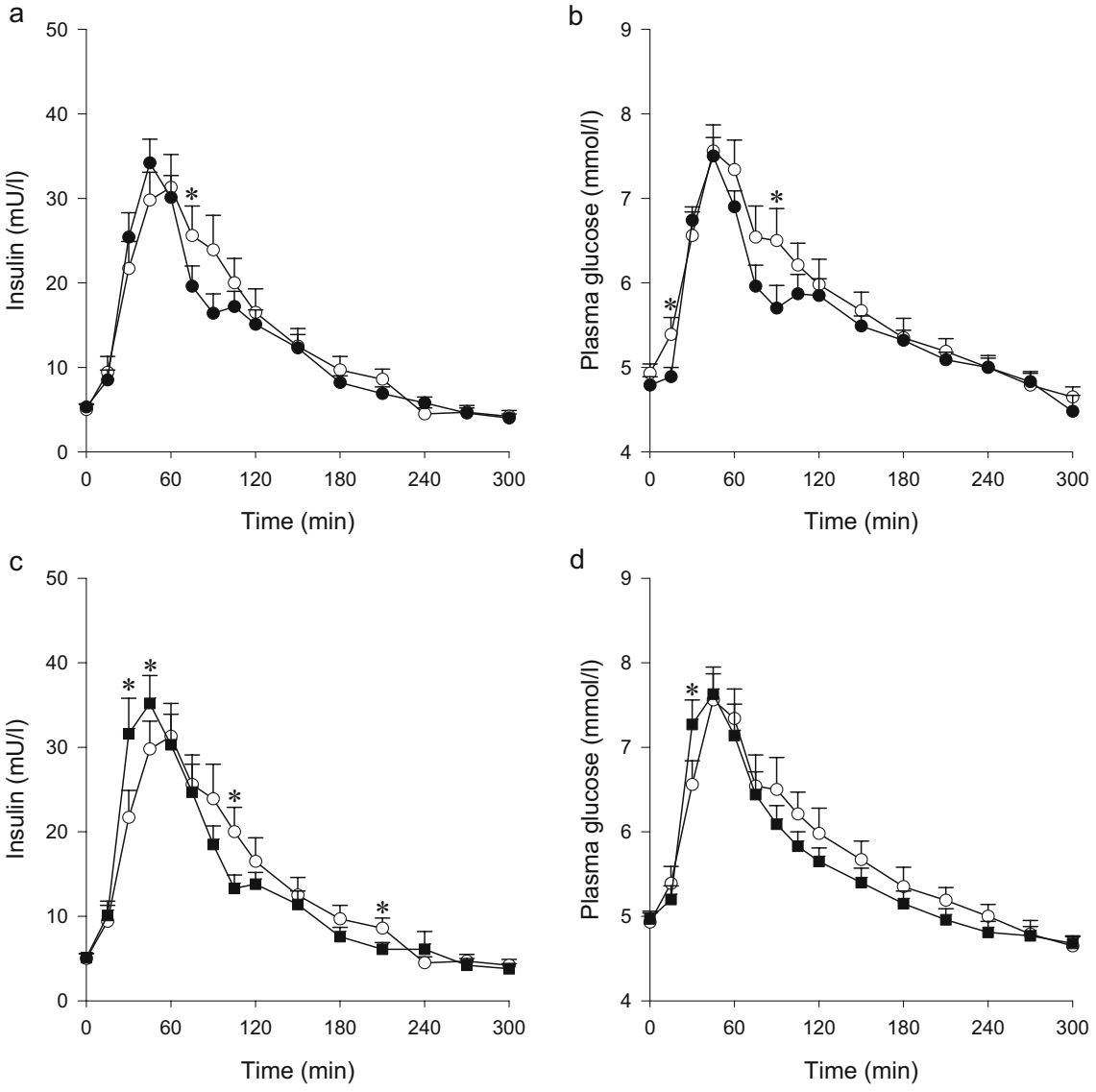

Fig. 2 Effects of fibre enrichment on the acute insulin and glucose responses on day 1. Mean (±SEM) serum insulin (**a** and **c**) and glucose (**b** and **d**) concentrations after consumption of control white bread (*white circles*) or fibre-enriched breads (wheat fibre, *black circles*; oat fibre, *black squares*) (n=14). Repeated measures ANOVA for insulin showed a significant effect of time (p<0.001) for wheat fibre and oat fibre and a fibre×time interaction (wheat fibre, p=0.026; oat fibre, p<0.001). Bonferroni correction was used to analyse significance at specific time points. *p<0.05 vs. control white bread

for RS, 2,219±370) and after all fibre-enriched test meals, measured as $AUC_{60–180\ min}$ (C 1,567±250, WF 1,223±170 [p=0.131], OF 1,220±161 [p=0.15], RS 1,557±263 [p=0.113; control for RS, 1,792± 342]).

A fibre×time interaction was observed for the acute glucose response after fibre enrichment with WF and OF (p=0.021) but not with RS (p>0.15). Glucose $AUC_{180\ min}$ (C 4,121±577, WF 3,699±369, OF 3,520±439, RS 3,467± 686; p>0.15; control for RS 4,130±872), C_{max} (C 8.0±0.3, WF 8.0±0.2, OF 7.9±0.4, RS 7.8±0.2 mmol/l; p>0.15; control for RS 8.1±0.4) and T_{max} (C 56.8±5.3, WF 49.3± 2.9, RS 60±13.0; p>0.15; control for RS 60±7.9; and OF 47.1±3.1 min; p=0.082) were not significantly changed by fibre enrichment.

Postprandial incretin responses, day 1

T_{max} for GIP was earlier after OF and in trend after WF fibre enrichment (C 83.6±7.2, OF 64.3±6.9 [p=0.022], WF 70.7±6.0 [p=0.054], RS 60.0±5.0 min [p>0.15]; control for RS 76.7±8.8 min). After OF the postprandial GIP response exceeded that of C at 30 min ($GIP_{30\ min}$, C 8.4±1.2, OF 13.1±1.3 pmol/l; p=0.001).

Responses of GIP measured as $AUC_{300\ min}$ were not significantly altered by fibre enrichment (C 1,570±163, WF 1,448±183, OF 1,872±247, RS 1,630±154; p>0.15; control for RS 1,550±193). Basal values of GIP were not significantly different (p>0.15). A fibre × time interaction observed when OF (p=0.020) failed to reach statistical significance after correcting for the baseline (p=0.057).

C_{max} for GIP was not altered by fibre enrichment (p>0.15). Basal levels of GLP-1 were significantly different only between C (9.4±2.3) and WF (12.2±3.2) (p=0.034) (OF 12.3±3.4 [p=0.139], RS 8.6±1.7 pmol/l [p>0.15]; control for RS 7.6±2.2). Responses of GLP-1 measured as $AUC_{300\ min}$ were not significantly altered by fibre enrichment (C 2,207±513, WF 1,880±415, OF 2,018±238, RS 2,388±346; p>0.15; control for RS 2,405±611). Fibre enrichment did not change C_{max} and T_{max} of GLP-1 (p>0.15).

Partial intraclass correlations (r_{ic}) with insulin as the dependent variable and GIP, GLP-1 and glucose as independent variables and with simultaneous adjustment for time, bread, GIP, GLP-1 and glucose were r_{ic}=0.268 (p<0.001) for glucose, r_{ic}=0.253 (p<0.001) for GIP and r_{ic}=0.084 (p=0.025) for GLP-1; adjusted R^2 for the total model was 0.716.

Postprandial GIP and GLP-1 responses

These are shown in Fig. 3.

Serum magnesium levels were not significantly different 300 min after intake of the fibre-enriched breads compared with control (C 0.88±0.02, WF 0.89±0.03, OF 0.89±0.05, RS 0.90±0.03 mmol/l; p>0.15; control for RS 0.87±0.03).

Delayed effects on consumption of control, day 2

Postprandial insulin and glucose responses, day 2

Postprandial insulin and glucose responses on day 2 are shown in Fig. 4. The postprandial insulin response, measured as $AUC_{180\ min}$, was not significantly changed by prior

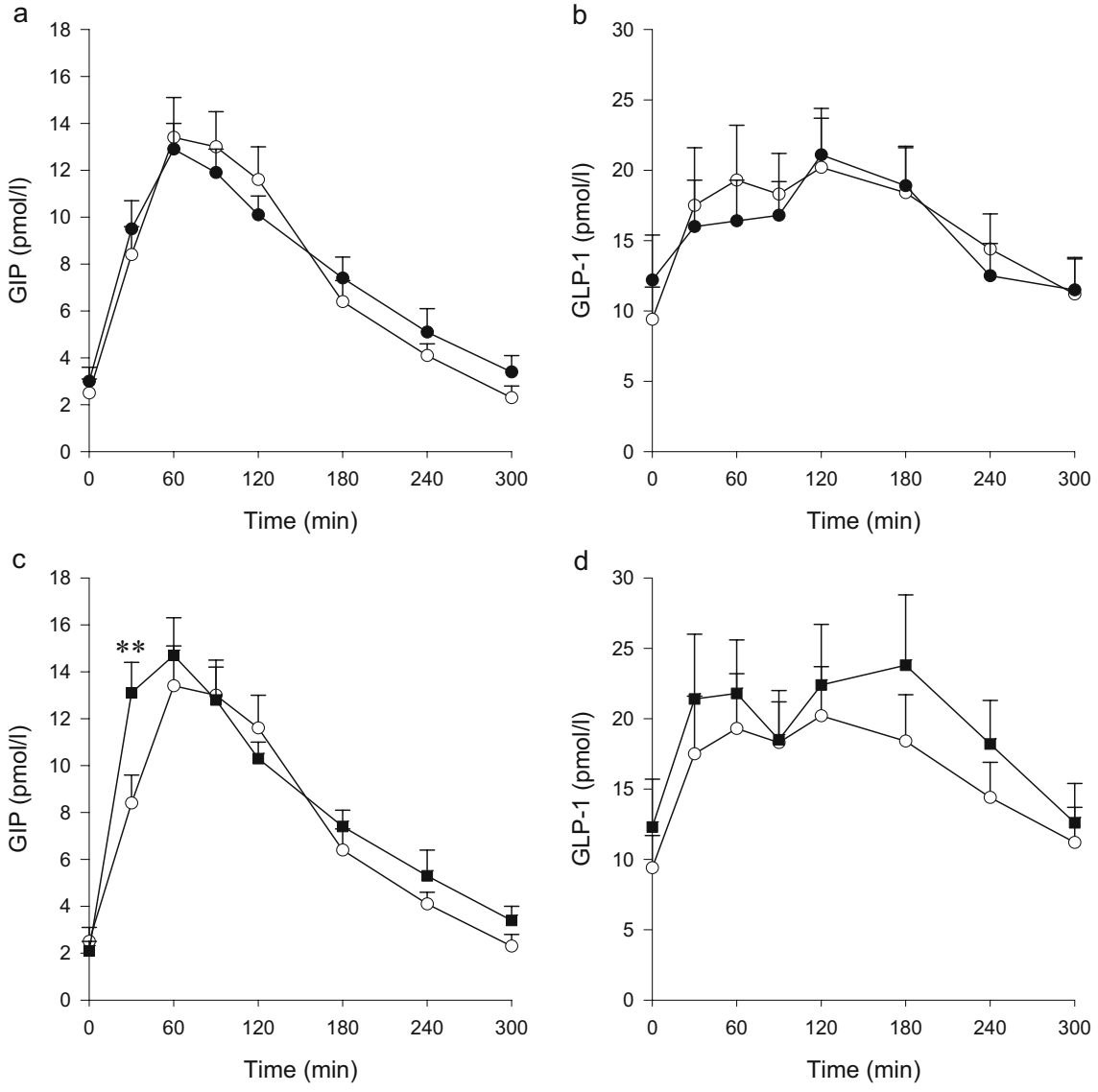

Fig. 3 Effects of fibre enrichment on the acute GIP (**a** and **c**) and GLP-1 (**b** and **d**) responses on day 1. Mean (±SEM) plasma GIP and GLP-1 concentrations after consumption of control white bread (*white circles*) or fibre-enriched breads (wheat fibre, *black circles*; oat fibre, *black squares*) (n=14). Bonferroni correction was used to analyse significance at specific time points. **p<0.01 vs control

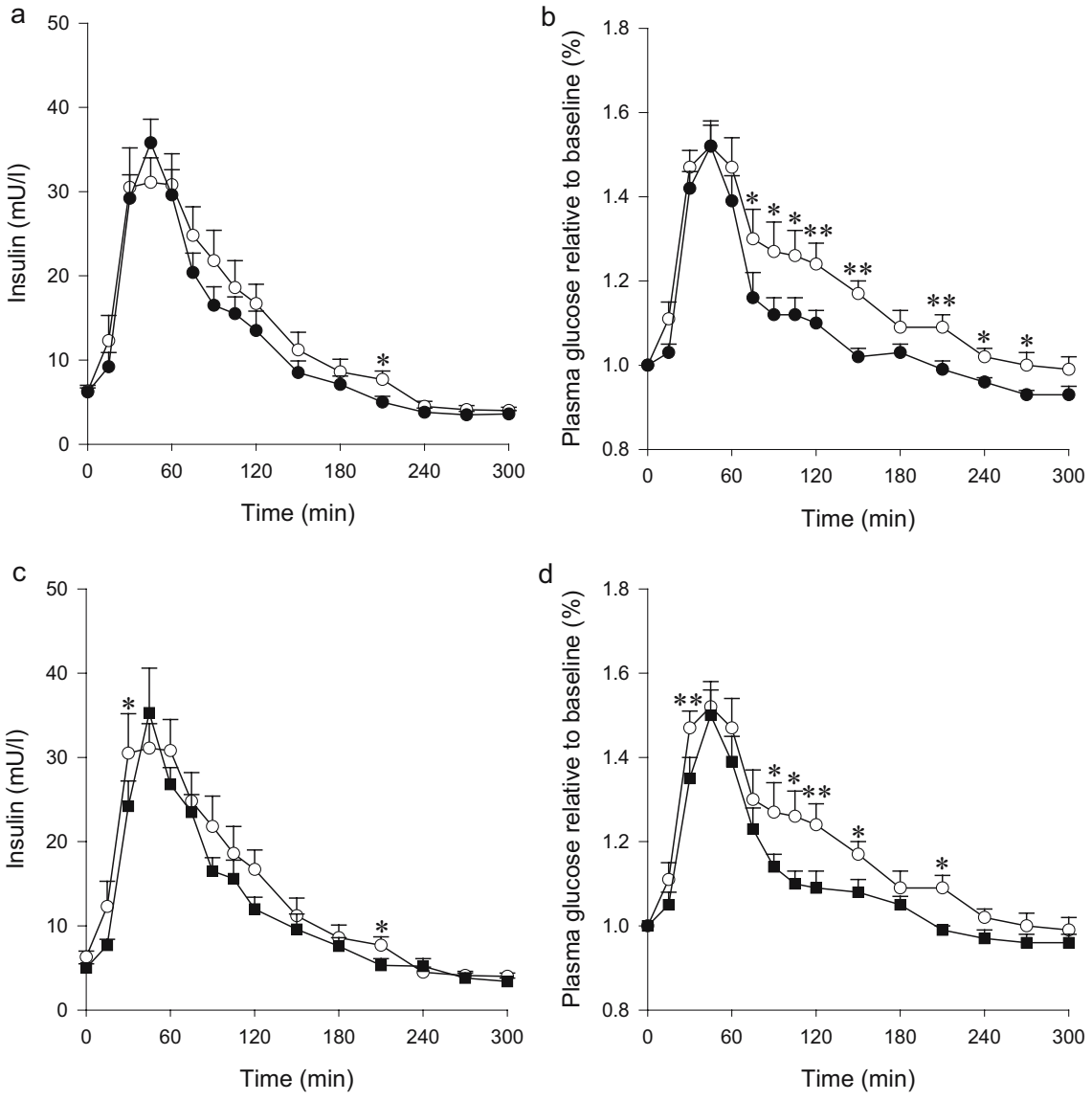

Fig. 4 Effects of prior fibre enrichment on the acute insulin (**a** and **c**) and glucose (**b** and **d**) responses on day 2. Mean (±SEM) serum insulin and glucose concentrations after consumption of control white bread, when preceded on the day before by three portions of white bread (*white circles*), by bread enriched with wheat fibre (*black circles*) or by bread enriched with oat fibre (*black squares*) (n=14). Because of significant differences in basal levels, plasma glucose responses on day 2 are presented relative to baseline. Bonferroni correction was used to analyse significance at specific time points. *p<0.05, **p<0.01 vs control

fibre enrichment (C-C 2,308±277, C-WF 1,959±240, C-OF 2,011±180, C-RS 2,557±287; p>0.15; control for C-RS 2,489±349). Basal insulin was significantly increased the day after the ingestion of three portions of control, when compared with the first study day (C-C 6.32±0.72, C 5.02± 0.55 mU/l; p=0.012), but not when compared with C-WF, C-OF or C-RS (p>0.15).

Capillary glucose $AUC_{180\ min}$ was reduced by 31% after the 24-h prior intake of WF ($AUC_{C\text{-}WF}$=2,850±331; p=0.007) and by 32% after OF ($AUC_{C\text{-}OF}$=2,830±277; p=0.011) compared with control ($AUC_{C\text{-}C}$=4,140±401). After consumption of RS, a trend towards lower glucose values was observed ($AUC_{C\text{-}RS}$=3,054±551 [29%]; control for RS 4,277±579; p=0.092). C_{max} (C-C 7.6±0.2, C-WF 7.9±0.2, C-OF 7.8±0.2, C-RS 7.6±0.3 mmol/l; p>0.15; control for C-RS 7.7±0.3) and T_{max} (C-C 46.1±4.8, C-WF 42.9± 3.1, C-OF 43.9±2.9, C-RS 45.0±5.6 min; p>0.15; control for C-RS 45.0±7.1) were unchanged. Basal plasma glucose was elevated the next day after consumption of OF (5.05± 0.10 mmol/l, p=0.016) and WF (5.07± 0.09 mmol/l, p=0.020), but not after RS (4.85±0.18, p>0.15), compared with control (C-C 4.72±0.11 mmol/l; control for C-RS 4.70±0.14 mmol/l).

C-peptide-to-insulin ratios were unaffected by prior fibre ingestion (C-C 14.1±1.3, C-WF 16.2±1.1, C-OF 14.1±1.4, C-RS 14.2±1.5; p>0.15; control for C-RS 123.7±1.5).

Postprandial incretin responses, day 2

Postprandial incretin responses on day 2 are shown in Fig. 5. Basal values of GLP-1 (C-C 9.7±2.6, C-WF 8.0±2.6, C-OF 11.8±3.1, C-RS 9.5±4.4; $p>0.15$; control for C-RS 9.3±3.3 pmol/l) and $AUC_{300\ min}$ (C-C 1,632±260, C-WF 2,790±682 [$p=0.104$]; C-OF=977±174 [$p=0.145$], C-RS=1,924±547 [$p>0.15$]; control for C-RS 1,452±279) were not significantly different.

Basal values of GIP and $AUC_{300\ min}$ GIP were not significantly different ($p>0.15$). GIP was significantly different from control after WF at 180 min (C-C 8.3±0.8, C-WF 6.3±0.6 pmol/l; $p=0.022$), but at no other time point.

There was also no significant fibre × time interaction regarding GIP and GLP-1 responses ($p>0.15$).

Effects on NEFA levels

NEFA levels were suppressed after ingestion of all first- and second-day test meals. There were no differences in fasting NEFA levels and $AUC_{300\ min}$ between the test days. Analysis of the last 2 h showed that there was an effect of time the day after WF and OF ($p<0.001$), an effect of time ($p<0.001$) and fibre ($p<0.020$) and a fibre × time interaction ($p=0.035$) the day after RS, with a delayed NEFA increase.

Other values tested

Differences in breath H_2 concentrations, measured as total $AUC_{300\ min}$, were observed on study days C-OF (5,700±

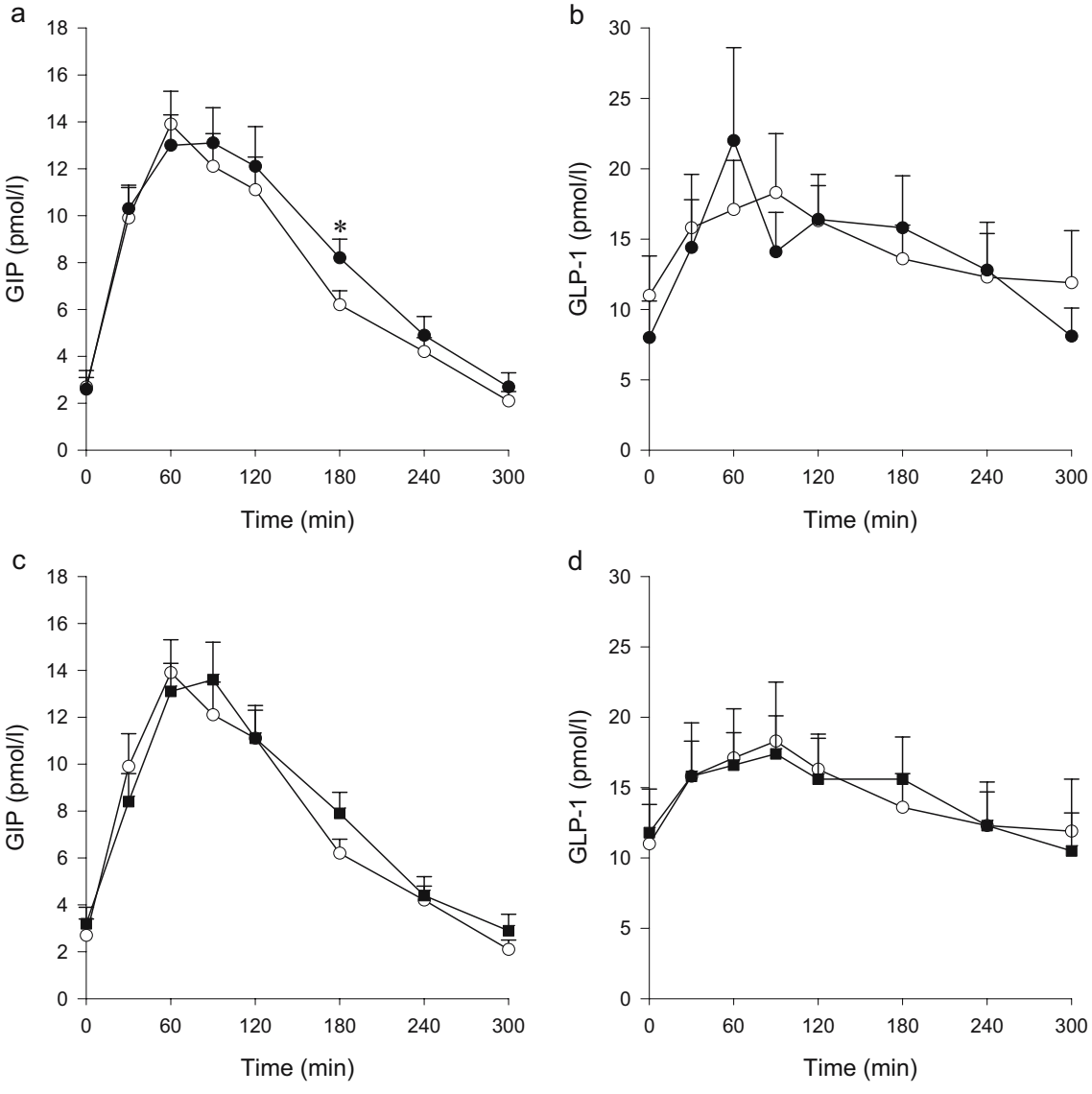

Fig. 5 Effects of prior fibre enrichment on the acute GIP (**a** and **c**) and GLP-1 (**b** and **d**) responses on day 2. Mean (±SEM) plasma GIP and GLP-1 concentrations after consumption of control white bread, when preceded on the day before by three portions of white bread (*white circles*), by bread enriched with wheat fibre (*black circles*) or by bread enriched with oat fibre (*black squares*) ($n=14$). Bonferroni correction was used to analyse significance at specific time points. *$p<0.05$ vs control

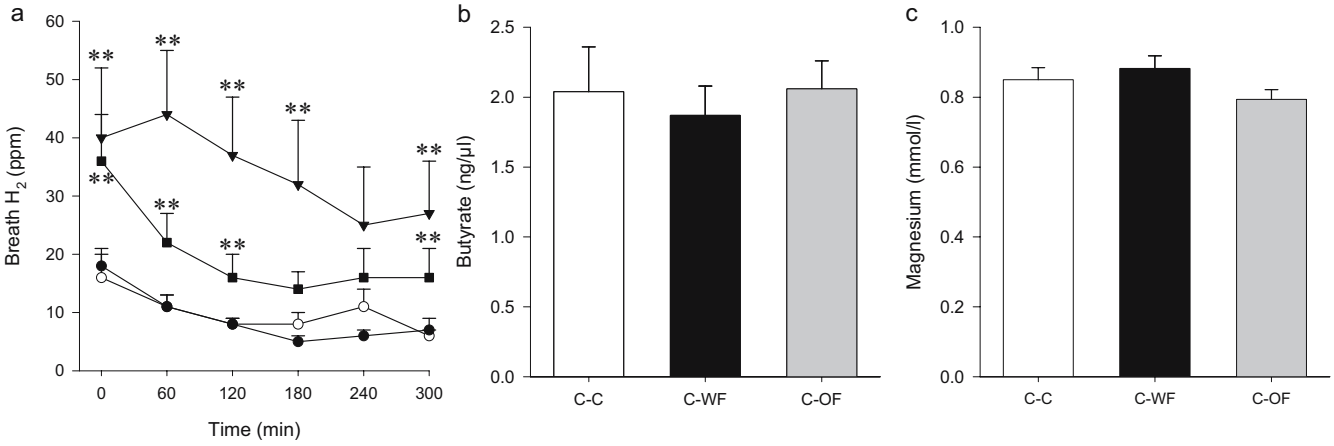

Fig. 6 **a** Mean (±SEM) breath hydrogen responses after consumption of control white bread when preceded on the day before by three portions of white bread (*white circles*) or by bread enriched with wheat fibre (*black circles*) or with oat fibre (*black squares*) (n=14) or with resistant starch (*black triangles* [substudy, n=9]). Bonferroni correction factor was used to analyse significance at specific time points. *p<0.05, **p<0.01 vs. control. **b** Mean (±SEM) basal values of serum butyrate on day 2, when preceded on the day before as for panel **a** (n=14). **c** Mean (±SEM) basal values of serum magnesium on day 2, when preceded on the day before as for panel **a**. C-C, control bread after control bread; C-WF, control bread after bread enriched with wheat fibre; C-OF, control bread after bread enriched with oat fibre. n=14

1,190; p=0.017) and C-RS (10,300±2,930; p=0.016; control for C-RS 3,037±630), but not on days C-WF (2,580±357; p>0.15) and C-C (2,730±463; p>0.15), compared with C (2,880±568) as measured on day 1. The time courses of breath H_2 are presented in Fig. 6a.

Fibre ingestion on the previous day did not significantly influence basal levels of butyrate (C-C 2.0±0.3, C-WF 1.9±0.2, C-OF 2.1±0.2, C-RS 1.6±0.2 ng/µl; p>0.15; control for C-RS 1.8±0.3) and magnesium (C-C 0.85±0.03, C-WF 0.88±0.04, C-OF 0.79±0.03, C-RS 0.91±0.04 mmol/l; p>0.15; control for C-RS 0.86±0.03) (Fig. 6b,c).

There were no significant changes in the estimated intestinal transit times and stool consistencies (p>0.15), as assessed by Bristol stool charts.

Body weight remained stable during the study period (63.4±6.5 [SD] vs 63.0±6.6; p>0.15).

Discussion

A primary finding of this study was an accelerated insulin response directly after the ingestion of insoluble dietary fibres, associated with an earlier response of biologically active GIP. In a recent study an earlier postprandial hyperinsulinaemia was shown after the intake of cereal bran with a high content of insoluble fibre compared with a low-fibre control meal [22]. The authors attributed the observed effect to different protein contents in the test meals, because adding protein to carbohydrates might have augmented insulin secretion [36]. However, in the present study protein and other macronutrient contents were virtually identical, and it is likely that differences in the time course of GIP explain the accelerated insulin response after fibre intake. This is supported by partial correlation analysis showing GIP to be independently correlated with postprandial insulin responses.

It has been hypothesised that a reduced insulinotropic effect of GIP precedes the development of type 2 diabetes [37], and mice with a GIP receptor knockout develop glucose intolerance [38]. GIP analogues have been suggested for possible therapeutic use in the treatment of type 2 diabetes [39]. An accelerated GIP response by insoluble fibre enrichment has not been described previously and might be a mechanism linking cereal fibre intake to the reduced risk of diabetes, as observed in population studies. However, additional larger and longer-term studies are needed, and other effects of GIP, for example on lipid metabolism, need further elucidation [39, 40].

A loss of early-phase insulin induced by a somatostatin infusion during an OGTT reduces glucose tolerance in healthy subjects [41], indicating that the shape of insulin secretion plays an important role in glucose metabolism. Insulin is secreted into the portal vein and targets the liver directly, and short-term alterations in insulin levels might change the metabolic outcome. The later postprandial insulin response in our study tended to be reduced after the ingestion of all fibre-enriched test meals compared with control. This might be relevant in the longer term, since binding of insulin to its receptor in the liver has been shown to activate IRS-1 and -2, which, via protein kinase B/Akt, phosphorylate the forkhead transcription factors Foxo1 and Foxa2 and thereby inhibit gluconeogenesis and fatty-acid oxidation in the liver [42]. Inhibition of liver fatty acid oxidation, regulated via both IRS-1 and IRS-2, is more sensitive to insulin than IRS-2-mediated inhibition of gluconeogenesis. This might promote the development of hepatic steatosis in (relatively) hyperinsulinaemic states, even in healthy subjects, before changes in fasting glucose levels can be observed. However, these possible mechanisms need to be investigated in further studies. It is likely that a complex network of other pathways is involved (for review see [43]).

The postprandial glucose response on day 1 was not significantly affected by fibre enrichment. A more pronounced effect might appear in glucose-intolerant subjects due to a hyperbolic function between early insulin response and glucose tolerance [44].

The postprandial glucose response on day 2 upon ingestion of control bread was significantly reduced after prior intake of insoluble fibres for 24 h. After RS this effect failed to reach statistical significance, probably because of the smaller size of this substudy. Enhanced carbohydrate handling on the second day after consumption of RS has recently been shown [45]. The authors attributed the observed effects to increased colonic fermentation with higher short-chain fatty acid concentrations in the portal vein, and thereby improved glucose metabolism, e.g. by reduced hepatic glucose production [23]. In our study, colonic fermentation was increased after the intake of OF or RS, but not after WF, compared with C. However, second-day glucose responses were comparable after the ingestion of either OF or WF, indicating that colonic fermentation was not responsible for the observed effects. In addition, postprandial GLP-1 levels 24 h after the intake of highly fermentable fibre compared with non-fermentable fibre were unchanged, a finding in accordance with the results of Robertson and colleagues [45], who concluded that 24 h might not be sufficient to upregulate GLP-1 mRNA.

Oral magnesium supplementation has been shown to improve insulin sensitivity [46], and a higher intake seems to be associated with a reduced risk of developing diabetes [47]. We analysed the test breads and serum samples for magnesium concentrations and found that the effects of fibre enrichment could not be attributed to differences in magnesium content of the test breads or magnesium absorption.

Subjects tended to need less insulin to handle significantly more glucose 24 h after the ingestion of cereal fibres. Cereal fibres are known not to hinder the absorption of macronutrients from the gut. This is in accordance with our findings that: (1) the postprandial glucose response on day 1 was not significantly reduced by fibre enrichment; (2) estimated gut transit times were not affected; and (3) maximal concentrations of insulin and the time points of maximal concentration were not influenced by previous fibre enrichment on day 2. An effect of fibre enrichment on gastric emptying is also unlikely as recent research has shown that GIP, which was influenced on day 1 upon fibre enrichment, does not inhibit gut motility [48, 49]. Therefore, improved glucose handling on day 2 was possibly induced by non-insulin-dependent glucose uptake or improved peripheral or hepatic insulin sensitivity. Fasting insulin was only increased after the 24-h consumption of the control bread, and fasting insulin in the presence of normal fasting glucose is inversely related to the insulin sensitivity index [44]. Improved peripheral insulin sensitivity (and unaffected hepatic insulin sensitivity) after a high-fibre diet has been reported in a study using the euglycaemic–hyperinsulinaemic clamp and stable isotope techniques [26]. However, the soluble fibre and fat contents were different in the control group. In our study insulin sensitivity was not measured and a different study design is needed to clarify the mechanisms involved.

In conclusion, the consumption of insoluble dietary fibres within the recommended daily fibre intake [50] significantly accelerated the early insulin response. This was associated with an earlier increase of postprandial active GIP values, whereas GLP-1 was unaffected. Fibre enrichment was further associated with a significant reduction of postprandial glucose values the following day, upon ingestion of the control bread. The short-term effects of cereal fibre do not seem to be influenced by colonic fermentation.

Our observations might be of relevance to public health, since they offer a non-pharmacological and safe way to improve the body's handling of carbohydrate. These findings need to be confirmed in larger and longer-term, well-controlled studies, also including subjects with impaired glucose metabolism. Stable isotope and clamp techniques, ideally together with muscle and fat biopsies (and the investigation of liver tissue in animal models), will be useful in further elucidating the mechanisms involved.

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