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ARTICLE

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Effects of whey protein and dietary fiber intake on insulin sensitivity, body composition, energy expenditure, blood pressure, and appetite in subjects with abdominal obesity

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Abstract

Background Recently, we demonstrated that whey protein (WP) combined with low dietary fiber improved lipemia, a risk factor for cardiovascular disease in subjects with abdominal obesity. In the present study, we investigated the effects of intake of WP and dietary fiber from enzyme-treated wheat bran on other metabolic parameters of the metabolic syndrome.

Methods The study was a 12-week, double-blind, randomized, controlled, parallel intervention study. We randomized 73 subjects with abdominal obesity to 1 of 4 iso-energetic dietary interventions: 60 g per day of either WP hydrolysate or maltodextrin (MD) combined with high-fiber (HiFi; 30 g dietary fiber/day) or low-fiber (LoFi; 10 g dietary fiber/day) cereal products. We assessed changes in insulin sensitivity, gut hormones (GLP-1, GLP-2, GIP, and peptide YY), body composition, 24-h BP, resting energy expenditure and respiratory exchange ratio (RER), and appetite.

Results Sixty-five subjects completed the trial. Subjective hunger ratings were lower after 12 weeks of WP compared with MD, independent of fiber content ($P = 0.02$). We found no effects on ratings of satiety, fullness or prospective food consumption for either of the interventions. Intake of WP combined with LoFi increased the postprandial peptide YY response. There were no effects of WP or fiber on insulin sensitivity, body composition, energy expenditure, incretins, or 24-h BP.

Conclusions WP consumption for 12 weeks reduced subjective ratings of hunger in subjects with abdominal obesity. Neither WP nor dietary fiber from wheat bran affected insulin sensitivity, 24-h BP, gut hormone responses, body composition, or energy expenditure compared with MD and low dietary fiber.

Introduction

Observational studies indicate inverse relationships between dairy product consumption and abdominal obesity [1] and the metabolic syndrome [2]. Whey protein (WP) is a dairy product constituent that has received growing interest regarding metabolic health, with studies showing beneficial effects on blood pressure (BP) [3, 4], insulin resistance [5], and body composition [6]. Furthermore, epidemiological evidence indicates that diets rich in cereal fiber are inversely associated with abdominal obesity [7] and several other metabolic risk factors [8, 9]. Evidence from randomized, controlled trials shows that dietary fiber supplementation may lower BP [10], and that high intake of wholegrains and

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cereal fiber may improve body composition [11] and insulin sensitivity [12, 13].

The positive metabolic effects of WP and cereal fiber may partly be an indirect result of enhanced satiety [14, 15]. Protein is more satiating than fat and carbohydrate [16], and additionally causes greater energy expenditure, possibly via increased diet-induced thermogenesis [17, 18]. The protein-induced satiety is correlated with the release of the gut hormone glucagon-like peptide 1 (GLP-1) [19]. Interestingly, acute studies have found that WP may enhance subjective measures of satiety and increase the secretion of GLP-1 and glucose-dependent insulinotropic polypeptide (GIP) compared with other protein sources [20, 21]. However, evidence on the long-term effects of WP on appetite is sparse [22]. Likewise, there are only a few long-term studies examining the effects of dietary fiber on appetite. Long-term intake of wheat bran and arabinoxylan—the main dietary fiber constituent of wheat bran—has been shown to promote GLP-1 release in subjects with hyperinsulinemia [23] and improve markers of glycemic control in subjects with type 2 diabetes (T2D) [24]. Arabinoxylan oligosaccharides (AXOS) from enzymatically treated wheat bran may increase the postprandial GLP-1 response [25] and improve insulin sensitivity in short-term settings [26].

Recently, we found that WP combined with low dietary fiber reduced postprandial and fasting TG and reduced fasting apolipoprotein B-48 [27]. The aim of the present study was to assess the separate and combined effects of 12 weeks intake of WP and high-fiber cereal products made with enzyme-treated wheat bran on other features of the metabolic syndrome than increased TG, i.e., insulin sensitivity, body composition, and BP as well as appetite and energy expenditure. We hypothesized that the combination of high-protein and high-fiber diets would have additive beneficial effects on these outcomes.

Methods

Study design and participants

The current study was part of the MERITS study, which primarily aimed to investigate the effects of WP and dietary fiber on the lipid profile. The study design is previously described in detail [27]. In brief, the study was a randomized, controlled, parallel, double-blind 12-week intervention. Seventy-three subjects with abdominal circumference ≥80 cm (women) or ≥94 cm (men) and age ≥40 years were recruited. Severe chronic conditions or treatment with medication known to directly affect glucose homeostasis were grounds for exclusion. Continued use of regular medication including antihypertensive- or lipid-lowering medication was allowed if no changes were made during the trial or within 3 months prior to enrollment. Participants were randomized by age and sex to receive either: WP + high fiber (WP-HiFi), WP + low fiber (WP-LoFi), maltodextrin + high fiber (MD-HiFi), or maltodextrin + low fiber (MD-LoFi). The WP and maltodextrin (MD) supplements were provided by Arla Foods Ingredients Group P/S (Viby, Denmark). Each serving contained either 30 g of WP hydrolysate (Lacprodan® HYDRO.REBUILD) or 30 g of MD (Glucidex® 19). The participants consumed the powder supplements (WP or MD) twice daily. All bread and breakfast cereals were provided by Lantmännen (Vaasan bakery, Vilnius, Lithuania)/ Lantmännen Cerealia AB (Järna, Sweden). The HiFi products contained a mixture of enzyme-treated wheat bran and refined wheat, while the LoFi products were based on refined wheat. The wheat bran was enzymatically treated with cell wall-degrading enzymes (xylanase, glucanase, cellulase) by DuPont Industrial Biosciences Aps (Brabrand, Denmark). The test products for the different intervention groups were iso-energetic. Nutritional composition of the test products is published elsewhere [27]. The participants were required to substitute any habitual intake of bread products and breakfast cereals with the provided test products, aiming at a minimum intake of 6 servings/day (corresponding to 30 g of dietary fiber/day from the HiFi products, or 10 g of dietary fiber/day from the LoFi products). In order to isolate the metabolic effects of protein and fiber intake not related to weight changes, an energy-balanced design was chosen in which weight stability throughout the study period was intended.

Study visits and experimental procedures

All study procedures were conducted before and after the intervention. On the first visit, participants attended the clinic following an overnight fast (minimum 10 h). Body composition (fat mass, lean body mass, fat percentage, and android/gynoid fat ratio) was estimated in the fasting state by Dual-Energy X-ray Absorptiometry scans (Hologic Horizon A scanner, Hologic, Inc., Massachusetts, USA) using Apex System Software (version 5.6.0.5). Waist circumference was measured at the horizontal level between the rim of the lower ribs and the iliac crest. Hip circumference was measured at the largest circumference at the level of trochanter major.

A standard oral glucose tolerance test (OGTT; 75 g of D-glucose) was performed and blood samples were collected at $t = -15$, -10, 0, 30, 60, 90, and 120 min for measurements of insulin, glucose and glucagon.

At the second visit, participants were equipped with a BP device (Arteriograph 24, type TD3, TensioMed Ltd, Budapest, Hungary) for 24-h ambulatory BP measurements. Systolic and diastolic BP, heart rate and aortic augmentation index were measured every 30 min during the day and

every 60 min during the night, and the 24-h means were calculated.

At the third visit, participants attended the clinic following an overnight fast. Resting energy expenditure (REE) and respiratory exchange ratio (RER) were assessed by indirect calorimetry using an Oxycon Pro calorimeter (Intramedic, Gentofte, Denmark) with a ventilated hood. Respiratory gases were collected for 30 min (initial 5 min were systematically discarded). Measurements were adjusted for urinary nitrogen. Subsequently, a high-fat mixed meal test (4700 kJ, 70 g of fat) was performed [27]. Blood samples were collected at $t = -10$, 0, 15, 30, 60, 90, 120, 240, and 360 min for analysis of GIP, GLP-1, and glucagon-like peptide 2 (GLP-2). Peptide YY (PYY) was analyzed at $t = 0$, 30, 60, 90, 120, and 240 min. Ten times during the meal test, participants rated their appetite by responding to an electronic questionnaire (ACQUI software, XYZT, Copenhagen) using visual analog scales [28]. Participants rated their hunger, satiety, fullness, and prospective food consumption before intake of the meal and at 30–60 min intervals during the 6-h meal test. Three hours after the meal, the indirect calorimetry measurement was repeated.

Adherence to the intervention diets was assessed by self-reported test product journals, 3-day weighed dietary records, and by plasma alkylresorcinols (cereal bran intake marker) and urinary carbamide (protein intake marker) [27].

The study was conducted at the Department of Endocrinology and Internal Medicine, Aarhus University Hospital, Denmark in accordance with the Declaration of Helsinki of 1975 as revised in 1983. All participants signed a declaration of informed consent. The study protocol was approved by the Central Denmark Region Committees on Health Research Ethics (Journal no. 1-10-72-370-15).

Blood sample analyses

Blood samples were centrifuged at $2000 \times g$ for 15 min at 4 °C and immediately frozen at −20 °C. Samples were moved to −80 °C within 8 h. Fasting levels were calculated as the mean of three fasting blood samples. Plasma insulin was measured by ELISA technique using commercial kits (cat. K6219, Dako Denmark A/S) with intra-/inter-assay precision of 5.1–7.5% and 4.2–9.3%. Plasma glucose was measured on Cobas c111-system by standard enzymatic colorimetric assays using commercial kits (cat. 04657527, Roche Diagnostics Gmbh). Intra-/inter-assay precision were between 0.8–1.1% and 0.5–0.6%. Glucagon was measured using radioimmunoassay (cat. GL-32K, EMD Milipore, Darmstadt, Germany). Intra-/inter-assay precision was 4.0–6.8% and 7.3–13.5%. GIP and GLP-1 samples were extracted in 70% ethanol. Total GLP-1 and total GIP were measured using radioimmunoassays (GLP-1: antibody code no. 89390, GIP: code no. 80867). Sensitivity for both assays was below 1 pmol/L, and intra-assay coefficient of variation < 10%. GLP-2 samples were extracted in 75% ethanol and intact GLP-2 was measured using radioimmunoassay [29]. The antiserum (code no. 92160) is directed against the N-terminus of GLP-2 and therefore measures only fully processed GLP-2 of intestinal origin. Sensitivity was below 5 pmol/L, and intra-assay coefficient of variation below 10%. Total PYY was determined by ELISA technique using commercial kits (cat. EKU06515, Biomatik, Corporation, Cambridge, Ontario, Canada) with intra-/inter-assay precision of <10% and <12%. HbA1c was analyzed at the Department of Clinical Biochemistry at Aarhus University Hospital (DS/EN ISO 15189:2013-approved).

Calculations and statistical analysis

The sample size calculation was based on the primary metabolic outcome of the MERITS trial, postprandial triglyceride incremental area under the curve (AUC) [27], for which 66 completers were needed (80% power, $\alpha = 0.05$).

In order to assess main treatment effects and interactions between protein and fiber, a two-factor ANOVA model was applied. Pairwise comparisons of groups were corrected for multiple comparisons using the Tukey-Kramer method. All estimates were adjusted for sex and age and reported as means and 95% confidence intervals (CI), unless otherwise stated. Normality was checked by diagnostic plots of residuals. If the assumptions of normality or equal variance across groups were not met, the dependent variable was log-transformed. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated by fasting plasma glucose (mmol/L) × fasting plasma insulin (mU/L)/22.5. The composite insulin sensitivity index ($ISI_{Composite}$) was calculated as described by Matsuda & DeFronzo [30]. $P < 0.05$ was considered statistically significant. All statistical analyses were performed using STATA/IC 15.1 (StataCorp LP College Station, TX, USA).

Results

A total of 65 participants completed the study. The participants (31 men and 34 women) had a median age of 64 years (25th–75th centile: 58–68). Both age and sex distribution were similar across groups. The average BMI was 29.4 kg/m^2 (SD: 3.7), and 52% of participants fulfilled the criteria for the metabolic syndrome. HbA1c at baseline was 39 mmol/mol (SD: 4) equal to 5.8% (SD: 0.3). Additional baseline characteristics and self-reported dietary intake for each group have previously been published [27]. In brief,

compliance was generally high, with participants consuming on average 88% of the target in terms of cereal products, and 94% of the test powders (no differences between groups). These self-reported data on test product intakes corresponded well with the biochemical markers of compliance (p-alkylresorcinols and u-carbamide) [27]. The total protein and fiber intake (calculated from 3-day weighed dietary records) differed significantly between the groups as intended. Average protein intake was 142 g/day (SD 16) or 1.7 g/kg/day (SD 0.3) vs. 87 g/day (SD 18) or 1.1 g/kg/day (SD 0.3) in the high/low protein groups. Dietary fiber intake averaged from 34 g/day (SD 5) vs. 16 g/day (SD 5) in the high/low-fiber groups.

Insulin sensitivity and gut hormones

We found no effects of the interventions on 2-h glucose, insulin and glucagon responses during the OGTT, nor did we observe any changes in fasting glucose, insulin, glucagon, HOMA-IR, or $ISI_{Composite}$ (Table 1). There was an interaction between protein and fiber level for postprandial PYY (Table 1). After 12 weeks, postprandial PYY increased for WP-LoFi compared with WP-HiFi and MD-LoFi. There were no effects of the interventions on fasting concentrations of PYY. Furthermore, we found no effects on fasting or postprandial levels of GIP, GLP-1 and GLP-2 (Table 1).

Body composition

Measurements of body composition are presented in Table 2. Total body weight did not change in any of the groups [27]. Waist- and hip circumference, lean body mass, fat mass, fat percentage and android/gynoid fat distribution ratio were unaffected by the intervention.

Appetite and energy expenditure

We found an effect of protein supplementation on hunger sensation (Table 2). Intake of WP for 12 weeks reduced subjective ratings of hunger during the high-fat meal test by -3233 mm × 360 min (95% CI: -5820, -646; $P = 0.02$) compared with MD. There were no differences between groups in ratings of satiety, fullness or prospective food consumption. Furthermore, we found no effects on fasting or postprandial REE and RER (Table 2).

Ambulatory blood pressure, heart rate, and arterial stiffness

We did not detect any impact of the interventions on 24-h systolic and diastolic BP, heart rate or augmentation index (Table 3).

Discussion

In the present study, we studied the combined effects of two dietary components, i.e., WP and dietary fibers with promising potential for improving metabolic risk factors.

We found no significant impact on insulin sensitivity, BP or body composition. The absence of effect of the high-fiber diet on insulin sensitivity is in contrast with a similar study by Weickert et al. [13], where a high cereal fiber diet improved insulin sensitivity in overweight subjects compared with a high-protein diet. The discrepant findings may rest upon differences in the amounts of fiber consumed (~49 g/day [13] vs. ~34 g/day [27]), or differences in the sources of dietary fiber (mixed cereal grains [13] vs. enzyme-treated wheat bran). It is possible that a larger amount of cereal fiber than what was used in the present study is necessary to affect insulin sensitivity. This might also be the case regarding the effect on appetite, where high fiber intake trended to reduce appetite. However, consumption of 29 g of cereal fiber/day from wholegrains for 12 weeks has been found sufficient to reduce postprandial insulin level compared with consumption of refined grains in subjects with the metabolic syndrome [31].

The type of dietary fiber is a topic of great debate regarding its influence on glucose metabolism. While observational evidence points toward an inverse relationship between T2D and insoluble fiber intake [32], predominantly soluble fibers are found to improve insulin sensitivity in long-term clinical intervention trials [33]. As wheat bran consists primarily of insoluble fiber, this might explain the lack of effect on glucose metabolism in the present study. Furthermore, the participants of the present study were non-diabetics, which might have made any improvements in insulin sensitivity difficult to detect. Nonetheless, our findings concur with the study of Jenkins et al. [34] comparing 3 months consumption of wheat bran with refined wheat in subjects with T2D, in which markers of glycemic control were unaffected.

The acute insulinotropic potential of WP, compared with other protein sources [35, 36], did not translate into any detectable long-term effects on HOMA-IR, insulin or incretin levels. In contrast, a previous long-term study [5] found that WP isolate reduced HOMA-IR and fasting insulin compared with glucose in overweight subjects. It is possible that the WP formulations, isolate and hydrolysate, may affect insulin sensitivity differentially; however, several other long-term interventions with WP isolate have similarly been unable to detect effects on insulin sensitivity [4, 37].

While several high-protein trials (between 1.2 and 1.6 g protein/kg/day or 25–30 g protein/meal) have reported promising effects on metabolic outcomes [38], longer-term studies of increased protein intake ≥ 12 months do not

Table 1 Insulin sensitivity and gut hormones at baseline and week 12.

	WP-LoFi		WP-HiFi		MD-LoFi		MD-HiFi		Two-factor ANOVA, *P*		
	(*n* = 15)		(*n* = 17)		(*n* = 16)		(*n* = 17)				
	Baseline	Week 12	Baseline	Week 12	Baseline	Week 12	Baseline	Week 12	Protein level	Fiber level	Interaction
Fasting glucose (mmol/L)[1]	5.6 (0.4)	5.6 (0.5)	5.6 (0.5)	5.6 (0.7)	5.7 (0.4)	5.8 (0.4)	5.6 (0.5)	5.6 (0.5)	0.32	0.57	0.78
Glucose iAUC (mmol/L × 120 min)[1]	276 (95)	265 (132)	282 (119)	274 (163)	281 (154)	307 (164)	267 (147)	275 (143)	0.33	0.85	0.68
Fasting insulin (pmol/L)[2]	51.0 (35.0–78.0)	43.4 (33.8–76.6)	51.7 (40.0–58.7)	42.5 (33.2–56.2)	60.5 (41.5–77.0)	64.3 (42.5–83.8)	38.4 (28.6–53.5)	39.5 (27.5–51.5)	0.07	0.25	0.68
Insulin iAUC (pmol/L × 120 min)[2]	36,198 (18,542–45,125)	26,513 (18,216–44,216)	33,278 (22,646–48,605)	32,908 (21,819–50,872)	28,753 (19,527–56,234)	31,569 (23,927–44,327)	29,939 (17,455–48,430)	30,578 (17,894–45,117)	0.65	0.89	0.64
Fasting glucagon (pg/mL)[1]	67.0 (17.9)	63.6 (16.2)	67.1 (14.7)	71.7 (18.1)*	67.1 (13.1)	71.1 (18.1)	64.9 (16.4)	66.7 (16.6)	0.41	0.25	**0.04**
Glucagon tAUC (pg/mL × 120 min)[a]	7860 (1824)	7678 (1563)	8619 (1995)	8620 (2374)	8115 (1881)	8363 (1924)	8428 (2076)	8473 (2441)	0.44	0.85	0.50
$ISI_{Composite}$[2]	6.5 (3.7–8.0)	6.7 (4.5–9.5)	5.3 (4.4–8.7)	7.3 (4.9–8.4)	5.5 (3.9–8.1)	5.4 (3.5–7.5)	7.8 (4.7–11.4)	8.2 (5.0–11.3)	0.44	0.34	0.90
HOMA-IR[2]	1.7 (1.4–2.8)	1.6 (1.2–3.0)	1.9 (1.4–2.0)	1.6 (1.1–1.9)	2.1 (1.5–2.8)	2.2 (1.6–3.2)	1.2 (1.0–1.8)	1.4 (1.0–1.8)	0.08	0.21	0.93
Fasting GLP-1 (pmol/L)[1]	21.5 (4.2)	19.6 (3.8)	19.3 (3.0)	20.2 (4.0)	21.0 (5.2)	20.7 (4.5)	21.2 (3.6)	21.1 (4.8)	0.91	0.19	0.23
GLP-1 iAUC (pmol/L × 360 min)[2]	4174 (2370–5422)	4264 (2955–6851)	6143 (3341–6911)	3791 (2471–5344)*	3471 (2646–4674)	3062 (2301–5608)	3896 (2531–4695)	4676 (2730–5603)	0.26	0.21	0.08
Fasting GIP (pmol/L)[1]	10.3 (5.6)	9.3 (4.3)	8.1 (3.8)	9.3 (3.8)	9.1 (5.4)	10.8 (5.0)	9.5 (6.0)	8.9 (6.0)	0.90	0.88	0.10
GIP iAUC (pmol/L × 360 min)[1]	22,225 (6316)	20,173 (4608)	21,675 (6573)	19,698 (5127)	23,263 (5928)	22,469 (4825)	22,034 (8488)	22.814 (7779)	0.14	0.51	0.61
Fasting PYY (pg/mL)[2]	841 (597–1099)	798 (697–1288)	826 (675–1252)	943 (561–1155)	871 (752–1153)	1091 (758–1211)	946 (647–1355)	846 (562–1234)	0.84	0.31	0.12
PYY tAUC (pg/mL × 360 min)[1]	212,183 (75,976)	292,444 (129,311)*[a]	244,325 (99,742)	244,072 (95,258)[b]	268,315 (125,116)	259,869 (89,691)[b]	269,414 (113,666)	283,222 (130,081)[a, b]	0.07	0.17	**0.01**
Fasting GLP-2 (pmol/L)[1]	11.6 (4.2)	15.3 (4.2)*	12.9 (4.1)	14.8 (4.7)	11.8 (4.3)	13.8 (3.7)	11.1 (3.0)	16.3 (6.9)*	0.69	0.72	0.09
GLP-2 iAUC (pmol/L × 360 min)[2]	7676 (4830–9881)	8258 (3113–12,158)	9938 (5460–12,454)	5831 (3641–9454)*	7059 (5366–7712)	6049 (3602–8469)	7391 (5933–8925)	5955 (4020–10,628)	0.44	0.13	0.06

Different superscript letters within a row indicate significant difference in Δ (week 12—baseline) between groups after Tukey correction ($P < 0.05$). Data were log-transformed for insulin iAUC and fasting PYY. Responses of 120 min are obtained during an OGTT. Responses of 360 min are obtained during a high-fat meal test.

ANOVA analysis of variance, *GIP* glucose-dependent insulinotropic polypeptide, *GLP-1* glucagon-like peptide 1, *GLP-2* glucagon-like peptide 2, *HiFi* high fiber, *HOMA-IR* homeostasis model assessment of insulin resistance, *iAUC* incremental area under the curve, *ISI* insulin sensitivity index, *LoFi* low fiber, *MD* maltodextrin, *OGTT* oral glucose tolerance test, *PYY* peptide YY, *WP* whey protein.

*Significantly different from baseline ($P < 0.05$). *P* values indicate overall main effects and interactions between protein and fiber levels.

P-values < 0.05 are written in bold.

[1]Mean (SD).

[2]Median (25th–75th centile).

Table 2 Body composition, fasting and postprandial energy expenditure, and subjective appetite ratings at baseline and week 12.

	WP-LoFi		WP-HiFi		MD-LoFi		MD-HiFi		Two-factor ANOVA, *P*		
	(*n* = 15)		(*n* = 17)		(*n* = 16)		(*n* = 17)				
	Baseline	Week 12	Baseline	Week 12	Baseline	Week 12	Baseline	Week 12	Protein level	Fiber level	Interaction
Waist circumference (cm)[1]	99.2 (11.4)	99.3 (12.0)	99.1 (10.8)	100.3 (10.3)	102.4 (10.1)[3]	104.0 (11.0)	98.1 (11.8)	99.0 (11.5)	0.75	0.57	0.34
Hip circumference (cm)[1]	105.0 (8.1)	104.1 (8.7)	109.8 (5.7)	110.0 (5.5)	110.7 (9.5)[3]	111.8 (11.1)	105.6 (9.1)	106.0 (8.1)	0.16	0.51	0.32
Lean body mass (kg)[1]	51.2 (11.4)	51.6 (11.9)	51.9 (11.5)	52.6 (11.5)*	53.1 (9.4)	53.6 (9.1)	48.0 (10.3)	48.6 (10.3)*	0.91	0.48	0.75
Fat mass (kg)[1]	30.8 (7.5)	30.6 (8.5)	34.1 (4.5)	34.0 (4.7)	34.5 (10.2)	34.9 (10.5)	32.0 (5.7)	32.2 (6.0)	0.14	0.77	0.56
Fat percentage (% of total body mass)[2]	33.7 (31.7–43.1)	33.5 (29.9–44.1)	39.9 (33.5–44.6)	37.8 (32.2–44.8)	37.2 (31.1–45.2)	37.6 (31.3–45.3)	38.8 (35.6–42.8)	38.2 (35.0–43.6)	0.19	0.72	0.63
Android/gynoid ratio[1]	1.11 (0.19)	1.08 (0.19)*	1.07 (0.15)	1.07 (0.16)	1.07 (0.18)	1.07 (0.16)	1.05 (0.18)	1.04 (0.19)	0.15	0.33	**0.03**
Fasting energy expenditure (kcal/d)[1]	1546 (322)[4]	1563 (268)	1630 (283)[5]	1588 (264)	1528 (234)	1560 (225)	1392 (338)	1433 (297)	0.23	0.45	0.34
PP energy expenditure (kcal/d)[1]	2006 (296)	2015 (299)	2028 (301)	2036 (329)	1902 (271)	2003 (312)	1784 (349)	1693 (359)	0.93	0.05	0.07
Fasting RER[1]	0.83 (0.04)[4]	0.82 (0.04)	0.81 (0.05)[5]	0.83 (0.06)	0.80 (0.04)	0.84 (0.07)	0.81 (0.07)	0.81 (0.05)	0.38	0.58	0.19
PP RER[1]	0.81 (0.03)	0.81 (0.04)	0.79 (0.04)	0.79 (0.05)	0.81 (0.03)	0.83 (0.03)	0.83 (0.04)	0.81 (0.04)	0.77	0.26	**0.04**
Hunger (mm × 360 min)[1]	15,022 (6845)	14,299 (7606)[a,b]	12,906 (6907)	9245 (6348)[a*]	8984 (6306)	10,924 (6918)[b]	12,538 (6743)	12,330 (4928)[a,b]	**0.02**	0.08	0.78
Satiety (mm × 360 min)[1]	21,216 (5605)	21,785 (7662)	22,927 (6492)	24,924 (6512)	25,677 (6553)	24,218 (6592)	20,402 (7108)	23,016 (4233)	0.56	0.06	0.35
Fullness (mm × 360 min)[1]	20,037 (5508)	20,638 (7139)	21,873 (7369)	24,835 (6992)*	24,802 (7036)	24,371 (5113)	20,307 (7327)	22,628 (4316)	0.47	0.07	0.92
Prospective food consumption (mm × 360 min)[1]	15,909 (6751)	14,809 (7973)	14,657 (7192)	10,852 (6387)*	11,544 (8310)	12,758 (7006)	16,363 (6483)	14,678 (3585)	0.15	0.07	0.94

ANOVA analysis of variance, *HiFi* high fiber, *LoFi* low fiber, *MD* maltodextrin, *PP* postprandial, *RER* respiratory exchange ratio, *WP* whey protein.

*Indicates significant within-group change from baseline ($P < 0.05$). *P* values indicate main effects of protein and fiber level and interactions.

P-values < 0.05 are written in bold.

[1]Means (SD).

[2]Median (25th–75th centile).

[3]$n = 15$.

[4]$n = 14$.

[5]$n = 16$.

Table 3 Ambulatory blood pressure, heart rate and augmentation index at baseline and week 12.

	WP-LoFi		WP-HiFi		MD-LoFi		MD-HiFi		Two-factor ANOVA, *P*		
	($n = 15$)		($n = 17$)		($n = 16$)		($n = 17$)				
	Baseline	Week 12[a]	Baseline	Week 12	Baseline	Week 12[b]	Baseline	Week 12	Protein level	Fiber level	Interaction
SBP (mmHg)	126.5 (10.8)	129.6 (10.5)	127.5 (12.6)	124.8 (12.7)	122.9 (10.1)	125.4 (9.9)	122.8 (10.4)	124.3 (10.1)	0.34	0.13	0.24
DBP (mmHg)	70.5 (11.0)	71.4 (8.8)	71.4 (11.6)	69.6 (11.0)	69.4 (9.2)	70.6 (8.9)	72.5 (8.9)	7.6 (7.2)	0.69	0.23	0.31
Heart rate (bpm)	72.5 (11.2)	71.3 (12.0)	71.5 (10.3)	69.8 (10.4)	68.6 (8.9)	68.1 (9.5)	70.4 (7.0)	70.2 (5.5)	0.41	0.99	0.84
Augmentation index (%)	29.7 (9.0)	31.4 (8.7)	30.7 (9.5)[c]	31.5 (8.6)[c]	29.8 (8.3)	28.9 (6.3)	32.3 (9.2)	33.7 (10.3)	0.68	0.91	0.33

Values are means (SD).

ANOVA analysis of variance, *bpm* beats per minute, *DBP* diastolic blood pressure, *HiFi* high fiber, *LoFi* low fiber, *MD* maltodextrin, *SBP* systolic blood pressure, *WP* whey protein.

[a]$n = 14$.

[b]$n = 15$.

[c]$n = 16$.

support any beneficial nor detrimental effects of high protein intake [39]. This discrepancy may relate to differences in protein amount but also differing protein sources. Thus, prospective studies on protein intake have reported a positive association between risk of T2D and total protein intake [40]. When protein type was assessed, however, red meats were closely associated with an increased risk of T2D, whereas dairy protein was found to be protective of T2D and CVD [41–43].

During weight-reducing diets, a high intake of WP may preserve muscle mass in obese subjects [44]. Likewise, intake of wholegrains as part of a hypocaloric diet may reduce abdominal fat mass, without differential effects on body weight [45]. Thus, in the present study we assessed whether there were beneficial effects of WP and fiber on body composition, even in the absence of weight loss. However, this was not the case. Accordingly, previous studies using comparable amounts of WP [4, 5, 46] or cereal fiber [31] found no effects on body composition under energy-balanced conditions. Furthermore, we found no effects on fasting or postprandial REE or RER, which also was found in a study by Kjolbaek et al. [46] comparing WP with MD supplementation for 24 weeks. Any minor increase in thermogenesis induced by protein and/or fiber may require a longer time period than 12 or 24 weeks to affect body composition.

In the present study, intake of WP supplements for 12 weeks reduced the subjective ratings of hunger compared with MD. This was, however, not supported by any change in fullness, desire to eat or prospective food consumption. The reduced postprandial hunger sensation after WP intake may be explained by an increase in the anorexogenic PYY, as observed in the WP-LoFi group. However, we found no increase in PYY in the WP-HiFi group, even though the greatest reduction in hunger sensation was reported in this group.

Dietary fiber did not affect appetite. A systematic review of acute studies concluded that the majority of dietary fiber interventions had no effects on satiety or food intake [47]. Because of their higher viscosity, soluble fibers may enhance satiety more than insoluble fibers [48, 49], although this is debated [47]. Wheat bran contains mainly insoluble fiber and is considered to possess viscous-forming capacity [50]. The wheat bran of the present study was enzymatically treated to increase the content of soluble AXOS and subsequently increase satietogenic gut peptides as found in mice [51], but it did not result in any effects on appetite.

We found no effects of the interventions on BP. Two previous studies applying similar doses of WP supplements reported reductions in BP compared with carbohydrate [3, 4]. The two previous studies used WP isolate. WP-derived peptides are believed to exert antihypertensive effects via inhibition of angiotensin-converting enzyme (ACE) [52]. However, the degree of hydrolysis affects the

ACE inhibitory activity of the resulting WP peptides [53]. The use of hydrolysate rather than isolate in the present study may have negated an effect on BP. Additionally, it should be noted that the average BP of the participants at baseline was within the normal range and that 17 out of the 65 participants received antihypertensive medication [27]. We cannot exclude that an effect on BP may exist in subjects with untreated hypertension.

A strength of our study is that the participants managed to remain weight stable during the trial—thereby eliminating any impact of weight loss per se. It should be noted, that since participants were urged to maintain weight stability, any potential metabolic changes induced by weight loss following long-term reduction in hunger sensation is not reflected in the present study. We may have had insufficient power to detect differences in the reported outcomes, since the sample size calculation was based on postprandial triglyceride responses [27].

In conclusion, intake of WP and high dietary fiber from enzyme-treated wheat bran for 12 weeks did not affect insulin sensitivity, body composition or BP in weight stable subjects with abdominal obesity compared with MD and low dietary fiber. The present study suggests that WP consumption may reduce appetite; however, further investigation is warranted to clarify this.

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Author contributions RF-N, ER, KEBK, SG, and KH conceived and designed the study. RF-N and ER conducted the study. BH, JJH, and BL provided essential analytical and diagnostic assistance. RF-N and ER analyzed the data and wrote the first draft of the paper. All authors reviewed and approved the final article.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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