The Combination of Whey Protein and Dietary Fiber Does Not Alter Low-Grade Inflammation or Adipose Tissue Gene Expression in Adults with Abdominal Obesity

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Abstract

BACKGROUND: Abdominal obesity is characterized by low-grade inflammation and plays a central role in the development of type 2 diabetes and cardiovascular diseases. Dietary factors can influence low-grade inflammation and affect adipose tissue function. AIM: To investigate the separate and combined effects of whey protein and cereal fiber on inflammatory markers and adipose tissue gene expression in abdominal obesity. METHODS: We performed a 12-week, double-blind, randomized controlled dietary intervention in 65 adults with abdominal obesity. The participants were randomized to 4 groups using a 2 × 2 factorial design; they received either 60 g/day of whey protein or maltodextrin in combination with high-fiber wheat bran products (30 g fiber/day) or low-fiber refined wheat products (10 g fiber/day). Plasma concentrations of tumor necrosis factor-α (TNF-α), high-sensitivity C-reactive protein (hs-CRP), monocyte chemoattractant protein-1 (MCP-1), interleukin 1 receptor antagonist (IL-1Ra), and adiponectin were measured before and after intervention. Changes in gene expression related to inflammation, insulin signaling, and lipid metabolism were measured in abdominal subcutaneous adipose tissue. RESULTS: After intervention, TNF-α was reduced for both high-fiber groups compared with baseline, but did not significantly differ from the low-fiber groups. There were no differences in fasting or postprandial inflammatory markers between the groups. The relative gene expression of ribosomal protein S6 kinase B1 (S6K1) was increased after whey protein consumption. CONCLUSION: Intake of whey protein in combination with high cereal fiber content did not differentially affect low-grade inflammation or adipose tissue gene expression compared with maltodextrin consumption. Keywords: whey protein · wheat bran · dietary fiber · inflammation · adipose tissue gene expression

1. Introduction

Low-grade inflammation is closely related to obesity and plays a central role in the development of type 2 diabetes (T2D) and cardiovascular diseases (CVD) [1-4]. A number of circulating pro-inflammatory markers are found to be elevated in abdominal obesity, e.g. C-reactive protein (CRP), monocyte chemoattractant protein-1 (MCP-1), and tumor necrosis factor-alpha (TNF-α) [5-7]. Furthermore, postprandial inflammation is exaggerated in individuals with obesity, and may be involved in the development of insulin resistance, which plays a major role in the development of both T2D and CVD [8-10]. A dietary pattern characterized by low-fat dairy and whole-grain products is positively associated with the anti-inflammatory marker adiponectin.
and high intake of dairy products is associated with lower levels of CRP and TNF-α [12]. Observational studies indicate that foods rich in cereal fiber, such as whole grains and bran, are associated with lower levels of pro-inflammatory markers [13, 14]. However, results from intervention studies are inconsistent [15]. Randomized, controlled trials have indicated a reduction in CRP concentration following consumption of whey protein (WP) supplements (≥20 g/day) [16]. Concurrently, both milk peptides and dietary fiber may have beneficial effects on low-grade inflammation [17]. However, little is known about the impact of the combination of milk proteins and dietary fiber.

Adipose tissue is an active endocrine organ that releases pro-inflammatory adipokines, including TNF-α, MCP-1, and interleukin-1 receptor antagonist (IL-1Ra), as well as the anti-inflammatory protein adiponectin. Adipose tissue dysfunction leading to disturbances in the production or secretion of adipokines may increase insulin resistance in abdominal obesity [18]. Dietary constituents have also been found to affect gene expression in adipose tissue; high-protein diets have been shown to downregulate subcutaneous adipose tissue (SAT) expression of genes involved in lipid metabolism [19], while a high-fiber/low-insulin-response diet has been shown to downregulate genes involved in insulin signaling [20].

In this article, we aimed to investigate the effects of WP supplements and high-fiber diets on circulating inflammatory markers (both fasting and postprandial) and to analyze SAT expression of genes involved in inflammation, lipid metabolism, and insulin signaling. We hypothesized that intake of WP and high-fiber cereal products (either in combination or separately) would have beneficial effects on inflammation compared with isoenergetic intake of maltodextrin (MD) and low-fiber cereal products. This was tested in a 12-week, randomized controlled, double-blind dietary intervention study in adults with abdominal obesity.

2. Methods

The present study is part of the MERITS study that investigates the effects of WP and cereal fiber on lipemia and metabolic changes. The study was conducted at the Department of Endocrinology and Internal Medicine, Aarhus University Hospital, Denmark, between May 2016 and June 2017. The study protocol was approved by the Central Denmark Region Committees on Health Research Ethics (Journal no. 1-10-72-370-15). Details of study design, participants, and dietary interventions have been published elsewhere [21].

2.1 Study design, participants, and dietary intervention

We conducted a double-blind, randomized controlled, parallel intervention with a 2 × 2 factorial design. Inclusion and exclusion criteria have been described in detail elsewhere [21]. In short, 73 men and women (age ≥40 years) with abdominal obesity (waist circumference ≥80 cm for women and ≥94 cm for men) were randomized to 1 of 4 intervention diets for 12 weeks preceded by one week of run-in.

The participants were provided with isocaloric powder supplements containing either 2 × 30 g/d WP (Lacprodan® HYDRO.REBUILD) or MD (Gluclux® 19), in combination with either wheat bran products containing 30 g/d dietary fiber (HiFi) or refined wheat products containing 10 g/d dietary fiber (LoFi). The powder supplements were provided by Arla Foods Ingredients Group P/S (Viby, Denmark) and the wheat bran by Lantmännen Cerealia AB (Malmö, Sweden). The wheat bran was treated with cell wall-degrading enzymes (xylanase, glucanase, cellulase) by DuPont Industrial Biosciences Aps (Brabrand, Denmark) in order to increase the content of arabinoxylan oligosaccharides (AXOS) and improve the baking properties of the bran [22].

The participants were instructed to replace their habitual intake of bread and cereal products with the products provided, and to consume the...
powder supplements twice daily. They were encouraged not to change other dietary habits during the intervention period and to maintain their physical activity level to ensure weight stability. Both participants and study personnel remained blinded to the group allocations throughout the study. Compliance was assessed by measuring plasma alkylresorcinols (a marker of cereal bran intake) and urinary carbamide excretion (a protein intake marker). Also, the participants filled out a daily test product journal that was used to monitor compliance during the trial.

Before and after the 12-week intervention, the participants underwent a standardized, high-fat meal test (4700 kJ, 70 g of fat) following an overnight fast, as described previously [21]. A catheter was placed in an antecubital vein for blood sampling. Fasting blood samples were collected for measurement of TNF-α, IL-1Ra, hs-CRP, MCP-1, and adiponectin. During the test meal, postprandial blood samples were collected at t = 30, 60, 120, and 240 min for measurement of MCP-1, and at t = 30, 60, 120, and 360 for measurement of adiponectin.

2.2 Measurement of inflammatory markers

Blood samples for measuring TNF-α, IL-1Ra, and adiponectin were immediately centrifuged at 2000 × g for 15 min at 4 °C. Serum samples for measuring MCP-1 and hs-CRP were left at room temperature for 30 min before being centrifuged for 10 min at 2000 × g. Plasma and serum samples were then frozen at -20 °C and moved to -80 °C within 8 h for storage.

TNF-α, MCP-1, IL-1Ra, and adiponectin were all measured using ELISA technique. TNF-α was determined using a human Quantikine™ High Sensitivity kit (cat. HSTA00E, R&D Systems, Minneapolis, USA), with assay range of 0.2-10 pg/ml, intra-assay precision of 1.9-2.2%, and inter-assay precision of 6.2-6.7%. MCP-1 was measured using LEGEND MAX™ Human MCP-1/CCL2 ELISA kit with pre-coated plates (cat. 438808, BioLegend, San Diego, USA), with assay range of 7.8-500 pg/ml, intra-assay precision of 6.0-6.4%, and inter-assay precision of 1.8-6.0%. Adiponectin was determined using a human adiponectin ELISA kit (cat. K1001-1, B-Bridge International, Inc., Santa Clara, USA), with assay range 0.375-12.0 ng/ml, intra-assay precision 4.6-5.8%, and inter-assay precision 3.2-7.3%. IL-1Ra was determined using a human Quantikine™ kit (cat. DRA00B, R&D Systems, Minneapolis, USA), with assay range of 31.2-2000 pg/ml, intra-assay precision of 3.7-7.3%, and inter-assay precision of 6.7-11.0%. Hs-CRP was measured on a Cobas c 111 system using a commercial kit (ref. 05401607, Roche Diagnostics GmbH, Mannheim), with assay range of 0.15-20.0 mg/l, intra-assay precision of 0.3-1.5%, and inter-assay precision of 0.7-2.0%.

2.3 Adipose tissue biopsies

Biopsies were collected from abdominal SAT before and after dietary intervention. The procedure was performed under local analgesia using a Bergström needle. The tissue biopsies were cleaned with saline and snap-frozen in liquid nitrogen before storage at -80 °C.

2.4 Gene expression analyses

RNA purification and gene expression analyses were performed by BioXpædia A/S (Aarhus, Denmark), using real-time qPCR with pre-designed primers and TaqMan gene expression assays (Applied Biosystems, Life Technologies, California, USA). An overview of the assay identification numbers is given in supplementary Table A1. We measured the cycle threshold (Ct) values for each sample in triplicate. The mean fold change in the target genes, normalized to the reference gene β-actin (ACTB) and relative to the expression at week 0, was calculated by the 2-∆∆CT method [23]. The expression of the reference gene ACTB was not different between groups at baseline, and was not affected by the different dietary interventions (assessed by one-way ANOVA).

2.5 Calculations and statistical analyses

The postprandial responses in MCP-1 and adiponectin before and after intervention were calculated as total areas under the curve (AUC) for 240 min (MCP-1) and 360 min (adiponectin) using the trapezoidal rule.

Statistical analyses were performed in Stata IC/15.1 (StataCorp LP College Station, TX, USA), and graphical elements were constructed in GraphPad Prism 7.04 (Graphpad Software, CA, USA). P-values < 0.05 were considered statistically significant. Variables were assessed for normal distribution by quantile-quantile plots and histograms, and homogeneity of variance was assessed by Bartlett’s test. The distribution of paired data was further assessed by Bland-Altman plots. If the data were found not to be normally distributed, log-transformation was applied and the data were reassessed.
3.1 Inflammation

Fasting inflammatory markers at baseline and week 12 are presented in Table 1. After intervention, we observed a decrease in fasting TNF-\(\alpha\) for the two HiFi groups, but this change did not significantly differ from the LoFi groups. Compared with baseline, TNF-\(\alpha\) was decreased by 12% for WP-HiFi (95% CI: 4-19%, \(p<0.05\)) assessed by Wilcoxon signed-rank test. Abbreviations: hs-CRP - high-sensitivity C-reactive protein, IL-1Ra - interleukin 1 receptor antagonist, MCP-1 - monocyte chemoattractant protein-1, TNF-\(\alpha\) - tumor necrosis factor \(\alpha\).

We tested between-group differences in inflammatory markers using a nonparametric Kruskal-Wallis test, and within-group changes in inflammatory markers from baseline using Wilcoxon signed-rank test because data did not fulfill criteria for normal distribution and/or homogeneity of variance after log-transformation. Differences in relative gene expression between diets were assessed by a two-factor ANOVA adjusted for age and sex. Pairwise comparisons of groups were corrected for multiple comparisons by the Tukey-Kramer method. Data were checked by diagnostic plots of residuals (quantile-quantile plots, histograms, and residuals versus fitted plots). We assessed within-group changes in relative gene expression from baseline by paired t-tests. We applied the Benjamini-Hochberg procedure with a false discovery rate (FDR) of 0.25 to account for multiple statistical testing of the gene expression data [24].

3.2 Gene expression

Relative changes in SAT gene expression after the 12-week intervention period are shown in Figures 1-3. There was a main effect of protein level on ribosomal protein S6 kinase B1 (S6K1) of 29.4 \(\pm\) 3.7 kg/m\(^2\), and 52% fulfilled the International Diabetes Federation criteria for the metabolic syndrome. The participants remained weight stable throughout the intervention [21].
gene expression ($p = 0.02$), showing an increase after WP consumption compared with MD consumption. After intervention, the relative gene expression of 56K1 was significantly increased for WP-HiFi compared with MD-HiFi ($p = 0.02$ after correction for multiple comparisons) (Figure 3). MD-HiFi had a greater increase in resistin (RETN) gene expression compared with MD-LoFi ($p = 0.01$ after correction for multiple comparisons) (Figure 1). Any within-group changes in gene expression from baseline were not statistically significant after FDR correction.

### 4. Discussion

In the present study, we investigated the effects of WP supplements or a carbohydrate control in combination with high-fiber or low-fiber cereal products on circulating inflammatory markers and SAT gene expression. We found a reduction in the pro-inflammatory cytokine TNF-$\alpha$ compared with baseline after consumption of the wheat bran-rich diet for 12 weeks. However, this change was not significantly different from that observed in the refined wheat groups.

The MERITS study was originally powered to detect differences in postprandial triglycerides, which was the primary outcome [21]. We cannot exclude that the sample size may have been too small to detect differential effects on plasma cytokines. However, previous studies of similar or smaller sample sizes and shorter durations have detected differences in circulating TNF-$\alpha$ between a wholegrain wheat diet and a refined wheat diet in subjects with overweight and obesity [25, 26]. This discrepancy in results could be due to differences in study populations; the previous studies included only individuals with low intakes of wholegrain and dietary fiber at baseline, while our study population was adapted to a relatively high dietary fiber intake, corresponding to the Danish national average of 22 g/day [27]. It is possible that we would have observed a significant impact of our interventions in individuals with a less healthy background diet.

We found no effects of the interventions on postprandial responses of MCP-1 or adiponectin. A previous study in adults with overweight and obesity found that consumption of wholegrain wheat for 12 weeks reduced the postprandial response of several inflammatory markers compared with consumption of refined wheat [28]; however, they investigated different inflammatory markers than we did in the present study.

While consumption of wholegrain and cereal bran products has been associated with lower levels of inflammation [13, 14, 29], the mechanisms behind this association remain largely unknown, and may be attributed to a range of constituents present in the grain [30, 31]. Wheat grain contains a number of phytochemicals and dietary fiber types, which are mainly located in the bran fraction [32]. In the present study, we treated the wheat bran with cell wall-degrading enzymes prior to incorporating it into the test products. This bioprocessing may potentially have enhanced the availability of bioactive compounds [32]. Interestingly, a previous study showed that bioprocessing of wheat bran with cell wall-degrading enzymes and yeast fermentation greatly increased the bioavailability of phenolic acids, which are considered to possess anti-inflammatory properties [33]. In the same study, bioprocessed wheat bran exerted anti-inflammatory effects in lipopolysaccharide (LPS) stimulated blood ex vivo compared with native wheat bran. In the present study, the enzymatic treatment of the wheat bran changed the composition of the soluble and insoluble dietary products on circulating inflammatory markers and SAT gene expression. We found a reduction in the pro-inflammatory cytokine TNF-$\alpha$ compared with baseline after consumption of the wheat bran-rich diet for 12 weeks. However, this change was not significantly different from that observed in the refined wheat groups. The MERITS study was originally powered to detect differences in postprandial triglycerides, which was the primary outcome [21]. We cannot exclude that the sample size may have been too small to detect differential effects on plasma cytokines. However, previous studies of similar or smaller sample sizes and shorter durations have detected differences in circulating TNF-$\alpha$ between a wholegrain wheat diet and a refined wheat diet in subjects with overweight and obesity [25, 26]. This discrepancy in results could be due to differences in study populations; the previous studies included only individuals with low intakes of wholegrain and dietary fiber at baseline, while our study population was adapted to a relatively high dietary fiber intake, corresponding to the Danish national average of 22 g/day [27]. It is possible that we would have observed a significant impact of our interventions in individuals with a less healthy background diet.

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Figure 1. Relative changes from baseline to week 12 in the expression of selected genes involved in inflammation (mean and SD). Gene expression was measured in subcutaneous adipose tissue of 61 participants (WP-LoFi: n = 15, WP-HiFi: n = 16, MD-LoFi: n = 14, MD-HiFi: n = 16), except for RETN, where N = 59 due to measurement error (WP-LoFi: n = 14, WP-HiFi: n = 16, MD-LoFi: n = 14, MD-HiFi: n = 15). * Significant difference between groups assessed by two-factor ANOVA (p < 0.05 after correction for multiple comparisons by the Tukey-Kramer method). # Within-group change from baseline assessed by paired t-test (p < 0.05 before FDR correction, p > 0.05 after FDR correction). Abbreviations: FDR – false discovery rate, MD-HiFi – maltodextrin + high fiber, MD-LoFi – maltodextrin + low fiber, WP-HiFi – whey protein + high fiber, WP-LoFi – whey protein + low fiber.

fiber fractions, particularly increasing the AXOS content. Enzyme-treated wheat bran with an increased AXOS content has been shown to induce anti-inflammatory effects in LPS-stimulated mice [34], but this effect is yet to be demonstrated in low-grade inflammation.

Except for the within-group reduction in TNF-α in the group receiving WP-HiFi, we found no effects of the WP intervention on low-grade inflammation. A study conducted in patients with acute ischemic stroke showed beneficial effects on inflammation after short-term enteral feeding with
investigated the long-term expression of selected genes involved in inflammation and lipid metabolism and correlated in lipid metabolism and certain genes involved in inflammation and lipid metabolism. Despite our previous studies having detected depot-specific differences in gene expression, we found no conclusive effects were attenuated after 18 weeks. We observed no difference in circulating insulin sensitivity (VAT) also, since studies have detected depot-specific differences in gene expression, we found no other indications of increased metabolism. A limitation of our study is that for most of the participants were weight stable during the interventions in the expression of genes related to lipid metabolism, despite our previous studies having detected depot-specific differences in gene expression. It would have been interesting to perform an acute study detecting a beneficial change in this group. In contrast, a control diet with low-fiber cereal products has previously been shown to increase IL-1Ra compared to a control diet with fat meal and WP compared with other protein products compared with a potato meal. We observed a within-group reduction in IL-1Ra after intake of a fiber-rich wheat-bran meal compared with a potato meal. The acute response in association with insulin resistance and inflammation in this group. A study from our previous studies has in our results corroborate the onset of T2D. A reduction in IL-1Ra elevated in obesity, and increased levels were strongly associated with insulin resistance and increased plasma levels of BCAAs are thought to block insulin signaling via inactivation of S6K1 through phosphorylation. We observed a reduction in IL-6 after intake of a fiber-rich high-fat meal with WP compared with other protein products compared with a potato meal.

Interestingly, we found an increase in SAT gene expression, while the acute response in CC chemokine ligand 5/RANTES or IL-6 in the present study.

Figure 2. Relative changes from baseline to week 12 in the expression of selected genes involved in lipid metabolism and cell differentiation (mean and SD). Gene expression was measured in subcutaneous adipose tissue of 61 participants (WP-LoFi: n = 15, WP-HiFi: n = 16, MD-LoFi: n = 14, MD-HiFi: n = 16). * Within-group change from baseline assessed by paired t-test (p < 0.05 before FDR correction, p > 0.05 after FDR correction). Abbreviations: FDR – false discovery rate, MD-HiFi – maltodextrin + high fiber, MD-LoFi – maltodextrin + low fiber, WP-HiFi – whey protein + high fiber, WP-LoFi – whey protein + low fiber.

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WP [35]. However, results from trials in healthier study populations, using a similar duration and WP dose to those in the present study, corroborate our results [36-38].

Furthermore, we found no long-term differences in postprandial responses of MCP-1 or adiponectin between the interventions. To our knowledge, few previous studies have investigated the long-term effects of dietary interventions on fat-induced postprandial inflammation. A study from our group previously reported no effects on postprandial MCP-1 after intake of WP or casein for 12 weeks [37]. However, an acute study detected a higher postprandial MCP-1 response following a high-fat meal with WP compared with other proteins, while the acute response in CC chemokine ligand-5 (CCL5/RANTES) was lower after the WP meal [39]. Another acute study found a reduction in postprandial IL-6 after intake of a fiber-rich wheat-bran meal compared with a potato meal [8]. However, we did not measure circulating CCL5/RANTES or IL-6 in the present study.

We observed a within-group reduction in IL-1Ra in the MD-LoFi group. Circulating IL-1Ra is elevated in obesity [40], and increased levels precede the onset of T2D [41]. A reduction in IL-1Ra after the intervention with MD and low-fiber products could therefore indicate an unexpected beneficial change in this group. In contrast, a control diet with low-fiber cereal products has previously been shown to increase IL-1Ra compared with a healthy, high-fiber Nordic diet [42]. In this regard, it is relevant to point out that the observed reduction from baseline for MD-LoFi was not significantly different compared with the other intervention groups in the present study.

Interestingly, we found an increase in SAT gene expression of S6K1 after WP compared with MD consumption. S6K1 encodes a kinase involved in the mammalian target of rapamycin (MTOR) signaling pathway, a key regulator of protein synthesis [43]. Intake of leucine—a branched-chain amino acid (BCAA) which is abundant in WP—induces activation of S6K1 through phosphorylation [44]. However, it is important to note that we did not measure phosphorylation of S6K1 in the present study. Activation of the MTOR pathway by BCAAs is thought to block insulin signaling via inactivation of insulin receptor substrate 1 (IRS1) [45, 46]. Furthermore, elevated plasma levels of BCAAs are strongly associated with insulin resistance and predict the onset of T2D [47, 48]. Consequently, it has been hypothesized that high-protein diets play a role in the de-

Figure 3. Relative changes from baseline to week 12 in the expression of selected genes involved in insulin signaling (mean and SD). Gene expression was measured in subcutaneous adipose tissue of 61 participants (WP-LoFi: n = 15, WP-HiFi: n = 16, MD-LoFi: n = 14, MD-HiFi: n = 16). *Significant difference between groups assessed by two-factor ANOVA (p < 0.05 after correction for multiple comparisons by the Tukey-Kramer method). # Within-group change from baseline assessed by paired t-test (p < 0.05 before FDR correction, p > 0.05 after FDR correction).

Abbreviations: FDR – false discovery rate, MD-HiFi – maltodextrin + high fiber, MD-LoFi – maltodextrin + low fiber, WP-HiFi – whey protein + high fiber, WP-LoFi – whey protein + low fiber.
velopment of insulin resistance [49, 50]. Interestingly, one study found a tendency for higher S6K1 protein expression following 6 weeks of high-protein diet, which was associated with reduced insulin sensitivity [51]. However, these effects were attenuated after 18 weeks. We found no changes in MTOR or IRS1 expression, in accordance with a previous study comparing two different high-protein diets [19]. It would have been interesting to determine the activation of the MTOR pathway in skeletal muscle tissue, since it has been demonstrated in humans that phosphorylation of both MTOR and S6K1 is increased in skeletal muscle following intake of a dairy protein-rich meal [52].

Furthermore, we found an increase in RETN gene expression in SAT after 12 weeks of MD-HiFi compared with MD-LoFi. RETN encodes resistin, a protein with pro-inflammatory properties which has been linked to abdominal obesity and insulin resistance. The role of resistin is, however, still inconclusive [53]. The increased RETN gene expression in MD-HiFi could indicate an unfavorable change in inflammation in this group. However, this finding should be interpreted with caution, since we found no other indications of increased inflammation in this group. We observed no differences between interventions in the expression of genes related to lipid metabolism, despite our previously reported findings regarding beneficial changes in the plasma lipid profile after the WP-LoFi diet [21].

A limitation of our study is that for most of the markers of inflammation, insulin signaling, and lipid metabolism we measured mRNA levels only; changes in gene expression do not necessarily correspond to changes in plasma protein levels [54]. Furthermore, we observed a large variation in mRNA levels of several of the genes related to inflammation. It is possible that some of this variability is due to viral infections during the trial, although the few cases of self-reported illnesses among our participants do not explain the outliers (data not shown). It would have been interesting to measure gene expression in visceral adipose tissue (VAT) also, since studies comparing SAT and VAT have detected depot-specific differences in gene expression, with VAT showing higher expression of certain genes involved in inflammation and lipid metabolism [55].

An important strength of our study is that the participants were weight stable during the intervention. Weight loss has great influence on low-grade inflammation [17], and differences in weight change between groups would have made it difficult to evaluate the effect of the test products themselves. Other strengths of our study include the use of a double-blind, randomized controlled design, and the fact that we measured biochemical markers in addition to self-reported data to verify a high degree of compliance with our interventions. However, the sample size may have been too small to detect differences in inflammatory markers.

In conclusion, intake of WP supplements and high-fiber wheat bran products for 12 weeks did not significantly influence fasting or postprandial markers of low-grade inflammation, and did not have a significant impact on SAT gene expression compared with MD and refined wheat products in participants with abdominal obesity.

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**Author contributions:** All authors designed the study; ER and RFN conducted the research. ER performed the statistical analyses and had primary responsibility for writing the manuscript; all authors contributed to, and approved of, the final manuscript.

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**Appendix**

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Table A1. Gene symbols (with their commonly used aliases in parentheses), gene names, and the corresponding TaqM an Gene Expression Assay IDs

| Gene symbol | Gene name | Assay ID          |
|-------------|-----------|-------------------|
| RPS6KB1(S6K1) | Ribosomal protein S6 kinase B1 | Hs00356367_m1 |
| CCL5 | C-C motif chemokine ligand 5 | Hs00982282_m1 |
| SLC2A4 | Solute carrier family 2 member 4 | Hs00168966_m1 |
| LPL | Lipoprotein lipase | Hs00173425_m1 |
| IL6 | Interleukin 6 | Hs00174131_m1 |
| LEP | Leptin | Hs00174877_m1 |
| IRS1 | Insulin receptor substrate 1 | Hs00178563_m1 |
| DGAT1 | Diacylglycerol O-acyltransferase 1 | Hs00201385_m1 |
| RETN | Resistin | Hs00220767_m1 |
| CCL2(MCP3) | C-C motif chemokine ligand 2 | Hs00234140_m1 |
| MTOR | M enhanic target of rapamycin | Hs00234522_m1 |
| PPARG | Peroxisome proliferator activated receptor gamma | Hs00234592_m1 |
| FGF1 | Fibroblast growth factor receptor 1 | Hs00241111_m1 |
| CD36 | CD36 molecule | Hs00354519_m1 |
| ADIPOR1 | Adiponectin receptor 1 | Hs00360422_m1 |
| ADIPQO | Adiponectin; C1Q and collagen domain containing | Hs00605917_m1 |
| FFAR4 | Free fatty acid receptor 4 | Hs00699184_m1 |
| IL1RN (IL1RA) | Interleukin 1 receptor antagonist | Hs00893626_m1 |
| FASN | Fatty acid synthase | Hs01005622_m1 |
| ACACA (ACC1) | Acetyl-CoA carboxylase alpha | Hs01460407_m1 |
| SCD (SCD1) | Stearoyl-CoA desaturase | Hs01682761_m1 |
| ACTB - reference gene | Actin beta | Hs99999903_m1 |

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