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# Concentrated Arabinoxylan in Wheat Bread Has Beneficial Effects as Rye Breads on Glucose and Changes in Gene Expressions in Insulin-Sensitive Tissues of Zucker Diabetic Fatty (ZDF) Rats

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**ABSTRACT:** The health-promoting effects of dietary fiber may vary with content, structure, and composition in the diet. The aim was to study how low-fiber wheat bread (WB), wheat bread supplemented with wheat arabinoxylan (AX) or oat $\beta$-glucan (BG), whole meal rye bread (RM), and rye bread with kernels (RK) affected central parameters of glucose and lipid metabolism and gene changes of Zucker diabetic fatty rats. Blood glucose response areas after an oral glucose tolerance test were significantly lower after AX (mean ± SEM; 2117 ± 170 mmol/L·180 min), RM (1978 ± 206 mmol/L·180 min), and RK (2234 ± 262 mmol/L·180 min) breads than after WB (3586 ± 100 mmol/L·180 min; $p < 0.0001$). AX, RK, and RM changed expressions of adipose GAPDH, AMPK, FAS, SREBP-1c, and hepatic PCG-1$\alpha$, whereas BG had similar effects as WB. Thus, arabinoxylan added to wheat bread had beneficial effects on glycemic control as whole grain rye bread in this animal model.

**KEYWORDS:** *arabinoxylan, $\beta$-glucan, glucose tolerance, lipids, genes*

## INTRODUCTION

The frequency of type 2 diabetes (T2D) and cardiovascular disease (CVD) is increasing globally. An epidemiological study in women from the Nurses Health Study[1] indicated that intake of cereal dietary fiber (DF) and the glycemic load are factors decreasing the risk of T2D. A meta-analysis reached the same conclusion; low glycemic index (GI) diets reduced both fasting glucose and HbA1c.[2] DF may contribute to the preventive effect on T2D and CVD by increasing intestinal viscosity, slowing glucose absorption, delaying gastric emptying, producing short-chain fatty acids during colonic fermentation, increasing the rate of bile excretion to reduce cholesterol, and beneficially changing inflammatory markers.[3−5] Other factors, such as n-3 fatty acids, polyphenols, vitamins, and minerals, may also contribute to the beneficial preventive role of DF, particularly when the DF is consumed as whole grain cereals.

The renewed interest in DF has inspired the food industry to produce DF-enriched foods by utilizing DF-rich ingredients from the processing of whole grains or as isolates. The predominating DF polysaccharide in wheat and rye is arabinoxylan, whereas oats and barley have a high content of DF in the form of $\beta$-glucan. Arabinoxylan and $\beta$-glucan are present in cereals in soluble and insoluble forms, but a soluble fraction can be concentrated after appropriate processing.[6,7] Most human studies show that arabinoxylan reduces postprandial glucose and insulin responses[8−10] without an effect on plasma cholesterol.[10,11] Intake of $\beta$-glucan is shown to reduce postprandial glucose and insulin responses[5] and exerts a cholesterol-lowering effect.[12] However, this is not a consistent finding and depends on the type of $\beta$-glucan used.[13] Most studies focusing on isolated DF have used refined wheat products as a control, and only a few have compared isolated DF with both refined wheat products and whole grain products to assess the potential beneficial effect. The aim of the present study was to evaluate the effect of five bread-based diets varying in content and composition of DF on glucose and lipid metabolism as well as on key regulatory genes involved in glucose and lipid metabolism from adipose, liver, and skeletal muscle tissue of Zucker diabetic fatty (ZDF) rats. ZDF rats develop hyperglycemia, hyperlipidemia, and insulin resistance progressing to overt diabetes due to $\beta$-cell exhaustion.[14] We tested the effects of four high-DF breads, that is, rye breads with kernels (RK) and whole meal rye bread (RM) (positive controls) and two experimentally designed white wheat breads with added concentrates of either soluble oat $\beta$-glucan (BG) or soluble wheat arabinoxylan (AX). The four breads were compared with white wheat bread (WB) as a negative control. The design allowed a comparison of bread supplemented with soluble concentrated DF with whole grain breads, where the DF included both soluble and insoluble DF either in milled form or with a high proportion of intact kernels. We hypothesized that the chemical composition and structural integrity of DF may play a role in the preventive effects on the abnormal glucose and lipid metabolism in ZDF rats as well as on changes in gene expressions in insulin-sensitive tissues.

## MATERIALS AND METHODS

**Chemicals.** All chemicals used were purchased from Millipore (Billerica, MA, USA), Roche Diagnostics (GmbH, Mannheim, Germany), Leo Pharma (Ballerup, Denmark), Wako Chemicals

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(Richmond, VA, USA), and Sigma-Aldrich (St. Louis, MO, USA) unless otherwise indicated.

**Experimental Diets.** The soluble concentrated wheat arabinoxylan fraction was isolated from the soluble fraction of wheat flour after extraction of starch and gluten, concentrated by evaporation, heat treated, further treated with $\alpha$-amylase and glucoamylase to deplete residual starch, precipitated with ethanol (1:3 v/v), filtered, and finally dried on a spray-dryer (Manildra Group, Nowra, Australia). The arabinoxylan concentrate contained 46.4% nondigestible carbohydrates (NDC), 31.2% soluble nonstarch polysaccharides (NSP), and 23.4% soluble arabinoxylan and had a protein content of 39.7%. The concentrate had an arabinose to xylose ratio of 0.9 and a weight average molecular weight ($M_w$) of 602 kDa.[15] Soluble $\beta$-glucan (PromOat, BioVelop AB, Kimstad, Sweden) was obtained from the subaleurone of oats by combining wet-milling and enzymatic hydrolysis of oat bran. PromOat contained 46.8% NDC, 40.5% soluble NSP, 35.2% $\beta$-glucan of which 34.6% was soluble, 4.3% protein and had a $M_w$ of 1978 kDa.[15]

The dry ingredients of the AX bread were 67.8% refined wheat flour, 24.4% arabinoxylan concentrate, 0.9% sugar, 1.7% salt, 1.8% margarine (80% vegetable fat), and 3.5% yeast. The dough with a dry ingredient to water ratio of 2.1 was proofed for 30 min at 30 °C with steam and baked for 40 min at 160 °C (hot air and steam). The BG bread was made with 71.0% refined wheat flour, 13.3% PromOat, 6.9% Vitacel WF600 (J. Rettenmaier & Söhne GmbH, Germany), 1.0% wheat gluten, 0.9% sugar, 1.7% salt, 1.8% margarine (80% vegetable fat), and 3.5% yeast. Vitacel WF600, a refined commercial fiber product produced from wheat straw, consisted of 72.7% cellulose and 16.9% arabinoxylan with an arabinose to xylose ratio of 0.09. Vitacel WF600 was added to achieve a DF level comparable to that of the other high-DF breads. The dry ingredient to water ratio in the BG dough was 1.5, and proofing and baking conditions were the same as described for the AX bread. AX and BG bread were baked at a local bakery (Oerum, Denmark). RK, RM, and WB breads were commercial products provided by Lantmännen Food R&D, Schulstad (Hvidovre, Denmark), under the commercial names of Mulitikernerugbrød, Mørkt rugbrød, and Hvede toast, respectively. The ingredients for the RK bread were, in decreasing order, rye kernels (49%), water, rye sourdough, rye bread crumbs, refined wheat flour, salt, yeast, vinegar, dried sourdough rye, canola oil, rye, and barley flour. RM bread was made of rye whole meal, water, rye sourdough, rye bread crumbs, salt, vinegar, dried sourdough rye, canola oil, yeast, and barley flour. WB bread was made of refined wheat flour (68%), water, yeast, sugar, salt, vinegar, canola oil, emulsifier (E471, E472e), rye flour, barley malt flour, and flour treatment agent (ascorbic acid). All breads were stored at −20 °C, minced in a Wenoken bowl cutter, and dried at 80 °C for 20−24 h. The bread crumbs were mixed with 20% rat chow (Altromin 1324), adjusted so that the vitamin and mineral contents were the same as in 100% Altromin 1324 and pelleted afterward (Brogaarden, Lynge, Denmark). The composition of the diet pellets is shown in Table 1. Detailed information on composition and physicochemical properties are available elsewhere.[15,16]

**Chemical Analysis.** All chemical analyses of breads were performed in duplicate on freeze-dried materials. Dry matter was determined by drying to constant weight at 103 °C for 20 h, and ash was analyzed according to the AOAC method.[17] Nitrogen was measured by DUMAS,[18] and protein was calculated as N × 6.25. Fat was extracted with diethyl ether after HCl hydrolysis according to the Stoldt procedure.[19] Gross energy was analyzed by use of an oxygen bomb calorimeter (Parr Instrument Co., Moline, IL, USA). Starch and NSP were analyzed as described by Bach Knudsen.[20] Klason lignin was measured gravimetrically as the sulfuric acid-insoluble residue as described by Theander and Åman.[21] The $\beta$-glucan content was determined according to AOAC method 995.16 using a Megazyme kit (Megazyme International Ireland Ltd.). The content of low molecular weight nondigestible carbohydrates (LMW-NDC) was calculated as (total carbohydrates − NSP − starch). Total carbohydrate was analyzed as NSP without prior starch removal. The content of resistant starch (RS) in breads was calculated as [$NSP_{glucose}$ − (cellulose + $\beta$-glucan]. Available carbohydrates were the sum of sugars and starch.

**Table 1. Composition of Feed Pellets Based on WB, AX, BG, RM, and RK Breads[a]**

| | diet[b] | | | | |
|---|---|---|---|---|---|
| | WB | AX | BG | RM | RK |
| energy, MJ/kg | 17 | 17 | 17 | 16 | 16 |
| g/kg diet | | | | | |
| ash | 71 | 82 | 72 | 80 | 66 |
| crude protein (N × 6.25) | 160 | 218 | 154 | 135 | 121 |
| crude fat | 24 | 30 | 29 | 23 | 20 |
| starch[c] | 546 | 403 | 453 | 446 | 486 |
| LMW NDC[d] | 68 | 63 | 60 | 58 | 49 |
| resistant starch[e] | 4 | 6 | 12 | 8 | 8 |
| NSP[f] | | | | | |
| cellulose | 15 | 17 | 57 | 25 | 25 |
| $\beta$-glucan | 3 | 4 | 42 | 17 | 18 |
| arabinoxylan | 22 | 71 | 34 | 74 | 74 |
| total NSP | 61 | 128 | 164 | 148 | 147 |
| soluble NSP | 24 | 81 | 62 | 49 | 46 |
| insoluble NSP | 37 | 47 | 102 | 99 | 101 |
| klason lignin | 4 | 0 | 5 | 6 | 8 |
| total dietary fiber[g] | 133 | 191 | 229 | 211 | 204 |

[a]Pellets were composed of 80% bread crumbs and 20% Altromin 1324 and adjusted with vitamin and minerals so that the vitamin and mineral contents were the same as in 100% Altromin 1324. [b]WB, white bread; AX, arabinoxylan bread; BG, $\beta$-glucan bread; RM, whole meal rye bread; RK, rye bread with kernels. [c]Starch including free glucose and maltooligosaccharides. [d]LMW NDC, low molecular weight nondigestible carbohydrates. [e]Calculated as $NSP_{glucose}$ − (cellulose + $\beta$-glucan). [f]NSP, nonstarch polysaccharides. [g]Total dietary fiber defined as the sum of NSP, LMW-NDC, and klason lignin.

**Animals and Housing.** Sixty 6-week-old (205.2 ± 2.9 g) ZDF rats (ZDF-*Lepr*[fa]/Crl) were purchased from Charles River Laboratories (Sulzfeld, Germany) and housed individually in cages maintained at 22 °C and a relative humidity at 75% with a 12 h light/dark cycle. Rats were acclimated for 5 days and had unlimited access to a control diet (Altromin 1324, Brogaarden) and water in that period. All procedures were carried out in compliance with the principles of laboratory animal care, and the experiment was approved by the Danish Animal Experiments Inspectorate, Danish Veterinary and Food Administration.

**Feeding Trial.** Rats were randomly divided into 5 groups of 12 rats each and fed the 5 diets shown in Table 1. The rats were allowed free access to diet and tap water. Body weight and fasting blood glucose were monitored every second week after an overnight fast (Figure 1). Food consumption (24 h) was measured every second week. Baseline blood samples were drawn after an overnight fast and collected from the tip of the tail after gently preheating the tail. According to the glycemic status of the rats, a 7 week intervention period was chosen, and in the seventh week an oral glucose tolerance test (OGTT) was performed. After week 7, the rats were fasted for 12 h before intraperitoneal injection of a lethal dose of sodium pentobarbital. Blood was collected from the retrobulbar plexus. Subsequently, the rats were sacrificed by cervical dislocation. A midline laparotomy was performed to obtain liver, white adipose tissue, and soleus muscle samples. The tissue samples were frozen immediately at −80 °C in liquid nitrogen for later real-time PCR analysis.

**Oral Glucose Tolerance Test.** Rats were fasted for 12 h before the OGTT. Blood was drawn from the tip of the tail, and whole blood glucose was measured using One-Touch equipment (Precision Xceed MediSense, Abbott Laboratories A/S, Denmark). Blood was drawn at time points −15, 0, 30, 60, 90, 120, and 180 min. Immediately after the 0 min sample, D-glucose (2 g D-glucose per kg body weight dissolved in 0.9% saline) was orally administered by gavage.

**Blood Sample Collection and Analysis.** Fasting blood glucose was measured on whole blood from the tail using the One-Touch

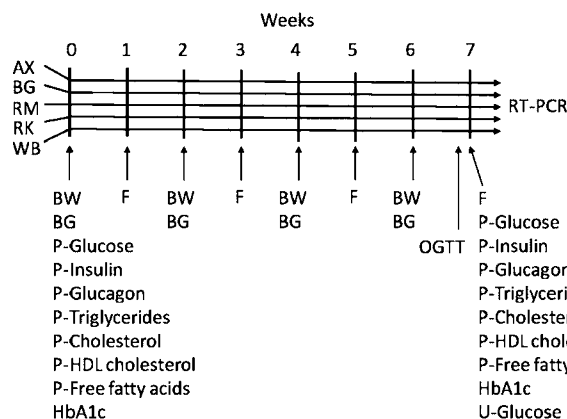


**Figure 1.** Study design of Zucker diabetic fatty rats fed on white bread (WB), arabinoxylan bread (AX), $\beta$-glucan bread (BG), whole meal rye bread (RM), or rye bread with kernels (RK) for 7 weeks. Oral glucose tolerance test (OGTT) was performed in the last week. Liver, muscle, and adipose tissues were used for real-time PCR (RT-PCR). BW, body weight; F, food intake; BG, blood glucose; U, urine.

equipment. Blood samples (baseline and week 7) were collected in chilled tubes containing a heparin/aprotonin solution and centrifuged at 4000 rpm at 4 °C for 10 min. Plasma was frozen at −80 °C for subsequent analysis. Whole blood was collected separately in ethylenediaminetetraacetic acid-preserved tubes for hemoglobin A1c (HbA1c) analysis. Plasma glucose (PG) was determined using the glucose oxidase method (GOD-PAP, Roche Diagnostics GmbH, Mannheim, Germany). Plasma insulin (PI) was analyzed by radioimmunoassay (RIA) using a sensitive rat insulin RIA kit (Millipore, Billerica, MA, USA). Plasma glucagon was analyzed by radioimmunoassay (RIA) kit (Millipore). Triglycerides (TG) and free fatty acids (FFA) were determined using enzymatic colorimetric kits (Boehringer Mannheim, Germany). HbA1c, total cholesterol, and HDL-cholesterol were analyzed using the Cobas c111 system (Roche Diagnostics GmbH, Mannheim, Germany). HbA1c was analyzed by HbA1c II kit (Roche Diagnostics GmbH), whereas an enzymatic colorimetric method (Roche) was used for the determination of total cholesterol and HDL-cholesterol.

**RNA Isolation from Rat Tissues.** Total RNA was extracted from frozen liver, adipose, and muscle tissue using the RNeasy Minikit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. RNA purity and concentration were examined by measuring absorbance at 260−280 nm (NanoDrop ND-8000 UV−vis spectrophotometer, NanoDrop Technologies, Wilmington, DE, USA). The 18S and 28S ribosomal bands were examined to evaluate the integrity of the RNA. The examination was done on a 0.7% nondenaturing agarose gel stained with SYBR green.

**Quantitative Real-Time PCR.** Quantitative real-time PCR was performed using the Fluidigm BioMark System (AROS, Applied Biotechnology AS, Denmark). The samples were analyzed for expression of 30 gene transcripts using rat-specific TaqMan assays (ABI, 4331182). Two specific assays were used as endogenous control: 18S (ABI, Hs99999901_s1) and Hprt1 (ABI, Rn01527840_m1). A list of the TaqMan assays used is given in Table 2. Samples were analyzed using Fluidigm 96.96 Dynamic (Fluidigm catalog no. BMK-M-96.96) arrays with assay triplicates in accordance with the manufacturer's protocol. One hundred nanograms of RNA was used as input in 20 $\mu$L reverse transcript reaction using the High Capacity cDNA Reverse Transcription Kit (ABI, PN4368813) in accordance with the manufacturer's protocol. After reverse transcription, the cDNA samples were amplified according to the instructions given in the *Fluidigm Specific Target Amplification Quick Reference Manual*. In short, the cDNA was amplified using a target-specific assay (diluted 1:100) and TaqMan PreAmp Master mix (2X) (ABI, PN 4391128) in a 14 cycle thermal cycler reaction: 95 °C 10 min and 14 cycles of 95 °C 15 s and 60 °C 4 min. Amplification was performed using the standard conditions: 50 °C 2 min, 95 °C 10 min, and 40 cycles of 95 °C, 15 s, and 60 °C, 1 min. The relative gene expression was calculated using the $(1 + \text{efficiencies})^{-\Delta CT}$ method, and the fold change (FC) was used to compare the expression levels.[22] ΔCT is the difference in cycle threshold ($C_T$) value between each target gene based on the average of the triplicates and the geometric mean $C_T$ values of 18S and Hprt1.

**Table 2. Applied Biosystem TaqMan Assays for Real-Time Quantitative RT-PCR**

| gene symbol | assay ID, TaqMan, applied biosystem | gene symbol | assay ID, TaqMan, applied biosystem |
|---|---|---|---|
| Adipoq | Rn00595250_m1 | Irs1 | Rn02132493_s1 |
| AdipoR1 | Rn01483784_m1 | Irs2 | Rn01482270_s1 |
| Akt2 | Rn00690901_m1 | Jnk1 | Rn01473307_m1 |
| AMPK | Rn00576935_m1 | LPL | Rn00561482_m1 |
| CD36 | Rn00580728_m1 | LXR$\alpha$ | Rn00581185_m1 |
| CPT1$\alpha$ | Rn00580702_m1 | PDK4 | Rn00585577_m1 |
| CRP | Rn00567307_g1 | PGC-1$\alpha$ | Rn00580241_m1 |
| FAS | Rn01463550_m1 | Pi3K | Rn01769520_m1 |
| GAPDH | Rn01462661_g1 | PPAR$\alpha$ | Rn00566193_m1 |
| Glut2 | Rn00563565_m1 | PPAR$\gamma$1 | Rn00440945_m1 |
| Glut4 | Rn00562597_m1 | SDH | Rn00590475_m1 |
| G6Pase | Rn00565347_m1 | Srebp-1c | Rn01495769_m1 |
| Gpr39 | Rn03037275_s1 | Srebp-2 | Rn01502638_m1 |
| GYS2 | Rn00565296_m1 | TNF-$\alpha$ | Rn99999017_m1 |
| IL6 | Rn00561420_m1 | 18S | Hs99999901_s1 |
| IR | Rn01637243_m1 | Hprt1 | Rn01527840_m1 |

**Statistical Analysis.** Statistical analyses were performed using StataIC software (version 11.2). Data are presented as the mean ± SEM. Data were evaluated prior to statistical analysis with Bartlett's test for equal variances. One-way ANOVA followed by post hoc Bonferroni's multiple-comparison test was used to determine the effects of the diets and to compare differences among group means. Whenever data were not normally distributed, data were logarithmically transformed before analysis. If data were not normally distributed after logarithmic transformation or Bartlett's test failed to satisfy equality of variance, the Kruskal−Wallis test was applied. Time × treatment interactions for body weight, food intake, and fasting glucose were analyzed using ANOVA with repeated measures. The significance between baseline and week 7 in each group was determined by use of Student's paired *t* test. The differences were considered to be significant when $p < 0.05$. The food efficiency was determined as body weight per food intake per week. A homeostasis model assessment of $\beta$-cell function (%) (HOMA-$\beta$) was calculated using the equation: $[(20 \times \text{PI}\ (\mu\text{U/mL}))/(\text{PG}\ (\text{mmol/L}) - 3.5)]$.[23] An OGTT was used to determine the glucose tolerance, and the increment in glucose was expressed as the total area under the curve (AUC) using the trapezoidal rule. Changes across time (7 weeks minus baseline) in body weight and food efficiency were correlated with changes in blood glucose by using Spearman's rank correlation test. The problem of multiple statistical testing of gene expression was addressed using false discovery rate (FDR) corrections,[24] and only significant results after FDR corrections are discussed and presented in the figures.

## ■ RESULTS

**Body Weight and Food Intake.** At baseline body weight was similar between groups ($p = 0.94$). For all groups, body weight increased gradually during the trial (Figure 2). However, after 3 weeks, a reduction in the rate of body weight gain was observed in the WB group. After 6 weeks, body weight in the WB group (326.2 ± 3.6 g) was lower than in the RK (378.3 ± 6.8g), AX (383.7 ± 3.7g), and RM (390.8 ± 9.1 g) groups ($p < 0.0001$), respectively, and a time × treatment interaction was observed ($p = 0.0001$). In the first week, rats in the AX group ate significantly less than the other four groups ($p < 0.0001$)

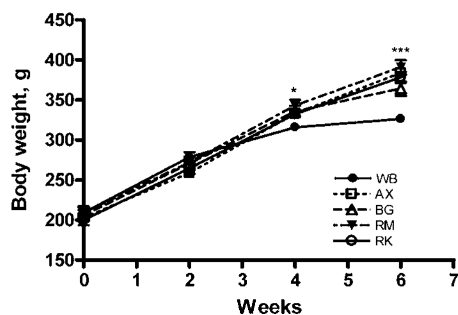


**Figure 2.** Body weight gain of Zucker diabetic fatty rats fed white bread (WB), arabinoxylan bread (AX), β-glucan bread (BG), whole meal rye bread (RM), or rye bread with kernels (RK) for 7 weeks. Data are presented as the mean ± SEM, $n = 12$. (*) $p < 0.05$; (***) $p < 0.001$. Week 4: RM significantly greater than WB; week 6: AX, RM and RK significantly greater than WB.

(Figure 3). After 7 weeks, 24 h food intake was significantly higher in the WB group (40.3 ± 1.5 g; 661 ± 27 kJ) compared

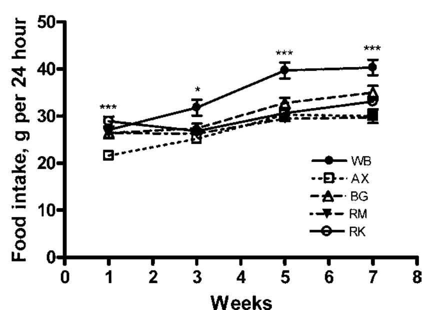


**Figure 3.** Food intake in grams per 24 h of Zucker diabetic fatty rats fed white bread (WB), arabinoxylan bread (AX), β-glucan bread (BG), whole meal rye bread (RM), or rye bread with kernels (RK) for 7 weeks. Data are presented as the mean ± SEM, $n = 12$. (*) $p < 0.05$; (***) $p < 0.001$. Week 1: WB, BG, RM and RK significantly greater than AX; week 3: WB significantly greater than AX; week 5: WB significantly greater than BG, AX, RM and RK; Week 7: WB significantly greater than AX, RM and RK; and BG significantly greater than AX and RM.

to the RM (29.7 ± 0.6 g; 481 ± 10 kJ), AX (30.0 ± 1.4 g; 503 ± 23 kJ), and RK (33.1 ± 0.9 g; 536 ± 15 kJ) groups, respectively ($p < 0.001$). Food intake for BG (35.0 ± 1.5 g; 577 ± 24 kJ) was also higher compared to the AX and RM groups ($p < 0.0001$), and a time × treatment interaction from 0 to 7 weeks ($p < 0.0001$) was observed. At the end of the study, a lower food efficiency was observed for the WB group (1.2 ± 0.1 g body weight per g food/week) compared to the AX (1.9 ± 0.1 g body weight per g food/week), RM (1.9 ± 0.1 g body weight per g food/week), and RK (1.6 ± 0.1 g body weight per g food/week) groups ($p < 0.0001$). Rats in the BG group had a food efficiency between WB and the AX, RK, and RM groups (1.5 ± 0.1 g body weight per g food/week) ($p < 0.0001$).

**Correlations between Parameters.** Changes in body weight between baseline and 7 weeks were negatively and significantly correlated with changes in blood glucose ($r = -0.730$, $p < 0.0001$). The same was observed for the correlation between food efficiency and blood glucose ($r = -0.611$, $p < 0.0001$).

**Fasting Glucose and HbA1c.** Baseline fasting glucose ($p = 0.32$) and HbA1c ($p = 0.14$) were similar among groups (Figure 4). During the intervention, fasting glucose rose markedly in rats on the WB diet followed by rats on the BG diet (Figure 4A), so that after 6 weeks, fasting glucose was significantly higher for the WB group compared to the RK, RM, and AX groups, respectively ($p = 0.0004$) and for the BG group compared to the RM group ($p = 0.0004$). A time × treatment interaction was found in the interval from 0 to 6 weeks ($p < 0.0001$). Similarly, there was an increase in HbA1c for all groups (Figure 4B). By week 7 HbA1c had increased significantly ($p < 0.0001$) in the RM, AX, RK, BG, and WB groups by 42, 50, 57, 91, and 122%, respectively. Between groups, HbA1c in the WB group was significantly higher than in the AX, RM, and RK groups ($p < 0.0001$), and HbA1c in the BG group was higher than in the RM group ($p < 0.0001$).

**Insulin and Glucagon.** Baseline insulin was similar between groups ($p = 0.80$) (Figure 4C). Insulin increased significantly during the intervention by 153% ($p = 0.005$), 176% ($p < 0.0001$), and 204% ($p < 0.0001$) in the RK, RM, and AX diets, respectively. At week 7, insulin was thereby significantly lower for the WB group than for the AX, RM, and RK groups ($p < 0.0001$) and for the BG group compared to AX and RM groups ($p < 0.0001$). At baseline no significant difference was seen in plasma glucagon between groups after multiple comparisons (Table 3) or after the interventions ($p = 0.055$).

**HOMA-β.** No significant difference was observed in baseline HOMA-β ($p = 0.38$) (Figure 4D). β-Cell function decreased significantly ($p < 0.0001$) during the intervention by 53 and 76% in the BG and WB groups, respectively. At week 7 HOMA-β was significantly lower for the WB group compared to the AX, RM, and RK groups ($p < 0.0001$) and for the BG group compared to the AX and RM groups ($p < 0.0001$).

**Oral Glucose Tolerance Test.** After 180 min, blood glucose remained elevated in the WB and BG groups with mean blood glucose levels of 17.7 and 12.4 mmol/L, respectively, whereas the glucose levels in the AX, RK, and RM groups were close to baseline (Figure 5A). The area under the curve ($AUC_{0-180\ min}$) was significant higher for the WB group (3586 ± 100 mmol/L·180 min) compared to RM (1978 ± 206 mmol/L·180 min), AX (2117 ± 170 mmol/L·180 min), and RK (2234 ± 262 mmol/L·180 min) groups, respectively ($p < 0.0001$), and also for BG (2930 ± 177 mmol/L·180 min) compared to AX and RM groups ($p < 0.0001$), respectively (Figure 5B).

**Plasma Lipid Profile.** No significant difference was observed in baseline levels of total cholesterol, HDL cholesterol, TG, or FFA (Table 3). RK and RM diets decreased total cholesterol by 13% ($p = 0.028$) and 28% ($p < 0.0001$), respectively, whereas the BG diet caused an increase in total cholesterol by 10% ($p = 0.046$). Thereby, at week 7, total cholesterol was significantly higher for the BG group compared to AX, RK, and RM ($p < 0.0001$) and also for the WB group compared to the RM group ($p < 0.0001$). HDL-cholesterol increased significantly in the WB group ($p < 0.0001$) and was significantly higher at 7 weeks than the AX, RM, and RK groups ($p = 0.0003$). TG decreased in the WB group ($p = 0.0087$), and at week 7 TG was significantly higher in the AX and BG groups compared to the WB group ($p = 0.0094$). FFA declined in all groups but with no significant differences between diets ($p = 0.78$).

**Expression of Key Genes Related to Insulin Signaling Cascade and Glucose Metabolism in Liver, Adipose, and Muscle Tissue.** Expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was up-regulated in adipose tissue for AX with 1.4 FC, RM with 1.4 FC, and RK with 1.5 FC

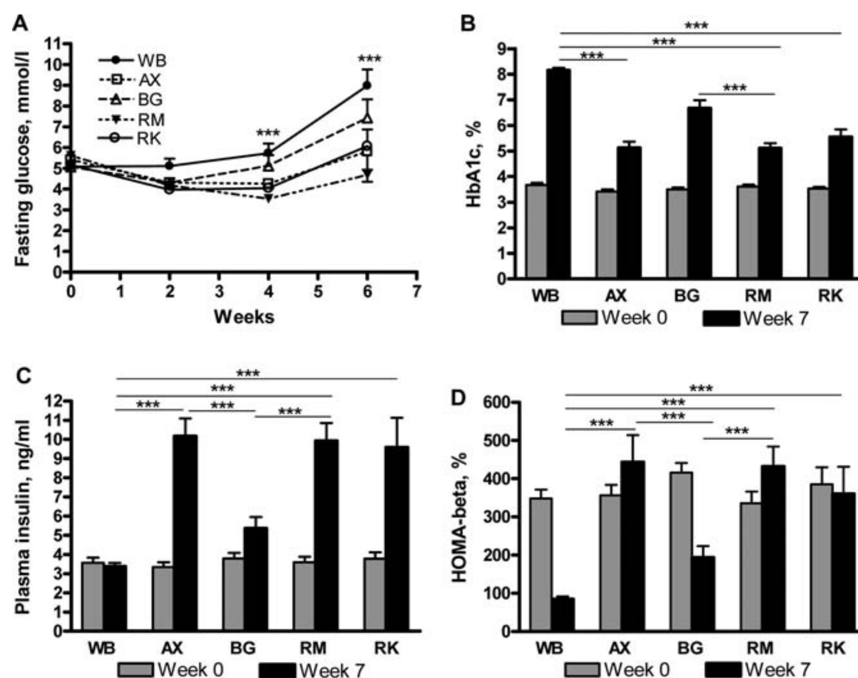


**Figure 4.** (A) Fasting glucose, (B) HbA1c (%), (C) plasma insulin, and (D) HOMA-$\beta$ of Zucker diabetic fatty rats fed white bread (WB), arabinoxylan bread (AX), $\beta$-glucan bread (BG), whole meal rye bread (RM), or rye bread with kernels (RK) for 7 weeks. Data are presented as the mean ± SEM, $n = 12$. $p$ values for HbA1c, plasma insulin, and HOMA-$\beta$ are calculated as the Δ (week 7 minus baseline) differences between groups. (***) $p < 0.001$. For fasting glucose: Week 4: WB and BG significantly greater than RM; week 6: WB significantly greater than AX, RM and RK; and BG significantly greater than RM.

**Table 3. Plasma Profile at Baseline and 7 Weeks in Rats Fed Diets Based on WB, AX, BG, RM, or RK[a]**

| | diet[b] | | | | | |
|---|---|---|---|---|---|---|
| | WB | AX | BG | RM | RK | $p$ value |
| 0 weeks | | | | | | |
| glucagon, pg/mL | 93.1 ± 4.4 | 79.3 ± 1.8 | 90.6 ± 1.9 | 83.1 ± 2.8 | 90.2 ± 3.7 | 0.014[c] |
| total cholesterol (TC), mmol/L | 4.33 ± 0.16 | 4.39 ± 0.23 | 3.94 ± 0.11 | 4.02 ± 0.16 | 4.04 ± 0.22 | 0.30 |
| HDL-cholesterol, mmol/L | 1.92 ± 0.04 | 1.89 ± 0.07 | 1.81 ± 0.11 | 1.95 ± 0.08 | 2.06 ± 0.08 | 0.17 |
| HDL/TC, mmol/L | 0.43 ± 0.03 | 0.44 ± 0.03 | 0.46 ± 0.03 | 0.50 ± 0.03 | 0.53 ± 0.03 | 0.30 |
| triglyceride, mmol/L | 3.64 ± 0.29 | 3.11 ± 0.43 | 2.72 ± 0.35 | 3.20 ± 0.30 | 2.77 ± 0.24 | 0.30 |
| free fatty acids, mmol/L | 1.75 ± 0.12 | 1.82 ± 0.13 | 1.81 ± 0.15 | 1.83 ± 0.16 | 1.83 ± 0.11 | 0.30 |
| 7 weeks | | | | | | |
| glucagon, pg/mL | 102.5 ± 3.2 | 99.1 ± 4.1 | 94.4 ± 3.5 | 78.4 ± 5.8 | 92.1 ± 5.4 | 0.055[d] |
| total cholesterol (TC), mmol/L | 4.54 ± 0.13ab | 3.92 ± 0.17b | 4.32 ± 0.17a | 2.88 ± 0.16c | 3.52 ± 0.23b | <0.0001[d] |
| HDL-cholesterol, mmol/L | 3.28 ± 0.20a | 1.92 ± 0.20b | 2.40 ± 0.27 | 1.95 ± 0.11b | 2.43 ± 0.15b | 0.0003[d] |
| HDL/TC, mmol/L | 0.75 ± 0.07 | 0.51 ± 0.06 | 0.55 ± 0.06 | 0.69 ± 0.04 | 0.70 ± 0.03 | 0.059[d] |
| triglyceride, mmol/L | 2.60 ± 0.23a | 3.71 ± 039b | 3.72 ± 0.40b | 3.46 ± 0.35 | 2.96 ± 0.36 | 0.0094[d] |
| free fatty acids, mmol/L | 1.42 ± 0.07 | 1.13 ± 0.11 | 1.17 ± 0.13 | 1.22 ± 0.18 | 1.20 ± 0.12 | 0.78[d] |

[a]Data are presented as the mean ± SEM, $n = 10-12$. Means in a row without a common letter differ, $p < 0.05$. [b]WB, white bread; AX, arabinoxylan bread; BG, $\beta$-glucan bread; RM, whole meal rye bread; RK, rye bread with kernels. [c]After multiple-comparisons test with Bonferroni post hoc analysis, no significant difference between groups. [d]$p$ values are calculated as Δ (week 7 minus baseline) differences between groups.

compared to the WB group ($p < 0.0001$) and for RM (1.2 FC) and RK (1.3 FC) compared to BG ($p < 0.0001$) (Figure 6A). In liver tissue the same tendency was seen, although this was not significant after FDR correction. No significant change was observed for the expression of hepatic glucose-6-phosphatase or glycogen synthase 2 after FDR correction. The five diets did not alter the expression of: insulin receptor (IR), AKT kinase, or insulin receptor 1 (Irs1) in any of the tissues; liver glucose transporter 2 (GLUT 2); liver or muscle succinate dehydrogenase (SDH); adipose or muscle glucose transporter 4 (GLUT4); insulin receptor 2 (Irs2); or muscle pyruvate dehydrogenase kinase 4 (PDK4). Small changes in liver Irs2, adipose SDH, and liver PDK4 were seen, but this was not significant after FDR correction. Phosphoinositide 3-kinase inhibitor was undetectable.

**Expression of Key Genes Related to Inflammation in Liver, Adipose, and Muscle Tissues.** Adipose tumor necrosis factor-alpha (TNF-$\alpha$) expression tended to increase in the WB group compared to the other four groups, but this did not reach statistical significance ($p = 0.074$). Adipose adiponectin expression was highest in the AX and WB groups, although again this difference was not statistically significant ($p = 0.097$). Expression of adipose adiponectin receptor 1 (adipoR1) was up-regulated in the RK group compared to

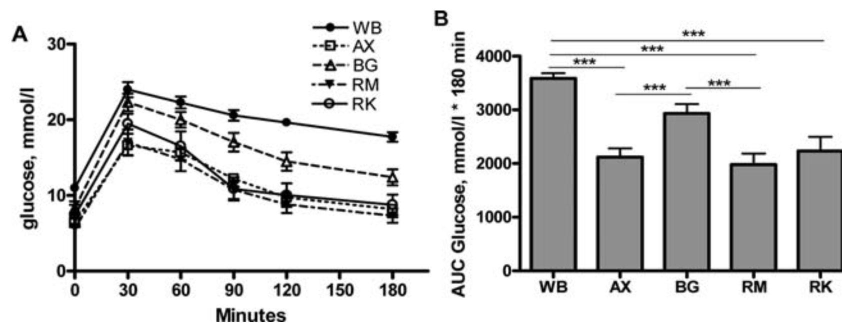


**Figure 5.** (A) Blood glucose and (B) glucose area under the curve (AUC) during an oral glucose tolerance test from Zucker diabetic fatty rats fed white bread (WB), arabinoxylan bread (AX), β-glucan bread (BG), whole meal rye bread (RM),or rye bread with kernels (RK) for 7 weeks. Data are presented as the mean ± SEM $n = 12$. (∗∗∗) $p < 0.001$.

the WB and BG groups ($p = 0.0006$) with 1.3 FC and 1.4 FC, respectively (Figure 6B), and for AX and RM compared to BG ($p = 0.0006$) with 1.3 FC each. In the liver the same effect was seen, but this was not significant after FDR correction. Adipose c-Jun N-terminal kinase (JNK-1) expression (Figure 6C) was up-regulated for the RK diet compared to the WB and BG diets with 1.3 FC each ($p = 0.0049$). Diets did not affect expression of liver or muscle JNK-1, TNF-α, or C-reactive protein or muscle adipoR1, whereas interleukin 6 was undetectable.

**Expression of Key Genes Involved in Lipid Metabolism in Liver, Adipose, and Muscle Tissues.** RK, AX, and RM diets significantly up-regulated adipose AMP-activated protein kinase (AMPK) expression (Figure 6D) compared to WB with 2.2, 1.9, and 1.8 FC, respectively ($p < 0.0001$), and also for RK and AX diets compared to BG diet with 2.0 and 1.8 FC, respectively ($p < 0.0001$). Adipose lipoprotein lipase (LPL) expression (Figure 6E) was up-regulated with 1.5 FC in both AX and RK groups compared to the WB group ($p = 0.0029$). Adipose fatty acid synthase (FAS) expression (Figure 6F) was up-regulated in AX, RM, and RK groups with 2.7, 2.8, and 3.6 FC, respectively, compared to the WB group ($p < 0.0001$). The same trend was observed in muscle tissue, although this was not significant ($p = 0.054$). Diets did not affect expression of liver and muscle AMPK or LPL; liver, adipose, or muscle carnitine palmitoyltransferase I; liver FAS; or adipose and muscle CD36. The intervention did tend to affect liver CD36; however, this was not significant after FDR correction.

**Key Nuclear Receptors Involved in Regulation of Glucose and Lipid Metabolism.** Hepatic peroxisome proliferator-activated receptor gamma coactivator 1-α (PGC-1α) expression (Figure 6G) was up-regulated in the WB group with 1.4, 1.5, and 1.8 FC in the RM, RK, and AX groups, respectively ($p < 0.0001$). Adipose sterol regulatory element binding protein (SREBP)-1c expression (Figure 6H) was suppressed in AX, RK, and RM groups with 1.4, 1.4, and 1.5 FC, respectively, compared to WB ($p = 0.0022$). Adipose SREBP-2 expression (Figure 6I) was up-regulated in RK and RM groups with 1.3 FC each compared to WB group ($p = 0.0009$). Diets did not affect liver X receptor-α, PPARγ1, or PPARα in any tissues. SREBP-1c and SREBP-2 were unaffected in muscle and liver tissues, and PGC-1α was unaffected in muscle and adipose tissues. We also investigated the potential impact of the five diets on the expression level of the G protein-coupled receptor 39 (GPR39), a member of the ghrelin receptor family. However, no significant difference was detected between groups in liver or adipose tissues.

## DISCUSSION

In the present study we demonstrated that feeding a wheat-based bread diet supplemented with concentrated soluble wheat arabinoxylan to ZDF rats for 7 weeks improved glycemic control similar to diets naturally rich in soluble and insoluble arabinoxylan from rye. The diet supplemented with soluble β-glucan, however, did not delay the development of T2D, as did other high-DF diets.

Body weight in the WB group was significantly lower than in the AX, RK, and RM groups. This most likely was an outcome of the more deranged control seen in the WB group. In support of this we found that by the end of the intervention, urinary glucose in rats fed WB was significantly higher than for the RK and RM groups (data not shown). The rats fed the low-DF diet also had a higher food intake than rats on the high DF diets, supporting that high-DF diets have a satiating effect.[25] We observed a negative correlation between body weight or food efficiency and blood glucose; that is, a lower body weight or food efficiency was correlated to a hyperglycemic control in the more severely metabolic deranged animals. The expression of GPR39 may be involved in energy and glucose homeostasis.[26] As we did not observe any differences in expression of GPR39 in liver and adipose tissue, other factors may play a larger role for the observed differences in blood glucose and food intake.

The glucose and insulin changes did not depend on whether arabinoxylan was supplemented to refined wheat bread or occurred naturally in the two rye breads. This is correlated to the high degree of similarity of concentrated arabinoxylan from wheat and arabinoxylan present in the rye cell walls. A previous study using the same bread types showed that the $M_w$ of purified extractable carbohydrate in the AX bread was reduced 2-fold after baking but remained almost unaffected during passage through the small intestine of pigs.[15] Similarly, the $M_w$ of purified extractable carbohydrate in RM and RK bread was not significantly changed in the ileum. Several human studies indicate that arabinoxylan improves glycemic control in subjects with T2D[10] or impaired glucose tolerance[9] and in healthy volunteers.[8] Möhlig et al.[7] did, however, not show a decreased glucose response to soluble arabinoxylan in healthy volunteers. Few studies in rats have focused on glycemic control in relation to arabinoxylan. In Wistar rats, a diet with 4% isolated arabinoxylan with low viscosity did not reduce postprandial glucose, whereas cross-linking of the arabinoxylan significantly increased viscosity and blunted the glucose response.[27] Raised intestinal viscosity is probably the reason for the improved glucose regulation obtained with the AX bread. Thus, the study with ileum-cannulated pigs fed the same bread types as in the

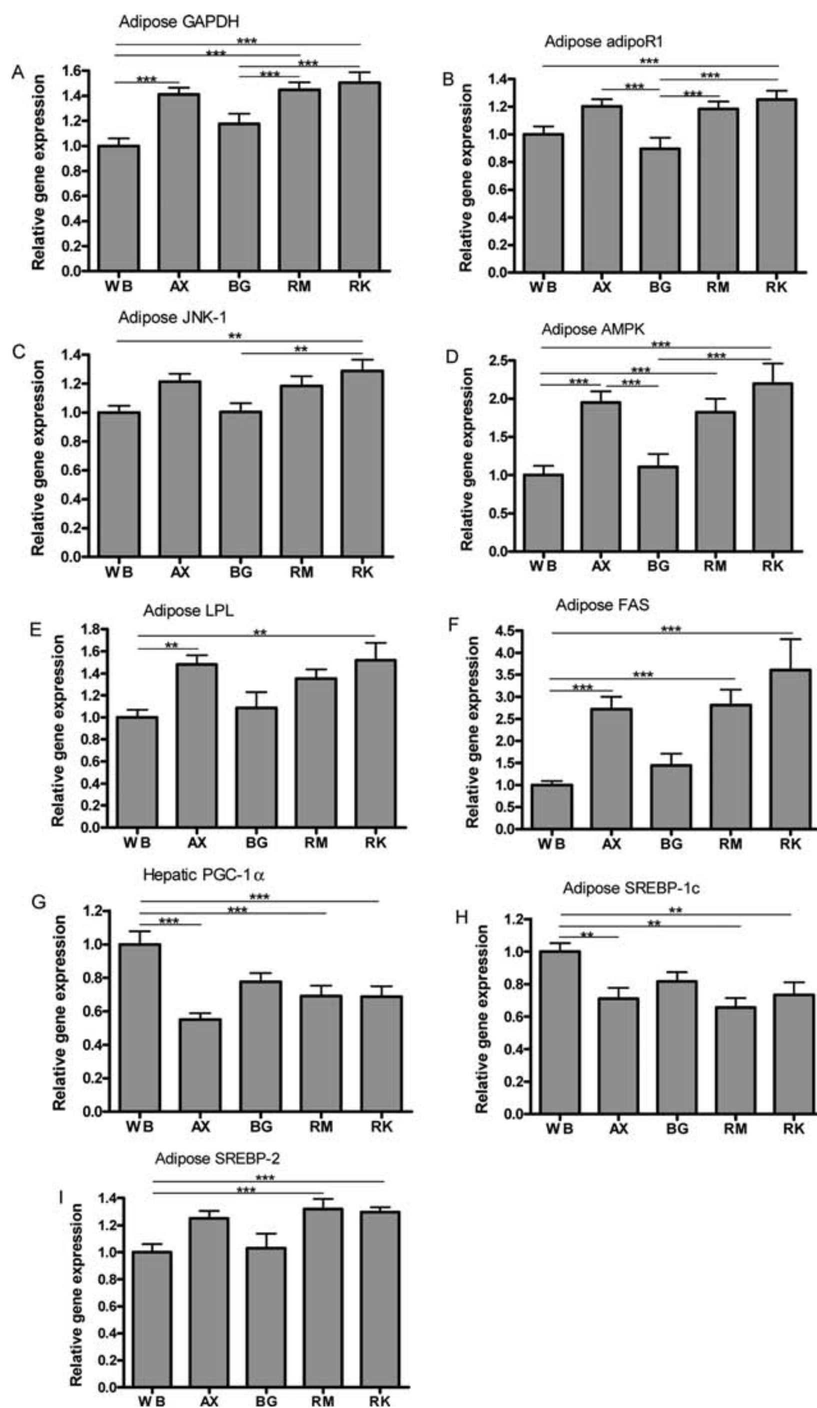


**Figure 6.** Effects of diets on gene expression in adipose and liver tissue of Zucker diabetic fatty rats fed white bread (WB), arabinoxylan bread (AX), $\beta$-glucan bread (BG), whole meal rye bread (RM), or rye bread with kernels (RK) for 7 weeks: (A) adipose GAPDH, (B) adipose AdipoR1, (C) adipose JNK-1, (D) adipose AMPK, (E) adipose LPL, (F) adipose FAS, (G) hepatic PGC-1$\alpha$, (H) adipose SREBP-1c, (I) adipose SREBP-2. Data are presented as the mean ± SEM relative to WB group. Data are presented for genes exposing significant differences between groups after the false discovery correction. $n = 12$. (**) $p < 0.01$; (***) $p < 0.001$.

current study observed that the AX bread resulted in the highest concentration of dissolved arabinoxylan in the ileum among all of the breads that caused a substantial increase in ileal AX viscosity.[16]

Rye is high in soluble arabinoxylan, and rye bread has previously been found to elicit a low postprandial insulin response[28] and prolonged low glucose profiles[28] in human studies. Although intact rye kernels, comprising 49% of the ingredients in the RK bread, are known to produce lower glycemic responses than milled kernels due to the potential protection of starch hydrolysis by the intact kernels,[29] this was not the case in the current study. The most likely reason is that the pelleting process acts as a grinding process, making the two diets, that is, RM and RK, intestinally similar.

In contrast to the arabinoxylan-rich diets, the $\beta$-glucan-rich BG diet did not improve glycemic control in this animal model. The lack of effect is most likely a consequence of the high sensitivity of $\beta$-glucan to food processing, that is, breadmaking,

where $M_w$ of $\beta$-glucan decreased 8 times,[15] and to the conditions in the gastrointestinal tract that lead to further reduction in $M_w$. In a parallel study in ileal-cannulated pigs, the BG bread produced ileal efflux with the lowest viscosity due to a high degree of depolymerization and degradation of the $\beta$-glucan during passage through the small intestine.[16] A study with healthy ileostomists has also shown significant degradation of $\beta$-glucan.[30] If similar degradation takes place in the rodent upper gut, this could also explain the absence of an effect on blood glucose in the current study. A factor that appears to influence the glycemic response is the dose level; the effect of $\beta$-glucan appears to be dose-dependent in human studies.[31] In rats, 2.5% $\beta$-glucan from barley and oat flour[32] and 6% from barley flour[33] has been shown to reduce fasting plasma glucose and HbA1c, respectively. However, 2.5% $\beta$-glucan was ineffective when animals had a more severely deranged glycemic control after 5 months of intervention.[32] Also, using 4.2% $\beta$-glucan in the BG diet did not cause beneficial effects in our study.

Insulin secretion tightly regulates glucose homeostasis by inhibiting hepatic gluconeogenesis and enhancing glycolysis in the periphery. In the AX, RM, and RK groups, an increased level of insulin was observed concomitantly with up-regulation of adipose GAPDH expression, indicating increased glycolysis in the adipose tissue. As up-regulation of adipose AMPK expression indicates increased mitochondrial fatty acid oxidation and uptake of glucose into adipocytes,[34] an increased expression of adipose adipoR1 and adipose AMPK also explain the improved glycemic control of the AX, RK, and RM groups. We did not find differences in expression levels related to hepatic gluconeogenesis or insulin sensitivity in any of the tissues, indicating no difference in peripheral insulin resistance. However, as indicated by HOMA-$\beta$, the dysfunction of the $\beta$-cells may be more severe in the WB and to a lesser degree in the BG group. Because pronounced insulinopenia was observed at the end of the study in the WB group and to a lesser degree in the BG group, we did not estimate HOMA-IR. This was chosen although the appropriate way is to assess HOMA-$\beta$ along with HOMA-IR. Islet PGC-1$\alpha$ expression is increased in animal models of diabetes resulting in defective insulin secretion.[35] Our results in the WB group corroborate an up-regulation of PGC-1$\alpha$ in the liver as previously shown.[35]

Diets with AX, RM, and RK lowered plasma total cholesterol compared to the BG diet, and the RM diet lowered total cholesterol compared to the WB diet. In human studies, arabinoxylan has not shown a cholesterol-lowering effect.[10,11] In Wistar rats, however, arabinoxylan has previously been shown to reduce cholesterol absorption from a cholesterol-rich diet, leading to reduced plasma cholesterol.[36] In humans[12] and animals,[37] $\beta$-glucan from oat and barley have repeatedly been shown to reduce total cholesterol and LDL-cholesterol. As for glycemic control, the BG diet did not reduce plasma cholesterol in the current study, presumably due to degradation during passage through the small intestine.

SREBPs are involved in both lipogenesis and cholesterol homeostasis. In transgenic mice with overexpression of adipose SREBP-1c, this overexpression causes insulin resistance and increased mRNA HMG CoA reductase.[38] This corroborates our study with increased expression of SREBP-1c in the glucose-intolerant WB group. A high level of HDL is considered to be protective in humans in relation to the risk of developing T2D and CVD. Interestingly, in contrast to humans, ZDF rats have an altered lipoprotein metabolism resulting in higher levels of HDL with increasing progression of diabetes.[39] This agrees with our findings of high plasma HDL level in the WB group. Rather puzzling, we found that the WB diet decreased fasting TG. Our results in rats are, however, in line with human studies showing decreased TG levels after a high-GI diet.[40]

Increase in insulin and subsequently in SREBPs[38] is found to increase lipogenesis. Increased expression of adipose SREBP-2 and FAS in the AX, RM, and RK groups is consistent with the higher insulin level observed in these groups. Increased lipogenesis in adipose tissue is associated with small adipocytes and enhanced insulin sensitivity in humans,[41] which agrees with the improved glucose tolerance observed in the AX, RM, and RK rat groups. Insulin also stimulates LPL activity, which fits with the increasing adipose LPL expression in the AX and RK groups. It is puzzling that the expression of JNK-1 was increased in the RK group compared to the WB and BG groups because an increased level of JNK-1 is normally associated with insulin resistance.[42] The reason for this is not known.

It is a strength that this randomized study looked at the effects of isolated DF compared to both negative (white wheat bread) and positive controls (two rye breads) to evaluate the importance of added fibers. Results from plasma measurements are compared to gene expressions to provide indication of the mechanism of action of fibers. We also analyzed the composition of breads, fibers, and pellets. A weakness of the study is that the pelleting process may have diluted the effects of the two diets (RM and RK) and may hide minor differences. It would have been of interest to measure concentrations of short-chain fatty acids in plasma to get a quantitative estimation of the fermentation process; however, we did not obtain a sufficient plasma volume for this.

Arabinoxylan is highly resistant to degradation due to its complex structure compared to $\beta$-glucan.[16] Consequently, we think that incorporation of arabinoxylan into a range of food products such as breads and breakfast products has great potential. Caution should, however, be exercised regarding translation of our results from the animal model to human clinical context. Thus, the doses of active ingredients are very high in the animal experiment and also a similar regular intake will be difficult to pursue in humans. Still more details of the physiochemical properties are needed to clarify the optimal chemical structure needed for beneficial effects.

In conclusion, 7 weeks of treatment with refined wheat bread enriched with concentrated arabinoxylan has similar beneficial effects as arabinoxylan provided from rye breads in delaying the development of T2D in ZDF rats. Bread enriched with $\beta$-glucan did not induce metabolic effects different from WB in the current study. Importantly, diet intervention caused considerable changes in gene expressions in insulin-sensitive tissues. It remains to be clarified if the bread types have similar metabolic impacts in humans as in rats.

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The authors declare no competing financial interest.

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## ■ ABBREVIATIONS USED

AdipoR1, adiponectin receptor 1; AMPK, AMP-activated protein kinase; AX, arabinoxylan bread; BG, $\beta$-glucan bread; CVD, cardiovascular disease; DF, dietary fiber; FAS, fatty acid synthase; FC, fold change; FDR, false discovery rate; FFA, free fatty acid; GAPDH, glyceraldehyde 3 phosphate dehydrogenase; GLUT2/4, glucose transporter 2/4; GPR39, G protein-coupled receptor 39; IR, insulin receptor; Irs1/2, insulin receptor 1/2; JNK-1, c-Jun N-terminal kinase; LPL, lipoprotein lipase; NDC, nondigestible carbohydrates; NSP, nonstarch polysaccharides; PDK4, pyruvate dehydrogenase kinase 4; PG, plasma glucose; PGC-1$\alpha$, peroxisome proliferator-activated receptor gamma coactivator 1-$\alpha$; PI, plasma insulin; PPAR$\gamma$1/$\alpha$, peroxisome proliferator-activated receptor gamma1/alpha; RM, whole meal rye bread; RK, rye bread with kernels; SDH, succinate dehydrogenase; SREBP-1c/2, sterol regulatory element binding protein 1C/2; T2D, type 2 diabetes; TG, triglyceride; TNF-$\alpha$, tumor necrosis factor-alpha; WB, white bread; ZDF, Zucker diabetic fatty

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