

Dietary carbohydrate modification induces alterations in gene expression in abdominal subcutaneous adipose tissue in persons with the metabolic syndrome: the FUNGENUT Study¹⁻⁴

Petteri Kallio, Marjukka Kolehmainen, David E Laaksonen, Jani Kekäläinen, Titta Salopuro, Katariina Sivenius, Leena Pulkkinen, Hannu M Mykkänen, Leo Niskanen, Matti Uusitupa, and Kaisa S Poutanen

ABSTRACT

Background: Diets rich in whole-grain cereals and foods with a low glycemic index may protect against type 2 diabetes, but the underlying molecular mechanisms are unknown.

Objective: The main objective was to test whether 2 different carbohydrate modifications—a rye-pasta diet characterized by a low postprandial insulin response and an oat-wheat-potato diet characterized by a high postprandial insulin response—affect gene expression in subcutaneous adipose tissue (SAT) in persons with the metabolic syndrome.

Design: We assessed the effect of carbohydrate modification on SAT gene expression in 47 subjects [24 men and 23 women with a mean (\pm SD) age of 55 ± 6 y] with the features of the metabolic syndrome in a parallel study design. The subjects had a mean (\pm SD) body mass index (kg/m^2) of 32.1 ± 3.8 and a 2-h plasma glucose concentration of 8.0 ± 2.3 mmol/L. Adipose tissue biopsies were performed, and oral-glucose-tolerance tests and other biochemical measurements were conducted before and after the intervention.

Results: We detected 71 down-regulated genes in the rye-pasta group, including genes linked to insulin signaling and apoptosis. In contrast, the 12-wk oat-wheat-potato diet up-regulated 62 genes related to stress, cytokine-chemokine-mediated immunity, and the interleukin pathway. The insulinogenic index improved after the rye-pasta diet ($P = 0.004$) but not after the oat-wheat-potato diet. Body weight was unchanged in both groups.

Conclusions: Dietary carbohydrate modification with rye and pasta or oat, wheat, and potato differentially modulates the gene expression profile in abdominal subcutaneous adipose tissue, even in the absence of weight loss. *Am J Clin Nutr* 2007;85:1417–27.

KEY WORDS Gene-nutrient interactions, metabolic syndrome, insulin resistance, microarray, adipose tissue, diet intervention, insulinemic response, rye, oat, wheat

INTRODUCTION

The pathogenesis of the metabolic syndrome is not well understood, but lifestyle, including diet, and genetic factors clearly interact in its development and progression. These interactions are likely to be reflected in gene expression. The metabolic syndrome, characterized by central obesity, abnormal insulin and glucose metabolism, dyslipidemia, and hypertension, predisposes to cardiovascular diseases and especially type 2 diabetes (T2DM) (1–3).

Abdominal obesity and insulin resistance are the core features of the metabolic syndrome; associated abnormalities include inflammation, endothelial function, sex hormone metabolism, and cortisol metabolism (4–6). Impaired first-phase insulin secretion is also an inherent feature in those who have impaired fasting glycemia or impaired glucose tolerance and is a strong risk factor for progression to T2DM (7–9).

Epidemiologic evidence suggests that diets rich in whole-grain cereals and foods with a low glycemic index may protect against T2DM (10, 11). Moreover, dietary resistant starch may improve insulin sensitivity (12). Rye bread generates a lower postprandial insulin response than does wheat bread, even though the postprandial glucose response remains unchanged (13). This response is not due to the fiber content of the bread, but may be due to the bread structure (14). Hypothetically, repeated lower postprandial insulinemic responses may allow β cell function to recover or decrease insulin resistance, which improves early insulin secretion over the long term (15). In line with this hypothesis, we found that high-fiber rye bread increased the acute insulin response, but insulin sensitivity remained unchanged (16). Furthermore, we recently showed that rye and pasta-based carbohydrate modification can enhance early insulin secretion in persons with the metabolic syndrome (17), although no changes in glucose tolerance or insulin resistance were observed. This effect was found to be independent of the fiber content of the diet.

Abdominal subcutaneous adipose tissue (SAT) produces a variety of secretory factors that have an important role in inflammation and insulin resistance via endocrine, paracrine, or autocrine signals (18, 19). Impaired insulin signaling occurs in

¹ From the Department of Clinical Nutrition, Food and Health Research Centre (PK, MK, and KSP), Department of Medicine (DEL and LN), Department of Computer Science (JK), and Department of Clinical Nutrition (TS, KS, LP, HMM, and MU), University of Kuopio, Kuopio, Finland, and VTT, Espoo, Finland (KSP).

² PK and MK contributed equally to this work.

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⁴ Address reprint requests to M Kolehmainen, Department of Clinical Nutrition, Food and Health Research Centre, University of Kuopio, Finland. E-mail: marjukka.kolehmainen@uku.fi.

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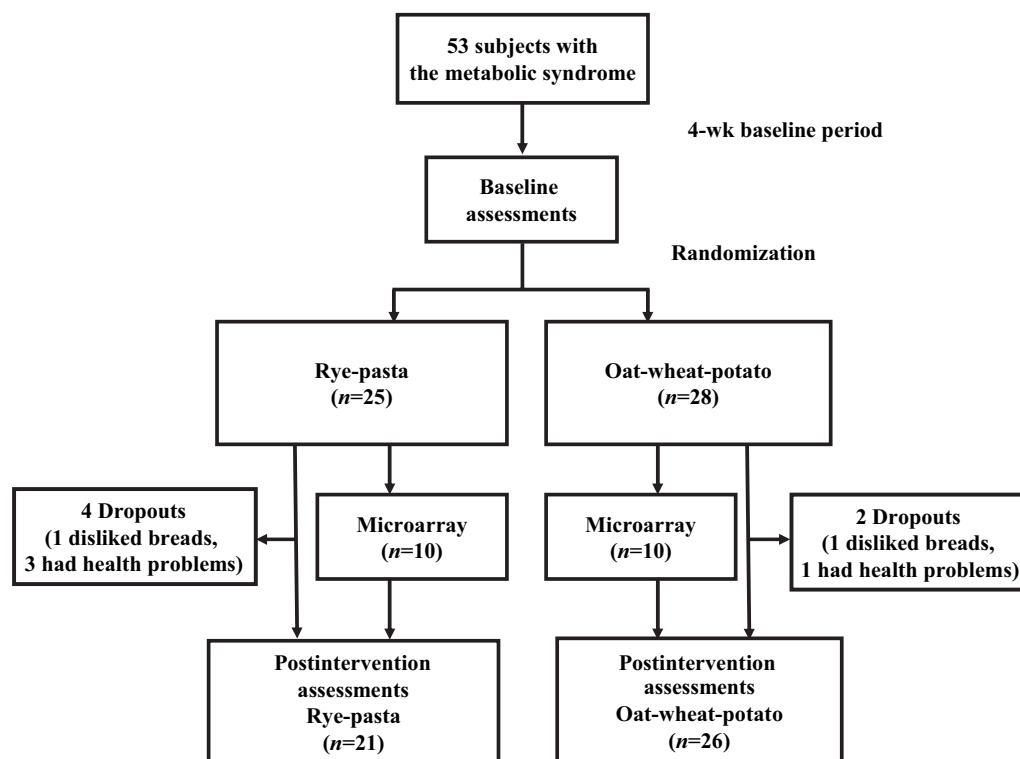


FIGURE 1. Flow diagram of the subjects' progress through the phases of the study.

adipocytes early in the development of insulin resistance, before overt glucose intolerance (20).

Gene expression profiling by microarrays is a valuable tool for finding new gene candidates and pathways in complex pathophysiological conditions and diseases such as the metabolic syndrome and T2DM. Microarrays have recently been applied to study changes in SAT gene expression occurring with caloric restriction and weight loss (21, 22). Microarray techniques have not been applied to human studies testing the effects of dietary interventions on gene expression in the absence of weight loss.

In this study, we examined the effects of 2 different carbohydrate modifications (low-insulin- and high-insulin-response diets) on gene expression in abdominal SAT and on glucose and insulin metabolism in persons with the metabolic syndrome. We hypothesized that the repeated lower postprandial insulin responses would be reflected to gene expression in SAT even in absence of weight loss.

SUBJECTS AND METHODS

Subjects

Fifty-three subjects were recruited to participate in the dietary intervention known as the Functional Genomics and Nutrition (FUNGENT) Study. The study population is a subpopulation of a previously reported dietary intervention (17). At screening, the health status and medical history of the subjects were examined by an interview, clinical examination, and laboratory examinations (hemoglobin, lipid profile, insulin, glucose, and liver, kidney, and thyroid functions). The inclusion criteria were an age of 40–70 y, a body mass index (kg/m^2) of 26–40, and ≥ 3 of the following 5 criteria for a diagnosis of the metabolic syndrome according to the National Cholesterol Education Program (23):

impaired fasting glucose (6.1–6.9 mmol/L), waist circumference >102 cm (men) or >88 cm (women), triacylglycerol concentration >1.7 mmol/L, HDL-cholesterol concentration <1.0 mmol/L (men) or <1.2 mmol/L (women), and blood pressure >130 (systolic)/85 (diastolic) mm Hg or antihypertensive medication use. Persons were excluded if they had overt diabetes or were taking cholesterol-lowering, corticosteroid, analgesic, or anticoagulant medication. Forty-seven subjects (24 men and 23 women) entered the postintervention analyses. Twenty subjects were selected for the microarray analysis of gene expression in SAT ($n = 10$ per group). These subjects were selected because they had both pre- and postintervention samples that were, at most, only slightly contaminated by blood for the microarray analysis. The subjects gave written informed consent before participating in the study. The Ethics Committee of the University of Kuopio and Kuopio University Hospital approved the study. The study was carried out according to the Declaration of Helsinki.

Study design

The study design had 2 parallel groups and consisted of a 4-wk baseline period and a 12-wk test period. At the end of the baseline period, the subjects were randomly assigned according to sex, median age (cutoff: 57 y), and 2-h plasma glucose (cutoff: 7.35 mmol/L) to either a rye-pasta diet or to an oat-wheat-potato diet (Figure 1). The subjects replaced their normal breads and baked products with the test breads during the intervention. The aim was to cover $>25\%$ of the daily energy intake with the test breads.

About 50% of the daily bread consumption in the oat-wheat-potato group was to be oat bread; similarly, 50% of the daily bread consumption in the rye-pasta group was to be endosperm rye bread. The aim was to achieve a similar fiber intake from the

breads because the fiber content of these breads was almost equal (5.4 g fiber/100 g for the oat bread and 5.7 g fiber/100 g for the endosperm rye bread). The subjects in the rye-pasta group were given a package (400 g) of dark pasta or spaghetti 1 time/wk and were advised to use ≥ 1 portion of pasta (70 g dry pasta) ≥ 3 times/wk as part of warm dishes. The subjects in the oat-wheat-potato group were advised to use mainly potatoes as part of warm dishes and were given a package (210 g) of powdered mashed potatoes 1 time/wk. Otherwise, the diet was to remain unchanged.

The subjects were especially advised not to change the amount and type of fat and cold cuts eaten with the bread. Another goal of the dietary counseling was that the subject's weight should not change $>5\%$ from their baseline weight. Compliance with the diets was assessed with the daily records of bread use and with 4-d food records. The subjects kept daily records of the number of portions of test breads, potato, and pasta eaten and the quantity, quality, and frequency of other cereals that were eaten. Four-day food records, which included one weekend day, were kept by the subjects twice during weeks 4–8 as described previously (17).

The test breads for this study were chosen on the basis of the results of our previous postprandial studies with whole-meal breads. In those studies, we showed that rye bread and pasta consumption produced relatively lower postprandial insulin responses than did wheat bread consumption (14, 24). Additionally, a subpopulation ($n = 19$) of the study underwent 2 postprandial challenges with the test breads presented in this study, in random order, during the screening phase of the study (17). For the postprandial tests, the subjects received the test meal, which contained the test bread (50 g available carbohydrates), 40 g cucumber, and 3 dL of a no-calorie orange drink. A fasting blood sample and 8 blood samples after the start of eating (15, 30, 45, 60, 90, 120, 150, and 180 min) were taken for the measurement of plasma glucose and insulin. The maximal responses and areas under the curve (AUCs) for glucose and insulin were calculated.

The subjects underwent a clinical investigation at baseline and at the end of the intervention period, including an oral-glucose-tolerance test, measurement of blood pressure, waist circumference, body weight, height, and body composition. Fasting blood samples were drawn for the biochemical measurements. An adipose tissue biopsy for gene expression studies and the determination of adipocyte cell size was taken.

Biochemical analyses and anthropometric and body-composition measurements

Plasma glucose was analyzed by using the glucose dehydrogenase photometric method (Merck Diagnostica, Darmstadt, Germany) and KonePro Clinical Chemistry Analyser (Thermo Clinical LabSystems; Konelab, Vantaa, Finland). Serum insulin was analyzed by using the chemiluminescent immunoassay (ACS 180 Plus Automated Chemiluminescence System; Bayer Diagnostics, Tarrytown, NY). Body weight was measured on the same calibrated electronic scale throughout the study. Waist circumference was measured halfway between the lowest rib and the iliac crest. Body composition was measured by bioelectrical impedance (BIA 101S with BODYGRAM software; Akern Srl Bioresearch, Florence, Italy). We used the insulinogenic index as a measure of early insulin secretion. The insulinogenic index was determined by the increment in insulin during the first 30 min after oral glucose ingestion divided by the corresponding increment in glucose. The quantitative insulin sensitivity check index

(QUICKI) was calculated as $1/(\ln \text{ insulin concentration} + \ln \text{ glucose concentration})$ (25).

Adipose tissue biopsy

An adipose tissue biopsy (0.5–5 g) sample was collected as a needle biopsy from the superficial abdominal SAT lateral to the umbilicus under local anesthesia (10 mg Lidocain/mL; Orion Pharma, Espoo, Finland) and was washed twice with Gibco phosphate-buffered saline (Invitrogen, Carlsbad, CA). Biopsy samples were collected after a 12-h fast. Part of the samples was stored in RNAlater (Ambion, Austin, TX) at 4 °C for later RNA extraction. After 24 h, RNAlater was removed and the samples were stored at -80 °C until RNA extraction. Adipocyte cell size was determined from the same samples as follows. After being washed, the adipocytes were isolated in the presence of collagenase (0.5 mg/mL) under constant shaking at 2 Hz at 37 °C in buffer containing 125 mmol NaCl/L, 5 mmol KCl/L, 1 mmol CaCl_2 /L, 2.5 mmol MgCl_2 /L, 1 mmol KH_2PO_4 /L, 4 mmol glucose/L, 2% bovine serum albumin, and 25 mmol Tris/L at pH 7.4 (26, 27). After 60 min, the cells were filtered through nylon cloth and washed 3 times with the same buffer without collagenase. Direct microscopic determination of the adipocyte diameter was performed by placing an aliquot of the cell suspension on the Bürker chamber and examining it with a light microscope (model CH-2; Olympus, Center valley, PA). The diameters of 100–200 cells were estimated, and the median of the diameters was used to calculate fat cell volume.

RNA extraction

Total RNA obtained before and after the intervention from adipose tissue of each subject was extracted initially with Trizol (Invitrogen) followed by further purification with RNeasy Mini-Kit (Qiagen, Valencia, CA). RNA isolation and purification were performed according to the manufacturer's instructions (Invitrogen and Qiagen). RNA concentrations and the ratio of A_{260} to A_{280} were determined with the use of a NanoDrop-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE); the acceptable ratio of A_{260} to A_{280} was 1.9–2.1. Integrity of the RNA was assessed by using agarose gel electrophoresis. RNA samples from 10 subjects each from both the rye-pasta group and the oat-wheat-potato group were chosen for microarray analyses based on the purity of the tissue (avoidance of blood contamination) and RNA. One tissue sample was used for each microarray analysis.

cDNA synthesis and oligonucleotide microarrays

Synthesis of biotin-labeled complementary RNA (cRNA), hybridization to DNA microarrays (Affymetrix HG-U133 Plus 2.0 GeneChip) and detection of hybridized cRNA were performed as recommended by the manufacturer (Affymetrix Inc, Santa Clara, CA). Briefly, 2 μg total RNA was used to generate double-stranded cDNA by reverse transcription with the use of the One-Cycle cDNA Synthesis Kit (Affymetrix Inc). Labeled cRNA was prepared by using double-stranded cDNA as a template by in vitro transcription with the use of the IVT Kit (Affymetrix Inc). Biotinylated cRNA was fragmented and added to hybridization cocktail. Two hundred microliters of this cocktail was used for the hybridization of HG-U133 Plus 2.0 array (Affymetrix Inc) in a hybridization oven at 60 rpm at 45 °C for 16 h, which was followed by washings using the GeneChip Fluidics Station 400



(Affymetrix Inc). The arrays were stained with streptavidin-phycoerythrin (Molecular Probes, Eugene, OR), incubated with biotinylated anti-streptavidin immunoglobulin (Vector Laboratories, Burlington, Canada), and restained with streptavidin-phycoerythrin.

Microarray data extraction and analysis

The arrays were scanned with the HP GeneArray Scanner 3000 (Affymetrix Inc). Data were extracted from the scanned images and autoscaled to median intensity by using Affymetrix GeneChip Operating Software. Gene expression profiles were compared by using dChip (Internet: www.dchip.org), a package for the statistical analysis of microarray data in samples with a large number of replicates. Invariant set normalization was used to normalize arrays at the probe level (28). Model-based expression index signals were then calculated according to the PM/MM-difference model. After these steps, only genes that were present in >50% of the replicates in ≥ 1 of the 2 time points were selected for further analysis. To compare gene expression profiles between the 2 groups, we used the false discovery rate (FDR) to determine the significant changes in the genes using Significance Analysis of Microarrays (SAM) (29). We used 300 permutations to obtain the FDR value. An FDR of 0.18 ($\Delta = 0.7$) in the rye-pasta group and of 0.24 ($\Delta = 0.6$) in oat-wheat-potato group were used. The FDR is presented as a q value for each gene in the final list of significant genes. Genes were additionally defined as differentially expressed when a P value < 0.05 was obtained by paired Student's t test. Further downstream analyses of gene annotations and pathways were conducted with PANTHER Classification System Version 6.0 (Internet: www.pantherdb.org) (30) and the Database for Annotation, Visualization and Integrated Discovery 2.1 (DAVID 2.1; Internet: <http://david.abcc.ncifcrf.gov/>) (31). In the PANTHER analysis, we compared our gene list with the *Homo sapiens* gene list of the National Center for Biotechnology Information using a paired t test (a P value < 0.01 was considered significant in the biological processes, and a P value < 0.05 was considered significant in the pathway analyses).

Real-time polymerase chain reaction

Real-time quantitative polymerase chain reaction (qPCR) was used to confirm the results obtained from oligonucleotide microarrays. For that purpose, 5 μg total RNA samples were used as a template for reverse transcriptase reactions to generate cDNA with the High Capacity cDNA Archive Kit according to the manufacturer's protocol (Applied Biosystems, Foster City, CA). qPCR was then performed with an ABI Prism 7500 real-time PCR system by using assays based on TaqMan chemistry (assay-by-design or assay-on-demand) and ABI Prism 7500 SDS software (Applied Biosystems). The endogenous control was chosen by using the Human Endogenous Control Kit (Applied Biosystems). Of the 11 possible candidates, cyclophilin A1 was chosen as the best candidate when cDNA pool constructed from isolated human adipose tissue RNA was used as a template. Each PCR consisted of 6 ng cDNA, 1X Assay Mix, and 1X TaqMan Universal PCR Master Mix with UNG (Applied Biosystems). qPCR data were collected during each extension phase of the PCR reaction by using ABI Prism 7500 SDS software. Threshold cycles were determined for each gene with the automated Ct option. For the standard curve, all samples were pooled to generate a representative cDNA for standard dilutions. A standard

curve with 5 concentrations (0.17, 0.5, 2, 6, and 12 ng/ μL) and calibrator (2 ng/ μL) were used on every plate and for every gene. This standard curve was used to determine the relative quantity of cDNA in each sample by comparison by using methods described in the ABI Prism User Bulletin no. 2. Quantities on each plate were first corrected by the calibrator on the plate. Furthermore, the relative amount per plate was corrected with the corresponding values of endogenous control cyclophilin A1. Analyses for the relative quantity of specific genes before and after the intervention were analyzed in triplicate.

Statistical analysis

The data were analyzed with SPSS for WINDOWS 11.5 (SPSS Inc, Chicago, IL). Variables with skewed distributions by Shapiro-Wilks test were normalized with logarithmic or reciprocal transformation. Untransformed values are reported as means \pm SDs unless otherwise mentioned. A general linear model (GLM) for repeated measures was used to determine differences between the groups (the interaction of time and group) during the intervention with the corresponding baseline variable included in the analysis as a covariate. GLM for univariate analysis was used to assess the difference in the relative change during the intervention (calculated as the percentage change from baseline) of the insulinogenic index and QUICKI between the groups, with the baseline variable as a covariate. A paired-samples t test was used to examine changes in variables within each group.

RESULTS

Biochemical and clinical characteristics at baseline

The baseline characteristics of the 2 groups did not differ significantly, except for systolic blood pressure ($P = 0.038$; **Table 1**).

Postprandial tests

In the postprandial challenge ($n = 19$), the insulin AUC and maximal insulin response to rye bread were lower than those to oat-wheat bread ($P = 0.004$ and $P < 0.001$, respectively). The glucose AUC ($P = 0.31$) and maximum glucose response ($P = 0.46$) did not differ significantly between the rye and oat-wheat bread portions.

Diet

Reported compliance with the diet was good. The portions of rye bread and oat-wheat bread consumed exceeded the minimum number recommended in the study (**Table 2**). During the intervention, carbohydrate, total fiber, and soluble and insoluble fiber intakes increased in the rye-pasta group; protein and soluble fiber intakes increased and the total fiber intake decreased in the oat-wheat-potato group (**Table 2**).

Oral-glucose-tolerance tests

There was a borderline significant difference ($P = 0.055$) in the insulinogenic index between the groups (**Table 3**). After the 12-wk intervention, we detected a significant improvement in the insulinogenic index ($P = 0.004$) in the rye-pasta group (**Table 3**). Moreover, the difference between groups in the relative percentage change in the insulinogenic index during the intervention was significant ($P = 0.027$). The insulinogenic index and the

TABLE 1Clinical characteristics of the subjects at baseline¹

	Rye-pasta group (n = 13 M, 8 F)	Oat-wheat-potato group (n = 11 M, 15 F)	P ²
Age (y)	54.0 ± 6.8	56.0 ± 6.0	NS
BMI (kg/m ²)	31.7 ± 0.5	32.2 ± 3.7	NS
Waist circumference (cm)	110 ± 11.4	108 ± 9.0	NS
Systolic blood pressure (mm Hg)	136 ± 15.4	145 ± 15.6 ²	≤0.05
Diastolic blood pressure (mm Hg)	88 ± 9.6	87 ± 9.0	NS
Fasting plasma glucose (mmol/L)	6.3 ± 0.7	6.3 ± 0.6	NS
Fasting serum insulin (pmol/L)	82.2 ± 41.4	82.2 ± 42.0	NS
Fasting serum total cholesterol (mmol/L)	6.1 ± 1.0	5.7 ± 0.8	NS
HDL cholesterol (mmol/L)	1.2 ± 0.3	1.3 ± 0.3	NS
LDL cholesterol (mmol/L)	3.8 ± 0.7	3.6 ± 0.6	NS
Triacylglycerol (mmol/L)	2.1 ± 1.2	1.9 ± 1.3	NS

¹ All values are $\bar{x} \pm$ SD.² Independent-samples *t* test for equal variances. Logarithmic transformed values were used when the distribution of a variable was not normal.

30-min insulin concentration during the oral-glucose-tolerance test seemed to decrease during the intervention in the oat-wheat-potato group, but these changes were not significant ($P = 0.151$ and $P = 0.160$, respectively). Other biochemical and clinical characteristics did not change during the intervention.

Adipocyte cell size

Adipocyte cell size decreased by 21% in the rye-pasta group during the intervention ($P = 0.011$), but remained unchanged in the oat-wheat-potato group. The change in adipocyte size did not correlate with the gene expression of validated genes.

Gene expression profiles

Rye-pasta group

We detected a list of 71 genes (**Table 4**) using an FDR of 0.18 and a P value <0.05 as selection criteria. After the 12-wk rye-pasta diet, the expression of all genes was down-regulated modestly, ie, mean changes of 0.71- to 0.93-fold. The list included

genes closely linked to the insulin signaling pathway, including insulin-like-growth-factor (IGF) binding protein-5 (*IGFBP5*; mean: 0.84; range: 0.71–1.02) and the insulin receptor (*IR*; mean: 0.73; range: 0.39–0.92). The gene expression for hormone-sensitive lipase (*LIPE*; mean: 0.85; range: 0.71–1.00) was also down-regulated. The Panther Classification System was used to detect clusters on the basis of gene ontology terms. We determined clusters for both biological processes and pathways. Clusters formed on the basis of biological processes included apoptosis ($P = 0.009$). Genes related to apoptosis were lectin, tumor necrosis factor receptor superfamily 1A, fragile X mental retardation, autosomal homolog, mitochondrial ribosomal protein S30, twist homolog 2, and secreted frizzled-related protein 1.

Oat-wheat-potato group

We detected a list of 62 genes (**Table 5**) using an FDR of 0.24 and a P value <0.05 as selection criteria. Interestingly, after the 12-wk oat-wheat-potato diet, we found results opposite to those

TABLE 2Daily energy and nutrient intakes in the rye-pasta and oat-wheat-potato groups at baseline and during the study and test bread intake during the study¹

Intake	Rye-pasta group (n = 21)		Oat-wheat-potato group (n = 26)		P ²
	Baseline	Test period	Baseline	Test period	
Energy (MJ)	8.9 ± 2.4	8.5 ± 2.1	7.2 ± 1.8 ³	7.7 ± 2.1	NS
Protein (% of energy)	17.7 ± 2.8	16.9 ± 2.0	17.7 ± 3.2	20.2 ± 3.2 ^{4,5}	<0.001
Total fat (% of energy)	33.2 ± 4.6	27.5 ± 4.7	32.1 ± 5.8	28.7 ± 4.9	NS
Carbohydrates (% of energy)	43.2 ± 6.2	49.38 ± 6.1 ⁴	47 ± 6.1 ⁶	47.7 ± 6.5	<0.05
Carbohydrates (g · MJ ⁻¹ · d ⁻¹) ⁷	25.8 ± 3.7	29.4 ± 3.7 ⁴	28.1 ± 3.6 ⁶	28.4 ± 3.9	<0.05
Total fiber (g · MJ ⁻¹ · d ⁻¹) ⁷	2.9 ± 1.1	3.4 ± 0.8 ⁸	3.4 ± 0.78 ⁶	2.9 ± 0.55 ^{3,4}	<0.001
Soluble fiber (g/d) ⁷	0.6 ± 0.2	1.0 ± 0.2 ⁴	0.8 ± 0.2 ⁶	0.9 ± 0.2 ³	<0.001
Insoluble fiber (g/d) ⁷	1.3 ± 0.5	2.0 ± 0.5 ⁴	1.5 ± 0.3	1.5 ± 0.3 ³	<0.001
Minimum amount of test breads to be consumed (portions/d)	—	8.2 ± 1.9	—	7.6 ± 2.0	—
Test breads (portions/d)	—	8.4 ± 2.1	—	7.9 ± 2.2	—
Oat bread or endosperm rye bread (portions/d)	—	4.4 ± 1.1	—	4.1 ± 0.8	—
Pasta (times/wk)	—	3.0 ± 0.9	—	0.7 ± 0.6	—
Rice (times/wk)	—	0.6 ± 0.6	—	0.6 ± 0.5	—

¹ All values are $\bar{x} \pm$ SD.² General linear model for repeated measures for the interaction of time and group corrected for the baseline variable.^{3–6,8} For variables in which the group × time interaction was significant, the within-group change was tested with the paired *t* test (⁴ $P < 0.01$, ⁸ $P < 0.05$), and the difference between the groups at certain time points was tested with the independent-samples *t* test (³ $P < 0.01$, ⁵ $P < 0.001$, ⁶ $P < 0.05$).⁷ Energy-adjusted values.

TABLE 3

Body weight, insulinogenic index, and quantitative insulin sensitivity check index (QUICKI) at the beginning (0 wk) and at the end (12 wk) of the intervention and plasma glucose concentrations in response to an oral-glucose-tolerance test at the beginning (0 wk) and at the end (12 wk) of the intervention¹

	Rye-pasta group (n = 21)	Oat-wheat-potato group (n = 26)	P ²
Body weight (kg)			
0 wk	94.3 ± 17.6	91.3 ± 12.5	NS
12 wk	94.8 ± 18.3	91.4 ± 12.6	
Insulinogenic index			
0 wk	90.89 ± 67.7	111.97 ± 81.9	0.055
12 wk	111.44 ± 87.2 ³	92.07 ± 43.1	
QUICKI			
0 wk	0.55 ± 0.08	0.56 ± 0.08	NS
12 wk	0.54 ± 0.07	0.55 ± 0.08	
Plasma glucose (mmol/L)			
0 wk			
0 min	6.41 ± 0.55	6.23 ± 0.59	NS
30 min	10.20 ± 1.38	9.92 ± 1.98	
120 min	8.16 ± 1.97	7.95 ± 2.47	
12 wk			
0 min	6.58 ± 0.87	6.22 ± 0.61	NS
30 min	10.48 ± 2.17	9.90 ± 1.93	
120 min	8.11 ± 2.67	7.67 ± 2.35	

¹ All values are $\bar{x} \pm$ SD. There were no significant differences between the groups at baseline.

² General linear model for repeated measures for the interaction of time and group corrected for the baseline variable.

³ $P < 0.01$ (within-group paired t test).

of rye-pasta group and detected only up-regulation of gene expression, with the changes ranging from 1.07- to 1.93-fold. Surprisingly, 11 of the genes had a close link to stress response: serum glucocorticoid regulated kinase, map kinase interacting serine/threonine kinase 2, dual specificity phosphatase 6 (*DUSP6*), chemokine (C-C motif) ligand 18 (pulmonary and activation-regulated), heat shock 10-kDa protein 1 (chaperonin 10), heat shock 70-kDa protein 8, peroxiredoxin 6, thioredoxin domain-containing transcriptional intermediary factor 1, B cell linker, CD86 antigen, and zinc finger protein 443. Clusters formed on the basis of biological processes included protein phosphorylation ($P = 0.006$), cytokine- and chemokine-mediated immunity ($P = 0.008$), and protein modification ($P = 0.009$). Furthermore, clusters for pathways including the oxidative stress pathway ($P = 0.005$) and the interleukin pathway ($P = 0.037$) and inflammation mediated by the chemokine- and cytokine-signaling pathway ($P = 0.059$) were formed. Genes closely linked to oxidative stress were *DUSP6* (mean: 1.32; range: 1.06–2.20) and MAP kinase interacting serine/threonine kinase 2 (*MKNK2*; mean: 1.15; range: 1.04–1.42).

Real-time polymerase chain reaction validation

Microarray results with qPCR were validated for 7 genes. Four genes were selected to validate gene expression in the rye-pasta group and 3 genes in the oat-wheat-potato group. The genes encoding *LIPE* (0.88-fold change), growth arrest-specific 7 (*GAS7*; 0.93-fold change), *IGFBP5* (0.86-fold change), and cyclin D2 (*CCND2*; 0.87-fold) were confirmed in the rye-pasta group. Genes encoding serum/glucocorticoid regulated kinase (*SGK*; 1.41-fold change), phosphoinositide-3-kinase (*PIK3C2B*; 0.92-fold change), and solute carrier family 40 (*SLC40A1*; 1.36-fold change) were selected in the oat-wheat potato group. For 6 genes (*LIPE*, *GAS7*, *IGFBP5*, *CCND2*, *SKG*, and *SLC40A1*), the

changes in gene expression were in accordance with microarray data. For one gene (*PIK3C2B*), the changes in expression from microarray findings were not confirmed by qPCR (**Figure 2**).

DISCUSSION

In the present randomized parallel study, we found that a 12-wk carbohydrate modification of the diet differentially modulated gene expression in the abdominal SAT of men and women with the metabolic syndrome, even in the absence of changes in body weight and insulin sensitivity. General down-regulation of abdominal SAT gene expression was evident in the rye-pasta group, including the genes related to insulin signaling and apoptosis. In contrast, the oat-wheat-potato diet up-regulated genes related to stress, including inflammation and interleukin cytokines, oxidative stress, and heat shock proteins.

As we showed earlier in a larger sample of 72 subjects (17), the main clinical finding in this substudy was that long-term rye bread and pasta diets increased early insulin secretion as measured by the insulinogenic index. Deterioration of early insulin secretion is crucial in the progression from normal glucose tolerance to impaired glucose tolerance and further to T2DM. In this and previous studies (13, 14), rye bread has consistently induced lower postprandial insulin responses than wheat bread, even though the glycemic responses have been similar. The rye-pasta diet may improve early insulin secretion by decreasing the excess demands placed on the β cells, which allows them to recover (15). Differences in fiber, fat, and protein contents do not explain the lower postprandial responses of insulin to rye bread (14) and do not seem to explain the differences in the change in insulin secretion between the rye-pasta and oat-wheat-potato groups during the intervention (17).

TABLE 4

Differentially expressed genes after 12 wk of the rye-pasta diet ($n = 10$)

Probe set identifier	Gene name	UniGene symbol	Accession	Fold change ¹	Range ¹	P ²	q ³
							%
208186_s_at	lipase, hormone-sensitive	<i>LIPE</i>	NM_005357	0.85	0.71–1.00	0.0006	0.0
211998_at	H3 histone, family 3B (H3.3B)	<i>H3F3B</i>	AW138159	0.82	0.72–0.91	0.0007	0.0
213311_s_at	KIAA1049 protein	<i>KIAA1049</i>	BF000251	0.83	0.68–0.96	0.0019	0.0
202996_at	polymerase (DNA-directed), Δ 4	<i>POLD4</i>	NM_021173	0.80	0.68–1.00	0.0023	7.1
208993_s_at	peptidyl-prolyl isomerase G (cyclophilin G)	<i>PP1G</i>	AW340788	0.74	0.61–1.11	0.0028	7.1
211628_x_at	ferritin, heavy polypeptide pseudogene 1	<i>FTHP1</i>	J04755	0.92	0.86–0.99	0.0029	0.0
200953_s_at	cyclin D2	<i>CCND2</i>	NM_001759	0.85	0.79–1.03	0.0033	0.0
208890_s_at	plexin B2	<i>PLXNB2</i>	BC004542	0.80	0.68–0.98	0.0036	9.7
35436_at	golgi autoantigen, golgin subfamily a, 2	<i>GOLGA2</i>	L06147	0.76	0.65–1.01	0.0040	9.9
230435_at	FLJ30851 protein	<i>FLJ30851</i>	BF108666	0.84	0.73–0.95	0.0043	0.0
224943_at	BTB (POZ) domain containing 7	<i>BTBD7</i>	A1580162	0.82	0.69–1.05	0.0043	10.7
222108_at	adhesion molecule with immunoglobulin-like domain 2	—	AC004010	0.79	0.55–1.07	0.0045	10.7
216037_x_at	transcription factor 7-like 2 (T cell specific, HMG-box)	<i>TCF7L2</i>	AA664011	0.85	0.68–0.98	0.0045	9.9
239523_at	tumor suppressor candidate 5	<i>TUSC5</i>	AW137636	0.82	0.63–0.96	0.0045	10.7
211959_at	insulin-like growth factor binding protein 5	<i>IGFBP5</i>	AW007532	0.87	0.71–1.02	0.0048	9.9
244026_at	Elongation factor, RNA polymerase II, 2	<i>ELL2</i>	BF063657	0.78	0.50–0.97	0.0048	10.7
212826_s_at	solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 6	<i>SLC25A6</i>	A1961224	0.91	0.86–1.01	0.0052	9.7
201231_s_at	enolase 1, (α)	<i>ENO1</i>	NM_001428	0.91	0.83–1.05	0.0054	7.1
202036_s_at	secreted frizzled-related protein 1	<i>SFRP1</i>	AF017987	0.81	0.63–1.03	0.0058	9.9
226656_at	cartilage associated protein	<i>CRTAP</i>	AW024741	0.89	0.77–0.98	0.0058	0.0
202191_s_at	growth arrest-specific 7	<i>GAS7</i>	BE439987	0.73	0.48–0.98	0.0061	14.5
221815_at	abhydrolase domain containing 2	—	BE671816	0.75	0.58–1.01	0.0062	9.7
1007_s_at	discoidin domain receptor family, member 1	—	U48705	0.77	0.56–1.00	0.0063	15.7
217899_at	hypothetical protein FLJ20254	<i>FLJ20254</i>	NM_017727	0.83	0.61–0.96	0.0063	10.7
202844_s_at	ralA binding protein 1	<i>CHST10</i>	AW025261	0.80	0.71–0.99	0.0068	7.1
226448_at	family with sequence similarity 89, member A	<i>MGC15887</i>	A1130705	0.90	0.81–1.02	0.0072	14.2
212248_at	metadherin	<i>LYRIC</i>	A1886796	0.89	0.83–0.97	0.0073	0.0
218472_s_at	pelota homolog (<i>Drosophila</i>)	<i>PELO</i>	NM_015946	0.83	0.62–0.97	0.0074	9.9
212457_at	transcription factor binding to immunoglobulin heavy constant mu enhancer 3	<i>TFE3</i>	AL161985	0.89	0.79–1.00	0.0076	9.9
213754_s_at	poly(A) binding protein interacting protein 1	<i>PAIP1</i>	AW613203	0.87	0.74–1.06	0.0077	10.7
230434_at	phosphatase, orphan 2	<i>MGC2610</i>	AA769615	0.81	0.65–0.97	0.0078	9.7
209543_s_at	CD34 antigen	<i>CD34</i>	M81104	0.89	0.77–1.05	0.0082	10.7
212073_at	casein kinase 2, α 1 polypeptide	<i>CSNK2A1</i>	A1631874	0.81	0.73–1.00	0.0085	0.0
227047_x_at	zinc finger and BTB domain containing 4	<i>ZBTB4</i>	N63748	0.84	0.63–1.00	0.0086	14.5
225232_at	myotubularin related protein 12	<i>PIP3AP</i>	AA524700	0.89	0.77–0.99	0.0089	7.1
212878_s_at	kinesin 2	<i>KNS2</i>	AA284075	0.86	0.69–0.99	0.0092	7.1
202550_s_at	VAMP (vesicle-associated membrane protein)-associated protein B and C	<i>VAPB</i>	NM_004738	0.87	0.73–0.97	0.0094	9.7
230180_at	DEAD (Asp-Glu-Ala-Asp) box polypeptide 17	<i>DDX17</i>	AA521056	0.76	0.46–1.46	0.0094	15.7
206209_s_at	carbonic anhydrase IV	<i>CA4</i>	NM_000717	0.78	0.51–1.01	0.0108	15.7
223337_at	serologically defined colon cancer antigen 10	<i>SDCCAG10</i>	AF039693	0.85	0.70–1.00	0.0109	9.9
201177_s_at	SUMO-1 activating enzyme subunit 2	<i>UBA2</i>	NM_005499	0.89	0.79–1.10	0.0109	14.2
1566558_x_at	FLJ90757 protein	<i>FLJ90757</i>	AK096609	0.71	0.47–1.00	0.0113	12.5
201105_at	lectin, galactoside-binding, soluble, 1 (galactin 1)	<i>LGALS1</i>	NM_002305	0.93	0.83–1.00	0.0117	9.7
212130_x_at	eukaryotic translation initiation factor 1	<i>SUI1</i>	AL537707	0.93	0.85–1.02	0.0118	9.7
212157_at	syndecan 2 (heparan sulfate proteoglycan 1, cell surface-associated, fibroglycan)	<i>SDC2</i>	BE048514	0.84	0.68–1.02	0.0120	9.9
213850_s_at	splicing factor, arginine/serine-rich 2, interacting protein	<i>SFRS2IP</i>	A1984932	0.82	0.70–1.08	0.0123	16.5
207966_s_at	golgi apparatus protein 1	<i>GLG1</i>	NM_012201	0.83	0.66–1.04	0.0127	15.7
229404_at	twist homolog 2 (<i>Drosophila</i>)	<i>TWIST2</i>	A1086614	0.86	0.70–1.04	0.0131	12.5
204710_s_at	WIPI49-like protein 2	<i>WIPI-2</i>	NM_016003	0.88	0.76–1.09	0.0134	10.7
201900_s_at	aldo-keto reductase family 1, member A1 (aldehyde reductase)	<i>AKRIA1</i>	NM_006066	0.92	0.87–1.01	0.0147	10.7
229398_at	RAB18, member RAS oncogene family	<i>RAB18</i>	A1769954	0.79	0.54–0.94	0.0151	14.2
235427_at	transcribed locus	—	AA418074	0.83	0.68–1.00	0.0176	14.5
209024_s_at	synaptotagmin binding, cytoplasmic RNA interacting protein	<i>SYNCRIP</i>	A1472757	0.86	0.67–1.07	0.0183	12.2
212053_at	KIAA0251 protein	<i>KIAA0251</i>	AK025504	0.93	0.87–1.04	0.0209	10.7

(Continued)

TABLE 4 (Continued)

Probe set identifier	Gene name	UniGene Symbol	Accession	Fold change ¹	Range ¹	P ²	q ³
221792_at	RAB6B, member RAS oncogene family	<i>RAB6B</i>	AW118072	0.75	0.44–1.16	0.0213	16.5
1557553_at	protein phosphatase 1, regulatory (inhibitor) subunit 12B	<i>PPP1R12B</i>	BF438357	0.77	0.51–1.05	0.0229	16.5
226216_at	insulin receptor	<i>INSR</i>	W84556	0.73	0.39–0.92	0.0232	10.7
235964_x_at	Chromosome 20 open reading frame 118	—	AA603344	0.86	0.73–0.97	0.0255	9.9
1553703_at	CDNA FLJ13242 fis, clone OVARC1000578	<i>FLJ90396</i>	NM_153358	0.86	0.72–1.02	0.0269	15.7
213227_at	progesterone receptor membrane component 2	<i>PGRMC2</i>	BE879873	0.88	0.76–1.03	0.0269	12.2
210950_s_at	farnesyl-diphosphate farnesyltransferase 1	<i>FDF1</i>	BC003573	0.93	0.86–1.01	0.0297	12.5
225719_s_at	mitochondrial ribosomal protein L55	<i>MRPL55</i>	BG497783	0.88	0.73–1.03	0.0297	10.7
204045_at	transcription elongation factor A (SII)-like 1	<i>TCEAL1</i>	NM_004780	0.91	0.83–0.98	0.0298	9.7
231968_at	Homo sapiens, clone IMAGE:6198912, mRNA	—	AK025416	0.91	0.85–0.99	0.0307	12.2
201637_s_at	fragile X mental retardation, autosomal homolog 1	<i>FXR1</i>	NM_005087	0.92	0.84–1.02	0.0333	16.5
209076_s_at	WDR45-like	<i>WDR45L</i>	BC000974	0.93	0.81–0.99	0.0340	14.5
226011_at	coiled-coil domain containing 12	<i>MGC23918</i>	BG107767	0.88	0.77–1.01	0.0344	12.2
218398_at	mitochondrial ribosomal protein S30	<i>MRPS30</i>	NM_016640	0.85	0.70–1.01	0.0379	14.2
243561_at	YY1 associated factor 2	<i>YAF2</i>	AA651631	0.82	0.64–1.11	0.0391	16.1
49878_at	peroxisomal biogenesis factor 16	<i>PEX16</i>	AA523441	0.90	0.84–1.00	0.0420	9.7
207643_s_at	tumor necrosis factor receptor superfamily, member 1A	<i>TNFRSF1A</i>	NM_001065	0.89	0.79–1.01	0.0499	12.5

¹ All values are means.

² Paired *t* test.

³ Represents the false discovery rate of each gene.

In the rye-pasta group, we detected changes in genes related to insulin signaling and apoptosis. The rye-pasta diet down-regulated *IGFBP-5* gene expression. This protein modulates the effects of IGF-I and IGF-II, but its function in human fat tissue is unknown. However, the up-regulation of the closely related *IGFBP-3* is known to induce insulin resistance in mouse adipocytes (32). Moreover, *IGFBP-3* has been shown to be up-regulated in human omental adipose tissue of obese individuals (33). Thus, assuming that the mechanism of *IGFBP-5* is similar to that of *IGFBP-3*, the down-regulation of *IGFBP-5* seen in the present study might promote insulin sensitivity at the molecular level in SAT.

The rye-pasta diet down-regulated the gene encoding the insulin receptor. Selective knockout of the insulin receptor in the white adipose tissue of mice paradoxically improves lipid and glucose homeostasis (34), but such a knockout model may not be physiologically relevant for modest down-regulation brought about by carbohydrate modification. It has been proposed that the activation of the full cohort of insulin receptors is not required for normal insulin action; thus, the concept of “spare receptors” has been introduced (35). Repeatedly lower postprandial hyperinsulinemia (16) and lower postprandial nonesterified fatty acid concentrations could hypothetically contribute to the down-regulation of adipose tissue expression of the insulin receptor through decreased demand for receptor binding.

Down-regulation of the gene expressing *LIPE* in the rye-pasta group may occur as a consequence of the decrease in adipocyte cell size that we found with the rye-pasta diet. Some studies have reported a close association of adipocyte cell size with *LIPE* mRNA expression (36) and hormone-sensitive lipase concentration and activity (37). Thus, the decrease in cell size in the rye-pasta group could contribute to down-regulation of *LIPE* mRNA expression. Hormone-sensitive lipase is the rate-limiting enzyme of lipolysis, which is a hallmark of insulin resistance in

adipose tissue. Therefore, down-regulation of *LIPE* and decreased adipocyte cell size suggest decreased lipolysis and enhanced insulin sensitivity in SAT after the rye-pasta diet (38). In this study, however, we did not find a significant correlation between *LIPE* gene expression and adipocyte cell size.

Interestingly, the 12-wk oat-wheat-potato diet seemed to especially activate genes responding to stress. The oxidative stress pathway, interleukin pathway, and inflammation mediated by the chemokine and cytokine signaling pathway were also activated. Moreover, the present data suggest that the oat-wheat-potato diet, which induced repeated high insulin responses, can provoke alterations in immune status and inflammation. It is well established that adipose tissue has a role in inflammation (39). Cross-sectional epidemiologic data suggest that whole grains and a low-glycemic-index diet may reduce systemic inflammation in women with T2DM (40). Up-regulation of gene expression for serum and glucocorticoid-regulated kinase suggests activation of the glucocorticoid axis, which can occur in response to various stress stimuli (cytokines, aldosterone, growth factors, oxidative stress, heat shock protein activation, and glucocorticoids) (41). Activation of the pituitary-adrenal glucocorticoid axis may be involved in the pathogenesis of the metabolic syndrome (42).

Enhanced gene expression for heat shock 10-kDa protein 1 and heat shock 70-kDa protein 8 indicate a reaction in response to any of a number of stresses, including oxidative stress and inflammation (43). Increased MAP kinase interacting serine/threonine kinase 2 gene expressions suggests activation of MAP kinases, which can be activated by heat shock proteins, inflammatory cytokines, oxidative stress, altered redox status, and other stresses. MAP kinase activation also contributes to the inflammatory response and oxidative stress and may play an important role in signal transduction (44).

TABLE 5

Differentially expressed genes after 12 wk of the oat-wheat-potato diet ($n = 10$)

Probe set identifier	Gene name	UniGene symbol	Accession	Fold change ¹	Range ¹	Paired P^2	q^3
222162_s_at	ADAM metallopeptidase with thrombospondin type 1 motif, 1	—	AK023795	1.62	1.02–2.73	0.0001	0.0
218611_at	immediate early response 5	<i>IER5</i>	NM_016545	1.46	1.06–2.85	0.0011	15.0
223044_at	solute carrier family 40 (iron-regulated transporter), member 1	<i>SLC40A1</i>	AL136944	1.38	1.03–2.10	0.0017	15.0
216858_x_at	gb:AL080112.1	—	AL080112	1.21	0.98–1.39	0.0030	20.0
208891_at	dual specificity phosphatase 6	<i>DUSP6</i>	BC003143	1.32	1.06–2.20	0.0032	15.0
205133_s_at	heat shock 10-kDa protein 1 (chaperonin 10)	<i>HSPE1</i>	NM_002157	1.11	0.97–1.21	0.0036	15.0
202581_at	heat shock 70-kDa protein 1B	<i>HSPA1B</i>	NM_005346	1.47	1.09–1.79	0.0042	20.0
217627_at	zinc finger protein 573	<i>ZNF573</i>	BE515346	1.28	1.02–2.13	0.0048	22.5
205801_s_at	RAS guanyl releasing protein 3 (calcium and DAG-regulated)	—	NM_015376	1.37	0.81–1.72	0.0048	22.5
218541_s_at	chromosome 8 open reading frame 4	<i>C8orf4</i>	NM_020130	1.93	0.66–4.16	0.0057	24.7
205932_s_at	msh homeo box homolog 1 (<i>Drosophila</i>)	<i>MSX1</i>	NM_002448	1.31	1.09–1.97	0.0067	15.0
204484_at	phosphoinositide-3-kinase, class 2, β polypeptide	<i>PIK3C2B</i>	NM_002646	1.24	0.89–1.72	0.0079	24.7
243431_at	BTB (POZ) domain containing 14A	<i>BTBD14A</i>	BF000597	1.53	0.86–2.13	0.0081	24.7
221031_s_at	hypothetical protein DKFZp434F0318	—	NM_030817	1.63	0.88–3.72	0.0081	24.7
201212_at	legumain	<i>LGMN</i>	D55696	1.18	1.00–1.53	0.0082	15.0
214415_at	CDNA FLJ43430 fis, clone OCBBF2027661	<i>AKAP8L</i>	N58120	1.35	1.06–2.59	0.0085	15.0
219178_at	queuine tRNA-ribosyltransferase domain containing 1	<i>QTRTD1</i>	NM_024638	1.47	1.06–2.99	0.0086	24.7
209924_at	chemokine (C-C motif) ligand 18 (pulmonary and activation-regulated)	<i>CCL18</i>	AB000221	1.72	0.66–3.22	0.0087	24.7
203072_at	myosin IE	<i>MYO1E</i>	NM_004998	1.29	1.01–1.84	0.0088	20.0
202437_s_at	cytochrome P450, family 1, subfamily B, polypeptide 1	<i>CYP1B1</i>	NM_000104	1.47	0.93–2.43	0.0088	24.7
229067_at	similar to Formin binding protein 2 (srGAP2)	—	BF977829	1.18	0.94–1.45	0.0090	22.5
201739_at	serum/glucocorticoid regulated kinase	<i>SGK</i>	NM_005627	1.51	1.02–3.35	0.0092	24.7
207655_s_at	B cell linker	<i>BLNK</i>	NM_013314	1.53	0.83–2.48	0.0095	24.7
216051_x_at	KIAA1217	<i>KIAA1217</i>	AK022045	1.17	0.93–1.40	0.0101	24.7
202345_s_at	fatty acid binding protein 5 (psoriasis-associated)	<i>FABP5</i>	NM_001444	1.14	0.96–1.42	0.0111	24.7
201088_at	karyopherin α -2 (RAG cohort 1, importin α 1)	<i>KPNA2</i>	NM_002266	1.15	1.01–1.39	0.0113	15.0
230712_at	hypothetical protein FLJ20719	<i>FLJ20719</i>	AI634549	1.25	1.02–1.50	0.0117	22.5
201436_at	eukaryotic translation initiation factor 4E	<i>EIF4E</i>	AI742789	1.24	0.97–1.60	0.0123	24.7
205928_at	zinc finger protein 443	<i>ZNF443</i>	NM_005815	1.35	0.87–2.96	0.0127	24.7
221841_s_at	Kruppel-like factor 4 (gut)	<i>KLF4</i>	BF514079	1.31	0.98–2.08	0.0129	24.7
204912_at	interleukin 10 receptor, α	<i>IL10RA</i>	NM_001558	1.27	0.99–1.56	0.0138	22.5
218205_s_at	MAP kinase interacting serine/threonine kinase 2	<i>MKNK2</i>	NM_017572	1.15	1.04–1.42	0.0140	24.7
201410_at	pleckstrin homology domain containing, family B (evectins) member 2	<i>PLEKHB2</i>	AI983043	1.16	1.00–1.68	0.0142	24.7
223235_s_at	SPARC-related modular calcium binding 2	<i>SMOC2</i>	AB014737	1.30	1.06–1.69	0.0143	22.5
242751_at	peroxiredoxin 6	<i>PRDX6</i>	N55072	1.49	0.83–2.50	0.0144	24.7
205105_at	mannosidase, α , class 2A, member 1	<i>MAN2A1</i>	NM_002372	1.19	1.01–1.39	0.0149	20.0
208836_at	ATPase, Na ⁺ /K ⁺ transporting, β 3 polypeptide	<i>ATP1B3</i>	U51478	1.13	1.06–1.33	0.0154	15.0
1554479_a_at	caspase recruitment domain family, member 8	<i>CARD8</i>	AF511652	1.18	0.94–1.38	0.0165	24.7
201218_at	C-terminal binding protein 2	<i>CTBP2</i>	N23018	1.20	0.94–1.59	0.0174	24.7
217752_s_at	CNDP dipeptidase 2 (metallopeptidase M20 family)	<i>CNDP2</i>	NM_018235	1.15	1.05–1.37	0.0179	15.0
209214_s_at	Ewing sarcoma breakpoint region 1	<i>EWSR1</i>	BC004817	1.07	0.97–1.17	0.0185	24.7
240135_x_at	TIMP metallopeptidase inhibitor 3	<i>TIMP3</i>	BF001514	1.42	1.00–3.17	0.0199	24.7
210895_s_at	CD86 antigen (CD28 antigen ligand 2, B7-2 antigen)	<i>CD86</i>	L25259	1.34	1.05–2.26	0.0199	24.7
209007_s_at	chromosome 1 open reading frame 63	<i>NP0014</i>	AF267856	1.18	0.99–1.73	0.0200	24.7
230734_x_at	striatin, calmodulin binding protein	<i>STRN</i>	AI279536	1.21	0.96–1.58	0.0212	22.5
213699_s_at	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, θ polypeptide	—	AA854017	1.11	0.96–1.25	0.0227	22.5
229528_at	hypothetical protein LOC283378	<i>SBN01</i>	AI670935	1.20	1.06–1.43	0.0234	15.0
202007_at	nidogen 1	<i>NID</i>	BF940043	1.15	1.01–1.36	0.0240	22.5
226008_at	necdin-like 2	<i>NDNL2</i>	AA627644	1.15	0.97–1.36	0.0243	24.7
226845_s_at	myeloma overexpressed 2	—	AL036350	1.14	0.92–1.38	0.0244	24.7
236699_at	muscleblind-like 2 (<i>Drosophila</i>)	<i>MBNL2</i>	AL566294	1.24	0.97–1.50	0.0281	24.7
223991_s_at	UDP-N-acetyl- α -D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 2 (GalNAc-T2)	<i>GALNT2</i>	AF130059	1.21	1.00–1.50	0.0316	24.7
212943_at	KIAA0528 gene product	<i>KIAA0528</i>	AB011100	1.13	0.95–1.29	0.0324	24.7

(Continued)

TABLE 5 (Continued)

Probe set identifier	Gene name	UniGene symbol	Accession	Fold change ¹	Range ¹	Paired <i>P</i> ²	<i>q</i> ³
234987_at	chromosome 20 open reading frame 118	<i>SAMHD1</i>	AV715309	1.12	1.01–1.45	0.0325	22.5
221891_x_at	heat shock 70-kDa protein 8	<i>HSPA8</i>	AA704004	1.08	0.99–1.22	0.0340	24.7
225707_at	ADP-ribosylation-like factor 6 interacting protein 6	<i>ARL6IP6</i>	AL581082	1.12	0.94–1.31	0.0349	24.7
209476_at	thioredoxin domain containing	<i>TXNDC</i>	AL080080	1.12	0.91–1.26	0.0363	24.7
201450_s_at	TIA1 cytotoxic granule-associated RNA binding protein	<i>TIA1</i>	NM_022037	1.15	0.96–1.34	0.0390	24.7
222207_x_at	CDNA: FLJ20949 fis, clone ADSE01902	—	AK024602	1.12	0.87–1.31	0.0397	24.7
200033_at	DEAD (Asp-Glu-Ala-Asp) box polypeptide 5 /// DEAD (Asp-Glu-Ala-Asp) box polypeptide 5	<i>DDX5</i>	NM_004396	1.08	0.98–1.22	0.0398	24.7
203478_at	NADH dehydrogenase (ubiquinone) 1, subcomplex unknown, 1, 6 kDa	<i>NDUFC1</i>	NM_002494	1.09	0.99–1.27	0.0409	24.7
224802_at	Nedd4 family interacting protein 2	<i>NDFIP2</i>	AA019338	1.16	1.03–1.29	0.0479	22.5

¹ All values are means.

² Paired *t* test.

³ Represents the false discovery rate of each gene.

The mechanisms by which the oat-wheat-potato diet induces gene expression of numerous manifestations of metabolic and oxidative stress and immune activation are unclear. One explanation could be the repeated mild postprandial hypoglycemia that follows the initial hyperglycemia induced by carbohydrates with a high-glycemic or insulinemic index (15). We also found that ingestion of wheat bread is followed by initial hyperinsulinemia and a subsequent transient drop in glycemia below fasting levels, which was not seen after the rye bread meals (14). Hypoglycemia results in activation of counterregulatory stress hormones such as cortisol, glucagons, and catecholamines, which restores its consequent restoration of fasting glucose concentrations and increased nonesterified fatty acid concentrations (15).

In this particular study, we used both *P* values (dChip) and FDR (SAM with 300 permutations) to interpret the microarray data. We used *q* values for each individual gene rather than fold

change thresholds; *q* values represent the FDR of each gene. Currently, there is no gold standard for microarray data analysis (45). However, the methods used in the present study are well established and are used widely elsewhere (21, 46). Real-time PCR was used to confirm selected results from the changes in gene expression found in the microarray analyses. In this study, changes in gene expression were moderate, but occurred even in the absence of weight loss. On the basis of findings from medium-term interventions of moderate weight loss in humans, dramatic changes would not be expected (21, 22).

It is possible that the changes in SAT gene expression might have indirectly influenced insulin secretion in the pancreas, but the mechanisms are unclear. It is unlikely that differences in intakes of protein, saturated fat, carbohydrate, and fiber in the rye-pasta and wheat-oat-potato groups differentially affected early insulin secretion (17). It is nonetheless possible that differences in macronutrient and micronutrient intakes contributed to the different patterns of changes in gene expression.

In conclusion, 2 dietary carbohydrate modifications differentially modulated the gene expression profile of mRNA expression in human SAT. Genes regulating insulin signaling and apoptosis were down-regulated during the rye-pasta diet, and genes related mainly to metabolic stress were up-regulated during the oat-wheat-potato diet. The changes in gene expression suggest that over the long term, such carbohydrate modifications may influence the risk of cardiovascular disease and T2DM, even in the absence of weight loss.

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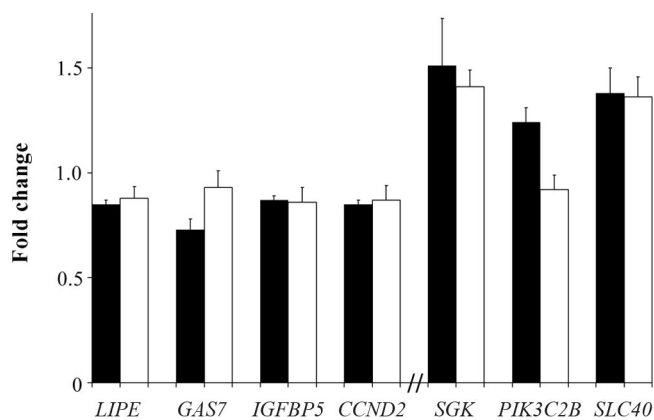


FIGURE 2. Mean (\pm SEM) changes in gene expression in response to the rye-pasta and oat-wheat-potato diets determined by microarray (■) and quantitative polymerase chain reaction (□) analyses. *n* = 10 per group. Target genes are related to endogenous control cyclophilin A1. *LIPE* (hormone-sensitive lipase), *GAS7* (growth arrest-specific 7), *IGFBP5* (insulin-like growth factor binding protein 5), and *CCND2* (cyclin D2) were confirmed in the rye-pasta group, and *SGK* (serum/glucocorticoid regulated kinase), *PIK3C2B* (phosphoinositide-3-kinase β), and solute carrier family 40 (iron-regulated transporter), member 1 (*SLC40A1*) were confirmed in the oat-wheat-potato group. The change in expression of *PIK3C2B* found in the microarray analysis was not confirmed by quantitative polymerase chain reaction analyses.

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