Healthy Nordic diet downregulates the expression of genes involved in inflammation in subcutaneous adipose tissue in individuals with features of the metabolic syndrome[1–4]

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ABSTRACT

Background: Previously, a healthy Nordic diet (ND) has been shown to have beneficial health effects close to those of Mediterranean diets.

Objective: The objective was to explore whether the ND has an impact on gene expression in abdominal subcutaneous adipose tissue (SAT) and whether changes in gene expression are associated with clinical and biochemical effects.

Design: Obese adults with features of the metabolic syndrome underwent an 18- to 24-wk randomized intervention study comparing the ND with the control diet (CD) (the SYSDIET study, carried out within Nordic Centre of Excellence of the Systems Biology in Controlled Dietary Interventions and Cohort Studies). The present study included participants from 3 Nordic SYSDIET centers [Kuopio (n = 20), Lund (n = 18), and Oulu (n = 18)] with a maximum weight change of ±4 kg, highly sensitive C-reactive protein concentration <10 mg/L at the beginning and the end of the intervention, and baseline body mass index (in kg/m^2) <38. SAT biopsy specimens were obtained before and after the intervention and subjected to global transcriptome analysis with Gene 1.1 ST Arrays (Affymetrix).

Results: Altogether, 128 genes were differentially expressed in SAT between the ND and CD (nominal $P < 0.01$; false discovery rate, 25%). These genes were overrepresented in pathways related to immune response (adjusted $P = 0.0076$), resulting mainly from slightly decreased expression in the ND and increased expression in the CD. Immune-related pathways included leukocyte trafficking and macrophage recruitment (e.g., interferon regulatory factor 1, *CD97*), adaptive immune response (interleukin*32*, interleukin 6 receptor), and reactive oxygen species (neutrophil cytosolic factor 1). Interestingly, the regulatory region of the 128 genes was overrepresented for binding sites for the nuclear transcription factor κB.

Conclusion: A healthy Nordic diet reduces inflammatory gene expression in SAT compared with a control diet independently of body weight change in individuals with features of the metabolic syndrome. The study was registered at clinicaltrials.gov as NCT00992641. *Am J Clin Nutr* 2015;101:228–39.

Keywords Nordic diet, adipose tissue, obesity, polyunsaturated fatty acids, transcriptome

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INTRODUCTION

A healthy Nordic diet (ND)[5] has been proposed as an alternative for the Mediterranean diet in Nordic countries (1–5). Recently, we showed in the pan-Nordic randomized dietary intervention study [Systems Biology in Controlled Dietary Interventions and Cohort Studies (SYSDIET)] that an isocaloric ND has beneficial influences on lipid metabolism, signs of systemic inflammation, and ambulatory blood pressure compared with the control diet (CD) (1, 6). The key dietary items of the ND are whole-grain cereal products; local berries, fruits, and vegetables; fish; low-fat or fat-free milk products; rapeseed oil; and vegetable oil–based margarines. All these foods have been shown to have beneficial effects on health and may potentially have an effect on adipose tissue metabolism (7–12).

Cereal products within the ND were local whole-grain products such as oats and rye. In cohort studies, whole-grain foods have been associated with a reduced risk of the metabolic syndrome, cardiovascular diseases, and type 2 diabetes (13–16). The evidence from interventions is, however, quite controversial (17–21). Moreover, the ND contains foods that are rich in polyphenols, such as local vegetables, fruits, and berries. Their consumption has also been associated with beneficial health effects and even reduced all-cause mortality (22–24). The evidence regarding the effects of berries on the risk markers is, however, somewhat controversial (10, 25–30). Replacing saturated fatty acids with unsaturated fatty acids in the diet has been shown to prevent the atherosclerotic process and risk of developing cardiovascular diseases (8, 31–34). However, the evidence is more controversial regarding the effects on low-grade inflammation and insulin sensitivity (31). The quality of dietary fatty acids has also been shown to have a direct effect on the adipose tissue function (35, 36).

Dysfunctional adipose tissue may contribute to systemic disturbances, including insulin resistance and dyslipidemia, and as an endocrine organ, it has local as well as systemic effects on metabolism (37, 38). In obese individuals, altered secretion of free fatty acids, adipokines, and cytokines from adipose tissue has been suggested to contribute to systemic insulin resistance (39). Low-grade inflammation has been proposed as an important link between obesity and its consequences (40, 41). Little is known about whether and how the composition of diet influences the function of adipose tissue and adipose tissue inflammation at the molecular level, because the few available dietary intervention studies have been too small to draw any definitive conclusions (12, 35, 42, 43).

In this substudy of SYSDIET, our aim was to examine if dietary-induced changes in gene expression in subcutaneous adipose tissue (SAT) occur in the absence of body weight changes. We further examined whether such changes in gene expression are associated with beneficial clinical and biochemical effects of the ND. Consequently, we performed global transcriptome analysis on SAT from individuals with features of the metabolic syndrome at the beginning and end of a randomized intervention study comparing the ND with CD.

SUBJECTS AND METHODS

Study design and participants

The SYSDIET study design and participants have been described previously (1). In short, the present study was a randomized controlled multicenter study performed in 6 centers within Nordic countries. The participants were randomly allocated according to sex and baseline medians of age, BMI, and fasting glucose concentration into 18- to 24-wk-long intervention diets—namely, the ND or CD—which were started after a 4-wk run-in period with habitual diet. The composition of the isocaloric diets has been described earlier (1). In the ND, participants consumed whole-grain products, berries, fruits and vegetables, rapeseed oil, 3 fish meals per week, and low-fat dairy products, and they avoided sugar-sweetened products in agreement with the Nordic Nutrition Recommendations for healthy diet (44). In contrast, the individuals with the CD consumed low-fiber cereal products and dairy fat–based spreads, and they limited the amount of fish consumed in accordance to the average nutrient intake in Nordic countries. A clinical nutritionist or a dietitian gave instructions about the diets. Dietary intake was monitored at baseline and during the intervention by 4-d food records and consumption records as described earlier. Furthermore, dietary biomarkers were used to verify the adherence to experimental diets (1). The study participants were advised to keep body weight and physical activity constant and not to change their smoking habits and alcohol consumption or drug treatment during the study.

Altogether, 309 individuals were originally contacted and screened, and they visited the study clinics as described earlier (1). After exclusions, 200 individuals started the intervention: 104 in the ND group and 96 in the CD group. Altogether, 96 individuals in the ND group and 70 in the CD group completed the trial. The inclusion criteria were age 30–65 y, BMI (in kg/m^2) 27–38, and 2 other International Diabetes Federation criteria for the metabolic syndrome (45). Antihypertensive and lipid-lowering medication, as well as inhaled corticosteroids, was allowed but without dosage changes during the trial. The exclusion criteria included any chronic disease and condition that could hamper adherence to the dietary intervention protocol.

For the transcriptome study, we included participants who had donated adipose tissue biopsy specimens (**Figure 1**) in Kuopio

[3]Supplemental Tables 1–3 are available from the "Supplemental data" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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[5]Abbreviations used: *BACH1*, BTB and CNC homology 1, basic leucine zipper transcription factor 1; CD, control diet; *CYBA*, cytochrome b-245, alpha polypeptide; *CYBB*, cytochrome b-245, beta polypeptide; FDR, false discovery rate; *GK*, glycerol kinase; IL-1Ra, IL-1 receptor antagonist; *IL6R*, interleukin 6 receptor; *IRF1*, interferon regulatory factor 1; *JUNB*, jun B proto-oncogene; *LILRB2*, leukocyte immunoglobulin-like receptor, subfamily B member 2; *LRP10*, low density lipoprotein receptor-related protein 10; *MMP25*, matrix metallopeptidase 25; *MYD88*, myeloid differentiation primary response 88; *NCF1*, neutrophil cytosolic factor 1; ND, Nordic diet; *NF-kB*, nuclear transcription factor kB; *PARVG*, parvin, gamma; *PIK3AP1*, phosphoinositide-3-kinase adaptor protein 1; *PIK3CG*, phosphoinositide-3-kinase, catalytic, gamma polypeptide; *PIK3R3*, phosphoinositide-3-kinase, regulatory subunit 3 (gamma); *PTPN6*, protein tyrosine phosphatase nonreceptor type 6; RT-qPCR, reverse transcribed quantitative real-time polymerase chain reaction; SAT, subcutaneous adipose tissue; *STAT1*, signal transducer and activator of transcription 1; *STXBP2*, syntaxin binding protein 2; *SYK*, spleen tyrosine kinase; SYSDIET, Systems Biology in Controlled Dietary Interventions and Cohort Studies; *TAP1*, transporter 1, ATP-binding cassette, sub-family B; TF, transcription factor; *TNF*, tumor necrosis factor; *TNFRII*, tumor necrosis factor receptor II.

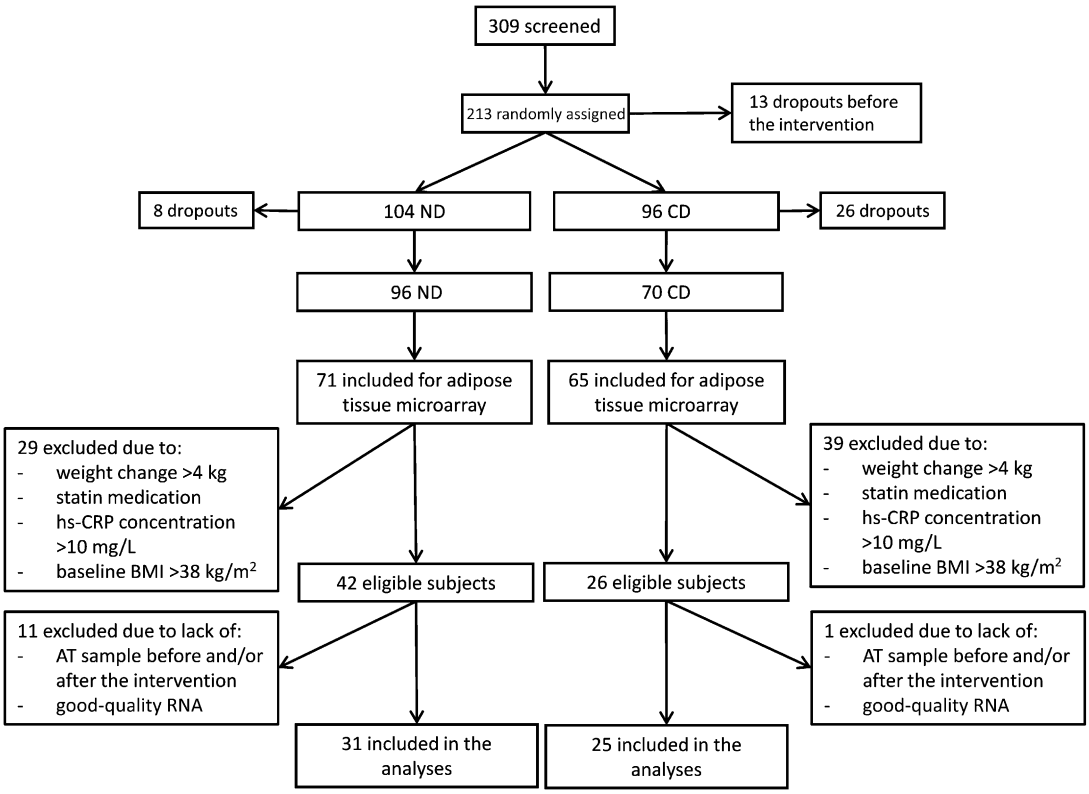

FIGURE 1 Flowchart for the selection of SYSDIET study participants for adipose tissue transcriptome analysis. AT, adipose tissue; CD, control diet; hs-CRP, highly sensitive C-reactive protein; ND, Nordic diet; SYSDIET, Systems Biology in Controlled Dietary Interventions and Cohort Studies.

(n = 20, with 24-wk intervention), Lund (n = 18, with 24-wk intervention), and Oulu (n = 18, with 18-wk intervention) with a maximum weight change of ±4 kg, no statin medication, highly sensitive C-reactive protein concentration <10 mg/L at baseline and at the end of the intervention, and baseline BMI <38. In our experience, it is difficult to discriminate dietary macronutrient effects from the effects of weight changes on adipose transcriptomic data (46, 47). Therefore, we selected the individuals who had a relatively stable body weight.

Clinical and biochemical measurements

Procedures regarding the clinical and biochemical measurements have been described earlier (1). Briefly, subjects were examined in the morning after an overnight fast. Anthropometric measurements were performed according to the standard operational procedures agreed on by all centers. Fasting plasma glucose, cholesterol, triglycerides, HDL cholesterol, and liver enzyme activities were analyzed locally by using a routine method. A standard 2-h oral glucose tolerance test (75 g D-glucose) was performed. Blood samples were taken at time points of 0, 30, and 120 min to measure the concentrations of plasma glucose and serum insulin. Indices for insulin sensitivity and secretion, as well as AUC for glucose and insulin, were calculated as described (48, 49). Blood samples from all study centers were analyzed for apolipoproteins A-I and B, cytokines, and adipokines at the University of Eastern Finland and Kuopio University Hospital and serum insulin at the Aarhus University Hospital by using routine automated clinical chemistry analyzers.

Adipose tissue biopsy

After clinical examination, an SAT biopsy specimen was obtained by needle aspiration just below and lateral to the umbilicus under local anesthesia (1% lidocaine) as described by Kolaczynski et al. (50). Tissue pieces were rapidly rinsed in saline and subsequently frozen in liquid nitrogen and kept at −70°C.

RNA isolation

All RNA work was performed centrally at the Karolinska Institute (Stockholm). SAT pieces (100 mg) were disrupted mechanically and RNA was extracted by using the miRNeasy mini kit (Qiagen). RNA samples were treated with RNase-free DNase (Qiagen). RNA concentration and purity were measured with a Nanodrop ND-1000 Spectrophotometer (Thermo Fisher Scientific). High-quality RNA was confirmed by using an Agilent 2100 Bioanalyzer (Agilent Technologies).

Transcriptome analysis

From total RNA, we prepared and hybridized biotinylated complementary RNA to Gene 1.1 ST Arrays (Affymetrix Inc.) and then washed, stained, and scanned the slides by using standardized protocols. We selected Affymetrix arrays because we believe that these arrays are more sensitive to detect small changes in gene expression than other common arrays. The microarray hybridizations were done at the local Bioinformatics and Expression Analysis core facility (www.bea.ki.se). The Gene 1.1 ST Arrays determine the expression of 28,869 transcripts.

Subsequent data analyses were performed with the Affymetrix Expression Console version 1.1. To allow comparisons of transcript levels between samples, we subjected all samples to an all–probe set scaling-to-target signal of 100. Of the 22,337 annotated transcripts on the array, the 11,258 transcripts with a mean signal >70 were taken forward for further analyses. Subsequent analysis was limited to the 50% (n = 5629) of these 11,258 transcripts with highest variation between samples, measured as SD adjusted for mean expression. The microarray data have been submitted to the Gene Expression Omnibus in a Minimum information about a microarray experiment-compliant format (GSE56716).

Quantitative real-time polymerase chain reaction

RNA was reverse transcribed by using the Omniscript RT kit (Qiagen) and random hexamer primers. The following genes and TaqMan assays (Applied Biosystems) were used for reverse-transcribed quantitative real-time polymerase chain reaction (RT-qPCR): interferon regulatory factor 1 (*IRF1*, Hs00971960_m1); jun B proto-oncogene (*JUNB*, Hs00357891_s1); phosphoinositide-3-kinase adaptor protein 1 (*PIK3AP1*, Hs00381030_m1); spleen tyrosine kinase (*SYK*, Hs00895377_m1); BTB and CNC homology 1; basic leucine zipper transcription factor 1 (*BACH1*, Hs00230917_m1); interleukin 6 receptor (*IL6R*, Hs01075666_m1); protein tyrosine phosphatase, nonreceptor type 6 (*PTPN6*, Hs00169359_m1); and the reference gene LDL receptor-related protein 10 (*LRP10,* Hs01047362_m1). Ten nanograms of complementary DNA was mixed with 2× TaqMan Universal PCR Master Mix and TaqMan primers (Applied Biosystems) in a final volume of 20 μL. Messenger RNA levels were normalized to *LRP10* expression by using a comparative cycle threshold (Ct) method calculated by the following formula: $2^{\Delta Ct\ -\ \text{target gene}}/2^{\Delta Ct\ -\ \text{reference gene}}$. *LRP10* was chosen as the most appropriate control gene following extensive PCR optimizations with several reported genes in the laboratory. Expression of LRP showed a low variability between samples, did not correlate with BMI, and did not differ significantly between groups.

Ethics

All study participants provided their written informed consent, and local ethics committees of all participating centers approved the study protocol. The study was registered at clinicaltrials.gov as NCT00992641.

Statistical analysis

The analyses of dietary, clinical, biochemical, and array gene expression variables were performed by applying linear mixed-effects models as described previously (1) by using SPSS version 19.0 (SPSS Inc.). The models included the outcome of interest as the dependent variable, subject identifier as a random effect, and body weight, $\log_{10}$-transformed age, sex, study center (i.e., also study duration), study group, time point, and study group × time point interaction as covariates. The age variable was $\log_{10}$-transformed before analyses to address the skewed distribution. Analyses of systolic and diastolic blood pressures included antihypertensive treatment as the covariate. Adjustment for weight change did not significantly affect the results.

In the analysis of gene expression, we used nominal $P < 0.01$ and a false discovery rate (FDR) of 25% as a threshold to define which transcripts were differentially regulated by the 2 diets. This threshold means that 1 of 4 significantly changed genes could be a false-positive finding. We used the WEB-based GEne SeT AnaLysis Toolkit (WebGestalt; http://bioinfo.vanderbilt.edu/webgestalt/) to assess the function of differentially regulated genes, and to visualize these results in Gene ontology (GO) slim, which is part of the web tool. We also analyzed whether the genes that were differentially regulated by the 2 diets are enriched for specific gene sets compared with all 5628 genes used in the adipose tissue analysis by applying default settings. oPOSSUM (http://opossum.cisreg.ca/oPOSSUM3/) (51, 52) was used to check for overrepresented transcription factor (TF) binding sites among 128 unique targets compared with 5628 control genes by using single TF binding site analysis and default settings. A 1-sided t test was used to test the difference in gene expression between the groups by using RT-qPCR because the experiments were confirmatory.

Fold change in gene expression levels between baseline and end of the intervention was correlated to changes in clinical and biochemical measurements by using Pearson correlation.

RESULTS

Dietary data

The dietary intake in the subset of participants chosen for microarray is shown in **Table 1** and is representative of the SYSDIET study as reported in the original analyses with the whole study population (1): the PUFA intake was higher and SFA intake lower in the ND group compared with the CD group. In addition, the intakes of dietary fiber, vitamin C, folate, and magnesium were higher in the ND group than in the CD group.

Clinical and biochemical measurements

The clinical and biochemical results for the participants chosen for transcriptome analyses are shown in **Table 2**. The results were similar to those published for the whole SYSDIET study (1). After the intervention, fasting serum LDL cholesterol and apolipoprotein B concentrations were lower in the ND group compared with the CD group. There were no significant differences in fasting plasma glucose or serum insulin concentrations (Table 2) or in the indices for insulin sensitivity and secretion or AUC values for glucose and insulin at baseline or in response to the intervention between the groups (data not shown). Moreover, there were no significant differences in circulating cytokine or high-molecular-weight adiponectin concentrations (**Supplemental Table 1**) between the groups at baseline or in response to the intervention, although there was a trend toward difference between the groups in IL-1 receptor antagonist (IL-1Ra). When the within-group changes were analyzed for these cytokines, IL-1Ra concentration increased significantly within the CD ($P <$ 0.01), as was found in the whole SYSDIET study population.

Adipose tissue transcriptomics

Of the 5628 gene transcripts quantified in SAT in relation to diet, 148 probe sets were differentially regulated by the ND and CD groups with nominal $P < 0.01$ and an FDR of 25%, together

TABLE 1
Dietary intake of the participants in the healthy ND and CD groups at the beginning (week 0) and end (week 18/24) of the intervention[1]

	ND (n = 31)		CD (n = 25)			
Variable	Week 0	Week 18/24	Week 0	Week 18/24	Estimate (95% CI)	Significance (P value)[2]
Energy, kJ	7915 ± 1605	8652 ± 1789[3]	7997 ± 2155	8110 ± 1772	594 (−181, 1370)	0.13
Protein, E%	17.2 ± 3.1	16.9 ± 1.9	17.3 ± 2.8	17.1 ± 2.2	−0.2 (−2.0, 1.6)	0.82
Carbohydrate, E%	45.3 ± 5.5	46.1 ± 5.4	45.1 ± 7.4	42.4 ± 5.7[4]	3.0 (−0.3, 6.2)	0.07
Sucrose, g	41.2 ± 17.3	40.6 ± 16.1	38.0 ± 17.9	31.6 ± 13.9	4.6 (−4.6, 13.8)	0.32
Fat, E%	32.6 ± 6.5	32.0 ± 5.2	33.0 ± 6.8	36.7 ± 4.8[3]	−3.5 (−7.3, 0.2)	0.06
SFA, E%	13.1 ± 3.0	10.2 ± 1.8[3]	13.4 ± 3.8	15.8 ± 2.7[3]	−5.0 (−7.0, −3.0)	0.01
MUFA, E%	11.6 ± 2.7	12.3 ± 2.5	11.7 ± 2.3	13.1 ± 2.1	−0.3 (−1.8, 1.2)	0.71
PUFA, E%	5.0 ± 1.2	6.9 ± 1.7[3]	4.6 ± 1.7	4.5 ± 1.0	2.2 (1.3, 3.2)	0.01
Linoleic acid, g	7.8 ± 2.8	8.7 ± 4.0	7.5 ± 2.5	7.7 ± 3.0	1.26 (−0.58, 3.12)	0.18
α-Linolenic acid, g	1.16 ± 0.52	2.09 ± 1.55[3]	1.28 ± 0.65	1.48 ± 0.77	0.91 (0.30, 1.51)	0.01
Fiber, g	22.3 ± 7.5	38.2 ± 9.8[3]	21.2 ± 5.5	15.9 ± 4.6[3]	21.3 (17.0, 25.6)	0.01
Cholesterol, mg	250 ± 93	214 ± 72	283 ± 129	305 ± 131	−57 (−113, −2)	0.04
Salt, g	7.1 ± 2.3	6.9 ± 2.8	7.2 ± 2.8	6.6 ± 2.2	0.2 (−1.1, 1.5)	0.76
β-Carotene, μg	2755 ± 1977	2932 ± 1690	2382 ± 1682	1637 ± 1020	877 (−325, 2080)	0.15
Vitamin C, mg	123 ± 58	148 ± 50[4]	102 ± 56	69 ± 31[4]	56 (21, 92)	0.01
Vitamin E, mg	9.8 ± 3.6	14.4 ± 3.2[3]	8.4 ± 2.7	8.1 ± 2.5	5.5 (3.7, 7.3)	0.01
Folate, μg	288 ± 95	374 ± 200[3]	247 ± 65	221 ± 66	117 (36, 198)	0.01
Sodium, mg	2831 ± 947	2782 ± 1141	2849 ± 1087	2610 ± 835	146 (−362, 653)	0.57
Potassium, mg	3891 ± 1135	4250 ± 991[4]	3607 ± 1227	3125 ± 940[4]	770 (224, 1315)	0.01
Magnesium, mg	366 ± 106	441 ± 104[3]	356 ± 114	292 ± 84[3]	134 (89, 179)	0.01
Calcium, mg	1053 ± 432	1043 ± 366	936 ± 395	927 ± 385	−20 (−194, 154)	0.82
Alcohol, E%	2.0 ± 3.2	1.8 ± 2.9	2.8 ± 3.2	3.1 ± 3.3	−0.6 (−2.3, 1.0)	0.44

[1]Values are means ± SDs. CD, control diet; E%, percentage of energy; ND, Nordic diet.
[2]Linear mixed-effects models adjusted for body weight, $\log_{10}$-transformed age, sex, study center, study group, time point, and study group × time point interaction. The ND and CD groups did not differ from each other at week 0.
[3]Within-group difference, $P < 0.01$.
[4]Within-group difference, $P < 0.05$.

representing 128 unique genes (**Supplemental Table 2**). The ND led to a downregulation of 118 genes and an upregulation of 10 genes. **Table 3** lists the genes with largest (>30%) differences in fold changes between groups. The effect size of the change in expression of these genes was larger in the CD group.

As a next step, we analyzed with the tool WebGestalt whether the 128 genes differentially regulated by the 2 diets were overrepresented for specific pathways. The most significantly overrepresented pathways were related to defense response, showing general downregulation of immune-related genes in the SAT of the ND, relative to the CD. Specifically, 22 genes in the "immune response" ontology were downregulated by the ND compared with the CD, with an adjusted P value of 0.0076 (**Table 4**). The 2 types of diets differentially regulated genes related to leukocyte trafficking and macrophage recruitment [matrix metallopeptidase 25 (*MMP25*), *IRF1*, parvin, gamma(*PARVG*), *CD97*, and phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit γ (*PIK3CG*)], the adaptive immune response [syntaxin binding protein 2 (*STXBP2*), interleukin 32 (*IL32*), leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 2(*LILRB2*), myeloid differentiation primary response 88 (*MYD88*), *PTPN6*, *SYK*, signal transducer and activator of transcription 1(*STAT1*), and transporter 1, ATP-binding cassette, sub-family B (*TAP1*)], and reactive oxygen species [neutrophil cytosolic factor 1(*NCF1*), *BACH1*, cytochrome b-245, beta polypeptide (*CYBB*), and cytochrome b-245, alpha polypeptide (*CYBA*)] (Table 4 and **Table 5**).

We also examined whether changes in expression of the immune-related genes were correlated with clinical and biochemical measurements, as well as with cytokines. However, we did not find significant correlations with the clinical or biochemical markers (data not shown).

Seven genes that were differentially regulated between the 2 groups were randomly selected for confirmation by RT-qPCR (**Supplemental Table 3**). None were differentially expressed between the groups at baseline. We confirmed that the genes *IRF1*, *JUNB*, *SYK*, *BACH1*, and *IL6R* were downregulated by the ND compared with the CD (**Figure 2**). For 2 genes, *PIK3AP1* and *PTPN6*, the expression changes were consistent but nonsignificant by RT-qPCR. Taken together, the RT-qPCR data confirmed the validity of the microarray transcriptome analysis, which has a 25% FDR.

Subsequently, we observed that 56 differentially regulated genes between the diets were annotated as belonging to the "metabolic process" and explored their impact on key pathways in adipose tissue (**Figure 3**). However, of 131 genes annotated as being involved in adipogenesis (www.wikipathways.org), only 1 was differentially regulated by the diets; the *STAT1* gene was downregulated (−0.18) in the ND group. Of the 67 genes involved in human lipolysis (41), only the *IL6R* gene was downregulated (−0.28) in the ND group. Among the 36 genes annotated to be involved in fatty acid β oxidation (www.wikipathways.org), only glycerol kinase (*GK*) was downregulated (−0.48) in the ND group. Finally, among the 168 genes annotated as being involved in insulin signaling (www.genmapp.org),

TABLE 2
Clinical and biochemical characteristics of the participants in the healthy ND and CD groups at the beginning and end of the intervention[1]

	ND (n = 31)		CD (n = 25)			
Variable	Week 0	Week 18/24	Week 0	Week 18/24	Estimate (95% CI)	Significance (P value)[2]
Sex (female), n (%)	21 (68)	—	16 (64)	—		—
Age, y	55.2 ± 7.8[3]	—	55.4 ± 7.5	—	—	—
Body weight, kg	87.0 ± 11.1	87.3 ± 12.3	90.8 ± 15.1	91.8 ± 15.1	−0.61 (−1.64, 0.41)	0.24
Waist, cm	102.9 ± 8.2	102.9 ± 9.3	104.9 ± 9.3	105.5 ± 9.0	−0.29 (−1.65, 1.08)	0.68
Total cholesterol, mmol/L	5.63 ± 0.83	5.40 ± 0.83	5.78 ± 0.75	5.76 ± 0.69	−0.21 (−0.47, 0.05)	0.1
LDL cholesterol, mmol/L	3.59 ± 0.73	3.33 ± 0.75	3.67 ± 0.70	3.69 ± 0.66	−0.29 (−0.52, −0.05)	0.02
HDL cholesterol, mmol/L	1.40 ± 0.28	1.50 ± 0.35	1.41 ± 0.50	1.42 ± 0.41	0.08 (−0.01, 0.18)	0.09
Triglycerides, mmol/L	1.42 ± 0.64	1.28 ± 0.54	1.55 ± 0.54	1.44 ± 0.49	−0.02 (−0.25, 0.20)	0.85
Apolipoprotein B, g/L	1.12 ± 0.28	1.07 ± 0.28	1.16 ± 0.22	1.18 ± 0.25	−0.07 (−0.13, −0.00)	0.04
Apolipoprotein A-I, g/L	1.44 ± 0.17	1.53 ± 0.24	1.45 ± 0.28	1.48 ± 0.24	0.07 (−0.00, 0.14)	0.06
Fasting plasma glucose, mmol/L	5.8 ± 0.6	5.7 ± 0.5	5.6 ± 0.5	5.6 ± 0.6	0.01 (−0.20, 0.23)	0.91
Fasting serum insulin, pmol/L	55.1 ± 28.5	57.8 ± 39.1	53.6 ± 23.9	60.8 ± 35.1	−4.48 (−17.2, 8.2)	0.48
2-h plasma glucose, mmol/L	6.1 ± 1.2	6.6 ± 1.8	6.0 ± 1.90	6.2 ± 2.0	0.38 (−0.30, 1.06)	0.27
2-h serum insulin, pmol/L	256.6 ± 186.9	284.3 ± 194.1	263.2 ± 200.5	272.2 ± 212.2	10.7 (−55.7, 77.2)	0.75
Systolic BP, mm Hg	126 ± 12	125 ± 15	133 ± 16	124 ± 13	7.25 (1.86, 12.63)	0.01
Diastolic BP, mm Hg	82 ± 10	81 ± 10	84 ± 10	80 ± 11	3.63 (0.26, 7.00)	0.04
Smoking, n (%)	1 (3)	1 (3)	1 (4)	1 (4)	—	—
Drug treatment of hypertension, n (%)	20 (65)	19 (61)	5 (20)	7 (28)	—	—

[1]BP, blood pressure; CD, control diet; ND, Nordic diet.
[2]Linear mixed-effects models adjusted for body weight, $\log_{10}$-transformed age, sex, study center (i.e., also study duration), study group, time point, and study group × time point interaction as covariates. Analyses of systolic and diastolic blood pressures included antihypertensive treatment as covariate.
[3]Mean ± SD (all such values).

3 genes—phosphoinositide-3-kinase, catalytic, γ polypeptide (*PIK3CG*); phosphoinositide-3-kinase, regulatory subunit 3 (γ) (*PIK3R3*); and syntaxin binding protein 2 (*STXBP2*)—were differentially regulated between the groups.

Finally, we used the web tool oPPOSSUM to explore TF binding sites within the regulatory regions of the 128 genes differentially expressed between the ND and CD. The binding sites of several TFs were overrepresented among the 128 genes (Table 5). Of particular interest is the increased occurrence of binding sites for nuclear transcription factor κB (NF-κB), which is the key TF in inflammatory pathways.

DISCUSSION

This study shows that the ND, compared with the CD, reduced the expression of genes related to inflammation within SAT in individuals with features of the metabolic syndrome independent of changes in body weight. To our knowledge, this is the first randomized controlled study in humans showing that dietary composition is associated with a reduction in the expression of a broad set of inflammatory genes in SAT, including those related to the adaptive immune response. It is noteworthy that the changes in adipose tissue gene expression were ascribed to dietary effects in the absence of weight loss. The results of the present study extend previous knowledge (1, 3, 5, 53–55) on health effects of the ND compared with the CD, especially in relation to the inflammatory-associated genes.

In the present study, the expression of a large number of inflammation-related genes was downregulated after the ND compared with the CD. Furthermore, we showed that NF-κB binding sites were overrepresented within the regulatory region of genes differentially expressed between the ND and CD. The ND contains many foods listed as key dietary factors having beneficial effects on health in the traditional Mediterranean diet (56, 57), such as high consumption of vegetables, fruits, and cereals; moderate consumption of fish; moderate consumption of low-fat dairy; and dietary fat source as vegetable fat–based margarines and rapeseed oil instead of olive oil. In the present study, we examined the complex nature of dietary compounds and their interactions in the whole diet in relation to gene expression in adipose tissue. Therefore, we cannot identify the specific food or dietary compound causing the effects shown here. The composition is, however, close to the Mediterranean diet because it was the inspiration for the ND (1). The evidence regarding the effect of the Mediterranean diet on gene expression in humans is mainly derived from studies on peripheral blood mononuclear cells as a target tissue (58, 59), which restricts the comparability with the adipose tissue. Bearing this in mind, the reported effects are mainly health-promoting downregulation in inflammation-related genes (59), which has been speculated to be mainly caused by polyphenolic compounds and fatty acid composition of the diet. The consumption of berries, fruits, and vegetables, as a source of polyphenols in the ND, has shown beneficial effects on immune function (10, 26–28, 30, 60, 61), but opposite results also exist (25). In addition to downregulation of expression of other inflammation-related genes in human peripheral blood mononuclear cells or monocytes, respectively (10), berries have been shown to inhibit the NF-κB activation in primary monocytes (60).

Based on observational studies (62–64), whole-grain consumption could be regarded as having an anti-inflammatory effect, but the evidence from dietary interventions is not consistent (18, 20, 49, 65). The studies on the effect of whole-grain cereals on adipose tissue gene expression are scarce. It has been

TABLE 3
Thirty-nine genes with the largest difference (>30%) in mRNA between the healthy ND and CD groups[1]

		ND (n = 31)		CD (n = 25)		
Probe set	Gene symbol	Week 0[2]	Week 24 − Week 0	Week 0[2]	Week 24 − Week 0	ND compared with CD[3]
7917576	*GBP5*	80 ± 57[4]	−14 ± 69	60 ± 30	41 ± 66	−0.85
7992811	*MMP25*	72 ± 60	−12 ± 67	69 ± 57	44 ± 76	−0.81
8133518	*NCF1*	233 ± 155	−35 ± 167	192 ± 124	107 ± 175	−0.71
7909214	*RASSF5*	104 ± 64	−16 ± 67	82 ± 39	35 ± 53	−0.58
8114010	*IRF1*	140 ± 66	−15 ± 59	118 ± 45	50 ± 77	−0.53
8073682	*PARVG*	111 ± 54	−11 ± 54	98 ± 47	40 ± 58	−0.50
8174103	*GK*	155 ± 69	−12 ± 73	125 ± 38	51 ± 69	−0.48
8042416	*ARHGAP25*	163 ± 73	−13 ± 70	134 ± 44	53 ± 70	−0.48
8025255	*STXBP2*	93 ± 46	−12 ± 47	77 ± 33	27 ± 38	−0.47
8042391	*PLEK*	254 ± 112	−26 ± 111	201 ± 83	72 ± 97	−0.46
8039212	*LILRB2*	147 ± 82	−21 ± 85	128 ± 57	40 ± 69	−0.46
7953569	*PTPN6*	245 ± 118	−25 ± 122	211 ± 87	72 ± 114	−0.44
7906757	*FCGR2A*	384 ± 182	−47 ± 168	315 ± 117	99 ± 180	−0.44
8035714	*GMIP*	93 ± 45	−11 ± 49	82 ± 33	26 ± 42	−0.43
8089911	*HCLS1*	163 ± 71	−17 ± 74	142 ± 51	46 ± 76	−0.43
7958202	*CHST11*	278 ± 122	−23 ± 129	247 ± 94	83 ± 117	−0.42
7971461	*LCP1*	1143 ± 484	−132 ± 498	983 ± 375	284 ± 472	−0.40
8026047	*JUNB*	355 ± 163	−52 ± 170	299 ± 106	75 ± 154	−0.40
8146500	*LYN*	413 ± 182	−40 ± 195	353 ± 104	105 ± 157	−0.40
8153959	*DOCK8*	332 ± 127	−25 ± 125	294 ± 85	87 ± 132	−0.37
7917516	*GBP1*	439 ± 112	−41 ± 91	389 ± 101	105 ± 215	−0.36
8150225	*RAB11FIP1*	88 ± 37	−11 ± 37	77 ± 22	18 ± 32	−0.35
7935337	*PIK3AP1*	147 ± 56	−16 ± 56	127 ± 44	31 ± 57	−0.35
7998637	*SEPX1*	313 ± 118	−34 ± 122	271 ± 68	66 ± 114	−0.35
8160346	*PTPLAD2*	158 ± 63	−15 ± 64	131 ± 37	33 ± 56	−0.34
7955908	*NCKAP1L*	222 ± 87	−24 ± 86	195 ± 72	44 ± 82	−0.33
8135363	*PIK3CG*	143 ± 54	−14 ± 56	127 ± 38	29 ± 46	−0.33
8026300	*CD97*	384 ± 149	−39 ± 148	340 ± 112	78 ± 138	−0.33
8156321	*SYK*	115 ± 46	−9 ± 39	104 ± 35	26 ± 44	−0.33
7927964	*SRGN*	811 ± 267	−61 ± 275	682 ± 168	173 ± 225	−0.33
7940191	*STX3*	172 ± 56	−7 ± 57	156 ± 47	45 ± 65	−0.33
8068105	*BACH1*	192 ± 61	−15 ± 70	168 ± 40	41 ± 52	−0.32
7927775	*NRBF2*	142 ± 39	−9 ± 43	129 ± 33	33 ± 45	−0.32
8174692	*SEPT6*	188 ± 65	−15 ± 59	163 ± 41	39 ± 72	−0.32
8079462	*NBEAL2*	185 ± 66	−13 ± 68	169 ± 50	41 ± 72	−0.31
8166730	*CYBB*	687 ± 232	−88 ± 245	610 ± 179	111 ± 186	−0.31
7905789	*IL6R*	246 ± 75	−16 ± 76	225 ± 61	49 ± 77	−0.28
7933192	*HNRNPA3P1*	138 ± 77	19 ± 72	161 ± 54	−28 ± 55	0.31
8165038	*SOHLH1*	66 ± 24	15 ± 35	79 ± 25	−10 ± 28	0.35
8065412	*CST1*	69 ± 26	16 ± 34	82 ± 20	−13 ± 31	0.39

[1]Statistical analyses have been made by using linear mixed-effects models adjusted for body weight, $\log_{10}$-transformed age, sex, study center (i.e., also study duration), study group, time point, and study group × time point interaction as covariates. All the values are significant at the level of $P < 0.01$. False discovery rate = 25%. *IL6R* was included as an interesting candidate gene. CD, control diet; ND, Nordic diet.

[2]Mean expression at week 0.

[3]ND week 18 or 24/week 0 minus CD week 18 or 24/week 0.

[4]Mean ± SD (all such values).

shown that a rye bread–based diet with a low postprandial insulin response resulted in the upregulation of genes linked to stress response and immunity in adipose tissue, compared with a wheat bread–based diet with a high postprandial insulin response (12, 65). Thus, our results are in line with these results by Kallio et al. (12, 65). However, the studies are not totally comparable, because in the present study, the whole dietary composition was modified.

It is also possible that dietary fatty acids may regulate the activity of NF-κB, which in turn influences inflammatory processes (8, 36, 66). The fatty acids of the ND group originated mainly from fatty fish and rapeseed oil as well as rapeseed oil–based margarine with a simultaneous decrease in the consumption of sources of saturated fat (1). Increasing the intake of n–3 PUFAs—namely, eicosapentaenoic, docosahexaenoic, and α-linolenic acids—has been shown to inhibit NF-κB signaling (8, 36, 66, 67). In previous intervention studies with fish oil, a diet enriched with SFAs, or overfeeding with either PUFA or SFA, effects were shown on the expression of inflammatory-related genes in adipose tissue (35, 43, 47).

TABLE 4
Fold changes of the expression of immune-related genes downregulated in the healthy Nordic diet group compared with the control diet group

Fold change[1]	Gene symbol	Gene name
−0.46	*LILRB2*[2]	Leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 2
−0.19	*TNFAIP3*	Tumor necrosis factor, α-induced protein 3
−0.23	*IL32*	Interleukin 32
−0.44	*PTPN6*	Protein tyrosine phosphatase, nonreceptor type 6
−0.27	*TAP1*	Transporter 1, ATP-binding cassette, subfamily B (MDR/TAP)
−0.28	*IL6R*	Interleukin 6 receptor
−0.47	*STXBP2*	Syntaxin binding protein 2
−0.4	*LYN*	v-yes-1 Yamaguchi sarcoma viral-related oncogene homolog
−0.85	*GBP5*	Guanylate binding protein 5
−0.4	*LCP1*	Lymphocyte cytosolic protein 1 (L-plastin)
−0.28	*VASP*	Vasodilator-stimulated phosphoprotein
−0.18	*CYBA*	Cytochrome b-245, α polypeptide
−0.19	*TRIM25*	Tripartite motif containing 25
−0.33	*NCKAP1L*	NCK-associated protein 1–like
−0.18	*STAT1*	Signal transducer and activator of transcription 1, 91 kDa
−0.33	*CD97*	CD97 molecule
−0.21	*MYD88*	Myeloid differentiation primary response gene (88)
−0.23	*CNPY3*	Canopy 3 homolog (zebrafish)
−0.33	*PIK3CG*	Phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit γ
−0.33	*SYK*	Spleen tyrosine kinase
−0.53	*IRF1*	Interferon regulatory factor 1
−0.31	*CYBB*	Cytochrome b-245, β polypeptide

[1]Calculated as [average of (healthy Nordic diet week 24/healthy Nordic diet week 0)] − [average of (control diet week 24/control diet week 0)].
[2]Differentially expressed genes between healthy Nordic diet and control diet, which are annotated as immune response according to Gene Ontology.

However, there is only little overlap between previously reported genes and the genes associated with the ND in the present study. This might be due to the limited power of each study (i.e., each study identifies and reports only part of the genes that actually are affected by the diet). Other factors related to study design might also be important, such as inclusion criteria, length of intervention, type of intervention, and transcriptome platform.

Adipose tissue inflammation has been associated with expansion of the fat mass (68, 69), although this is not always seen (70, 71). The present data strongly suggest that the composition of diet (i.e., the quality of diet), independently of weight change, is an important factor for regulating adipose tissue inflammation at the molecular level. Interestingly, concerning adipose tissue gene expression, there is little overlap between the genes reported to be associated with

TABLE 5
Overrepresentation of binding sites for the nuclear transcription factor κB[1]

Transcription factor	Target gene hits[2]	Target gene nonhits[3]	Background gene hits	Background gene nonhits	Target TFBS hits[4]	Background TFBS hits	z score[5]
SPI1	91	22	3644	1102	667	26,585	13.05
NFYA	39	74	1462	3284	75	2515	12.608
GABPA	64	49	2383	2363	157	5635	12.277
RXR::RAR_DR5	9	104	319	4427	15	366	11.023
FEV	90	23	3571	1175	557	22,895	10.662
Evi1	12	101	495	4251	23	651	9.613
REL	74	39	2579	2167	209	8177	9.532
Pax4	2	111	30	4716	2	30	9.439
ELK1	81	32	3242	1504	355	14,720	8.971
ELF5	93	20	3753	993	749	32,449	8.801
NF-κB	58	55	2093	2653	136	5186	8.627
MAX	54	59	1991	2755	112	4272	7.813
MZF1_1–4	95	18	3797	949	1204	53,511	6.914
RELA	51	62	1869	2877	101	3926	6.81

[1]In total, 128 differentially expressed genes were compared with all 5628 analyzed genes. TFBS, transcription factor binding site.
[2]Total number of genes among the 128 differentially expressed genes containing the TFBS; 123 genes were included in the database analysis.
[3]Total number of genes among the 128 differentially expressed genes not containing the TFBS; 123 genes were included in the database analysis.
[4]Total number of TFBSs for the 128 differentially expressed genes; 123 genes were included in the database analysis.
[5]The z score statistic reflects the occurrence of the TFBS in the target set of genes compared with background.

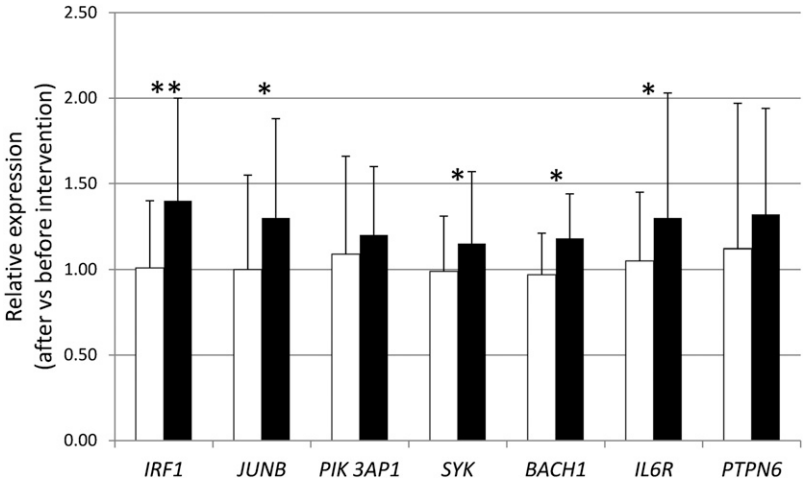

FIGURE 2 Confirmation of the genes regulated by the ND, as measured by reverse-transcribed quantitative real-time polymerase chain reaction. Values are mean and SD of relative expression levels before and after the intervention. The values for the ND (n = 31) are presented as white bars and the values for control diet (n = 25) as black bars. Statistics were done with the 1-sided t test: $^{*}P < 0.05$ and $^{**}P < 0.01$. ND, Nordic diet.

weight loss and those responding to dietary changes in the present study. Thus, although both weight loss and the ND seem to downregulate the inflammatory gene expression in adipose tissue, there are marked differences in the precise nature of the regulated genes (46, 65, 68, 72). In particular, a hypocaloric diet is accompanied by a reduction in adipose tissue macrophages and in genes related to macrophage function (69, 73). By contrast, we demonstrate for the first time in humans that dietary composition is associated with a reduction in expression of a broader set of inflammatory genes in SAT also related to the adaptive immune response (*STXBP2*, *IL32*, *LILRB2*, *MYD88*, *PTPN6*, *SYK*, *STAT1*, and *TAP1*). Our results are in line with the results from the experimental animal studies showing that T cells in adipose tissue are important mediators of local inflammation and metabolic dysfunction (74).

The clinical relevance of the observed reduction in inflammatory gene expression in adipose tissue is so far unclear. It is possible that adipose tissue inflammation, via secretion of inflammatory mediators, increases the levels of these mediators in the circulation and thereby contributes to insulin resistance and atherosclerosis (40, 75). Admittedly, this speculation needs to be verified by measuring the secretion of inflammatory proteins from adipose tissue. Moreover, in the original SYSDIET study, only IL-1Ra plasma concentration was differently changed between the 2 diets, and no influence on insulin sensitivity was observed (1). A wider screen of systemic inflammatory markers (e.g., cell surface markers and proteomics) and an examination of patients in the postprandial state might have detected additional systemic effects.

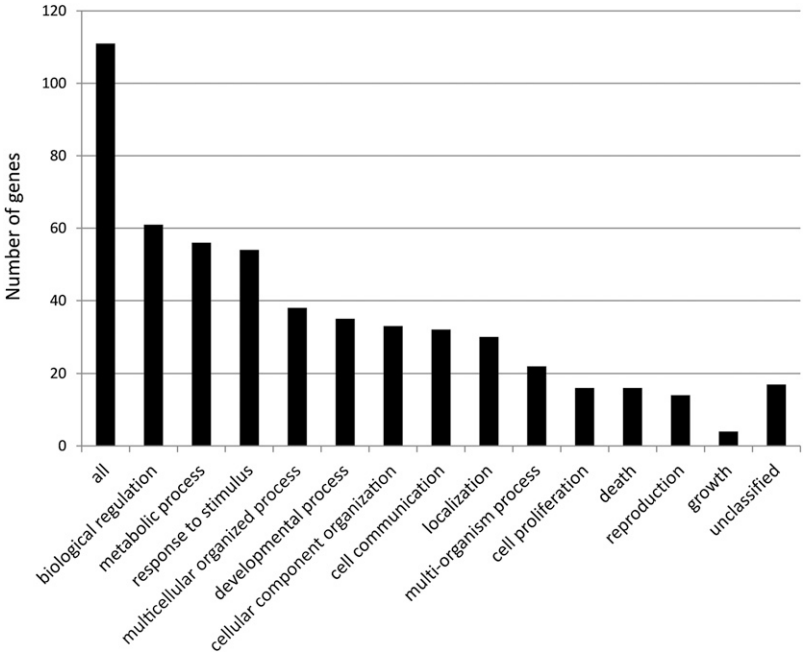

FIGURE 3 Annotated biological processes using WebGestalt software.

There are some weaknesses in the present study. The study population was still quite small, although it was larger than in most previous studies in this field (12, 35, 42, 72, 76, 77). Some participants of the original SYSDIET study were not willing to donate adipose tissue samples, which might potentially interfere with the interpretation of the results. Furthermore, the aim of the present study was to examine the effects of the ND on the gene expression in the SAT, and thus, we also excluded subjects with elevated C-reactive protein concentration, statin use, and a BMI >38. In addition, we studied the gene expression only in the fasting state, and therefore it was not possible to assess the potential effects on insulin sensitivity, for example, seen in the postprandial phase only. Another issue is that the volunteers participating in the present study may have had healthy eating habits before the study. When these volunteers were randomized in the CD, they may have actually modified their diet to an unhealthy direction (i.e., they had more changes in the diet than those randomized in the ND). Consequently, the changes in gene expression seemed to be more evident in the CD than in the ND.

In conclusion, the 18- to 24-wk ND reduces inflammatory gene expression in SAT compared with the CD in individuals with features of the metabolic syndrome, showing that the ND has an impact on white adipose tissue independent of body weight changes.

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