Healthy Nordic diet modulates the expression of genes related to mitochondrial function and immune response in peripheral blood mononuclear cells from subjects with the metabolic syndrome – a SYSDIET sub-study

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Abbreviations: ND: healthy Nordic diet, CD: control diet, PBMCs: peripheral blood mononuclear cells, T2DM: type 2 diabetes mellitus, CVD: cardiovascular diseases, IR: insulin resistance, MetS: metabolic syndrome, PUFA: polyunsaturated-fatty acid, SFA: saturated fatty acid, ROS: reactive oxygen species, FDR: false discovery rate, ETC: electron transport chain, OXPHOS: oxidative phosphorylation, SCFAs: short-chain fatty acids.

Keywords: Healthy Nordic Diet, Metabolic syndrome, PBMCs, Transcriptome, Gene-expression

Abstract

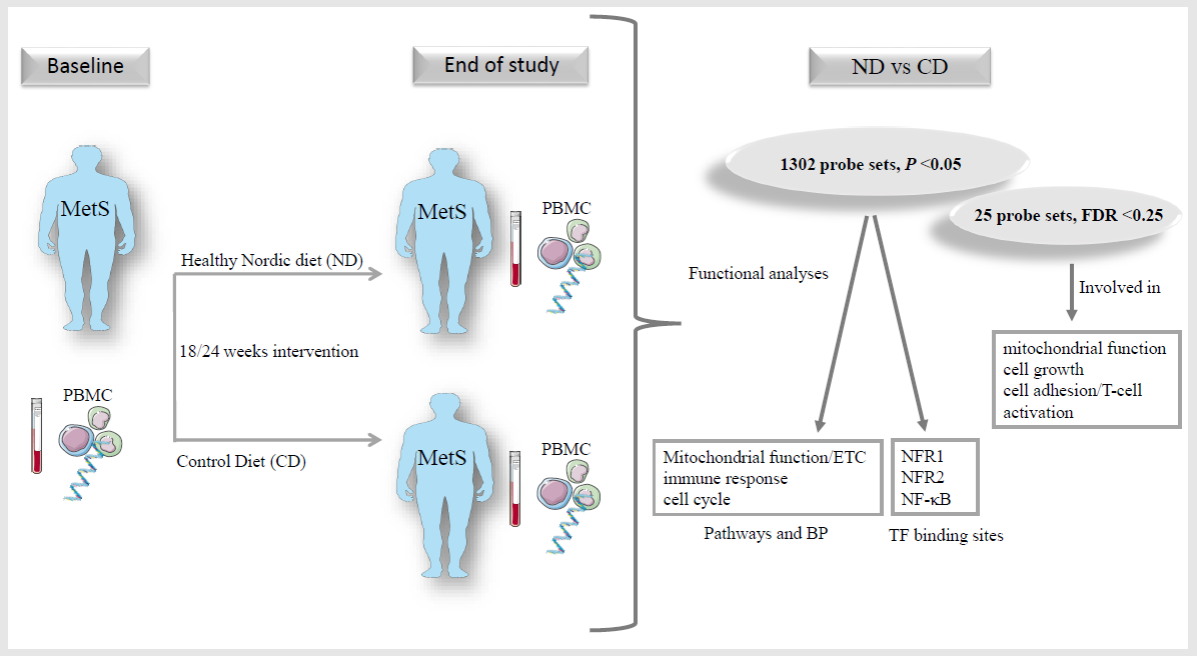
Scope: To explore the effect of a healthy Nordic diet on the global transcriptome profile in peripheral blood mononuclear cells (PBMCs) of subjects with the metabolic syndrome.

Methods and Results: Subjects with the metabolic syndrome underwent an 18/24 week randomized intervention study comparing an isocaloric healthy Nordic diet with an average habitual Nordic diet served as control (SYSDIET study). Altogether, 68 participants were included. PBMCs were obtained before and after intervention and total RNA was subjected to global transcriptome analysis. 1302 probe sets were differentially expressed between the diet groups (p-value < 0.05). Twenty-five of these were significantly regulated (FDR q-value < 0.25) and were mainly involved in mitochondrial function, cell growth and cell adhesion. The list of 1302 regulated probe sets was subjected to functional analyses. Pathways and processes involved in the mitochondrial electron transport chain, immune response and cell cycle were down-regulated in the healthy Nordic diet group. In addition, gene transcripts with common motifs for 42 transcription factors including NFR1, NFR2 and NF-κB, were down-regulated in the healthy Nordic diet group.

Conclusion: These results suggests that benefits of a healthy diet may be mediated by improved mitochondrial function and reduced inflammation.

Graphical abstract-Figure legend

Subjects (n=68) with the metabolic syndrome underwent an 18/24 week randomized intervention study comparing an isocaloric ND with an average habitual Nordic diet served as control (CD). Total RNA isolated from PBMCs was subjected to microarray analysis. 1302 probe sets were differentially expressed between the diet groups (p-value < 0.05). 25 of these were significantly regulated (FDR q-value < 0.25) and were mainly involved in mitochondrial function, cell growth and cell adhesion/T-cell activation. The list of 1302 regulated probe sets was subjected to functional analyses. Pathways and processes involved in the mitochondrial function, immune response and cell cycle were down-regulated in the ND compared to the CD group. In addition, gene transcripts with common motifs for 42 transcription factors including NFR1, NFR2 and NF-κB, were down-regulated. A healthy Nordic dietary pattern modulates the expression of genes involved in mitochondrial function and immune response in PBMCs of subjects with the metabolic syndrome. ND: healthy Nordic diet, CD: control diet, PBMCs: peripheral blood mononuclear cells, MetS: metabolic syndrome, ETC: electron transport chain, NRF1: nuclear respiratory factor 1, NRF2: nuclear respiratory factor 2. BP: biological process, TF: transcription factor, FDR: false discovery rate.



Introduction

Metabolic syndrome (MetS) represents a cluster of related risk factors, including glucose intolerance, hypertension, dyslipidemia and central obesity, which increase the risk of type 2 diabetes mellitus (T2DM) and cardiovascular diseases (CVD) [1]. Obesity, insulin resistance (IR) and T2DM are associated with chronic low-grade inflammation, characterized by abnormal cytokine production, and increased levels of acute-phase reactants [2-4]. Furthermore, increased reactive oxygen species (ROS) production caused by mitochondrial dysfunction has recently shown to be involved in the pathogenesis of IR, T2DM and MetS [5-7]. Mitochondria have also been recognized as key players in the inflammatory responses by linking ROS to production of proinflammatory signaling and inflammasome activation [8].

Excessive energy intake, unhealthy diet and sedentary lifestyle, all modifiable factors, increase the risk of developing both the MetS and T2DM [9]. Therefore, lifestyle changes are important when attempting to reduce the risk of these metabolic diseases [10, 11]. Epidemiological and dietary intervention studies have demonstrated that a traditional Mediterranean dietary pattern rich in whole-grains, fruits, vegetables, nuts, and olive oil, is associated with lower risk of MetS [9, 12, 13]. A healthy Nordic diet resembling the Mediterranean diet has also shown to improve cardiovascular risk factors in obese and hypercholesterolaemic subjects [14, 15]. We have previously shown that a healthy Nordic diet (ND) improved risk factors, such as cholesterol, blood pressure and inflammation related to the development of T2DM and atherosclerosis in subjects with the MetS when compared to the current average habitual Nordic diet defined as the control diet (CD) (The SYSDIET study) [16, 17]. The ND was based on Nordic and local food items, such as whole-grain products, abundant use of berries, fruit and vegetables, rapeseed oil, three fish meals per week and low-fat dairy products. In a sub-study of SYSDIET, the transcriptome profile of subcutaneous adipose tissue was altered and genes related to inflammation were significantly

different expressed after intake of ND [18]. There is, however, a lack of clarity in relation to the molecular mechanisms involved in the beneficial effect of a healthy diet on cardiovascular risk factors.

The peripheral blood mononuclear cells (PBMCs) are immune cells consisting mostly of monocytes and lymphocytes. These cells are exposed to many of the same environmental factors as metabolically active tissues and the arterial wall, and can give us additional information on how the diet influences metabolic regulation. Given the notion that PBMCs are central in the process of inflammation, these cells may constitute a link between systemic inflammation, diet, metabolic changes in peripheral tissues and the development of atherosclerosis and IR [19]. Nutrition-related metabolic changes such as fasting have been shown to alter the PBMC gene expression profile [20]. Furthermore, diets and dietary factors have been reported to modulate expression of genes involved in inflammation, oxidative stress and lipid metabolism in PBMCs [21-26]. A traditional Mediterranean diet was shown to modulate the transcriptome profile related to a reduced CVD risk in PBMCs [27].

In the present sub-study of the SYSDIET, our specific aim was to further explore the effect of a ND by investigating the global transcriptome profile of PBMCs in subjects with features of the MetS. We demonstrate that a ND significantly modulates the transcriptome profile compared to the current average habitual diet in the Nordic countries.

Materials and Methods

Design of the SYSDIET study

The SYSDIET study was a randomized controlled multi-center study performed in six centers (Kuopio and Oulu (Finland), Lund and Uppsala (Sweden), Aarhus (Denmark) and Reykjavik (Iceland)) as previously described [16]. The primary outcome was insulin sensitivity

and glucose tolerance, and among the main secondary outcomes were other traits related to MetS, i.e. blood pressure and serum lipid profile, inflammatory markers and transcriptome data. Details of the study design and the main measurements performed are described in the original publication [16]. Briefly, after a one-month run-in period during which all participants followed their habitual diet, participants fulfilling the inclusion criteria were randomized into the CD group or the ND group for the next 18 to 24 weeks. Randomization was performed at each center by matching according to gender and medians of age, BMI and fasting plasma glucose at screening, resulting in equal amounts of certain strata classes amongst the groups. The major visits were in the beginning (0 week) and at 12 and either 18 or 24 weeks (end of the study). The study participants were advised to keep their body weight and physical activity level constant and not to change their smoking or drinking habits or drug treatment during the study. PBMCs were not collected from the study center of Aarhus, and few subjects donated PBMCs from the study centers at Uppsala and Reykjavik. Therefore only subjects from the study centers in Kuopio and Oulu, Finland and from Lund, Sweden, were included in this current SYSDIET sub-study. All study participants provided written informed consent and local Ethics committees (Research Ethics Committee of the Hospital District of Northern Savo and Northern Ostrobothnia Hospital District, Oulu, Finland and Regional Ethical Review Board, Lund) approved the study protocol in accordance with the Helsinki Declaration. The study was registered at clinicaltrials.gov as NCT00992641.

Screening of study participants and inclusion/exclusion criteria

A screening examination was carried out 4 weeks before the study. This visit included medical history, and clinical examination [16]. The inclusion criteria were age 30 to 65 years, BMI 27- 39 kg/m^2, and two other of the International Diabetes Federation (IDF) criteria for MetS [28]. Anti-hypertensive and lipid lowering medication were allowed but without changes

during the trial. The main exclusion criteria included any chronic disease and condition, which could hamper the adherence to the dietary intervention protocol, chronic liver, thyroid and kidney diseases, alcohol abuse (> 40 g/d), diabetes, fasting triglycerides > 3.0 mmol/l, total cholesterol > 6.5 mmol/l and blood pressure > 160/100 mmHg.

For this sub-study of SYSDIET, we included participants who had donated PBMCs in Kuopio, Lund and Oulu (n=68, 42 in the ND group and 26 in the CD group) with a maximum weight change during the study of +/- 4 kg, no use of statins, high-sensitivity (hs) C-reactive protein (CRP) < 10 mg/L at baseline and at the end of the intervention (Figure 1). The study was originally designed to last for 24 weeks. Due to problems with recruitment, the original protocol was changed and a shorter intervention period at some of the study centers was included after the trial was started. Therefore, two of the centers included had 24 weeks of study length (Kuopio and Lund) and one center 18 weeks of study length (Oulu) [16]. For verification of selected target genes with RT-qPCR, the same criteria for inclusion was used (Figure 1). The three remaining study centers (Aarhus, Uppsala and Iceland) were not included in the transcriptome analyses or the RT-qPCR analyses as they did not isolate PBMCs (Aarhus n=31) or included a limited number of participants and PBMC samples in the study (Uppsala n=9 and Reykjavik n=15).

Diet

The nutrient composition of the isocaloric diets is extensively described elsewhere [16]. Briefly, Nordic nutrition recommendations formed the basis for the ND [29]. The main emphasis was on food items such as whole-grain products, abundant use of berries, fruits and vegetables, rapeseed oil, three fish meals per week, low fat dairy products and avoidance of sugar-sweetened products. The current average diet in the Nordic countries formed the basis for the CD and the individuals in the CD group received low fiber cereal products, e.g. breads

with fiber content less than 6 g/100 g, and dairy fat based spread e.g. a butter-vegetable oil mixture. Key food products were provided to the study participants in both groups.

A clinical nutritionist or a dietician instructed about the diets at the first visit (week 0). The study participants kept 4-day dietary records during the run-in period (baseline intake) and three times (weeks 2, 11 and 17 or 23) during the intervention period to assess the total diet.

Biochemical and anthropometric measurements

Screening laboratory measurements, fasting glucose, total lipid and lipoprotein concentrations and anthropometric measurements were performed locally according to the standard operational procedures agreed by all centers.

Sampling of PBMCs, RNA extraction, microarray hybridization

PBMCs were isolated within 30 to 45 min from blood samples collected after overnight fasting (12h) at each study center using cell preparation tubes (CPT) according to a standard operational procedure (SOP) based on the manufacturer's instructions (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). This is a standardized method to collect mononuclear cells with high purity (above 90%), and according to the manufacturer, approximately 80% of the cells are lymphocytes and 12% are monocytes. The PBMC pellet was immediately stored at -80^{o}C. At the end of the study, all samples were shipped on dry-ice to Karolinska Institute, Sweden. The number of monocytes and lymphocytes were counted and did not change before and after intervention in ND compared to the CD group (data not shown).Total RNA from the PBMCs from all centers was isolated and prepared at the same time in the same laboratory (Karolinska Institute, Stockholm). Total RNA was extracted using the RNeasy Mini Kit according to the manufacturer's instructions (Qiagen, Valencia, CA, USA). Contamination of DNA was removed during RNA isolation with DNase. RNA

integrity was checked using Bioanalyzer device (Agilent 2100 Bioanalyzer, Agilent Technologies, Santa Clara, CA, USA). RIN value below 8 was consider poor RNA quality. Two samples were excluded due to low RNA yield. Therefore, RNA samples from 40 participants from the ND group and 26 from the CD group were labeled (cRNA) and served as template for hybridization to the Human Gene ST 1.1 Arrays (Affymetrix, Inc., Santa Clara, CA, USA) according to the manufacturer's standard protocols. The Affymetrix CEL files (containing probe intensities) were imported into Partek Genomics Suite software (Partek, Inc. MO, USA) for further processing. Robust microarray analysis (RMA) yielding normalized log2 transformed signal intensities was applied for normalization. Gene transcripts with maximal signal values of less than 5 (Log2 values) across all arrays were removed to filter for low and non-expressed genes, reducing the number of probe sets from 33,297 to 16,831.

RT-qPCR

RNA was reverse transcribed by a high-capacity cDNA reverse transcription kit (Applied Biosystems). The concentrations of RNA ranged between 71.9 and 247.1 ng/ul and 500 ng of RNA was used in the cDNA reactions to a final yield of 25 ng/ul cDNA.RT-qPCR was performed on an ABI PRISM 7900HT (Applied Biosystems) with inventoried TaqMan gene expression assays for carbohydrate sulfotransferase 10 (*CHST10*, Hs01556595_m1), dihydrofolate reductase (*DHFR*, Hs00758822_s1), DAN family BMP antagonist (*NBL1*, Hs01556595_m2) and ubiquinol-cytochrome c reductase complex assembly factor 3 (*UQCC3*, Hs03054640_g1) (Thermo Fisher Scientific). TATA-binding protein (*TBP*, Hs00427620_m1) and glucuronidase beta (*GUSB*, Hs00939627_m1) was chosen as reference genes due to previous experience with these genes in PBMCs [30]. The assays used for the selected genes were chosen due to best coverage according to thermos Fischer.

The relative mRNA level for each transcript was calculated by the ΔΔcycle threshold (Ct) method [31]. Ct values for each target gene were normalized to the average Ct value of the reference genes ($Ct_{reference} - Ct_{target} = \Delta Ct$) and the relative change from baseline to the end of study visits was calculated and expressed as log ratio ($\Delta Ct_{end\ of\ study} - \Delta Ct_{baseline} = \Delta\Delta Ct$).

Statistical and functional analyses

For the microarray analyses, diet-induced changes in gene expression were obtained by calculating the signal log2 ratio between the 18/24 weeks and baseline intensities for each probe set. Differentially expressed probe sets between ND and CD were determined in R (version 3.5.1) with a linear regression model adjusted for age, gender and study center and with log ratio as the dependent variable. To account for multiple testing, we applied false discovery rate (FDR) analyses and a q-value < 0.25 was considered significant. Furthermore, a list of 1302 probe sets differently regulated by the two diets with a p-value < 0.05 were subjected to functional analyses with the software tool MetaCore (GeneGo, division of Thomson Reuters, St. Joseph, MI, USA) and the Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.8 (https://david.ncifcrf.gov/).

For RT-qPCR analyses, differences in gene expression between diets were tested with a linear regression model adjusted for age, gender and study center with log ratio as the dependent variable. Statistical analyses were performed in R version 3.5.1. Gene transcripts with a p-value < 0.05 were defined as significant changed between diets.

For the biochemical,anthropometric measurements, and dietary changes linear mixed-effects models (nlme package version 3.1-108) were used to analyze the differences between the two groups as previously described [16].

Results

Subject characteristics, dietary data and clinical and biochemical variables

A total of 66 subjects (n=40 ND group, n=26 CD group) were included in the analyses of the present study (Figure 1) and their baseline characteristics are shown in Table 1. The baseline characteristics did not differ between the two groups. During the intervention, the nutrient intakes in this subpopulation of participants were in agreement with the results obtained for the whole study population [16]. The polyunsaturated fatty acid (PUFA), β-carotene and fiber intake were higher, and the saturated fatty acid (SFA), intake were lower in the ND group compared to the CD group. However, in contrast to the whole study population, protein, salt and alcohol intakes did not differ significantly between the groups in the present subpopulation during the intervention (Table 2). Changes in clinical and biochemical variables were in agreement with the whole study population [16]. In particular, the IL1Ra plasma concentration was markedly decreased in the ND group compared to the CD group (Table 3). In the ND group, we also observed a decrease in LDL cholesterol concentration compared to the CD group in line with the main study (Table 3).

Gene expression profiling in PBMCs

Microarray hybridization was performed on RNA from PBMCs collected at baseline and at the end of the intervention (18 or 24 weeks). From the 33,297 probe sets presented on the microarray, 16,831 were defined as expressed and included in the statistical analyses. Diet-induced changes in gene expression were obtained by calculating the log2 ratio between the 18/24 weeks and baseline intensities for each probe set. The log2 ratio for ND was then compared to the log2 for the CD in the linear regression analyses. Overall, 1302 probe sets

were identified to be differentially expressed between the two intervention groups (p-value < 0.05) (Table S1) and used for further functional analyses to identify groups of regulated gene transcripts (see below). In order to identify significantly expressed gene transcripts between the two diet groups we adjusted the p-value for multiple testing and 25 probe sets were significantly altered with a FDR q-value < 0.25 (Table 4). The ND diet led to a down-regulation of 22 gene transcripts and up-regulation of three gene transcripts compared to the CD diet (Table 4). The down-regulated gene transcripts were related to cellular processes such as cell division, -proliferation and -growth (*DHFR, KNSTRN, OGFR, MRGBP, PPAI*), DNA damage and -repair and apoptosis (*UBE2A, ZBTB33, FAAP20, ATRAID*), cell adhesion (*CHRIST10, CLDN15*), ribosomal biogenesis (*RPL14*), protein modification such as palmitoylation (*ZDHHC5*) and glycosylation (*ALG14, POMGNT2*). In addition, several of the down-regulated gene transcripts were involved in mitochondrial functions such as the respiratory chain (*UQCC3*), protein folding (*CHCHD5*), mitochondrial transport (*SLC25A19*) or located within the mitochondria (*TRUB2, ALDH1B1*).

Four genes (*DHFR, CHST10, UQCC3* and *NBL1*) among the most regulated (FDR q-value < 0.25) and involved in different cellular processes (Table 4) were selected for confirmation by RT-qPCR analyses. The mRNA level of *CHST10* (p = 0.02) and *UQCC3* (p-value = 0.01) were significantly decreased in the ND group compared to the CD group. In addition, the mean log ratio level measured with RT-qPCR was similar for these gene transcripts compared to the level obtained with the microarray analyses (Figure 2). The mRNA level of *DHFR* was also decreased in the ND group compared to the CD group. Although the difference was not significant (p-value = 0.08), the mean effect was in the same direction as in the microarray analyses (Figure 2). In contrast to the microarray results, we were not able to detect any expression level of *NBL1* with the RT-qPCR method.

Pathways and Processes modulated by the intervention diets

To further identify differences across the two groups, we analyzed functional relationships among the 1302 differentially expressed probe sets (p-value < 0.05) using the software tools Metacore and DAVID. The gene list was divided into down- and up- regulated gene transcripts in the ND group when compared to the CD group and subjected to the software tools, separately. Altogether, five pathway maps were significantly modulated (FDR q-value < 0.05) between the groups among the 927 down-regulated gene transcripts using Metacore. The down-regulated pathways were involved in immune response, ubiquinone metabolism, neurophysiological process, regulation of degradation of deltaF508-CFTR and cytoskeleton remodeling (Table 5). In addition, we identified ten significantly enriched biological processes (FDR q-value < 0.05) in the list of down-regulated gene transcripts, by the use of the annotation tool DAVID (Table 6). These were related to mitochondrial function, cell cycle, transcription and translation processes.

To further explore the the changes in gene expression, transcription factor analyses were performed using Metacore with the list of down-regulated probe sets. Genes with binding sites for 42 transcription factors were overrepresented among the 927 probe sets (Table 7). Of particular interest is the increased occurrence of genes with binding sites for both the NF-κB subunits RelB and RelA (p65), SREBP1 and 2, IRF2, LXRα and β, YY1, NRF2, NRF1 and p53. The transcription factor p53 was also present in the list of the down-regulated gene transcripts (Table 7 and Table S1), in addition to Miz-1 and NRF1 (Table 7).

One pathway map involved in cytoskeleton remodeling was significantly modulated (FDR q-value <0.05) among the 375 up-regulated gene transcripts in the ND compared to CD group (Table 8). In addition, binding sites for two transcription factors were overrepresented

among the 375 up-regulated probe sets (CBFA2T3 and CREB1) as shown in Table 9. No significant enriched biological processes were identified among the up-regulated probe sets.

Discussion

We have investigated the impact of a ND compared to a CD on the global transcriptome profile of PBMCs in subjects with MetS participating in a multi-center dietary intervention study for 18/24 weeks. The significant changes in gene transcripts involved in mitochondrial function, cell growth and cell adhesion were of particular interest. Furthermore, functional annotation analyses showed that pathways and processes related to immune response and mitochondrial electron transport chain (ETC) were enriched among the down-regulated gene transcripts after intake of ND compared to CD.

In the current study, *UQCC3* was significantly down-regulated after the intake of ND compared to CD (Table 3 and Figure 2). The decreased mRNA expression in the ND group was also confirmed with the use of RT-qPCR. The protein encoded by *UQCC3* is associated with the bc_1 complex of the ETC [32]. Furthermore, several processes and one pathway related to ETC and respiratory chain in addition to genes with regulatory motifs for the transcription factors NRF1 and 2, were down-regulated in the ND group. NRF1 and 2 are nuclear transcription factors that, together with the transcriptional co-activator PGC1-a, stimulate the expression of a broad set of genes involved in mitochondrial biogenesis and functions [33]. In accordance to our results, decreased expression of genes linked to mitochondrial function has previously been observed in PBMCs in human dietary intervention studies. Intake of a diet rich in monounsaturated fat or a Mediterranean diet was found to decrease expression of genes linked to oxidative phosphorylation (OXPHOS) in PBMCs compared with a diet rich in SFA [22]. In addition, caloric restriction, which is considered beneficial for longevity and CVD, decreased expression of genes involved in OXPHOS in a human study [34]. The ETC is

the major site of production of ROS in the cells. Mitochondrial alterations may lead to increased production of mitochondrial-derived ROS, promote inflammation and thereby contribute to the development of metabolic disorders such as IR, T2DM and obesity [35-37]. A healthy dietary pattern, such as a healthy Nordic diet, may thus improve health by altering expression of genes related to mitochondrial function and thereby reduce ROS production. The mechanism behind this change is currently unknown. In animal studies, dietary fat has shown to influence the composition of the mitochondrial membrane and thereby the mitochondrial function [38-40]. Furthermore, intake of n-3 PUFA has been shown to reduce ATP production and respiratory chain activity in mice [41, 42].

In the present study, the ND down-regulated a pathway related to immune response as compared to the CD. In addition, gene transcripts with binding sites for both the NF-κB subunits RelB and RelA were overrepresented within the regulatory region of genes significantly down-regulated in the ND compared to the CD group. ND significantly down-regulated the gene expression of toll-like receptor 4, which is central in NF-KB signaling, in PBMC during a glucose challenge test in the same study [43]. In addition, NF-κB target genes and several immune related gene transcripts were down-regulated in the adipose tissue after intake of ND compared to CD [18]. In the current study, we also identified gene transcripts related to immune function that were down-regulated, such as *CHST10*. Furthermore, the decreased mRNA expression of CHST10 was confirmed with RT-qPCR analyses. *CHST10* encodes a protein with sulfotransferase activity, involved in T-cell adhesion and activation [44]. Activated T cells in adipose tissue are important mediators of local inflammation and metabolic dysfunction [45]. Regulation of gene transcripts in PBMCs related to immune response and inflammation have previously been reported in dietary intervention studies. In a randomized controlled trial, a traditional Mediterranean diet supplemented with olive oil decreased the expression of inflammatory genes in PBMCs in

subjects with CVD and overall modulated biological pathways related to CVD [27]. Furthermore, intake of olive oil in a postprandial study down-regulated *IFNG* gene expression in PBMCs [46] and sustained consumption of olive oil with high polyphenol content showed similar effect [47]. The healthy Nordic diet is in many respects similar to the Mediterranean diet, where the intake of SFA is reduced and the intake of n-6 and n-3 PUFAs and polyphenols is increased. Thus, genes related to immune response and inflammation represent possible gene targets modulated by a healthy dietary pattern in metabolically active tissues. Components of the ND, such as n-3 PUFAs and phytochemicals may decrease the expression of inflammation-related genes through attenuation of the NF-κB pathway [48, 49]. In addition, the fiber intake was higher in the ND compared to the CD group [16]. Dietary fibers may be fermented to short-chain fatty acids (SCFAs) by the gut microbiota. Recent studies suggest that SCFAs exert multiple beneficial effects on mammalian energy metabolism and may beneficial influence inflammation status in the circulation [50].

Gene transcripts related cellular processes such as cell division, -proliferation and –growth and apoptosis were down-regulated by ND compared to CD (Table 4, 6), indicating that intake of ND has an impact on basic cellular processes.. Interestingly, intake of fish oil has previously been shown to modulate expression of genes related cell cycle and apoptosis [51]. Whether the effects reported in the current study are specific to n-3 fatty acids remains to be elucidated. It is also tempting to speculate whether changes in these basic cellular processes by ND are linked to altered mitochondrial function and thereby ROS production. Intracellular ROS are known to act as signaling molecules and are essential for regulation of physiological functions such as cell cycle progression and proliferation, differentiation and apoptosis [52, 53].

The two groups differed in the consumption of fiber, fatty acids and a large number of micronutrients and non-nutritive phytochemicals and the limitation of the study is that it is

not possible to determine which dietary component specifically explains the effect on the transcriptome. The observed changes in the transcriptome profile may actually not be linked to a direct effect of the dietary components on PBMCs, but rather reflect metabolic changes due to long-term nutritional adaptation in the whole body. Long-term metabolic adaptations have been linked to a favorable change in the transcriptome profile of PBMCs after the intake of fish oil (1.8 g EPA + DHA/d) for 26 weeks in elderly subjects [54].

The majority of the regulated probe sets were down-regulated in the ND group compared to the CD group. This is in line with the transcriptome data obtained from the abdominal subcutaneous adipose tissue samples in the same study, where a similar regulation pattern was observed [18]. The magnitude of the gene expression changes observed in the ND compared to the CD group ranged from +47 % and -28 % (Table S1). The effect size is small, but are in line with the findings of other dietary transcriptome studies [19, 51]. It is however, recognized that this effect over time will have an impact on the disease development [55]. The intervention varied from 18 weeks to 24 weeks and we cannot rule out the possibility that differences in duration time may have influenced the results. In order to remove effects that correlates to study center (duration time) and get a more precise estimate of the dietary effect, we have therefore adjusted the linear regression analyses for study centers as a covariate. In the present study, the changes in gene expression in the ND group were compared to the changes in a control group. The reported effects are therefore directly linked to the ND and changes in gene expression caused by the ritual of the intervention or to daily fluctuations in the transcriptome are excluded. Previous dietary intervention studies that examined the transcriptome profile have often reported solely within- group changes [27, 54] or did not have a control arm at all [56].

The current data must be interpreted in relation to PBMCs and whether the observed changes in gene expression do have any impact on whole-body metabolic regulation is not

known. There are however, studies indicating that gene expression in PBMCs seem to reflect changes occurring in metabolically active organs such as the liver, adipose tissue and skeletal muscle [20, 57-59]. Furthermore, PBMCs are directly involved in the complex inflammation processes that underlie many lifestyle-related diseases.

In conclusion, we have shown that a healthy Nordic dietary pattern modulates the expression of genes involved in mitochondrial function and immune response in PBMCs of subjects with the MetS. In particular, we observed a reduced expression of genes related to ETC after a prolonged intake of a healthy Nordic diet. Although the impact of reduced expression of ETC genes at the cellular level in PBMCs needs to be addressed, the current findings may offer new mechanistic insight into the effects of a healthy diet on disease prevention.

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Statement regarding conflicts of interest

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Author Contribution

MCWM: planning, conducting statistical analysis (bioinformatics), interpretation and reporting of data, writing of the manuscript. VDM: planning, interpretation and reporting of data, writing of the manuscript. ID: planning, conducting, interpretation and reporting of data, responsibility of transcriptomic analyses laboratory, statistical analysis (bioinformatics), critically reviewed the manuscript. MK: planning, conducting, interpretation and reporting of data, critically reviewed the manuscript. JPa: planning, conducting and reporting statistical analysis (bioinformatics), critically reviewed the manuscript. AR: conducting and reporting statistical analyses, critically reviewed the manuscript. CC: planning, interpretation of data, critically reviewed the manuscript. OKO: conducting and reporting statistical analysis (bioinformatics), critically reviewed the manuscript. JPi: planning, interpretation of data, critically reviewed the manuscript. KBH: planning, interpretation of data, critically reviewed the manuscripts. KH: planning, conducting, critically reviewed the manuscript. LOD: planning, critically reviewed the manuscript. IG: planning, conducting, critically reviewed the manuscript. LC: planning, conducting, critically reviewed the manuscript. MUS: planning, conducting, critically reviewed the manuscript. BÅ: planning, conducting, reporting of data, critically reviewed the manuscript. FR: planning, conducting, critically reviewed the manuscript. JH: planning, conducting, critically reviewed the manuscript. KHH: planning, conducting, critically reviewed the manuscript. UR: planning, conducting, critically reviewed the manuscript. IT: planning, conducting, critically reviewed the manuscript. KP: planning, conducting, critically reviewed the manuscript. MJS: planning, conducting, critically reviewed the manuscript. US: planning, responsibility of conducting the intervention, reporting of data, critically reviewed the manuscript. PA: planning, conducting, interpretation and reporting of data, critically reviewed the manuscript. MU: responsibility of coordination

of the consortium, planning, funding, conducting, interpretation and reporting of data, critically reviewed the manuscript. SMU: planning, conducting, interpretation and reporting of data, funding, responsibility for coordination of PBMC samples, writing of the manuscript

Planning includes planning the intervention and/or the RNA analyses. Conducting includes conducting the intervention and/or the RNA analyses

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Supporting Information

Table S1. Changes in gene expression calculated with a linear regression model.

Table 1. Baseline characteristics of individuals in the ND and CD groups participating in the PBMC transcriptomic SYSDIET sub-study.

	ND (n=40)	**CD (n=26)**
Sex (female)	29 (73%)	17 (65%)
Age (years)	54.0 (8.9)	54.9 (7.7)
BMI (kg/m^2)	32.0 (3.2)	31.8 (2.7)
Total cholesterol (mmol/L)	5.54 (1.01)	5.76 (0.74)
LDL cholesterol (mmol/L)	3.52 (0.86)	3.67 (0.68)
HDL cholesterol (mmol/L)	1.35 (0.28)	1.39 (0.50)
Triglycerides (mmol/L)	1.50 (0.73)	1.54 (0.53)
0 h Glucose (mmol/L)	5.8 (0.7)	5.7 (0.5)
2 h Glucose (mmol/L)	6.3 (1.3)	6.2 (2.1)
Systolic BP (mmHg)	127 (14)	133 (16)
Diastolic BP (mmHg)	83 (11)	85 (11)

The results are given as mean (SD) values and female as number (%). ND: Healthy Nordic diet, CD: Control diet, PBMC: Peripheral blood mononuclear cells, BP: Blood pressure.

Table 2. Dietary data at baseline (week 0) and end of the study (week 18/24) in the ND and in CD group, and the differences in dietary variables at the end of the study with 95% CI.

	ND (n=40)		**CD (m=26)**		**Estimate**	**P-value[a]**
	Baseline	**End**	**Baseline**	**End**	**(95% CI)**	
Energy (kJ)	8127 (1779)	8346 (1743)	7914 (2152)	8133 (1816)	-8 (-617 to 601)	0.98
Carbohydrate (E%)	45.3 (6.0)	46.2 (5.9)	45.5 (7.5)	43.2 (6.5)	3.20 (0.64 to 5.75)	0.014
Sucrose (g)	42.0 (15.5)	38.2 (15.8)	38.8 (18.1)	35.6 (16.6)	-0.54 (-6.97 to 5.89)	0.87
Protein (E%)	17.0 (2.8)	17.0 (2.3)	17.3 (2.8)	16.4 (2.2)	0.88 (-0.26 to 2.01)	0.13
Fat (E%)	33.0 (6.6)	32.2 (5.0)	32.8 (6.7)	36.0 (5.6)	-3.88 (-6.47 to -1.30)	0.0034
SFA (E%)	13.1 (3.0)	10.4 (2.2)	13.2 (3.8)	15.3 (3.1)	-4.84 (-6.18 to -3.50)	2.1e-11
MUFA (E%)	11.6 (2.7)	12.2 (2.3)	11.7 (2.3)	12.8 (2.1)	-0.49 (-1.56 to 0.58)	0.37
PUFA (E%)	5.1 (1.3)	6.7 (1.8)	4.6 (1.7)	4.6 (1.1)	1.70 (0.95 to 2.44)	1.2e-05
Fiber (g)	22.4 (7.8)	35.7 (11.2)	21.0 (5.5)	15.7 (3.8)	18 (15 to 22)	2.1e-20
Beta-carotene (µg)	2502 (1938)	3423 (2521)	2328 (1671)	1744 (1029)	1498 (507 to 2488)	0.0032
Folate (µg)	279 (96)	323 (132)	245 (64)	225 (76)	63.6 (11.2 to 116.0)	0.018
Alcohol (E%)	2.6 (3.9)	2.3 (3.9)	2.7 (3.2)	3.5 (4.1)	-1.22 (-2.65 to 0.22)	0.096

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Data are given as mean (SD) and the differences in variables (Estimate) at the end of the study with 95% CI. [a] Linear mixed-effects models as previously described [16]. Estimate: the difference between the changes in the ND compare to the CD group when adjusted for covariates. ND: Healthy Nordic diet, CD: Control diet, SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids

Table 3. Main clinical and biochemical characteristics at baseline (week 0) and at the end of the study (18/24 week).

	ND (n=40)		**CD (n=26)**			
	Baseline	**End**	**Baseline**	**End**	**Estimate (95% CI)**	***p*-value[#]**
Body weight (kg)	88.2 (13.4)	88.8 (14.1)	90.6 (14.9)	91.60 (14.8)	-0.52 (-1.45 to 0.41)	0.27
Total cholesterol (mmol/l)	5.54 (1.01)	5.34 (0.95)	5.76 (0.74)	5.76 (0.68)	-0.21 (-0.47 to 0.05)	0.12
LDL cholesterol (mmol/l)	3.52 (0.86)	3.28 (0.79)	3.67 (0.68)	3.71 (0.66)	-0.28 (-0.50 to -0.05)	0.018
HDL cholesterol (mmol/l)	1.35 (0.28)	1.43 (0.35)	1.39 (0.50)	1.40 (0.41)	0.06 (-0.03 to 0.15)	0.19
Triglyceride (mmol/l)	1.50 (0.73)	1.41 (0.78)	1.54 (0.53)	1.44 (0.48)	0.02 (-0.20 to 0.23)	0.88
IL-1Ra (ng/l)	376 (219)	381 (228)	340 (157)	430 (270)	-82 (-162 to -1)	0.047
hsCRP (mg/l)	2.5 (2.1)	2.7 (2.0)	2.3 (2.0)	2.2 (1.7)	0.30 (-0.53 to 1.13)	0.48
0h Glucose (mmol/l)	5.83 (0.65)	5.79 (0.64)	5.66 (0.47)	5.59 (0.63)	0.04 (-0.16 to 0.23)	0.72
2h Glucose (mmol/l)	6.25 (1.34)	6.78 (1.74)	6.22 (2.06)	6.23 (1.98)	0.51 (-0.14 to 1.16)	0.12

Data are given as mean (SD) and the differences in variables at the end of the study with 95% CI. [#] Linear mixed-effects models adjusted as previously described [16]. Estimate: the difference between the changes in the ND compare to change in the CD group when adjusted for covariates. ND: Healthy Nordic diet, CD: Control diet

ID	Gene Symbol	CD (change)	ND Change)	ND vs CD (estimate)	95% CI	FDR	BP or protein function
8043036	*DHFR*	0.38	-0.09	-0.46	(-0.66, -0.26)	0.19	Dihydrofolat reductase metabolism, cell cycle
7982712	*KNSTRN*	0.21	-0.07	-0.28	(-0.42, -0.14)	0.20	Cell division, kinetochore
8022106	*LINC00526*	0.13	-0.13	-0.26	(-0.39, -0.13)	0.21	Non-coding RNA
7957530	*LOC400590*	0.21	-0.02	-0.23	(-0.34, -0.11)	0.24	Unkown
7940669	*UQCC3*	0.12	-0.10	-0.22	(-0.32, -0.12)	0.19	Mitochondrial electron transport, ATP biosyntetic process
8054297	*CHST10*	0.16	-0.06	-0.21	(-0.32, -0.11)	0.21	Cell adhesion, T- cell adhesion, sulfo-transferase activity
7978114	*EMC9*	0.10	-0.10	-0.21	(-0.30, -0.12)	0.19	ER membrane, unknown function
8018352	*SLC25A19*	0.14	-0.07	-0.20	(-0.30, -0.09)	0.23	Mitochondrial transport, transports thiamine pyrophosphates into mitochondria
8155327	*ALDH1B1*	0.14	-0.06	-0.19	(-0.28, -0.1)	0.20	Aldehyd dehydrogenase (NAD) activity, mitochondrial matrix
8063955	*OGFR*	0.10	-0.08	-0.18	(-0.27, -0.09)	0.20	Regulation of cell growth, opioid receptor-signalling
7917896	*ALG14*	0.17	-0.02	-0.18	(-0.27, -0.09)	0.23	Protein modification, N-linked glycosylation
8086462	*POMGNT2*	0.10	-0.08	-0.18	(-0.27, -0.09)	0.23	Protein modification, protein O-linked glycosylation
8044491	*CHCHD5*	0.11	-0.06	-0.17	(-0.26, -0.08)	0.24	Mitochondrial respiratory chain, mitochondrial import
8169645	*UBE2A*	0.14	-0.02	-0.17	(-0.25, -0.08)	0.23	DNA repair, histone ubiquitination
8063949	*MRGBP*	0.11	-0.06	-0.17	(-0.24, -0.09)	0.19	Histone acetylation, regulation of cell growth
8040831	*ATRAID*	0.08	-0.07	-0.16	(-0.23, -0.09)	0.19	Cell differentiation, apoptosis, regulation of gene expression
7934133	*PPA1*	0.10	-0.05	-0.15	(-0.23, -0.07)	0.23	Diphosphate metabolic process, cell division
8078984	*RPL14*	0.07	-0.08	-0.15	(-0.22, -0.07)	0.23	Ribosomal biogenesis
8164428	*TRUB2*	0.05	-0.10	-0.14	(-0.21, -0.07)	0.23	Pseudouridine synthesis in mitochondria
8141708	*CLDN15*	0.09	-0.05	-0.14	(-0.21, -0.07)	0.23	Cell-cell adhesion, tight junction
8169683	*ZBTB33*	0.14	0.00	-0.14	(-0.20, -0.07)	0.21	Wnt-signaling pathway, apoptosis, transcription factor activity
7940051	*ZDHHC5*	0.07	-0.03	-0.10	(-0.15, -0.06)	0.19	Protein modification, protein

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							palmitoylation
7911683	*FAAP20*	-0.16	0.02	0.17	(0.08, 0.25)	0.21	Cellular response to DNA damage stimulus
7898585	*NBL1*	-0.21	0.11	0.31	(0.16, 0.47)	0.20	Regulation of monocyte chemotaxis
8108470		-0.21	0.12	0.33	(0.16, 0.5)	0.23	unknown

Table 4. Gene transcripts differently expressed in the ND group compared to the CD group (FDR *q*-value < 0.25).

Differential expressed gene transcripts were calculated with a linear regression model adjusted for age, gender and study center and with log2 ratio as the dependent variable. Change in ND or CD is given as log2 ratio from baseline (log2(18/24 weeks) – log2(baseline)). Change in ND vs CD is given as adjusted log2 ratio (log2 ratio ND-log2 ratio CD). FDR with q-value < 0.25 was considered significant. ND: healthy Nordic diet, CD: control diet, FDR: False discovery rate, BP: Biological Process.

Table 5. The probe sets significantly down-regulated in the ND vs CD group (*p*-value 0.05, Table S1) are visualized using Metacore Pathway Maps. Significant Pathway Maps (FDR *q*-value <0.05).

PATHWAY MAPS	RATIO	FDR
IMMUNE RESPONSE_*ANTIGEN PRESENTATION BY MHC CLASS I, CLASSICAL PATHWAY*	11/54	0.00
UBIQUINONE METABOLISM	11/74	0.00
NEUROPHYSIOLOGICAL PROCESS_*DYNEIN-DYNACTIN MOTOR COMPLEX IN AXONAL TRANSPORT IN NEURONS*	9/54	0.00
REGULATION OF DEGRADATION OF DELTAF508-*CFTR IN CF*	7/39	0.01
CYTOSKELETON REMODELING_*NEUROFILAMENTS*	5/25	0.04

Ratio indicates the number of genes among down-regulated/number of genes represented in the pathway maps.
ND: healthy Nordic diet, CD: control diet, FDR: False discovery rate.

Table 6: Enriched biological processes in the ND group compared to the CD group among the 927 down-regulated probe sets (*p*-value < 0.05

BIOLOGICAL PROCESS	GENE SYMBOLS	FOLD ENRICHMENT	FDR (%)
GO:0006364~RRNA PROCESSING	*RPL17, NAF1, RPL14, RPL15, UTP15, RPLP2, WBP11, PIN4, RPP14, EXOSC10, EBNA1BP2, RPS26, RPS28, RPL8, RPL10, MTERF4, CCDC86, RPP21, RRP36, PNO1, RPL26, ISG20L2, FBL, PWP2, SENP3, RCL1, NOLC1, RPL13A, RPS14, YBEY*	3.22	0.00
GO:0032981~MITOCHONDRIAL RESPIRATORY CHAIN COMPLEX I ASSEMBLY	*ND1, NDUFAF5, NDUFB11, NDUFA2, ND5, NDUFB8, NDUFA6, ND2, ND3, NDUFS5, NDUFS4, FOXRED1, NDUFS3, ND6*	5.10	0.00
GO:0006120~MITOCHONDRIAL ELECTRON TRANSPORT, NADH TO UBIQUINONE	*ND1, NDUFB11, NDUFA2, NDUFS5, NDUFS4, NDUFB8, ND5, NDUFA6, ND2, ND3, NDUFS3, ND6*	5.63	0.01
GO:0006412~TRANSLATION	*MRPS36, RPL17, RPL14, RPL15, RPLP2, IGHMBP2, RPS26, RPS28, MRPL14, MRPL17, SLC25A22, RPL8, MRPL19, RPL10, SLC25A43, RPL26, GTF2H3, MRPS21, MRPS7, SLC25A12, RSL1D1, MRPS18A, SLC25A33, RPL13A, RPS14, SLC25A19, PELO, DHPS*	2.54	0.03
GO:0008033~TRNA PROCESSING	*ADAT1, PUS3, ELAC2, RPP21, TRMT2B, TRMT10C, FDXACB1, TRUB2, POP7, FBL*	6.21	0.04
GO:0007049~CELL CYCLE	*ARL2, ERH, LIN52, STK10, LIN9, CINP, THAP5, TP53, HCFC1, ARF6, SENP5, GAK, MAPK6, NOLC1, CCDC124, MAP3K8, PELO, THAP1, SUPT5H, CDK20, CHAF1B, RAD17, CCAR2*	2.43	0.35
GO:0006368~TRANSCRIPTION ELONGATION FROM RNA POLYMERASE II PROMOTER	*TAF2, ENY2, CCNK, POLR2J, CDK9, GTF2H3, CDK7, ADRM1, GTF2F1, SUPT4H1, TCEB1, NELFE, SUPT5H*	3.47	0.58
GO:0007005~MITOCHONDRION ORGANIZATION	*TFAM, ALAS1, ESRRA, NRF1, FXN, SLC25A33, RAB29, HCFC1, STOML2, FANCG, DNAJA3*	3.28	3.08
GO:0001522~PSEUDOURIDINE SYNTHESIS	*NAF1, PUS3, RPUSD3, RPUSD1, TRUB2*	8.84	3.18
GO:0006614~SRP-DEPENDENT COTRANSLATIONAL PROTEIN TARGETING TO MEMBRANE	*RPL17, RPS26, SRP54, RPS28, RPL14, RPL13A, RPS14, RPL15, RPL8, RPL26, RPL10, RPLP2*	2.93	4.31

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Enriched biological processes were identified using DAVID (FDR q-value < 0.05). ND: healthy Nordic diet, CD: control diet, GO, Gene ontology term; FDR, false discovery rate.

Table 7. Overrepresentation of regulatory motives for transcription factors among the gene transcripts significantly down-regulated *(p*-value < 0.05) in the ND group compared to the CD group.

Transcription Factor	Actual	n	R	N	Expected	Ratio	z-score
ATF-6 alpha (90kDa)	5	908	24	38951	0.56	8.94	6.01
THAP11	7	908	41	38951	0.96	7.32	6.26
STAT4	8	908	64	38951	1.49	5.36	5.40
LXR-beta	5	908	41	38951	0.96	5.23	4.19
PXR	5	908	41	38951	0.96	5.23	4.19
SREBP1 precursor	7	908	61	38951	1.42	4.92	4.74
SREBP2 (nuclear)	8	908	71	38951	1.66	4.83	5.00
MEIS1	10	908	92	38951	2.15	4.66	5.43
BLIMP1 (PRDI-BF1)	10	908	95	38951	2.22	4.52	5.30
SREBP1 (nuclear)	14	908	144	38951	3.36	4.17	5.89
LXR-alpha	10	908	104	38951	2.42	4.13	4.93
Miz-1	11	908	116	38951	2.70	4.07	5.11
FOXK1	7	908	75	38951	1.75	4.00	4.02
IRF2	7	908	76	38951	1.77	3.95	3.98
ZNF143	89	908	984	38951	22.94	3.88	14.14
ZNF217	9	908	101	38951	2.35	3.82	4.39
YY1	89	908	1157	38951	26.97	3.30	12.27
c-Myc	185	908	2544	38951	59.30	3.12	17.08
NRF2	16	908	227	38951	5.29	3.02	4.72
NF-kB1 (p50)	32	908	476	38951	11.10	2.88	6.39
NRF1	12	908	181	38951	4.22	2.84	3.84
STAT6	26	908	398	38951	9.28	2.80	5.58
RelB	13	908	208	38951	4.85	2.68	3.76
XBP1	10	908	160	38951	3.73	2.68	3.29
CTCF	11	908	184	38951	4.29	2.57	3.29
IRF4	26	908	440	38951	10.26	2.54	5.00
CREB1	289	908	5124	38951	119.40	2.42	16.84
GATA-3	37	908	657	38951	15.32	2.42	5.65
DBP	21	908	388	38951	9.05	2.32	4.04
MYOG	16	908	301	38951	7.02	2.28	3.45
CUX1 (p110)	72	908	1387	38951	32.33	2.23	7.19
HSF1	15	908	298	38951	6.95	2.16	3.10
PU.1	20	908	406	38951	9.46	2.11	3.48
E2F1	42	908	863	38951	20.12	2.09	4.99
SRF	20	908	414	38951	9.65	2.07	3.39
EGR1	22	908	470	38951	10.96	2.01	3.40
NANOG	33	908	730	38951	17.02	1.94	3.96

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p53	56	908	1318	38951	30.72	1.82	4.69
Oct-3/4	52	908	1245	38951	29.02	1.79	4.39
SP1	70	908	1684	38951	39.26	1.78	5.08
ESR1 (nuclear)	54	908	1321	38951	30.79	1.75	4.31
RelA	59	908	1541	38951	35.92	1.64	3.98

The analyses was performed in Metacore. ***Transcription factor in italics*** are presented in the list of down-regulated probe sets. ND: healthy Nordic diet, CD: control diet.
Actual; number of network objects in the dataset, which interact with the chosen object
n; number of network objects in the dataset.
R; number of network objects in the background list, which interact with the chosen object
N; total number of gene-based objects in the background list
Expected; mean value for hypergeometric distribution (n*R/N)
Ratio; connectivity ratio (Actual/Expected)
z-score; Actual/Expected/sqrt(variance)

Table 8. The gene transcripts significantly up-regulated in the ND vs CD group (p-value < 0.05, Table S1) are visualized using Metacore Pathway Maps. Significant Pathway Maps (FDR q-value < 0.05).

PATHWAY MAPS	RATIO	FDR
CYTOSKELETON REMODELING_*KERATIN FILAMENTS*	4/36	0.023

Ratio indicates the number of genes represented in the pathway maps/number of genes among up-regulated. ND: healthy Nordic diet, CD: control diet, FDR: False discovery rate.

Table 9. Overrepresentation of regulatory motives for transcription factors among the gene transcripts significantly up-regulated *(p*-value < 0.05) in the ND group compared to the CD group.

Transcription factor	Actual	n	R	N	Expected	Ratio	z-score
MTG16 (CBFA2T3)	3	273	17	38951	0.12	25.18	8.38
CREB1	66	273	5124	38951	35.91	1.84	5.41

The analyses was performed in Metacore. ND: healthy Nordic diet, CD: control diet.
Actual; number of network objects in the dataset, which interact with the chosen object
n; number of network objects in the dataset.
R; number of network objects in the background list, which interact with the chosen object
N; total number of gene-based objects in the background list
Expected; mean value for hypergeometric distribution (n*R/N)
Ratio; connectivity ratio (Actual/Expected)
z-score; Actual/Expected/sqrt(variance)

Figure legends

Figure 1. Flow chart showing the number of subjects included in the microarray analyses. The inclusion of study participants from the three study centers Kuopio, Oulu and Lund was based on the following criteria: weight change during the study < 4 kg, no use of statins, high-sensitivity (hs) CRP < 10 mg/L at baseline and at the end of the intervention, and BMI < 39 kg/m^2. ND: healthy Nordic diet, CD: control diet

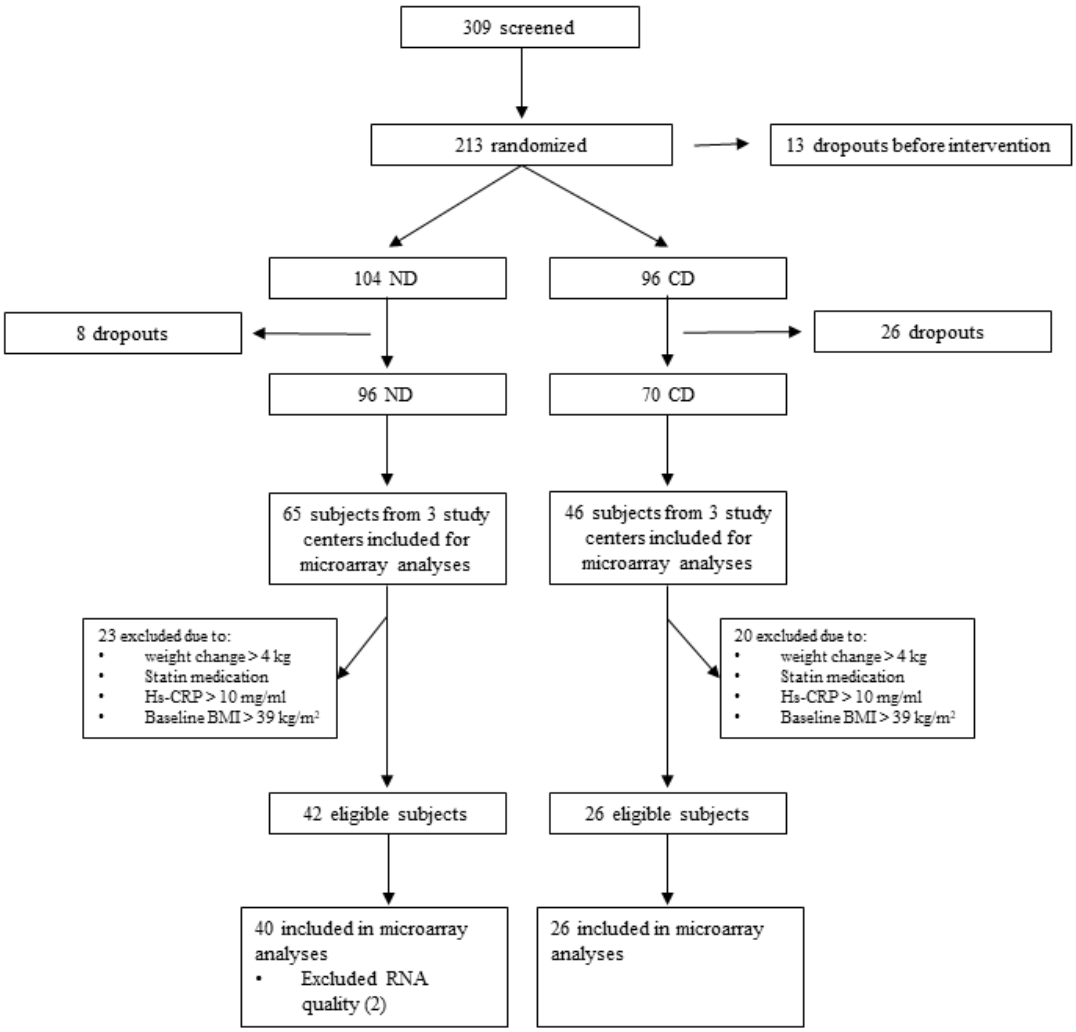

Figure 2. RT-qPCR validation of microarray findings. The changes in mRNA expression in the ND group compared to the CD group, adjusted for age, gender and study center, were analyzed with microarray (pink) and qPCR (green). Dots represent the log ratio (β-coefficient) and the lines represent the 95 % CI. Solid dots represent a significant change (*p*-value < 0.05), while empty dots represent a non-significant change (*p*-value ≥ 0.05).