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RESEARCH PAPER

Short-term fatty acid intervention elicits differential gene expression responses in adipose tissue from lean and overweight men

Marjan J. van Erk · Wilrike J. Pasman · Heleen M. Wortelboer · Ben van Ommen · Henk F. J. Hendriks

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Abstract The goal of this study was to investigate the effect of a short-term nutritional intervention on gene expression in adipose tissue from lean and overweight subjects. Gene expression profiles were measured after consumption of an intervention spread (increased levels of polyunsaturated fatty acids, conjugated linoleic acid and medium chain triglycerides) and a control spread (40 g of fat daily) for 9 days. Adipose tissue gene expression profiles of lean and overweight subjects were distinctly different, mainly with respect to defense response and metabolism. The intervention resulted in lower expression of genes related to energy metabolism in lean subjects, whereas expression of inflammatory genes was down-regulated and expression of lipid metabolism genes was up-regulated in the majority of overweight subjects. Individual responses in overweight subjects were variable and these correlated better to waist–hip ratio and fat percentage than BMI.

Keywords Subcutaneous adipose tissue · Microarray · Polyunsaturated fatty acids (PUFA) · Conjugated linoleic acid (CLA) · Medium chain triglycerides

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M. J. van Erk (✉) · W. J. Pasman · H. M. Wortelboer · B. van Ommen · H. F. J. Hendriks
Businessunit Biosciences, TNO Quality of Life, P.O. Box 360, 3700 AJ Zeist, The Netherlands
e-mail: marjan.vanerk@tno.nl

Introduction

Adipose tissue not only functions as a fat storage organ, it also has an important metabolic and endocrine function. Adipose tissue plays a role in insulin sensitivity and inflammatory status, e.g., through release of fatty acids and adipokines [1, 27]. In obesity, adipose tissue mass is increased and as a result, endocrine and metabolic functions of adipose tissue are affected. Moreover, obesity is associated with systemic inflammation due to macrophage infiltration in adipose tissue. Complications of obesity include insulin resistance, cardiovascular disease and metabolic syndrome [1, 38, 39].

It has been shown that energy restriction, changes in dietary macronutrient composition and weight loss result in gene expression changes in adipose tissue from overweight or obese subjects [6, 22, 35]. The goal of this study was to investigate the effects of a short-term nutritional intervention on gene expression in adipose tissue and to investigate whether the gene expression response differs between lean and overweight subjects. Earlier, we showed that dietary effects can be monitored by gene expression profiling in white blood cells [32].

The nutritional intervention consisted of a spread with increased levels of medium chain triglycerides, short-chain polyunsaturated fatty acids (PUFA: C18:2 and C18:3) and conjugated linoleic acid (CLA: C18:2) (further referred to as intervention spread). This composition was chosen to elicit an effect on food intake and satiety. Medium chain triglycerides, PUFA and CLA are reported to have satiating power [13, 18, 29]. The two spreads did not differ in their effect on satiety (described in detail elsewhere: Pasman et al., in preparation). To a large extent, it is unknown how the fatty acids can influence food intake, energy expenditure and eventually body mass [17]. Analysis of adipose

tissue gene expression is of interest because of its central role in fat and energy metabolism in response to nutritional interventions and because of its role in inflammation. Furthermore, PUFA have been reported to influence adipose tissue function [5, 21] and CLA can play a role in reduction of adipose tissue mass [3, 37, 40].

We found that in adipose tissue fatty acid transporter gene expression was slightly higher in lean subjects and slightly lower in overweight subjects in response to the intervention spread compared to the control spread. Also, fatty acid uptake showed a tendency to change in response to the intervention (details in Pasman et al., in preparation). Here we study in greater detail the response of adipose tissue to the different fatty acid intake by whole genome gene expression profiling of subcutaneous adipose tissue biopsies taken from the subjects after consumption of the intervention or control spread with an equal amount of fat for 9 days.

Effects of the short-term nutritional intervention were studied both in overweight and lean subjects, by focusing on enriched functional groups of genes and putting these in the context of the intervention, thereby aiming to shed a light on the potential mechanisms of action of the fatty acids.

Subjects and methods

Study design and subjects

The study was conducted at TNO Quality of Life, where subjects were recruited from a pool of volunteers. Each subject was informed about the study orally and in writing before they signed the informed consent forms. Subjects met the inclusion criteria: (1) healthy male subjects (lean: BMI between 18 and 25 kg/m^2 or overweight/obese: BMI between 27.5 and 35 kg/m^2), (2) normal Dutch eating pattern and used to consuming breakfast, (3) non-restrained eaters according to the Dutch Eating Behavior Questionnaire (lean: <2.5 and overweight/obese: <3.25) [33, 34]. Subjects with a disorder, using medication or having a history of medical or surgical events that may have affected study outcomes were excluded.

A total of 25 subjects (all of Dutch ancestry) were eligible of which 20 subjects were randomly selected as well as four reserves. Ten lean (BMI mean ± SD 21.2 ± 1.5 kg/m^2, age range 19–41 years) and ten overweight men (BMI 29.7 ± 1.9 kg/m^2, age range 21–58 years) started and completed the study. Body fat percentage was 12.2 ± 4.2% in the lean sub-group compared to 27.8 ± 3.7% in the overweight sub-group. Waist circumference was 79 ± 6 cm in the lean sub-group compared to 102 ± 8 cm in the overweight sub-group and waist–hip ratio (WHR) was 0.86 ± 0.05 in the lean sub-group compared to 0.96 ± 0.07 in the overweight subgroup.

The study was approved by the Medical Ethics Committee of the University Medical Centre of Utrecht (20 September 2005) and conducted according to the ICH Guideline for Good Clinical Practice in October–November 2005.

The study was designed as a randomized cross-over, placebo-controlled, double blind study. Each subject received two treatments. The treatment period lasted nine days and the wash-out period was 2 weeks. The two treatments consisted of a spread containing fatty acids known for their satiating effect [increased levels of medium chain triglycerides, short-chain polyunsaturated fatty acids (PUFA: C18:2 and C18:3) and conjugated linoleic acid (CLA: C18:2) (further referred to as intervention spread)] and a control spread containing fatty acids normally eaten with breakfast. The exact composition of the spreads is presented in Table 1. Blends with the fatty acids of interest were mixed by Lipid Nutrition (Wormerveer, The Netherlands). Romi Smil (Heerenveen, The Netherlands) prepared the spreads. The subjects used the spread with breakfast and lunch; 26 grams in the morning, and 26 g in the afternoon (in total 52 g of spread daily, containing 40 g of fat). The spreads replaced the spread normally consumed in the diet. The amount of fat supplied in the spread was similar in both interventions and energy intake was kept constant, resulting in a difference in fatty acid composition intake only.

No carry-over effect of treatments was found for bodyweight (data not shown).

Adipose tissue biopsies

Two fat biopsies were taken from each subject, one after consumption of the placebo spread for 9 days and one after consumption of the intervention spread for 9 days. Biopsies were taken in the evening and time of sampling was

Table 1 The composition of the spreads

	Intervention spread (%)	Control spread (%)
Lauric acid (MCT, C12:0) (and C14:0)	17.2	0
Palmitic acid (C16:0)	17.9	17.5
Stearic acid (C18:0)	3	10
Oleic acid (C18:1)	4.5	40
Conjugated linoleic acid (C18:2)	12.4	0
Linoleic acid (C18:2)	40.3	30
Pinolenic acid (C18:3)	2.5	0
Other fatty acids	2.2	2.5

kept constant as much as possible for the two treatments. Subcutaneous adipose tissue was obtained from the abdominal region by needle aspiration under local anesthesia using 2× 50 ml 8.4% $NaHCO_3$, 0.05% lidocaine and 1.0 mg/ml epinephrine (according to Kolaczynski et al. [16]). Part of the fat suspension was immediately frozen in liquid nitrogen and stored at −80°C for RNA isolation.

RNA isolation, labeling and hybridization

RNA was isolated by homogenizing the adipose tissue biopsies in Trizol (Life Technologies S.A., Merelbeke, Belgium). After chloroform extraction the RNA isolation was continued using NucleoSpin RNA II kit (Bioké, Leiden). The protocol included a DNase digestion step.

The isolated RNA samples were processed according to Affymetrix protocols by Scienion Ag (Berlin, Germany). In brief, RNA quality and integrity were verified using the RNA 6000 Nano assay on the Agilent 2100 Bioanalyzer (Agilent Technologies), resulting in RNA samples from ten lean and eight overweight subjects to be included in the microarray analysis (RNA samples from two overweight subjects did not pass the RNA quality control).

Next, 1.5 μg of high-quality total RNA was used with the Affymetrix Eukaryotic One-Cycle Target Labeling and Control reagents to generate Biotin-labeled antisense cRNA. The labeled cRNA was further used for the hybridization to the NuGO Affymetrix Human Genechip NuGO_Hs1a520180 (custom designed by the European Nutrigenomics Organization NuGO, consisting of 23,941 probesets including 71 control probesets, for details see http://blog.bigcat.unimaas.nl/~martijn/NuGO/). After an automated process of washing and staining, absolute values of expression were calculated from the scanned array using the Affymetrix GCOS software.

Data analysis

Quality control of microarray data was performed using BioConductor packages (a.o. simpleaffy and affyplm, through the NuGO MadMax pipeline: https://madmax.bioinformatics.nl). One microarray did not pass quality control criteria due to high background values and low percentage of present calls. Therefore, microarray data for nine lean and eight overweight subjects were included in data analysis.

Raw signal intensities (from CEL files) were normalized using the GCRMA algorithm (gc-rma slow). Probesets were filtered on at least one present call and at least one expression value above 5 (resulting in a set of 15,762 probesets). Expression data were log-transformed (base 2). The cross-over design of the study ensured measurement of gene expression profiles in adipose tissue of each subject after consumption of the placebo spread and after consumption of the intervention spread, allowing for analysis of gene expression changes in response to intervention within each subject [calculated as 2log ratio (intervention vs placebo)].

First, effects of BMI (lean sub-group or overweight subgroup), treatment (intervention spread or control spread) as well as the interaction component BMI × Intervention was tested using two-way ANOVA in SAS (SAS Institute Inc., Cary, USA). The null hypotheses (no difference) were rejected at the level of 0.01 of probability. When testing 15,762 probesets with these conditions, 158 probesets are expected to be called significant.

Second, a paired *t* test for treatment effect in the lean and in the overweight subgroup was performed in BRB ArrayTools (software for microarray data analysis developed by Dr. Richard Simon and Amy Peng Lam, http://linus.nci.nih.gov/BRB-ArrayTools.html), using multiple testing correction by estimation of false discovery rate (FDR). Threshold for significance was set by taking into account *P* value (<0.01) as well as estimated FDR (<20%). Finally, in case of responders and non-responders within a sub-group, differentially expressed genes were selected based on showing a consistent response in the majority of the subjects (defined as ≥25% expression change up in more than 50% of the subjects or ≥25% expression change down in more than 50% of the subjects).

Subsets of differentially expressed genes were analyzed further in GenMAPP to find functional groups of genes (based on Gene Ontology) that were overrepresented in the subsets [7]. Other tools used for data analysis include Gene Set Enrichment Analysis [31] and T-profiler [2] for enrichment analysis of functional groups of genes. The tissue-specific gene sets were derived from SymAtlas (http://symatlas.gnf.org/SymAtlas/) [30] and from Lyons et al. [20]. Cluster and treeview (http://rana.lbl.gov/EisenSoftware.htm, [8]) were used for generation of cluster trees, using average linkage clustering and uncentered correlation as similarity metric. A biological network was generated in MetaCore version 4.3 (GeneGo Inc., St Joseph, MI, USA). MetaCore™ is based on a proprietary manually curated database of human protein–protein, protein–DNA and protein compound interactions, metabolic and signaling pathways and the effects of bioactive molecules in gene expression. Only curated interactions were used.

Data from the placebo intervention were used for comparison of gene expression levels in adipose tissue from lean and overweight subjects.

The gene expression data are available through ArrayExpress, a public repository for microarray data (http://www.ebi.ac.uk/arrayexpress/, accession numer E-TABM-377).

Results

Subcutaneous adipose tissue gene expression profiles of lean and overweight subjects were distinctly different (3,069 probesets selected with $P < 0.01$ for BMI-effect; Fig. 1). An overview of enriched functional groups of genes (based on Gene Ontology) in this set of 3,069 probesets is shown in Table 2. Genes involved in energy metabolism were expressed at a higher level in adipose tissue from lean subjects (functional groups 'generation of precursor metabolites and energy', 'lipid metabolism', 'glucose metabolism'). Furthermore, genes encoding mitochondrial proteins were expressed at a higher level in adipose tissue from lean subjects than in adipose tissue from overweight subjects.

On the other hand, genes involved in defense response, intracellular signaling and cell adhesion were expressed at higher levels in adipose tissue from overweight subjects than in adipose tissue from lean subjects. Within these groups of genes, gene expression of specific macrophage markers [e.g., CD14, CD163 and macrophage scavenger receptor 1 (MSR1)] was significantly higher in adipose tissue of overweight subjects ($P < 0.001$ for BMI-effect in two-way ANOVA), pointing towards presence of macrophages or inflammatory cells in the adipose tissue biopsies from overweight subjects. This is further illustrated in supplementary Fig. 1, which clearly shows higher expression of genes specific for CD14-positive monocytes in adipose tissue from seven overweight subjects than in adipose tissue from lean subjects. Enrichment analysis of tissue-specific gene sets, by comparing the lean sub-group to overweight sub-group (GSEA) and by analyzing overweight subjects individually (T-profiler), showed that this set of genes specific for CD14-positive monocytes was significantly enriched in the adipose tissue from overweight subjects compared to lean subjects and that this gene set was among the two most enriched gene sets in seven out of the eight overweight subjects. Other inflammatory markers that were expressed at higher level in adipose tissue from overweight subjects included leptin, complement factor D (CFD), interleukin receptor 6 (IL6R), interleukin receptor 10 alpha (IL10RA), interleukin 1 receptor antagonist (IL1RN) and a number of interleukins (IL15, IL17D, IL18) (data not shown).

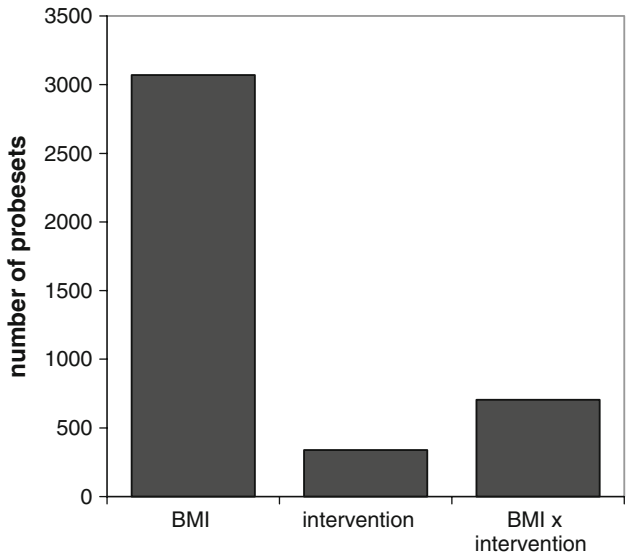

Fig. 1 Results of 2-way ANOVA investigating effect of BMI [lean ($n = 9$) or overweight ($n = 8$) group], intervention (intervention spread or placebo) and BMI × intervention interaction. Threshold for significance was set at $P < 0.01$. Testing 15,762 probesets with P value threshold of 0.01 would result in 158 probesets detected as significant by chance

Consumption of the intervention spread resulted in a differential gene expression response in adipose tissue from lean and overweight subjects, as indicated by the BMI-intervention interaction effect (706 probesets selected with $P < 0.01$ for BMI × intervention interaction effect; Fig. 1). When plotting the expression changes (intervention spread versus control spread) of this set of 706 genes in the lean and overweight subjects (Fig. 2), it can be seen that the expression changes are more pronounced (larger fold changes) in the lean subjects than in the overweight subjects. In addition, statistical analysis of treatment effects in the lean and overweight sub-group individually resulted in no probesets significantly differentially expressed due to the intervention in overweight subjects compared to 813 differentially expressed probesets due to the intervention in lean subjects.

Enrichment analysis of the set of differentially expressed genes in lean subjects indicated that in lean subjects consumption of the intervention spread led to down-regulation of genes involved in functional groups indicated as 'mitochondrion', 'generation of precursor metabolites and energy', 'lipid metabolism', 'carbohydrate metabolism' (Table 3), together pointing towards a general effect on energy metabolism. These selected 'energy metabolism' genes showed considerable variation in their response among the overweight subjects (supplementary Fig. 2).

The results indicate that in the overweight subjects the response to the intervention is less strong and more variable than in the lean subjects. We further explored the possibility that a selection of the overweight subjects did show a consistent gene expression response for a number of genes or processes. Therefore, genes were selected based on showing a consistent response in the majority of the subjects (defined as ≥25% expression change up in more than 50% of the subjects or ≥25% expression change down in more than 50% of the subjects). In total, 501 probesets fulfilled these criteria. Enriched functional groups in this set of genes were 'lipid metabolism', 'response to stress',

Table 2 Enriched functional groups (based on Gene Ontology) in total set of differentially expressed genes in lean versus overweight subjects (3,069 probesets), in genes expressed at high levels in lean than in overweight (1,325 probesets) and in genes expressed at higher levels in lean than overweight (1,744 probesets)

GO group	Functional group of genes	Number of genes measured	All genes		Higher in lean		Higher in overweight	
			Percent changed	*Z* score[a]	Percent changed	*Z* score	Percent changed	*Z* score
CC	Mitochondrion	522	38.5	14.4	34.3	25.7		
BP	Generation of precursor metabolites and energy	508	29.5	8.6	21.5	13.6		
BP	Oxidative phosphorylation	77	36.4	4.9	31.2	8.6		
BP	Lipid metabolism	503	27.6	7.3	17.9	10.2		
BP	Carbohydrate metabolism	392	25.5	5.3	12.5	4.7	13.0	2.6
BP	Glucose metabolism	77	28.6	3.0	22.1	5.4		
BP	Mitochondrial transport	17	41.2	2.9	41.2	5.7		
BP	Cell adhesion	502	19.1	2.0			13.3	3.3
BP	Cell-matrix adhesion	60	28.3	2.6			25.0	4.2
BP	Defense response	723	24.2	6.3			21.5	11.8
BP	Inflammatory response	183	26.2	3.8			24.6	7.2
BP	Response to stress	829	20.6	3.8			17.0	8.0
BP	Intracellular signaling cascade	930	19.1	2.8			13.8	5.0
BP	I-kappaB kinase/NF-kappaB cascade	98	28.6	3.4			22.4	4.5
MF	Scavenger receptor activity	28	32.1	2.4			32.1	4.2

Enrichment analysis was performed in GenMAPP, by comparing the three selected sets of genes (all genes differentially expressed in lean versus overweight subjects, genes expressed at higher levels in lean than in overweight subjects and genes expressed at higher levels in overweight than in lean) to all genes on the microarray. Data from the placebo intervention were used for comparison of gene expression levels in adipose tissue from lean and overweight subjects

GO Gene Ontology groups, *CC* cellular component, *BP* biological process, *MF* molecular function

[a] All *Z* scores in table: $P < 0.05$ (PermuteP)

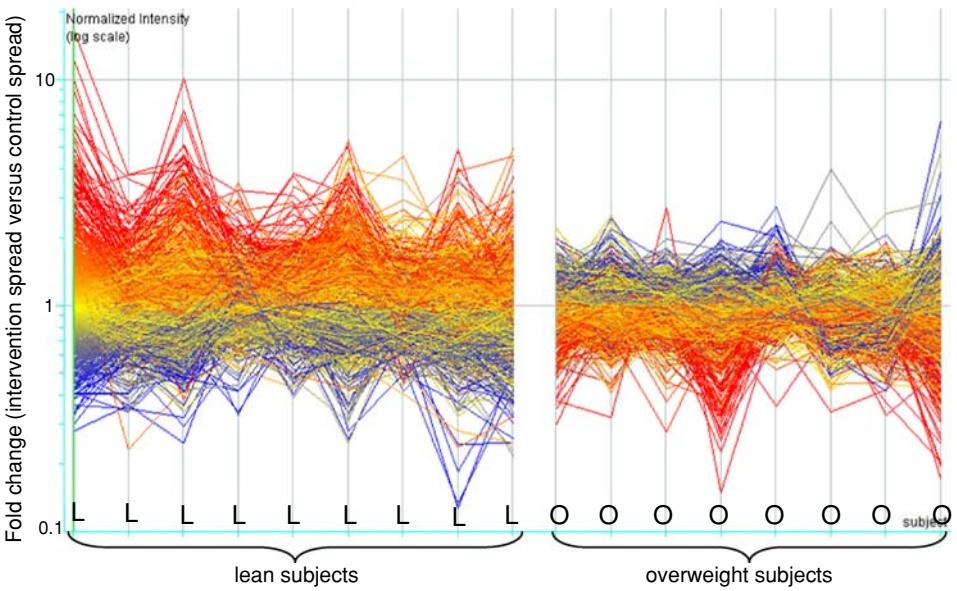

Fig. 2 Overview of expression changes (intervention spread vs. control spread) of 706 probesets ($P < 0.01$ for BMI × treatment effect) in nine lean (*L* left side) and eight overweight (*O* right side) subjects

'extracellular matrix', 'apoptosis' and 'cell adhesion'. Clustering of the genes in these functional groups (Fig. 3) illustrate variation in response among the overweight subjects, but also show that six of the overweight subjects show a similar response for most functional groups, whereas two other respond differently. Interestingly, expression of a number of the lipid metabolism and inflammatory genes (response to stress) in the clusters was

Table 3 Enriched functional groups (based on Gene Ontology) in set of differentially expressed genes (813 probesets) and in sub-set of down-regulated genes (509 probesets) in sub-group of lean subjects in response to treatment

GO group	Functional group of genes	Number of genes measured	All		Down-regulated	
			Percent changed	*Z* score	Percent changed	*Z* score
CC	Mitochondrion	522	6.5	3.1	5.7	4.1
BP	Generation of precursor metabolites and energy	508	6.9	3.5	5.5	3.7
BP	Lipid metabolism	503	6.2	2.7	5.8	4.0
BP	Carbohydrate metabolism	391	6.1	2.3	5.9	3.7
BP	Glucose metabolism	77	10.4	2.9	10.4	4.0
BP	Mitochondrial transport	17	23.5	4.2	23.5	5.1
BP	Regulation of cell cycle	367	6.27	2.36	4.9	2.4
BP	Complement activation\, classical pathway	22			13.6	3.0
BP	Oxygen and reactive oxygen species metabolism	65			7.7	2.4

Enrichment analysis was performed in GenMAPP, by comparing the two selected sets of genes (all genes differentially expressed in lean subjects in response to intervention and sub-set of down-regulated genes in lean subjects in response to treatment) to all genes on the microarray

GO Gene Ontology groups, *CC* cellular component, *BP* biological process

[a] All *Z* scores in table: $P < 0.05$ (PermuteP)

significantly different in overweight compared to lean subjects (BMI-effect) and changed in response to consumption of the intervention spread in direction of expression levels in lean subjects (genes marked with asterisk in Fig. 3).

Finally, individual response in overweight subjects was studied on level of functional groups of genes, using T-profiler [2], resulting in an enrichment score for functional groups in individual subjects based on gene expression changes in response to the intervention. A number of functional gene groups showed considerable variation in response among the overweight subjects, with up-regulation in one or more subjects and down-regulation in one or more other subjects. These functional gene groups include 'mitochondrion', 'cell adhesion' and 'extracellular matrix', 'immune response' and 'inflammatory response'. Interestingly, the individual response with regard to these functional groups correlated significantly to obesity phenotype markers WHR or fat percentage (Fig. 4). WHR correlated negatively with response of mitochondrial genes and positively with response of genes involved in cell adhesion. In addition, fat percentage correlated positively with response of inflammatory genes.

Discussion

To our knowledge, this study is one of the first to perform large-scale gene expression analysis in human adipose tissue biopsies in response to a short-term nutritional intervention, in both lean and overweight subjects. The study is exploratory in nature and describes the results on a pathway or functional group level with interpretation in relation to the intervention and literature data.

Our study data indicated that a considerable part of the differences in gene expression profiles could be attributed to the difference in BMI between the two sub-groups. Expression of inflammation-related genes was significantly higher in subcutaneous adipose tissue biopsies from overweight subjects than those from lean subjects. Specifically, markers specific for monocytes in blood were significantly higher expressed in the adipose tissue from overweight subjects, as was shown by different enrichment analysis approaches. As tissue macrophages originate from monocytes in blood that migrate into tissue, this finding is consistent with increased macrophage content of adipose tissue from overweight subjects, as previously described by others [38]. In addition to macrophage markers, also expression of specific adipokines was different between lean and overweight subjects. Consistent with other reports [14, 23], expression of leptin was higher and expression of adiponectin was lower (although not significantly) in adipose tissue from overweight subjects than in adipose tissue from lean subjects.

Expression of metabolism-related genes and genes encoding mitochondrial proteins was higher in subcutaneous adipose tissue from lean subjects than in adipose tissue from overweight subjects. A recent study of transcription profiles in adipose tissue from monozygotic twins discordant for BMI also reported lower expression of genes related to energy metabolism in obese subjects [26]. In addition, they report a reduction in mitochondrial DNA in adipose tissue from obese subjects. Overall, this indicates a

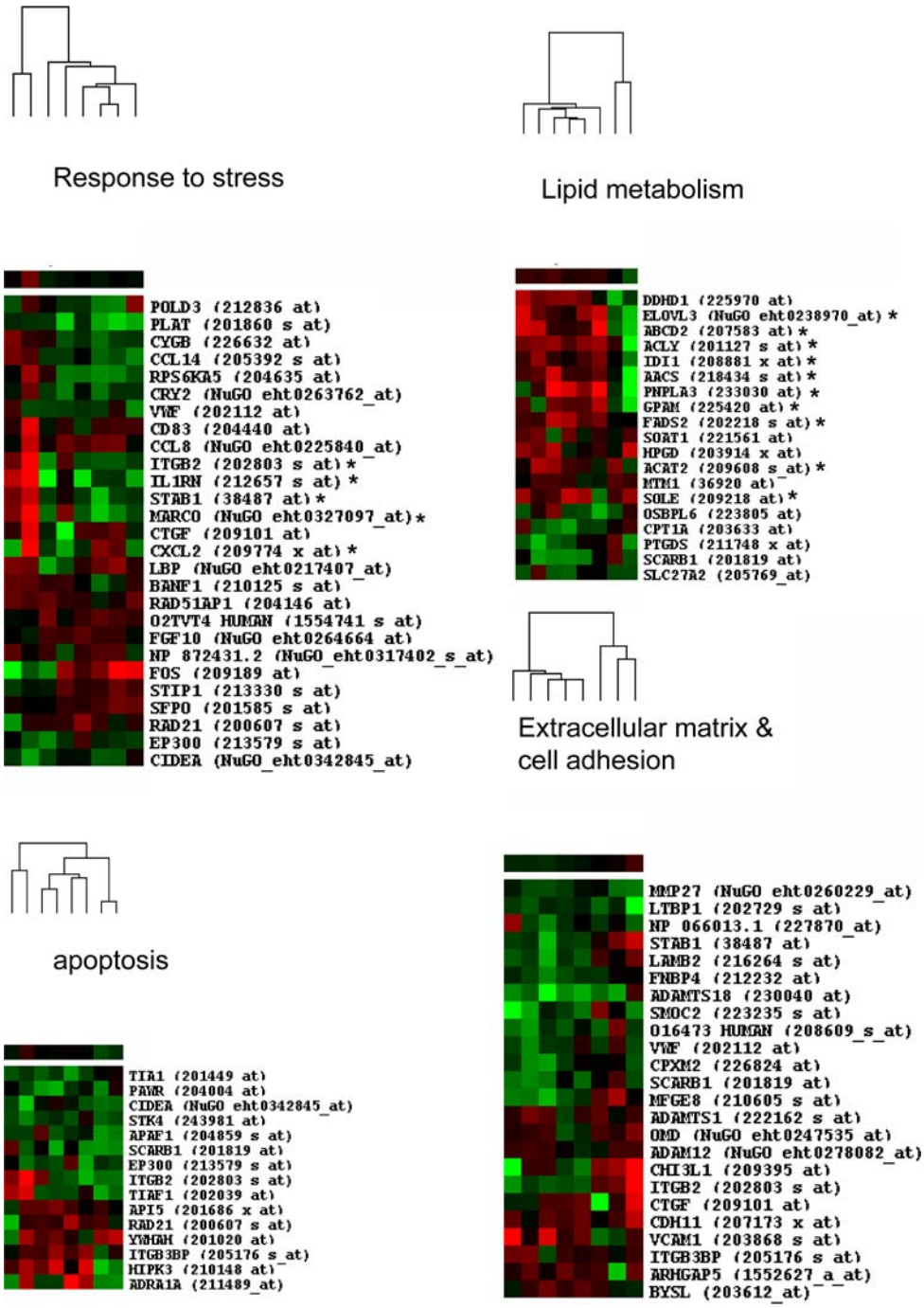

Fig. 3 Cluster maps of genes in biological processes enriched in set of genes with consistent expression change ($\geq$25%) in the majority of overweight subjects. Expression changes (2log ratio) of intervention spread versus placebo spread in eight overweight subjects were submitted to gene and sample clustering (using average linkage clustering and uncentered correlation as similarity metric). *Red* color indicates up-regulation of expression in response to intervention spread compared to placebo spread. *Green* color indicates down-regulation of expression in response to intervention spread compared to placebo spread. *Asterisks* are significantly different expression in overweight compared to lean subjects (BMI-effect) and expression changed in response to the intervention spread in direction of expression levels in lean subjects (colour in online)

possible difference in mitochondrial content or mitochondrial activity in adipose tissue from lean subjects and overweight subjects. However, it can not be excluded that the increased mitochondrial activity actually reflects a difference in cell number in the adipose tissue biopsies, as increased adipose tissue mass can be due to increased adipocyte cell size as well as increased number of adipocytes.

The dietary intervention consisted of replacement of oleic acid and stearic acid in the placebo spread by short-chain polyunsaturated fatty acids (PUFA: C18:2 and C18:3), conjugated linoleic acid (CLA: C18:2) and medium chain triglycerides (C12:0) in the intervention spread. Thus, the gene expression differences can be related to differences in intake of all these fatty acids and should be interpreted in that context.

Both CLA and PUFA (specifically n-3 PUFA) can exert anti-inflammatory effects [4, 24]. In the majority of overweight subjects, dietary intervention resulted in down-regulation of expression of a number of inflammation-related genes (in enriched category 'response to stress')

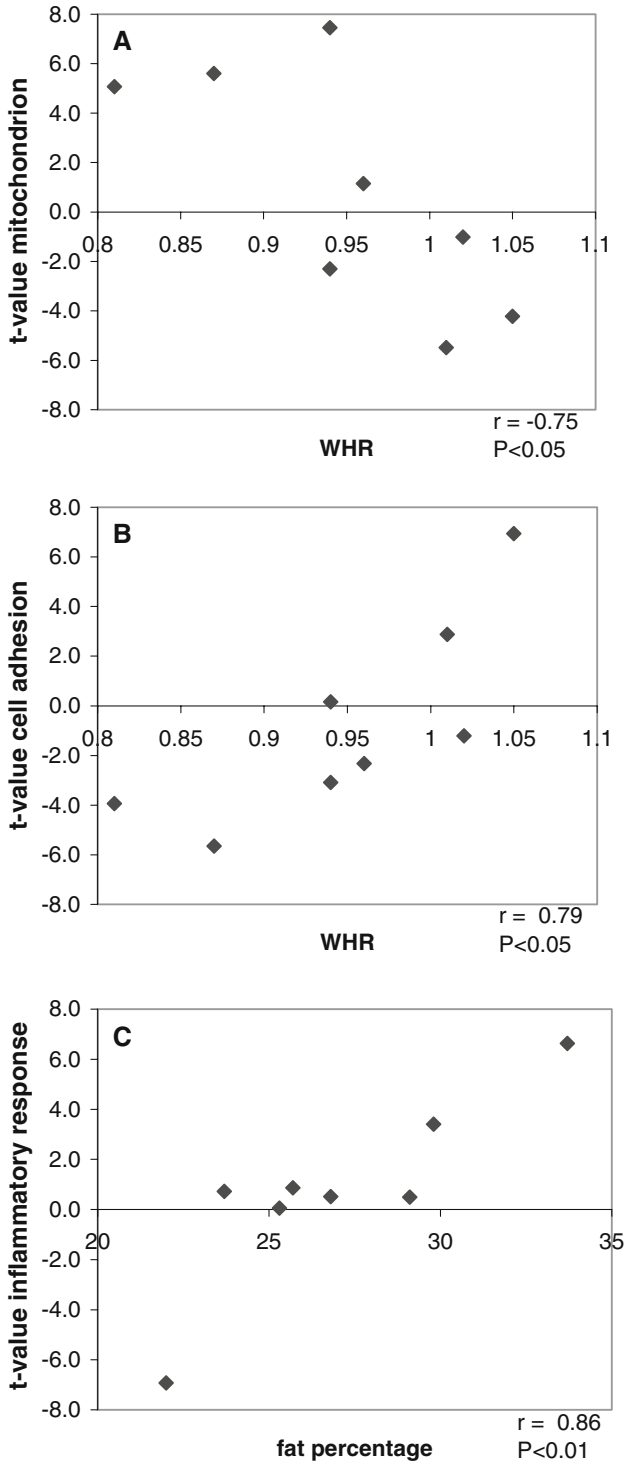

Fig. 4 Scatter plots of obesity phenotype parameters (waist–hip ratio *WHR* or fat percentage) and scores (*t* value) for enrichment of functional processes (based on Gene Ontology) that were affected by dietary intervention. **a** Score for enrichment of genes encoding mitochondrial proteins for each overweight subject plotted against WHR; **b** Score for enrichment of cell adhesion genes plotted against WHR; **c** Score for enrichment of inflammatory response genes for each overweight subject plotted against fat percentage

that were expressed at significantly higher level in adipose tissue from overweight subjects than in adipose tissue from lean subjects. This anti-inflammatory effect of the intervention was, e.g., seen for macrophage inflammatory protein 2 alpha (CXCL2), macrophage receptor MARCO (MARCO) and interleukin-1 receptor antagonist (IL1RN). In adipose tissue from lean subjects, the anti-inflammatory effect of the dietary intervention was restricted to down-regulation of genes involved in complement activation pathway (three subunits of complement C1q) and genes involved in reactive oxygen species metabolism.

Additionally, in adipose tissue from lean subjects the dietary intervention resulted in a down-regulation of genes involved in energy metabolism-related processes (generation of precursor metabolites and energy, lipid metabolism, carbohydrate metabolism). Biological network analysis revealed that a number of the differentially expressed energy metabolism genes are targets of HNF4-alpha in liver and/or pancreas [25, 37]. Thus, possibly the expression of genes related to energy metabolism could have been regulated through transcription factor HNF4-alpha (Fig. 5). PUFA can bind to and repress activity of HNF4-alpha [11, 28, 37]. This implicates that, in addition to liver, HNF4-alpha could be an important regulator of gene expression and energy metabolism in adipose tissue, at least in lean subjects.

In the majority of overweight subjects, dietary intervention affected expression of genes involved in lipid metabolism, resulting mostly in up-regulation of expression (Fig. 3). Expression of lipid metabolism genes was lower in adipose tissue from overweight subjects than in adipose tissue from lean subjects, therefore increased expression of lipid metabolism genes might be a beneficial effect of the intervention in overweight subjects. Moreover, increased energy expenditure as a result of up-regulation of lipid metabolism could be one of the mechanisms through which CLA decreases adipose tissue mass [4, 24, 37] and it could play a role in the proposed increase of energy expenditure by medium chain triglycerides [29]. Additional effects of the dietary intervention in the majority of overweight subjects included effects on genes involved in apoptosis and genes involved in cell adhesion and extracellular matrix. Effects on these processes are consistent with recently described effects of CLA and PUFA [9, 12, 37].

In overweight subjects the response to the dietary intervention was not as strong as in the lean subjects and moreover, the response among the overweight subjects was variable. The consistent response in a selection of overweight subjects was not related to treatment order. Possibly, adipose tissue in overweight subjects is not as flexible and responsive to dietary intervention as adipose

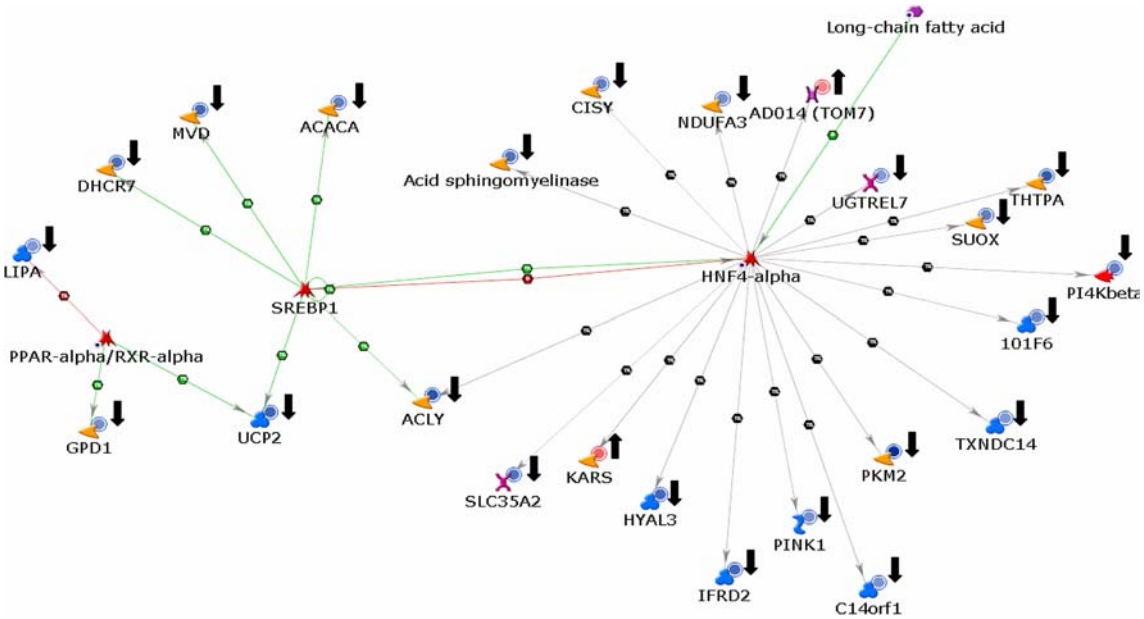

Fig. 5 Network of direct interactions between transcription factors HNF4alpha, SREBP1 and PPARA and genes involved in energy metabolism that were differentially expressed in lean subjects in response to intervention spread compared to control spread. *Arrow* pointing down indicates down-regulation of expression in response to intervention spread compared to control spread, arrow pointing up indicates up-regulation of expression in response to intervention spread compared to control spread. *Lines* between *symbols* indicate connections described in literature. This figure was made using MetaCore™ (v4.3) from GeneGo Inc. *LIPA* Lipase A, *GPD1* glycerol-3-phosphate dehydrogenase 1, *UCP2* mitochondrial uncoupling protein 2, *DHCR7* 7-dehydrocholesterol reductase; *MVD* diphosphomevalonate decarboxylase; *ACACA* acetyl-coA carboxylase 1; *ACLY* ATP citrate lyase; *CISY* citrate synthase; *NDUFA3* NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 3; *AD014(TOM7)* translocase of outer mitochondrial membrane 7 homolog (yeast); *UGTREL7* UDP-glucuronic acid/UDP-N-acetylgalactosamine transporter; *THTPA* thiamine-triphosphatase; *SUOX* Sulfite oxidase, mitochondrial precursor; *PI4Kbeta*: Phosphatidylinositol 4-kinase beta; *101F6*: Cytochrome b561 domain-containing protein 2; *TXNDC14*: thioredoxin domain containing 14; *PKM2* Pyruvate kinase 2/3; *C14orf1*: Probable ergosterol biosynthetic protein 28; *PINK1* Serine/threonine-protein kinase PINK1, mitochondrial precursor; *IFRD2* Interferon-related developmental regulator 2; *HYAL3* Hyaluronidase-3 precursor; *KARS* Lysyl-tRNA synthetase; *SLC35A2* UDP-galactose translocator

tissue in lean subjects. Also, the intervention effect might have been diluted due to increased adipose tissue mass in overweight subjects compared to the lean subjects, therefore resulting in less pronounced expression changes. Lastly, genotype and polymorphisms can not be exluded as a factor in the variation in response.

The overweight subjects were selected based on BMI, an obesity marker that does not take fully into account adipose tissue distribution in the body. In contrast, WHR and waist circumference are more specific markers for body fat distribution and presence of central or upper-body obesity [15, 19]. Interestingly, the individual response of the overweight subjects to the intervention correlated to the obesity phenotype markers WHR and fat percentage. For example, expression of mitochondrial genes was up-regulated in response to dietary intervention in the overweight subject with the lowest WHR and down-regulated in response to intervention in the overweight subject with the highest WHR. Interestingly, these two subjects have a similar BMI. Overall, these results show that the subjects, classified as overweight on the basis of their BMI, are heterogeneous in their response to dietary intervention and that this response is associated with obesity phenotype markers WHR and fat percentage, suggesting a link between body fat distribution and response to nutritional intervention.

The fat biopsies analyzed were subcutaneous biopsies, taken from the abdomen. Abdominal fat, mostly visceral abdominal fat, is often linked to complications and disease progression [36]. However, some studies report a role for subcutaneous abdominal tissue in obesity related diseases [10]. The finding that subcutaneous adipose tissue responds to nutritional intervention is encouraging for further research, as this is the only easily accessible source of adipose tissue from humans. Thus, whole genome expression profiling in adipose tissue may help to identify relevant mechanisms and targets of nutritional intervention in obesity prevention or treatment.

In summary, response to the dietary intervention was different in lean and overweight subjects. Increased short-chain polyunsaturated fatty acids (PUFA: C18:2 and C18:3), conjugated linoleic acid (CLA: C18:2) and medium chain triglycerides (C12:0) consumption resulted in lower expression of genes related to energy metabolism in lean subjects, whereas in the majority of

overweight subjects expression of inflammatory genes was down-regulated and expression of lipid metabolism genes was up-regulated. Individual responses in overweight subjects were variable and these correlated better to obesity phenotype markers WHR and fat percentage than BMI.

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