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ORIGINAL ARTICLE

Adipose tissue gene expression is differentially regulated with different rates of weight loss in overweight and obese humans

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BACKGROUND/OBJECTIVES: Moderate weight loss (WL) can ameliorate adverse health effects associated with obesity, reflected by an improved adipose tissue (AT) gene expression profile. However, the effect of rate of WL on the AT transcriptome is unknown. We investigated the global AT gene expression profile before and after two different rates of WL that resulted in similar total WL, and after a subsequent weight stabilization period.

SUBJECTS/METHODS: In this randomized controlled trial, 25 male and 28 female individuals (body mass index (BMI): 28–35 kg m^{-2}) followed either a low-calorie diet (LCD; 1250 kcal day^{-1}) for 12 weeks or a very-low-calorie diet (VLCD; 500 kcal day^{-1}) for 5 weeks (WL period) and a subsequent weight stable (WS) period of 4 weeks. The WL period and WS period together is termed dietary intervention (DI) period. Abdominal subcutaneous AT biopsies were collected for microarray analysis and gene expression changes were calculated for all three periods in the LCD group, VLCD group and between diets (ΔVLCD − ΔLCD).

RESULTS: WL was similar between groups during the WL period (LCD: − 8.1 ± 0.5 kg, VLCD: − 8.9 ± 0.4 kg, difference $P = 0.25$). Overall, more genes were significantly regulated and changes in gene expression appeared more pronounced in the VLCD group compared with the LCD group. Gene sets related to mitochondrial function, adipogenesis and immunity/inflammation were more strongly upregulated on a VLCD compared with a LCD during the DI period (positive ΔVLCD − ΔLCD). Neuronal and olfactory-related gene sets were decreased during the WL period and DI period in the VLCD group.

CONCLUSIONS: The rate of WL (LCD vs VLCD), with similar total WL, strongly regulates AT gene expression. Increased mitochondrial function, angiogenesis and adipogenesis on a VLCD compared with a LCD reflect potential beneficial diet-induced changes in AT, whereas differential neuronal and olfactory regulation suggest functions of these genes beyond the current paradigm.

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INTRODUCTION

Obesity is characterized by an expansion of adipose tissue (AT) mass and it has been recognized that AT dysfunction contributes to the development of co-morbidities observed in obesity.[1–3] These adverse health effects ameliorate upon moderate weight loss (WL).[4,5] A better understanding of the processes responsible for the beneficial effect of WL on AT function is therefore needed.

Microarray analysis revealed that hundreds to thousands of genes are differentially regulated during a dietary intervention (DI) in human AT.[6,7] Also, gene expression varies strongly between the WL and weight stabilization phase of a DI,[6,7] which stresses the need to separate these phases. Several studies have focused on which specific genes or gene sets contribute to improved AT function after WL. Studies in animals showed an increased expression of genes important for mitochondrial biogenesis when animals were kept on an energy-restricted diet.[8,9] Genes related to mitochondrial biogenesis were already upregulated shortly after Roux-en-Y gastric bypass in obese individuals, before any significant WL.[10] These data indicate that WL and/or energy restriction might improve mitochondrial function. Furthermore, WL has been shown to reduce AT expression of pro-inflammatory cytokines.[11] Temporal aspects seem to have an important role here, as many genes involved in innate immunity were upregulated after acute WL but were downregulated during a subsequent weight stabilization period.[6] Although these studies provide important insights into the potential of energy restriction and/or WL to influence AT function, a plethora of different DIs are available. Diets typically differ with respect to macronutrient composition, level of energy restriction, types of fat or carbohydrates used and/or total WL. Interestingly, one study revealed a predominant impact of energy restriction over macronutrient composition on AT gene expression in humans.[12] However, the effect of level of energy restriction, and thus rate of WL, on the human AT transcriptome has never been studied.

In this study, we investigated the global AT gene expression profile before and after two different rates of WL that resulted in similar total WL, either via a low-calorie diet (LCD) or very-low-calorie diet (VLCD), and then after a short weight stabilization period (no energy restriction) to distinguish between the effects of WL with and without energy restriction. We sought to identify genes and gene sets that were differentially expressed in response to these different rates of WL.

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MATERIALS AND METHODS

Subjects

Sixty-one overweight and obese individuals (body mass index (BMI): 28–35 kg m^{-2}) were recruited by advertisement via local media. Exclusion criteria, as described previously,[13] were smoking, cardiovascular disease, type 2 diabetes mellitus, liver or kidney disease, use of medication that influences body weight regulation, pregnancy, marked alcohol consumption (>21 alcoholic units per week for men and >14 alcoholic units per week for women), elevated fasting glucose (>6.1 mmol l^{-1}), total cholesterol (>7 mmol l^{-1}) or triacylglycerol (>3 mmol l^{-1}) concentrations, or blood pressure (>160/100 mmHg). Furthermore, volunteers were excluded if their weight had not been stable (weight change >3 kg) for 2 months before the start of the study. All participants gave their written informed consent before participation in the study. The study was performed according to the Declaration of Helsinki and was approved by the Medical Ethics Committee of Maastricht University Medical Centre.

Experimental protocol

The experimental protocol has been described previously.[13] Briefly, the participants in our study followed a DI program that was divided into two phases. First, participants were randomized to a 12-week LCD period or a 5-week VLCD period (WL period) and subsequently underwent a 4-week WS period. The WL period and WS period taken together was named the DI period (Figure 1). In the LCD group, participants followed a 12-week diet providing 1250 kcal day^{-1} designed by the dietician. One meal was replaced by meal replacements (Modifast; Nutrition et Santé Benelux, Breda, The Netherlands), two meals were prepared by the participants themselves and three small in-between meal snacks were consumed. In the VLCD group, participants followed a 5-week diet in which three meals per day were replaced by meal replacements, providing 500 kcal day^{-1}. During this period participants were allowed to consume an unrestricted amount of low-calorie vegetables. Both groups subsequently underwent a 4-week WS period with a diet based on the energy requirement of the participants. The study dietician provided dietary advice to both groups to assist in WL during the WL period (five meetings) and in remaining WS throughout the WS period (four meetings).

At the start of the study and at the end of each period fasting blood samples were drawn, abdominal subcutaneous AT biopsies were collected, body composition was determined and body weight, height and waist circumference were measured after an overnight fast of at least 10 h.

Because of the obvious differences between the DIs in this study, the researchers, study participants and dietician were not blinded to the intervention. This trial is registered with www.clinicaltrials.gov as NCT01559415.

Anthropometric measurements

Participants were weighed on the same scale (Seca model 861, Hamburg, Germany) accurate to the nearest 0.1 kg in light clothing. Body volume was determined with the Bod Pod device (Cosmed, Italy, Rome), as described previously.[13]

Biochemistry

Blood samples were collected into EDTA tubes and centrifuged for 15 min at 1000 *g* at 4 °C. Aliquots were immediately frozen in liquid nitrogen and subsequently stored at −80 °C. Plasma glucose, free fatty acid (FFA) and total cholesterol were analyzed with standard enzymatic methods (ABX Pentra 400 autoanalyzer, Horiba ABX, Montpellier, France). Plasma insulin concentrations were analyzed with commercially available RIA kits (Human insulin specific RIA, Millipore Corporation, Billerica, MA, USA).

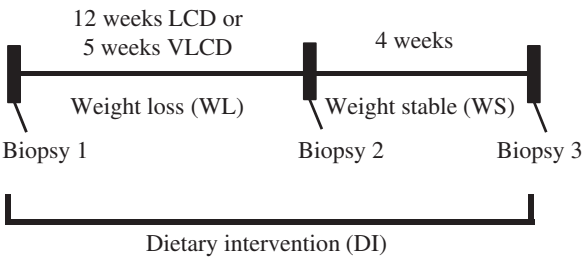

Figure 1. Study overview. Subcutaneous abdominal AT biopsies were taken at study start, end of the WL period and end of the WS period.

AT biopsy

Abdominal subcutaneous AT needle biopsies (≈1 g) were collected 6–8 cm lateral from the umbilicus under local anesthesia (2% lidocaine) by needle biopsy. Biopsies were immediately rinsed with sterile saline and visible blood vessels were removed. AT biopsies were snap-frozen in liquid nitrogen and stored at −80 °C for further analysis (see below).

Sample preparation and microarray analysis

Total RNA was extracted from frozen AT specimens (~150 mg) using TRIzol reagent (Invitrogen, Breda, The Netherlands). Total RNA (100 ng per sample) was labeled by Whole-Transcript Sense Target Assay and hybridized to human whole-genome Affymetrix Gene 1.1 ST arrays targeting 19654 unique genes (Affymetrix, Santa Clara, CA, USA). Sample labeling, hybridization to chips and image scanning was performed according to manufacturer's instructions.

Microarray data analysis

Microarray signals were normalized using robust multichip average. Genes with normalized signals >20 on at least 15 arrays were defined as expressed (11 532 genes) and selected for further analysis. Significant differences of individual genes were tested using the limma R library.[14] *P*-values were adjusted using false discovery rate (FDR).[15] A *q*-value below 0.05 was considered significant. To assess the differences in changes in gene expression during a certain period (WL period, end of WL—study start; WS period, end of WS—end of WL; or DI period, end of WS—study start) between diet groups, the changes in logSLR values of the LCD group were subtracted from the changes in logSLR values of the VLCD group during the same time period, resulting in ΔVLCD − ΔLCD. For example, a significantly negative ΔVLCD − ΔLCD comparison means that the change in gene expression in the VLCD group was significantly lower compared with the LCD group during a certain period. Between ΔVLCD and ΔLCD changes were significant when the *q*-value was <0.05 in a *t*-test with Bayesian correction (Limma). Changes within groups during a certain period (WL period, WS period or DI period) were defined as significantly different when the *q*-value was <0.05 in a paired *t*-test with Bayesian correction (Limma). Gene set enrichment analysis[16] was performed on the unfiltered data set (19 654 genes) and gene sets with a FDR *q*-value <0.05 were defined as significantly regulated. Array data have been submitted to the Gene Expression Omnibus (number GSE77962).

ASCA (ANOVA Simultaneous Component Analysis)[17] was performed to account for different sources of biological variation. ASCA is a multivariate method that partitions variation in the data and enables to interpret these partitions by simultaneous component analysis. ASCA was extended with a permutation procedure (500 times) to validate ASCA models.[18] Analysis was performed with diet, sex, time and their interactions in the model as factors. No significant interaction between sex and diet or sex and time was observed. Analyses were performed using Matlab (version R2012a).

Categories of gene sets

To make the data more representable we classified individual gene sets from the gene set enrichment analysis (databases used: KEGG, Reactome, Biocarta and WikiPathways) into gene set categories. For example, the gene set 'TNF alpha signaling pathway' was classified to the immunity/inflammation category. A category of gene sets was differentially regulated when at least one gene set within a category was differentially regulated.

Statistics

Data are presented as mean ± s.e.m. Comparisons of variables between time-points within the same group were made with the paired-sample *t*-test. Between-group comparisons were made with the independent-samples *t*-test. Statistical calculations were performed with SPSS for Macintosh, Version 21 (Chicago, IL, USA). $P < 0.05$ was considered statistically significant.

RESULTS

Four participants withdrew from the study during the DI, for four participants we could not collect enough biopsy material. Characteristics of the remaining 53 participants are displayed in Table 1.

Clinical characteristics

The WL-induced change in body weight was similar in the LCD and VLCD group (−8.1 ± 0.5 kg vs −8.9 ± 0.4 kg, respectively, $P = 0.25$, Table 1). Body weight did not change significantly in either group in the subsequent 4 weeks of the WS period (Table 1). However, fat mass (%) further decreased in both groups in this period (LCD: −0.6 ± 0.2%, $P = 0.032$; VLCD: −1.1 ± 0.3%, $P = 0.001$, no difference between groups, Table 1). Total cholesterol decreased more strongly in the VLCD compared with LCD group during WL and subsequently increased more strongly during the WS period in the former group (Table 1). Glucose, insulin and HOMA-IR decreased significantly during WL in both groups (Table 1). During the WS period glucose, insulin and HOMA-IR increased significantly in the VLCD group, whereas no significant changes were observed in the LCD group during this same period (Table 1).

Microarray gene expression

The WL period resulted in the differential expression of 512 genes in the LCD group, 6135 genes in the VLCD group and 23 genes were differentially regulated between the two diets (ΔVLCD–LCD comparison; Figure 2). In the VLCD group, 154 genes were significantly regulated during all dietary periods. During all periods more genes were regulated on a VLCD compared with a LCD. The expression pattern appeared more pronounced (both upregulation and downregulation) in the VLCD group compared with the LCD group during WL (Figure 3). This could be observed for significantly regulated genes in both the LCD group and VLCD group (Figure 3a) and for significantly upregulated (Figure 3b) or downregulated (Figure 3c) genes for each group separately.

Significantly regulated gene sets (FDR q-value < 0.05) in the KEGG, Reactome, Biocarta and WikiPathways databases were found in the LCD group and VLCD group. Significantly changed gene sets were subsequently categorized according to function (that is, the gene set 'TNF alpha signaling pathway' was classified to the immunity/inflammation category and so on) and the most prominent gene sets are shown for each category (Table 2).

An overview of all regulated gene set categories and gene sets during all three time periods in the LCD group, VLCD group and ΔVLCD–LCD comparison are displayed in Figure 4 and Supplementary Table 1, respectively. A category of gene sets

Table 1. Clinical characteristics at study start, the end of WL and the end of WS in the LCD group and VLCD group

	Study start		*End of WL*		*End of WS*	
	LCD	*VLCD*	*LCD*	*VLCD*	*LCD*	*VLCD*
Sex (male/female)	13/14	12/14				
Age (years)	51.7 ± 2.1	50.4 ± 1.5				
Weight (kg)	92.8 ± 2	92.1 ± 1.9	84.6 ± 2**	83.1 ± 1.6**	84.5 ± 2**	82.9 ± 1.7**
BMI (kg m^{-2})	31.5 ± 0.5	30.8 ± 0.4	28.7 ± 0.5**	27.8 ± 0.4**	28.7 ± 0.5**	27.7 ± 0.4**
Waist circumference (cm)	102.5 ± 2.1	101.3 ± 1.6	95.2 ± 1.9**	93.5 ± 1.4**	94.4 ± 2.1**	94.6 ± 1.4**^{1}
Fat mass (%)	40.1 ± 1.9	39.5 ± 1.6	34.7 ± 2.2**	34.8 ± 2**	34.1 ± 2.3**$^{,\#}$	33.7 ± 2**$^{,\#\#}$
Fat-free mass (kg)	55.5 ± 2.3	55.7 ± 2.4	55 ± 2.3*	54.2 ± 2.3**‡	55.3 ± 2.3	54.9 ± 2.4**$^{\dagger\#\#}$
Total cholesterol (mmol l^{-1})	6 ± 0.2	5.9 ± 0.2	5.5 ± 0.2**	4.9 ± 0.1**†	5.7 ± 0.2**	5.7 ± 0.2$^{\#\#2}$
Glucose (mmol l^{-1})	5.3 ± 0.1	5.2 ± 0.1	5.1 ± 0.1*	4.8 ± 0.1**‡	5.1 ± 0.1*	5 ± 0.1*$^{,\#\#2}$
Insulin (μU ml^{-1})	17.6 ± 1.1	14.7 ± 1.2	12.8 ± 0.7**	10.6 ± 1.1**	13.5 ± 0.9**	13.1 ± 1.4$^{\#}$
HOMA-IR	4.1 ± 0.3	3.4 ± 0.3	2.9 ± 0.2**	2.3 ± 0.3**	3.1 ± 0.2**	3 ± 0.3$^{\#}$

Abbreviations: FFM, fat-free mass; HOMA-IR, homeostatic model assessment insulin resistance; LCD, low-calorie diet; VLCD, very-low-calorie diet; WL, weight loss; WS, weight stable. Values are mean ± s.e.m. *$P < 0.05$, **$P < 0.01$, change from start of study within diet groups. $^{\dagger}P < 0.05$, $^{\ddagger}P < 0.01$ change from start of study between diet groups. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ change between end of WS and end of WL, within diet groups. $^{1}P < 0.05$, $^{2}P < 0.01$ change between end of WS and end of WL, between diet groups.

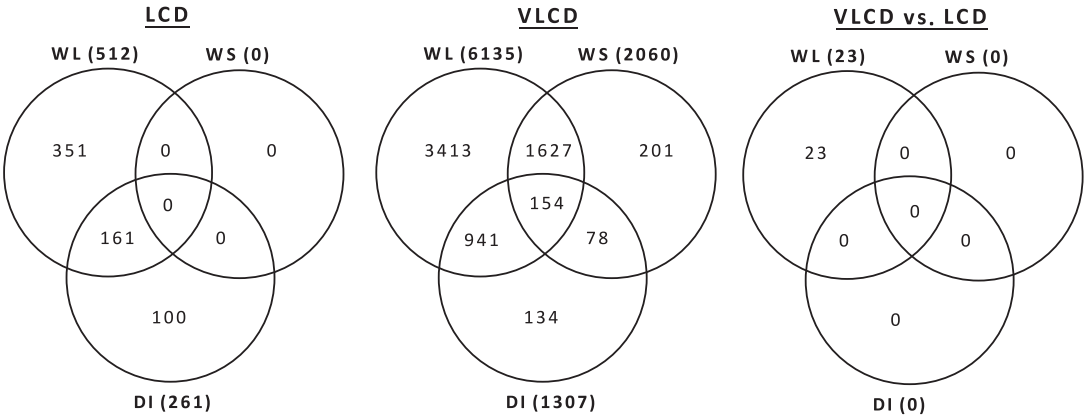

Figure 2. Venn-diagram of differentially regulated genes (FDR q-value < 0.05) in the LCD group, VLCD group and VLCD–LCD comparison.

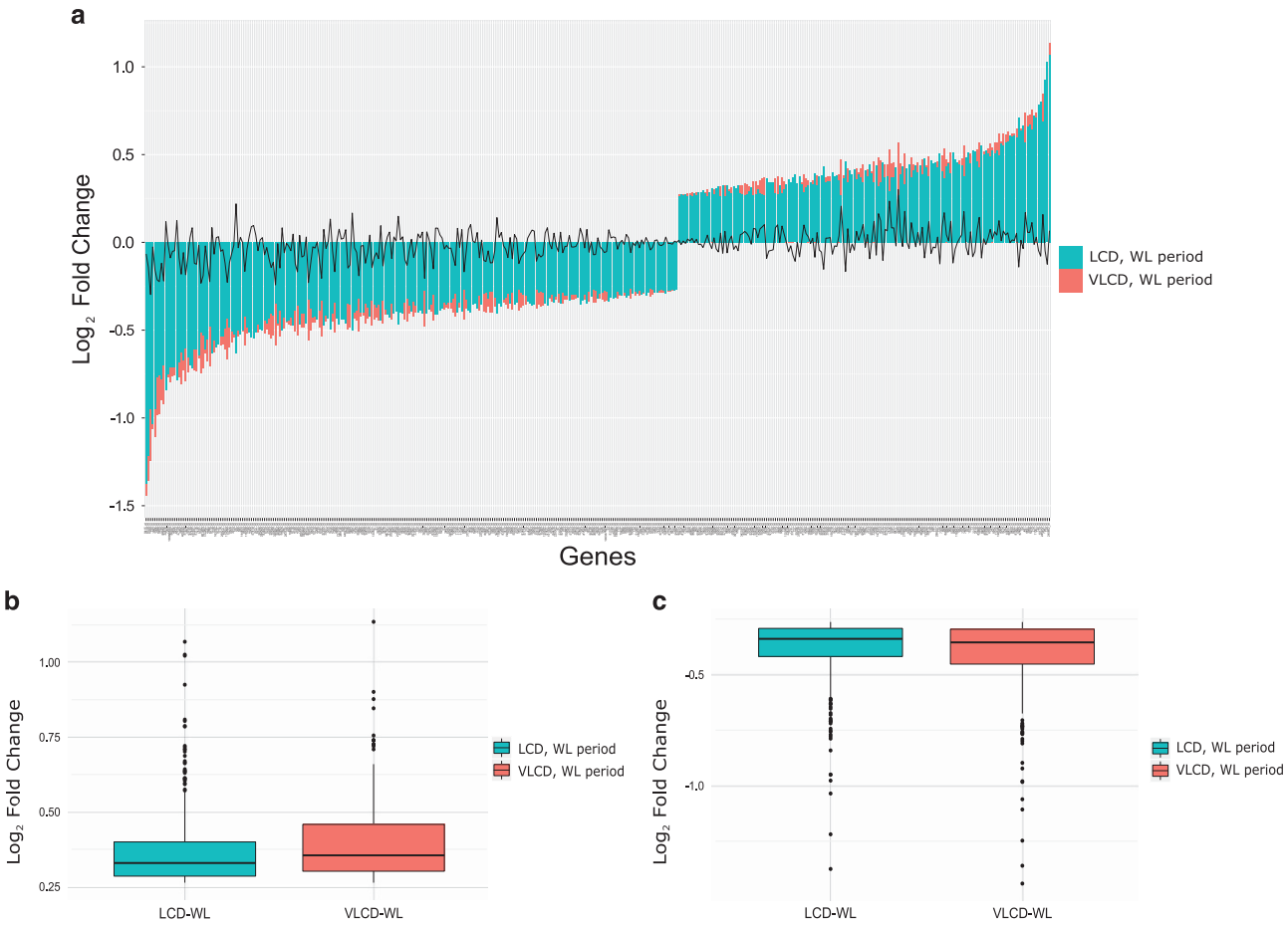

Figure 3. The WL period induces a more pronounced gene expression pattern (both upregulation and downregulation) in the VLCD group compared with the LCD group. (**a**) Log2 fold changes (FC) of significantly regulated genes ($\log_2(|FC| \geqslant 1.2)$, *P*-value and *q*-value < 0.05) that were significantly regulated in both the LCD group and VLCD group during the WL period. The line in the center represents the difference between the VLCD group and LCD group. (**b**) Median and outliers of significantly upregulated genes ($\log_2(|FC| \geqslant 1.2)$, *P*-value and *q*-value < 0.05) in the LCD group and VLCD group during the WL period. (**c**) Median and outliers of significantly downregulated genes ($\log_2(|FC| \geqslant 1.2)$, *P*-value and *q*-value < 0.05) in the LCD group and VLCD group during the WL period.

was determined as differentially regulated when at least one gene set within this category was differentially regulated. Overall, the regulation of expression of categories of gene sets were mostly opposite for the WL period and WS period (Figure 4). Furthermore, the direction (up or down) of regulation of gene sets was often similar between the LCD group and VLCD group, but was generally stronger in the VLCD group (Figures 3 and 4). Gene sets involved in transcription, protein modifications, epigenetic modifications and immunity/inflammation were upregulated during WL and then downregulated during WS in both the LCD group and VLCD group (Figure 4; Supplementary Table 1). Although the pattern of regulation was the same in the LCD group and VLCD group, the changes of these gene sets were more pronounced in the VLCD group, reflected in the ΔVLCD–LCD comparison that was positive during WL and negative during WS (Figure 4). Gene sets related to oxidative phosphorylation (OXPHOS)/tricarboxylic acid (TCA) cycle, lipid metabolism (other) and glucose metabolism were downregulated during WL in both groups, whereas the latter two were more strongly downregulated on a VLCD compared with a LCD (negative ΔVLCD–LCD comparison). Two gene sets of mitochondrial functioning, 'Mitochondrial biogenesis' and 'Mitochondrial gene expression', were upregulated in the VLCD group, remained unchanged in the LCD group and were more upregulated in the VLCD group compared with the LCD group (Figure 4). Adipogenesis-related gene sets were upregulated on a VLCD and remained unchanged on a LCD. Neuronal and olfactory regulation gene sets were significantly downregulated during WL and during DI in the VLCD group (Figure 4).

Several differentially regulated gene sets are not reported in Figure 4 and included gene sets related to cancer, proteasomal degradation and circadian rhythm. A closer inspection of gene sets related to cancer showed a strong overlap with genes involved in cell cycle and transcription. Cancer development in AT is extremely rare and therefore these gene sets are not discussed. The proteasome has many functions including protein degradation, cell cycle control, immune reaction and cellular response to stress. Gene sets that are related to the proteasome and a second category (for example: 'APC C-mediated degradation of cell cycle proteins') were categorized to the proteasomal degradation category. Finally, many individual genes in the gene sets 'circadian clock' and 'mitochondrial biogenesis' overlapped and were attributed to the 'Mitochondrial biogenesis' gene set, as this intervention did not include any changes to the chronobiology of the study participants. A number of categories showed both upregulated and downregulated gene sets at a certain time point (Figure 4, in purple). For example, the 'Assembly of collagen fibrils and other multimeric structures' gene set was upregulated, whereas the 'molecules associated with elastic fibers' gene set was downregulated during WS in the VLCD group (Supplementary

Table 2. List of frequently observed gene sets per category

Category	*Frequently observed gene sets*	*Category*	*Frequently observed gene sets*
Adipogenesis	Transcriptional regulation of white adipocyte differentiation (reactome)	Lipid metabolism (cholesterol/ sphingolipid)	Cholesterol biosynthesis (WP1795) Sphingolipid metabolism (reactome)
Amino acid metabolism	Metabolism of amino acids and deriviates (WP2693) Branched chain amino acid catabolism (reactome) Lysine degradation (KEGG)	Lipid metabolism (other)	Biosynthesis of unsaturated fatty acids (KEGG) Triglyceride biosynthesis (reactome) Lipid digestion mobilization and transport (WP2764)
Angiogenesis	VEGFA/VEGFR2 pathway (reactome) Angiogenesis overview (WP1993)	Mitochondrial functioning	Transcriptional activation of mitochondrial biogenesis (reactome) Mitochondrial gene expression (WP391) Mitochondrial biogenesis (reactome)
Cell cycle	M phase (reactome) Cell cycle checkpoints (reactome) Regulation of DNA replication (reactome)	Neuronal regulation	Neuroactive ligand receptor interaction (KEGG) Dopminergic neurogenesis (WP2855) Neuronal system (reactome)
Extracellular matrix organization	Collagen biosynthesis and modifying enzymes (reactome) Laminin interactions (reactome) Extracellular matrix organization (reactome)	Olfactory regulation	Olfactory transduction (KEGG) Olfactory signaling pathway (reactome)
Epigenetic organization	Chromatin modifying enzymes (reactome) HATS acetylates histones (reactome) Epigenetic regulation of gene expression (reactome)	Oxidative phosphorylation (OXPHOS)/ tricarboxylic acid (TCA) cycle	Oxidative phosphorylation (KEGG) The citric acid TCA cycle and respiratory electron transport (reactome) Pyruvate metabolism (reactome)
Glucose metabolism	Glycolysis and gluconeogenesis (WP534) Glucose metabolism (reactome) Pentose phosphate pathway (KEGG)	Proteasomal degradation	Proteasome (KEGG) Proteasome degradation (WP183) Ubiquitin mediated proteolysis (KEGG)
G-protein coupled receptor	GPCR downstream signaling (WP1824) GPCRS other (WP117) GPCR ligand binding (reactome)	Protein modifications	Protein processing in endoplasmic reticulum (KEGG) Ribosome (KEGG) Trans-golgi network vesicle budding (reactome)
Growth factors	SPRY regulation of FGF signaling (reactome) EGF-EGFR signaling pathway (WP437) Signaling by TGF-beta receptor complex (reactome)	Stress response	Cellular response to heat stress (reactome) Attenuation phase (reactome) Unfolded protein response UPR (reactome)
Immunity/ inflammation	TNF alpha signaling pathway (WP2808) Interferon gamma signaling (WP1836) LAIR pathway (Biocarta)	Transcription	RNA transport (KEGG) Spliceosome (KEGG) Eukaryotic translation initiation (reactome)

Abbreviations: KEGG, Kyoto Encyclopedia of Genes and Genomes; WP, WikiPathways. A maximum of three gene sets are shown per category. Gene sets were deemed significant when FDR q-value < 0.05.

Table 1), which both belong to the extracellular matrix (ECM) modifications category.

DISCUSSION

The current study showed that, with similar total WL, the rate of WL (LCD vs VLCD) has a major impact on abdominal subcutaneous AT gene expression. This is reflected both by the higher number of differentially regulated genes and the generally more pronounced changes in gene expression and gene sets in the VLCD group compared with the LCD group.

The observation that gene sets related to transcription, protein modifications and epigenetic modifications are strongly upregulated, whereas gene sets related to OXPHOS/TCA cycle, lipid metabolism (other) and glucose metabolism are strongly downregulated during the WL period in both groups is largely in line with other studies.[6,7] These processes reflect the necessary changes for the human body to adapt to the negative energy balance and decreased body weight. Interestingly, changes in these cellular processes were more pronounced in the VLCD group compared with the LCD group. Although WL was similar in both groups, the time to adapt was shorter and the negative energy balance was stronger on the VLCD, which apparently leads to more and stronger regulated genes. Interestingly, whereas the gene sets 'triglyceride biosynthesis' and 'glycolysis and gluconeogenesis' were more downregulated on a VLCD compared with a LCD during WL, there was no differential expression of pathways of OXPHOS/TCA cycle between groups. Apparently, energy requirements remain similar in the two diet groups.

Genes involved in inflammation and innate immunity were increased[6] or unchanged[7] in human AT directly after diet-induced WL, but were downregulated during a subsequent weight stabilization period. In line, gene sets involved in immunity/ inflammation were upregulated during WL and downregulated during WS in both groups in this study. Stimulation of these gene sets directly after WL could be the result of a negative energy balance, as stimulation of AT lipolysis has been shown to drive AT macrophage recruitment.[19] Moreover, the negative energy balance is larger on a VLCD compared with a LCD and could explain the stronger upregulation of immunity/inflammation gene sets in the former group. This adverse inflammatory profile is likely a transient process however, since sustained WL is associated with

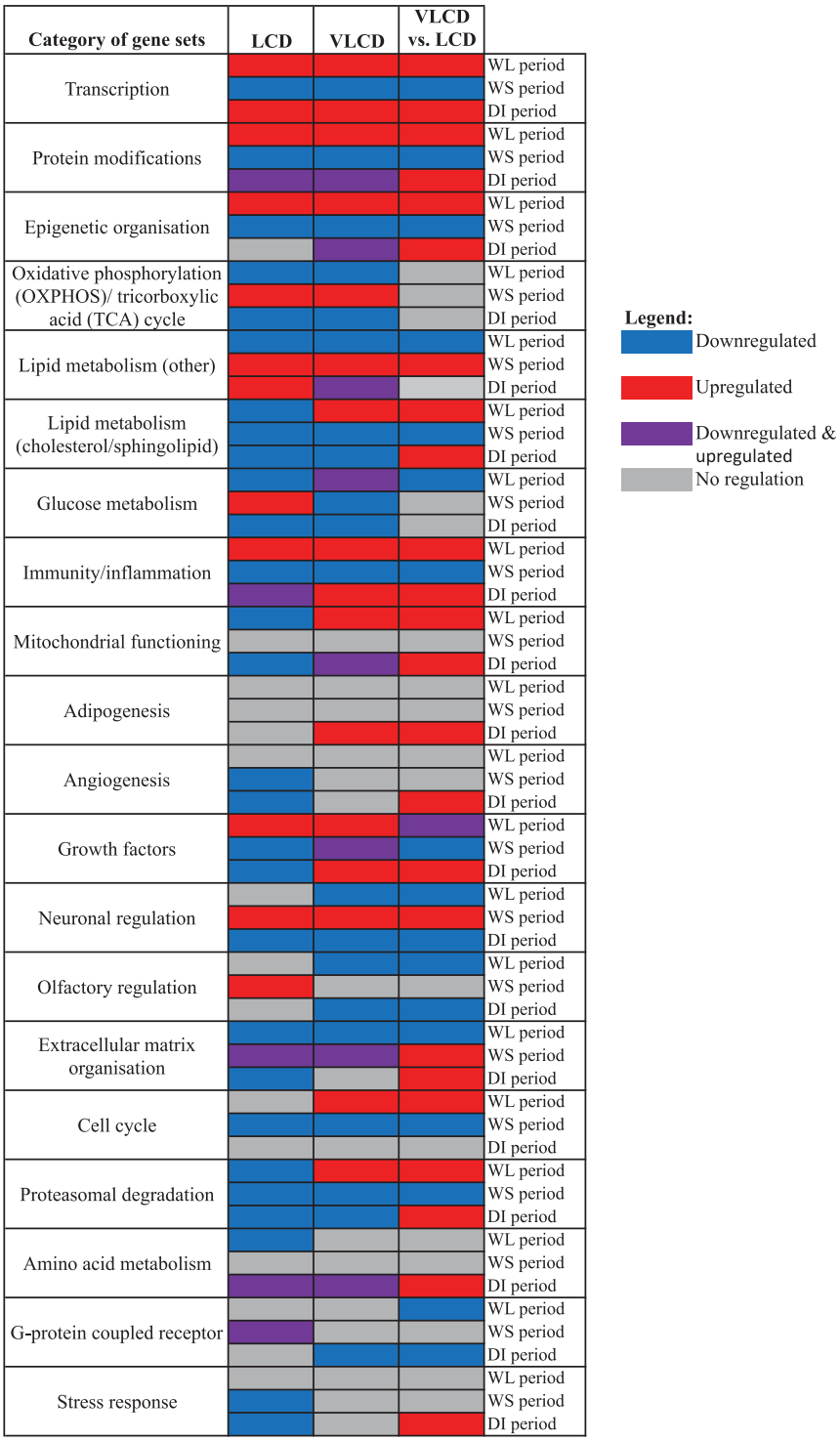

Figure 4. Categories of significantly regulated gene sets in the WL period, WS period, and DI period in the LCD group, VLCD group and ΔVLCD–LCD comparison. Each regulated category contains at least one gene set. Bars in purple represent categories that contain both downregulated and upregulated gene sets. Individual regulated gene sets for all time periods and groups can be found in Supplementary Table 1.

a reduction in infiltrating macrophages.[20,21] In this study, however, many inflammatory gene sets were still upregulated after the complete DI. The WS period in our study lasted 4 weeks and might have been too short to observe the improvement in inflammatory profile with WL maintenance (several months) that was reported in other studies.[6,7] Also, the reduction in body fat during the WS period in both groups suggests the presence of a negative energy balance, with the potential to simulate immunity/inflammation-related gene sets.

Interestingly, two gene sets related to mitochondrial function, 'Mitochondrial biogenesis' and 'Mitochondrial gene expression', were upregulated on a VLCD and unchanged on a LCD during DI. Two studies in mice showed increased expression of mitochondrial biogenesis genes in AT after calorie restriction.[8,9] Also, a

lower BMI was associated with increased mitochondrial function, whereas genes related to mitochondrial biogenesis were downregulated in subcutaneous AT of obese monozygotic twins compared with their leaner co-twins.[22] Intriguingly, induction of genes important for mitochondrial biogenesis was already observed 7–8 days after Roux-en-Y gastric bypass surgery, before the onset of WL. The present study showed that improvements in gene expression of mitochondrial biogenesis and function also occur after rapid WL followed by weight stabilization, which could assist in alleviating adipocyte dysfunction. Gene sets related to adipogenesis were upregulated in the VLCD group, remained unchanged in the LCD group and was upregulated on a VLCD compared with a LCD during DI. It was previously shown that diet-induced WL increases the differentiation capacity of human preadipocytes.[23] Gene sets related to adipogenesis were, however, only upregulated in the VLCD group. Growth factors are important stimulators of adipogenesis, especially transforming growth factor-β (TGF-β).[24] Indeed, growth factor-related gene sets, including TGF-β-related gene sets, were downregulated on a LCD and upregulated on a VLCD during DI and might explain the group differences in adipogenesis regulation.

The observation that gene sets involved in neuronal and olfactory regulation were differentially regulated in the LCD group and VLCD group is remarkable. Human white adipose tissue is innervated by the sympathetic nervous system, which is essential for stimulation of lipolysis and it was shown that white adipose tissue contains sensory neurons that convey information to the central nervous system.[25] After a 3-day fast norepinephrine spillover was observed in human AT, suggesting that sympathetic nervous system activity is increased upon calorie restriction.[26] However, neuronal gene sets were downregulated during WL on a VLCD. Besides the fact that these interventions differ strongly with respect to study length and severity of calorie restriction, it should be mentioned that the gene set category neuronal regulation contains pathways, such as 'dopminergic neurogenesis' and 'nicotine addiction' that are not directly involved in lipolysis. The main function of the olfactory system is to recognize volatile chemicals and to transport these signals to the olfactory bulb to create the perception of smell.[27] Smell is important for food perception and preference and is associated with metabolic status and BMI.[28,29] Recent studies have shown that the olfactory receptors are also expressed in the duodenum and lung epithelium.[30–32] To our knowledge, this is the first observation of differentially regulated olfactory genes after WL in human AT. Furthermore, the differences between the LCD and VLCD group with respect to olfactory and neuronal gene sets might have been caused by the decreased exposure to smells and tastes from the meal replacements in the VLCD group compared with the 'regular' meals in the LCD group. Overall, our findings need to be confirmed and it remains to be established how the differential regulation of olfactory and neuronal genes in adipocytes relates to AT functioning.

The ECM of an adipocyte provides protection against external stress and has a pivotal role in biological functions. ECM-related gene sets were downregulated during WL and were both upregulated and downregulated during WS in both groups. In accordance, long-term weight reduction induced by bariatric surgery resulted in downregulation of ECM-regulated genes.[33–35] Collagen synthesis is an energy-demanding process,[36] and it is therefore not surprising that the gene set related to this process, 'Collagen biosynthesis and modifying enzymes', was downregulated more strongly during a stronger energy deficit on a VLCD compared with a LCD during WL. Subsequently, these gene sets were upregulated more strongly in the VLCD group during WS when energy was readily available. Disturbed regulation of ECM genes could also have contributed to the increased inflammation of AT found during WL, because of the strong relationship between ECM components and inflammatory processes.[37]

An important question that remains is whether the differences in gene expression between groups persists on a long-term basis and what the physiological implications would be. If the mitochondrial gene expression changes are translated into improved mitochondrial function in the VLCD group, this might be associated with long-term increased energy expenditure and this might limit weight regain. Furthermore, if the increased adipogenesis and angiogenesis gene sets in the VLCD group resulted in the replacement of large dysfunctional adipocytes with smaller functional cells and a better capillarization of AT, this would be a beneficial outcome. A continued expression of inflammation/immunity and stress response gene sets could adversely affect the long-term AT inflammatory profile in the VLCD group. The long-term implications of a change in olfactory regulation in AT is unclear as their precise function in body weight management is largely unknown.

A strength of this study was the relatively high number of individuals tested with microarray analysis. Also, the unique study design allowed for a comparison of AT gene expression between WL on a LCD or VLCD, with similar total WL. A limitation of our study is that the use of whole tissue biopsies does not allow us to discriminate between adipocytes and the stromal vascular fraction. We therefore cannot exclude that some of the changes in gene sets are more representative of changes in the stromal vascular fraction than in the adipocytes. Study results are based on transcriptomics data, which have not been confirmed by PCR or functional validation. However, RT-PCR has not been shown to be an adequate method for microarray validation.[38] Moreover, validation is usually recommended in case of limited number of microarrays, but here we have used data from ~25 microarrays for every time point and diet group. Owing to the large amount of differentially regulated genes and gene sets, we were forced to categorize the gene sets, with potential unwanted selection bias. Furthermore, we were not able to discuss and show all relevant gene sets. Although there is no significant difference in WL between the LCD and VLCD group in the WL period, we cannot exclude that the slightly higher WL in the VLCD group may have somehow influenced our gene expression results. Also, estimated micronutrient intake was somewhat lower in the VLCD group compared with the LCD group (data not shown), thus we cannot exclude the fact that differences in micronutrient intake could have influenced AT gene expression.

In conclusion, we showed that the human AT gene expression profile was strongly differentially expressed with different rates of WL. Gene sets related to transcription, protein modifications and epigenetic regulation were more upregulated on a VLCD compared with a LCD to accommodate the more rapid weight change. Increased mitochondrial function, angiogenesis and adipogenesis on a VLCD compared with a LCD reflect potential beneficial changes in AT. Induction of an inflammatory profile directly after WL likely reflects a state of negative energy balance and is expected to improve in the long-term. Finally, we observed an unexpected change of gene sets involved in neuronal and olfactory regulation in AT that requires confirmation, but suggests a function of these genes beyond the current paradigm.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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