

## ORIGINAL ARTICLE

## Weight reduction modulates expression of genes involved in extracellular matrix and cell death: the GENOBIN study

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**Objective:** Lifestyle and genetic factors interact in the development of obesity and the metabolic syndrome. The molecular mechanisms underlying the beneficial dietary modifications are, however, unclear. We aimed to examine the effect of the long-term moderate weight reduction on gene expression in adipose tissue (AT) and to identify genes and gene clusters responsive to treatment and thereby likely contributing to the development of the metabolic syndrome.

**Design:** Randomized controlled and individualized weight reduction intervention.

**Subjects:** Forty-six subjects with impaired fasting glycemia or impaired glucose tolerance and features of metabolic syndrome, aged  $60 \pm 7$  years were randomized either to a weight reduction (WR) ( $n = 28$ ) or a control ( $n = 18$ ) group lasting for 33 weeks.

**Measurements:** Oral and intravenous glucose tolerance tests and subcutaneous AT biopsies were performed before and after the intervention. Gene expression of AT was studied using microarray technology in subgroups of WR (with weight reduction  $\geq 5\%$ ,  $n = 9$ ) and control group ( $n = 10$ ). The results were confirmed using quantitative PCR.

**Results:** In the WR group, glucose metabolism improved. Moreover, an inverse correlation between the change in  $S_1$  and the change in body weight was found ( $r = -0.44$ ,  $P = 0.026$ ). Downregulation of gene expression ( $P < 0.01$ ) involving gene ontology groups of extracellular matrix and cell death was seen. Such changes did not occur in the control group. The tenomodulin-gene was one of the most downregulated genes ( $-39 \pm 16\%$ ,  $P < 0.0001$ ). Moreover, its expression correlated with insulin sensitivity ( $r = -0.34$ ,  $P = 0.005$ ) before the intervention and with body adiposity both before ( $r = 0.42$ ,  $P = 0.007$ ) and after ( $r = 0.30$ ,  $P = 0.056$ ) the intervention.

**Conclusion:** Genes regulating the extracellular matrix and cell death showed a strong downregulation after long-term weight reduction. This likely reflects a new stable state at the molecular level in AT. Further studies are warranted to elucidate the mechanisms of these genetic factors.

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**Keywords:** insulin resistance; weight reduction; adipose tissue; gene-expression profile; extracellular matrix

## Introduction

Lifestyle and genetic factors interact in the development of obesity, the metabolic syndrome and progression to type 2

diabetes (T2DM).<sup>1</sup> These interactions are likely to be reflected in gene expression. The metabolic syndrome, characterized by central obesity, insulin resistance and abnormal glucose metabolism,<sup>2</sup> dyslipidemia and hypertension, predisposes to cardiovascular diseases<sup>3</sup> and T2DM.<sup>4,5</sup> Besides insulin resistance, T2DM is preceded by impaired first-phase insulin secretion, which is an inherent feature of impaired fasting glycemia and impaired glucose tolerance.<sup>6–8</sup>

Weight loss and beneficial dietary changes have been shown to decrease insulin resistance and prevent T2DM in

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overweight and obese individuals with impaired glucose tolerance,<sup>6,9–11</sup> but the effect of these interventions on genome-wide gene expression is largely unknown. Several cross-sectional studies have shown that expression of individual genes in adipose tissue (AT) differs between obese and lean subjects.<sup>12–14</sup> AT secretes numerous active agents that contribute to fat storage and distribution, lipolysis, inflammation and insulin resistance, typical processes, which are involved in the pathogenesis of metabolic syndrome, T2DM and cardiovascular diseases.<sup>15–18</sup> Weight reduction induces changes in the expression of various genes in AT.<sup>19–23</sup> A few studies have applied microarray technologies to study gene expression in AT of massively obese subjects either in comparison to lean subjects cross-sectionally<sup>24,25</sup> or after short-term weight reduction.<sup>26,27</sup> These studies have demonstrated downregulation in the expression of genes involved in inflammation and desaturation of fatty acids.<sup>26,27</sup>

In the present work, we studied the effect of long-term weight reduction on gene expression in AT of overweight or obese individuals with impaired fasting glycemia or impaired glucose tolerance and other features of the metabolic syndrome. We aimed to examine the effect of the long-term moderate weight reduction on gene expression in AT and to identify genes and gene clusters responsive to treatment and thereby likely contributing to the development of the metabolic syndrome.

## Methods

### Study design

Altogether 46 overweight or obese (BMI 28–40 kg m<sup>-2</sup>) subjects aged 40–70 years were recruited to the study. The subjects had impaired fasting glycemia (fasting plasma glucose concentration 5.6–7.0 mmol l<sup>-1</sup>) or impaired glucose tolerance (2-h plasma glucose concentration 7.8–11.0 mmol l<sup>-1</sup>) and at least two other features of the metabolic syndrome according to the Adult Treatment Panel III criteria<sup>28</sup> as modified by the AHA:<sup>29</sup> waist circumference >102 cm (males)/>88 cm (females), fasting serum triacylglycerol concentration ≥1.7 mmol l<sup>-1</sup>, fasting serum HDL-cholesterol <1.0 mmol l<sup>-1</sup> (males)/<1.3 mmol l<sup>-1</sup> (females), blood pressure ≥130/80 mm Hg. Subjects were randomized to a weight reduction (WR) (*n*=28) or a control group (*n*=18). The subjects were randomized at the Department of Clinical Nutrition (*n*=6 and 7, respectively) and at Kuopio Research Institute of Exercise Medicine (*n*=22 and 11, respectively). Subjects were matched for age, gender, BMI and status of glucose metabolism. The duration of the study varied between 32 and 38 weeks, the mean duration being 33.3±1.1 weeks.

At screening, the health status and medical history of the subjects were examined by an interview. Laboratory examinations included measurements of the liver, kidney and

thyroid function. All subjects kept a 4-day food record at baseline. At baseline and at the end of the intervention, the subjects underwent an oral glucose tolerance test (OGTT), frequently sampled intravenous glucose tolerance test (FSIGT), blood pressure measurement, anthropometric measurements (body weight, height, body composition (Bioelectrical impedance by STA/BIA Body Composition Analyzer, Akern Bioresearch Srl, Florence, Italy), waist circumference), AT biopsy and the following biochemical measurements: serum total and lipoprotein lipids, serum-free fatty acids and leptin (see Table 1). Blood pressure was measured twice on the right arm. The systolic and diastolic blood pressure was calculated as an average of the two measurements.

### Weight reduction program

The subjects underwent a 12-week intensive weight reduction program. The minimum aim for the study, weeks 12–33 was to maintain the achieved weight loss. A clinical nutritionist gave detailed individual counseling based on an interview and 4-day food records kept by the subjects, aiming to decrease energy intake. The subjects kept 4-day food records two times during the intensive period and two times during weeks 20–32. After the 12-week program, the

**Table 1** Baseline characteristics of the subjects

|  | Weight reduction<br>( <i>n</i> = 28) | Control<br>( <i>n</i> = 18) |
|--|--------------------------------------|-----------------------------|
| Gender (M/F)   | 12/16                                | 8/10                        |
| Age (years)  | 59±7                                 | 61±7                        |
| Weight (kg)  | 92.8±15.1                            | 87.9±8.3                    |
| Body mass index (kg m <sup>-2</sup> )  | 32.9±3.2                             | 32.4±2.5                    |
| <i>Blood pressure (mm Hg)</i>  |                                      |                             |
| Systolic   | 137±17                               | 137±12                      |
| Diastolic  | 90±10                                | 87±10                       |
| Waist circumference (cm)   | 108±9                                | 105±7                       |
| Lean body mass (kg)  | 58.1±12.0                            | 55.1±9.6                    |
| Body fat mass (kg)   | 34.7±8.9                             | 32.9±6.7                    |
| Body fat (%)   | 38±7                                 | 38±8                        |
| <i>Plasma glucose (mmol l<sup>-1</sup>)</i>  |                                      |                             |
| 0 min  | 6.4±0.5                              | 6.5±0.4                     |
| 30 min   | 9.8±1.5                              | 10.0±1.2                    |
| 120 min  | 6.9±2.0                              | 8.0±2.4                     |
| S <sub>i</sub> ((mU l <sup>-1</sup> ) <sup>-1</sup> × min <sup>-1</sup> ) <sup>a</sup> | 2.31±1.38                            | 2.43±0.79                   |
| AIR ((mU l <sup>-1</sup> ) <sup>-1</sup> × min <sup>-1</sup> ) <sup>a</sup>            | 5.04±4.04                            | 4.60±5.0                    |
| Serum cholesterol (mmol l <sup>-1</sup> )  | 5.2±1.0                              | 5.5±1.1                     |
| VLDL cholesterol (mmol l <sup>-1</sup> )   | 0.51±0.27                            | 0.59±0.30                   |
| LDL cholesterol (mmol l <sup>-1</sup> )  | 3.41±0.89                            | 3.62±0.92                   |
| HDL cholesterol (mmol l <sup>-1</sup> )  | 1.24±0.21                            | 1.26±0.17                   |
| Serum triacylglycerol (mmol l <sup>-1</sup> )  | 1.51±0.63                            | 1.78±0.64                   |
| Adipocyte cell size (pl)   | 718±362                              | 634±318                     |
| Serum leptin (ng ml <sup>-1</sup> )  | 20.5±11.3                            | 20.4±12.6                   |
| No. of smokers (regular/occasional)  | 1/1                                  | 3/2                         |

Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein. Mean ± s.d. Groups did not differ between each other based on general linear model for univariate analysis. <sup>a</sup>S<sub>i</sub>=insulin sensitivity index, AIR=acute insulin response.

subjects met the clinical nutritionist for checking the food records. During this visit they also had a possibility to discuss possible difficulties they had in maintaining the weight. Subjects were asked to maintain their habitual level of physical exercise. Physical activity was monitored by a questionnaire at the beginning and the weeks 12 and 33 of the study.

#### *Control group*

The subjects in the control group were advised to continue their normal lifestyle during the study, and to keep their diet and exercise habits unchanged.

#### *Glucose tolerance tests*

A 2-h OGTT was performed with 75 g of glucose. Blood samples for plasma glucose and serum insulin concentrations were drawn at 0, 30 and 120 min. The FSIGT was performed according to the Minimal Model method as described previously.<sup>30</sup> Insulin sensitivity index ( $S_I$ ), glucose effectiveness ( $S_G$ ) or acute phase insulin response to glucose (AIR) were calculated by the MINMOD Millennium software.<sup>31</sup>

#### *Biochemical analyses*

Biochemical analyses were performed in the Clinical Laboratory Centre of the Kuopio University Hospital and in the Clinical Unit of the University of Kuopio. Plasma glucose concentration was analyzed by the hexokinase method (Thermo Clinical LabSystems, Vantaa, Finland). Insulin was determined by chemiluminescence sandwich method (ACS, Bayer A/S, Tarrytown, NY, USA). VLDL, LDL and HDL were separated by ultracentrifugation (Beckman Optima L-90 K). The concentrations of total and lipoprotein cholesterol and total triacylglycerol were analyzed with a Kone Pro Clinical Chemistry Analyzer (Thermo Clinical LabSystems, Konelab, Espoo, Finland) using enzymatic methods (Roche Diagnostics, Mannheim, Germany). Concentration of serum-free fatty acids was measured by a turbidometric analyzer (Kone Ltd, Espoo, Finland). Commercial radioimmunoassay kit was used for the analysis of serum leptin concentration (Linco Research Inc., St Louis, MO, USA).

#### *Adipose tissue biopsy*

After an overnight fast, AT samples for RNA extraction and cell size measurements were taken by needle biopsy from subcutaneous abdominal AT under local anesthesia (lidocaine 10 mg ml<sup>-1</sup> without adrenaline). AT samples for the mRNA extraction were washed two times with PBS, treated with RNA later according to the instructions provided by the manufacturer (Ambion, Austin, TX, USA), and stored in -80 °C until RNA extraction.

Part of the sample was taken for the cell size measurement. After washing, adipocytes were incubated in the presence of

collagenase (0.5 mg ml<sup>-1</sup>) under constant shaking at 2 Hz and 37 °C in buffer containing 125 mmol l<sup>-1</sup> NaCl, 5 mmol l<sup>-1</sup> KCl, 1 mmol l<sup>-1</sup> CaCl<sub>2</sub>, 2.5 mmol l<sup>-1</sup> MgCl<sub>2</sub>, 1 mmol l<sup>-1</sup> KH<sub>2</sub>PO<sub>4</sub>, 4 mmol l<sup>-1</sup> glucose, 2% bovine serum albumin and 25 mmol l<sup>-1</sup> Tris at pH 7.4 as described earlier.<sup>32,33</sup> After 60 min, cells were filtered through a nylon cloth and washed three times with the same buffer without collagenase. Direct microscopic determination of the adipocyte diameter was performed by placing an aliquot of cell suspension on the Bürker chamber and examining with a light microscope (Olympus CH-2). The diameters of 100–200 cells were estimated. The median of diameters was used for the calculation of fat cell volume.

#### *RNA extraction*

Total RNA from fat tissue was extracted using the TRIzol method followed by further purification with RNeasy Mini Kit columns according to the instructions provided by the manufacturers' (Invitrogen, Carlsbad, CA, USA and Qiagen, Valencia, CA, USA). The RNA concentration and the  $A_{260}/A_{280}$  ratio was measured using the NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA), an acceptable ratio being 1.9–2.1.

#### *Microarray probe preparation*

The subjects were chosen for microarray experiments with the following criteria: 10 subjects from the WR group ( $n=28$ ), who had the most pronounced weight reduction during the intervention and 10 subjects from the control group ( $n=18$ ) with the most stable body weight during the study. Owing to technical problems in hybridization in one of the microarray chips, the samples from one of the subjects had to be excluded. Thus, the final number of subjects in the WR group was nine.

Synthesis of biotin-labeled cRNA, hybridization to DNA microarrays (HG-U133 Plus 2.0 GeneChip) and detection of hybridized cRNA were performed as recommended by the manufacturer (Affymetrix Inc., Santa Clara, CA, USA).

#### *Array data extraction and analysis*

The arrays were scanned using HP GeneArray Scanner 3000 (Affymetrix Inc.). Primary data extraction was performed with Affymetrix GeneChip Operating Software. The software produces a one-cell intensity file that contains probe-level intensities for each chip. In the WR group one of the microarray chips was damaged during procedures, and consequently this subject was excluded from the analyses. The detection calls were calculated using the Affymetrix detection algorithm (Affymetrix, Statistical algorithms description document, 2002). Microarray data analysis was performed with dChip (www.dchip.org) software. All chips were normalized by using Invariant Set Normalization.<sup>34</sup> Model-Based Expression indexes<sup>35</sup> were calculated to

summarize expression levels. A perfect match/mis-match difference model was used in Model-Based Expression indexes calculation, and outlier detection and correction was applied. After preprocessing steps, only genes that were called 'Present' in more than 50 percent of the replicates in at least one of the two time points were selected for further analysis. Differentially expressed genes were identified by using paired *t*-test with  $P < 0.01$  producing slightly different false discovery rate-values for the WR and control group. Differential expression of genes was examined within the groups. The set of differentially expressed genes was clustered with dChip's hierarchical clustering function. Before clustering, redundant probe sets were removed. Correlation was used as distance metric and the centroid-linkage method was applied. Finally, the differentially expressed genes were grouped by their Gene Ontology annotations.

#### Quantitative real-time PCR analysis of gene expression

Quantitative real-time PCR (QPCR) was used for the confirmation of microarray gene-expression results. QPCR analyses were performed with TaqMan chemistry-based assays according to the instructions by the manufacturer by using ABI Prism 7500 analyzer (Applied Biosystems). The analysis for the relative quantity of a specific gene before and after the intervention in 27 subjects of the WR group and in 18 subjects of the control group was done in triplets. A standard curve with points 0.025, 0.075, 0.3, 0.9 and  $1.8 \text{ ng } \mu\text{l}^{-1}$  of cDNA, respectively and calibrator at a concentration of  $0.3 \text{ ng } \mu\text{l}^{-1}$  was used on every plate. Relative quantity was analyzed using ABI Prism 7500 SDS software. Quantities on each plate were first corrected by the calibrator on the plate. The ratio of the amount per plate to the corresponding values of endogenous control was then calculated. The endogenous control was chosen using Human Endogenous Control Kit (Applied Biosystems). Among 11 possible candidates, cyclophilin A1 was chosen as the best candidate using cDNA synthesized from isolated human AT RNA.

#### Statistical analyses of the clinical data

The clinical data were analyzed using the SPSS software for Windows version 11.5.1 (SPSS Inc., Chicago, IL, USA). Data are given as mean  $\pm$  s.d., unless otherwise indicated. The normality of distributions of the study variables was tested with the Kolmogorov-Smirnov test with Lilliefors' significance correction. Logarithmic transformation was used to achieve normal distribution whenever needed (indicated in tables and/or figures). General Linear Model (GLM) for univariate analysis was used to test the difference in the baseline characteristics and in the changes among the groups. GLM for repeated measures was used for analyzing the interaction of time and group for each variable. Adjustments for age, gender and baseline body weight was

performed, when appropriate. Paired samples *T*-test was used for comparing the baseline and endpoint measurements within the study group. Correlation analyses were done using Pearson's method. Partial correlation analysis with adjustment for weight at baseline and gender was used when appropriate. For the clinical and biochemical measurements  $P < 0.05$  was considered as statistically significant.

#### Ethical considerations

The study was in accordance with the standards of the Helsinki Declaration. The Ethics Committee of the District Hospital Region of Northern Savo and Kuopio University Hospital approved the study plan. All participants gave their written informed consent.

We certify that all applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during this study.

## Results

#### Clinical and metabolic characteristics

The WR and control groups did not differ in any of the clinical variables at baseline (Table 1). The 33-week intervention decreased the measures of body adiposity and fat distribution (Table 2) as well as fasting concentrations of plasma glucose and serum leptin in the WR group. The differences in the changes of anthropometric measures between the groups during the intervention were significant (Table 2). No significant differences in the changes in blood pressure, percent of body fat, concentrations of plasma glucose, serum insulin, serum total and lipoprotein lipids,  $S_1$ ,  $S_G$  or acute phase insulin response to glucose were seen between the groups. The dietary data of the groups are shown in Table 3. Dietary fat intake decreased as aimed at in the WR group. Fiber intake maintained at the level before the intervention in spite of the slight, although non-significant decrease in energy intake in the WR group, whereas fiber content of the diet clearly decreased in the control group.

In the WR group with some improvement in  $S_1$ , the change in  $S_1$  correlated inversely with the change in body weight after adjustment for baseline body weight ( $r = -0.44$ ,  $P = 0.026$ ). When combining the groups a similar, although lower correlation was found ( $r = -0.23$ ,  $P = 0.057$ ).

#### Weight reduction group

To select subjects for microarray analyses, the WR group was initially divided into those who lost  $\geq 5\%$  of their body weight ( $n = 11$ , Group 1) and those who lost  $< 5\%$  ( $n = 17$ , Group 2) (Figure 1, Table 4). Finally, nine (4 males/5 females) subjects from the Group 1 were included in the microarray analyses. They lost more weight than the rest of the WR group ( $7.8 \pm 2.9$  vs  $3.3 \pm 3.3\%$ ,  $P = 0.002$ ). They also had significant increase in  $S_1$  and decrease in fasting serum insulin and in 2-h glucose concentrations, along with

**Table 2** Changes in clinical characteristics during the intervention in the study groups.

|  | Weight reduction (n = 28) | Control (n = 18)      | P <sup>a</sup> |
|--|---------------------------|-----------------------|----------------|
| Body weight (kg)   | -4.6 (-6.1 to -3.1)**     | -0.2 (-1.0 to 0.6)    | 0.0002         |
| Body mass index (kg m <sup>-2</sup> )  | -1.62 (-2.15 to -1.10)**  | -0.09 (-0.36 to 0.18) | 0.0002         |
| Waist circumference (cm)   | -3.6 (-4.9 to -2.3)**     | 0.6 (-0.5 to 1.7)     | 0.0001         |
| Lean body mass (kg)  | -1.6 (-2.6 to -0.5)*      | 0.3 (-0.3 to 1.0)     | 0.025          |
| Body fat mass (kg)   | -3.0 (-4.2 to -1.8)**     | -0.5 (-1.1 to 0.1)    | 0.009          |
| Body fat (%)   | -1.5 (-2.5 to -0.6)*      | -0.6 (-1.1 to 0.0)    | ns             |
| Fasting plasma glucose (mmol l <sup>-1</sup> )   | -0.3 (-0.49 to -0.15)**   | -0.26 (-0.55 to 0.02) | ns             |
| Fasting serum insulin (pmol l <sup>-1</sup> )  | -4.4 (-16.0 to 7.1)       | 4.1 (-7.1 to 15.2)    | ns             |
| S <sub>I</sub> ((mU l <sup>-1</sup> ) <sup>-1</sup> × min <sup>-1</sup> ) <sup>b</sup> | 0.24 (-0.05 to 0.53)      | -0.01 (-0.46 to 0.44) | ns             |
| AIR ((mU l <sup>-1</sup> ) <sup>-1</sup> × min <sup>-1</sup> ) <sup>b</sup>            | -0.11 (-0.85 to 0.63)     | 0.48 (-0.57 to 1.52)  | ns             |
| S <sub>G</sub> (min <sup>-1</sup> × 10 <sup>2</sup> ) <sup>b</sup>                     | -0.05 (-0.22 to 0.13)     | 0.12 (-0.23 to 0.47)  | ns             |
| Fasting serum leptin (ng ml <sup>-1</sup> )  | -3.18 (-4.93 to -1.43)*   | -0.51 (-1.73 to 0.72) | 0.050          |
| Adipocyte cell size (pl)   | 28.0 (-182 to 238)        | 106.5 (-52 to 265)    | 0.039          |

Abbreviation: CI, confidence interval. Δ (95% CI) Paired samples *t*-test for the change from baseline to the end of the study within the group: \**P* < 0.01, \*\**P* < 0.001. <sup>a</sup>General linear model for univariate analysis for the differences between the groups corrected with age, gender and weight at baseline. <sup>b</sup>S<sub>I</sub> = insulin sensitivity index, AIR = acute insulin response, S<sub>G</sub> = glucose effectiveness index.

improvements in measures of body adiposity when compared to those selected for microarray analysis from the control group (*n* = 10; 4 men/6 women) (Table 4).

#### Changes in gene expression in adipose tissue during the intervention

The expression of 105 genes (false discovery rate of 13.3% for the list of genes) in the WR group changed after the long-term moderate weight reduction program (Table 5). The expression of 82% (86/105) of genes was downregulated in the WR group. Overall, the changes were modest, with fold changes ranging from 0.67 to 1.68. The genes were then analyzed for the cluster formation according to the Gene Ontology (GO) biological process categories (Table 5). Gene Ontology clusters for biological processes were assessed for enrichment of genes into each cluster with *P* < 0.001. Altogether seven Gene Ontology biological process clusters were found. These clusters were associated with the function of the extracellular matrix (ECM) and cell death. The expression of 62 genes changed in the control group (false discovery rate of 12.9% for the list of genes) and no clusters were formed (Supplementary Information). None of the genes were the same as in the WR group.

To confirm the microarray analysis, seven genes showing either up- or downregulation in the microarray analysis were tested by QPCR analyses (Figure 2). The QPCR analysis confirms and extends the data obtained by microarray for the whole population. In the WR group, significant downregulation was observed by QPCR with genes encoding tenomodulin-gene (*TNMD*) (-39 ± 16%, *P* < 0.0001), *ADAM12* (-15 ± 18%, *P* = 0.023) and *CCND2* (-24 ± 28%, *P* = 0.040) (Table 6). Also the other genes confirmed by QPCR showed up- or downregulation as in microarray analyses (Table 6), but statistical significance was not reached in the QPCR analyses due to large inter-individual variation. In the control group, there were no significant changes in

the genes confirmed by QPCR, except in *PDGFRL* (27 ± 35%, *P* = 0.011).

One of the genes that showed most pronounced change during the intervention was the X-chromosomal *TNMD* gene. The transcript levels of the *TNMD* in AT in women before and after the intervention were about two times as high as in men (in women before the intervention 125 ± 45 AU, 99 ± 42 AU and after the intervention 110 ± 63 AU, 95 ± 32 AU for the WR and control group, respectively; in men 77 ± 32 AU, 51 ± 21 AU and 59 ± 27 AU, 53 ± 20 AU for the WR and control group, respectively; *P* = 0.014–0.005 for gender difference within the group). This is in an agreement with the fact that the *TNMD* gene is located in the X-chromosome. Interestingly, the *TNMD* expression level correlated significantly with fasting serum insulin (Figure 3) adjusted for baseline body weight, when the WR and control groups were combined. When correlation was adjusted for both gender and baseline body weight, association remained for fasting serum insulin before (*r* = 0.38, *P* = 0.014) and after (*r* = 0.29, *P* = 0.066) the intervention. *TNMD* expression correlated also with S<sub>I</sub> (*r* = -0.39, *P* = 0.012) before the intervention as well as body fat mass and with lean body mass both before (*r* = 0.42, *P* = 0.007; *r* = -0.42, *P* = 0.007, respectively) and after the intervention (*r* = 0.30, *P* = 0.056; *r* = -0.33, *P* = 0.037, respectively).

## Discussion

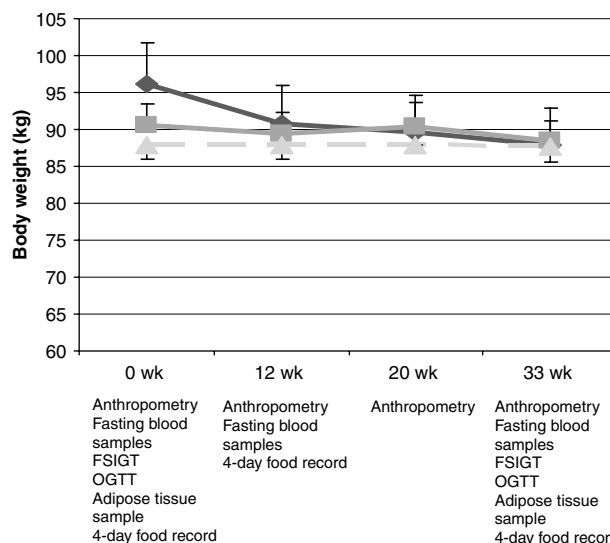
In the present study, we examined the effect of long-term moderate weight reduction on the gene expression in AT of overweight or obese subjects with the features of metabolic syndrome. Of the 105 genes responding to weight reduction, most were downregulated. The major gene clusters that were downregulated are involved in the function of the ECM and cell death.

**Table 3** Intake of energy, energy nutrients, cholesterol, fiber and calcium from the diet

|                              | Weight reduction (n = 28) | Control (n = 18) | P <sup>a</sup> |
|------------------------------|---------------------------|------------------|----------------|
| <b>Energy (kJ)</b>           |                           |                  |                |
| 0 week                       | 7202 ± 2285               | 7589 ± 1783      |                |
| 12 weeks                     | 6516 ± 1690               | 7426 ± 1944      |                |
| 32 weeks                     | 6717 ± 1458               | 7752 ± 1974      | Ns             |
| <b>Protein (E%)</b>          |                           |                  |                |
| 0 week                       | 18.1 ± 3.1                | 17.8 ± 2.8       |                |
| 32 weeks                     | 19.9 ± 4.0                | 17.7 ± 4.0       | Ns             |
| <b>Fat (E%)</b>              |                           |                  |                |
| 0 week                       | 33.0 ± 6.1                | 31.3 ± 8.3       |                |
| 32 weeks                     | 30.5 ± 6.0*               | 32.8 ± 4.9       | 0.051          |
| <b>SFA (E%)<sup>b</sup></b>  |                           |                  |                |
| 0 week                       | 12.3 ± 3.4                | 11.9 ± 3.5       |                |
| 32 weeks                     | 11.0 ± 3.1                | 12.0 ± 2.6       | ns             |
| <b>MUFA (E%)<sup>b</sup></b> |                           |                  |                |
| 0 week                       | 11.1 ± 2.4                | 10.1 ± 3.3       |                |
| 32 weeks                     | 10.3 ± 2.4                | 11.6 ± 2.2       | 0.013          |
| <b>PUFA (E%)<sup>b</sup></b> |                           |                  |                |
| 0 week                       | 5.9 ± 1.6                 | 5.3 ± 1.6        |                |
| 32 weeks                     | 5.7 ± 1.7                 | 5.3 ± 1.2        | ns             |
| <b>Carbohydrates (E%)</b>    |                           |                  |                |
| 0 week                       | 46.2 ± 6.6                | 47.4 ± 8.3       |                |
| 32 weeks                     | 46.5 ± 6.6                | 45.6 ± 8.4       | ns             |
| <b>Cholesterol (mg)</b>      |                           |                  |                |
| 0 week                       | 227 ± 98                  | 235 ± 108        |                |
| 32 weeks                     | 233 ± 114                 | 250 ± 131        | ns             |
| <b>Fiber (g)</b>             |                           |                  |                |
| 0 week                       | 23.0 ± 8.5                | 24.7 ± 6.2       |                |
| 32 weeks                     | 21.3 ± 7.7                | 19.7 ± 6.7**     | ns             |
| <b>Calcium (mg)</b>          |                           |                  |                |
| 0 week                       | 970 ± 383                 | 1133 ± 399       |                |
| 32 weeks                     | 1059 ± 356                | 1094 ± 477       | ns             |

Paired samples *t*-test for the change from baseline to the end of the study within the group: \**P* < 0.05, \*\**P* < 0.01. Mean ± s.d. <sup>a</sup>General linear model for repeated measures analysis for the differences between the groups during the intervention. <sup>b</sup>SFA = saturated fatty acids, MUFA = mono-unsaturated fatty acids, PUFA = poly-unsaturated fatty acids.

As expected, in the present study, successful weight reduction resulted in an improvement in glucose and insulin metabolism and in measures of body adiposity. The improvement of *S*<sub>1</sub> was related to the degree of weight reduction. Individual genetic patterns also modify the effect of lifestyle factors on clinical and biochemical parameters, which explain the large inter-individual variation that was also seen in the present study. Such variation may complicate attempts to find patterns in gene expression resulting from dietary or other lifestyle changes,<sup>36</sup> especially in studies investigating outpatient clinic like programs. In the present study, we, therefore, focused on the changes in gene expression within the WR group after a moderate weight



**Figure 1** The weight loss (kg) in the weight reduction group divided to those who lost ≥5% ♦ and to those who lost <5% ■ of their original weight and in the control group ▲ (mean ± s.e.m.) with the measurements done in the main study visits. FSIGT = frequently sampled intravenous glucose tolerance test, OGTT = oral glucose tolerance test. The visit on week 20 was not included in the program of the control group, and thus, this point has been calculated as a mean of body weight measured at weeks 12 and 33.

reduction. For gene-expression studies, we selected those subjects who responded best to weight reduction program.

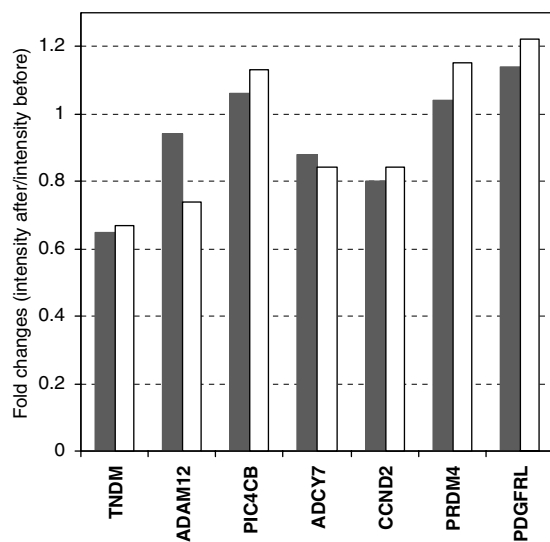
Weight loss and a reduction in fat mass decreased the expression of genes associated with the ECM in subcutaneous AT. Recent studies implicate that the ECM is involved in inflammation,<sup>37</sup> angiogenesis<sup>38</sup> and in the development of cardiovascular dysfunction in the metabolic syndrome and T2DM.<sup>39,40</sup> The ECM contributes to cell-matrix interactions, and in the context of the metabolic syndrome, is characterized by abnormal glucose and lipid metabolism and inflammation in adipose and other tissues, the capability of the ECM to adapt to potentially toxic signals may be of importance for maintaining healthy homeostasis.<sup>39</sup> Since AT consists also of connective tissue, it is expected that the ECM components participate in remodeling and function of AT through cell-matrix interactions. It has been shown that, in addition to the ECM remodeling, matrix metalloproteinases and their inhibitors (MMP/TIMP system) regulate proteolytic events and adipogenesis during obesity-mediated fat mass development.<sup>41</sup> In addition, increased formation of the ECM constituents has been found in the white AT of rats, which were exposed to early-life under-nutrition and subsequently developed visceral obesity.<sup>42</sup> Transcription of the ECM constituents has recently been shown to be regulated by insulin in 3T3-L1 adipocytes.<sup>43</sup> In accordance with our study, long-term energy restriction has suppressed genes associated with the ECM in mice.<sup>37</sup>

Weight reduction also decreased the expression of genes involved in terminal differentiation of fat cells, that is cell

**Table 4** Changes in clinical characteristics by the degree of weight loss (%) in the weight reduction group and in the subjects selected for the microarray analysis

|  | Weight reduction                           |                                       |                | Microarray analysis      |                       |                |
|--|--|---------------------------------------|----------------|--------------------------|-----------------------|----------------|
|  | Weight loss $\geq$ 5%,<br>Group 1 (n = 11) | Weight loss < 5%,<br>Group 2 (n = 17) | P <sup>a</sup> | Weight reduction (n = 9) | Control (n = 10)      | P <sup>b</sup> |
| Body weight (kg)   | -8.2 (-10.4 to -6.1)                       | -2.2 (-3.2 to -1.2)                   | 0.0004         | -7.7 (-10.2 to -5.1)     | 0.3 (-0.5 to 1.1)     | 0.0002         |
| Body mass index (kg m <sup>-2</sup> )  | -2.9 (-3.6 to -2.2)                        | -0.7 (-1.1 to -0.4)                   | 0.0006         | -2.7 (-3.5 to -1.9)      | 0.1 (-0.2 to 0.4)     | 0.0001         |
| Waist circumference (cm)   | -6.2 (-8.3 to -4.2)                        | -1.9 (-3.1 to -0.8)                   | 0.0003         | -5.7 (-8.5 to -2.9)      | 0.9 (-1.1 to 2.9)     | 0.003          |
| Lean body mass (kg)  | -2.8 (-5.3 to -0.4)                        | -0.7 (-1.5 to 0.04)                   | 0.067          | -2.4 (-3.9 to -0.8)      | 0.3 (-0.8 to 1.4)     | 0.038          |
| Body fat mass (kg)   | -5.3 (-7.4 to -3.3)                        | -1.5 (-2.6 to -0.4)                   | 0.001          | -5.3 (-7.3 to -3.3)      | 0.01 (-0.8 to 0.8)    | 0.0007         |
| Body fat (%)   | -2.7 (-4.7 to -0.8)                        | -0.7 (-1.7 to 0.2)                    | 0.041          | -2.6 (-4.4 to -0.9)      | -0.2 (-1.1 to 0.7)    | 0.003          |
| Fasting plasma glucose (mmol l <sup>-1</sup> )   | -0.3 (-0.61 to 0.01)                       | -0.3 (-0.56 to -0.11)                 | ns             | -0.25 (-0.58 to 0.08)    | -0.08 (-0.54 to 0.38) | ns             |
| 2h-plasma glucose (mmol l <sup>-1</sup> )  | -1.77 (-3.56 to 0.02)                      | -0.39 (-1.15 to 0.36)                 | 0.072          | -1.04 (-2.25 to 0.16)    | 0.52 (-1.07 to 2.11)  | 0.068          |
| Fasting serum insulin (pmol l <sup>-1</sup> )  | -19.3 (-38.8 to 0.18)                      | 4.3 (-9.7 to 18.4)                    | 0.032          | -17.6 (-39.3 to 4.08)    | 3.53 (-11.2 to 18.3)  | 0.003          |
| S <sub>i</sub> ((mU l <sup>-1</sup> ) <sup>-1</sup> × min <sup>-1</sup> ) <sup>c</sup> | 0.51 (-0.13 to 1.14)                       | 0.07 (-0.23 to 0.38)                  | 0.037          | 0.56 (-0.12 to 1.24)     | -0.11 (-0.94 to 0.72) | 0.093          |
| S <sub>G</sub> (min <sup>-1</sup> × 10 <sup>2</sup> ) <sup>c</sup>                     | -0.05 (-0.44 to 0.35)                      | -0.05 (-0.24 to 0.14)                 | ns             | -0.08 (-0.54 to 0.38)    | 0.12 (-0.40 to 0.63)  | ns             |
| AIR ((mU l <sup>-1</sup> ) <sup>-1</sup> × min <sup>-1</sup> ) <sup>c</sup>            | -0.96 (-2.33 to 0.41)                      | 0.37 (-0.52 to 1.25)                  | ns             | -1.03 (-2.93 to 0.87)    | -0.09 (-1.78 to 1.60) | 0.099          |
| Fasting serum leptin (ng ml <sup>-1</sup> )  | -5.68 (-9.37 to -2.0)                      | -1.56 (-3.06 to -0.06)                | 0.013          | -6.06 (-10.47 to -1.66)  | -0.37 (-2.38 to 1.63) | 0.005          |
| Adipocyte cell size (pl)   | 41 (-287 to 369)                           | 19 (-287 to 325)                      | ns             | 134 (-263 to 531)        | 106 (-99 to 311)      | ns             |

Δ (95 % CI). <sup>a</sup>General linear model for univariate analysis for the differences between Groups 1 and 2 corrected with age, gender and weight at baseline. <sup>b</sup>General linear model for univariate analysis for the differences between the subjects selected for the microarray analysis from the weight reduction and control groups corrected with age, gender and weight at baseline. <sup>c</sup>S<sub>i</sub> = insulin sensitivity index, AIR = acute insulin response, S<sub>G</sub> = glucose effectiveness index.



**Figure 2** The fold changes using quantitative real-time PCR (black bars) and microarray (white bars) analysis in subjects whose adipose tissue samples were chosen for microarray analysis from the weight reduction group (n = 9). TNMD = tenomodulin, ADAM12 = A disintegrin metalloproteinase domain 12, PIC4CB = phosphatidylinositol 4-kinase, catalytic  $\beta$  polypeptide, ADCY7 = adenylate cyclase 7, CCND2 = cyclin D2, PRDM4 = pr domain containing 4, PDGFR = platelet-derived growth factor receptor-like.

death. AT apoptosis has not been extensively studied, but loss of weight involves both decrease in adipocyte cell size and the number of adipocytes.<sup>44</sup> Cell number can decrease by the reduction in the rate of production of new adipocytes or by apoptosis.<sup>45</sup> However, there are reports from animal studies showing that short-term food restriction alone might not cause apoptosis.<sup>46,47</sup> In our study, the subjects were examined in the phase of weight maintenance, which

naturally may decrease the apoptotic activity in the tissue. AT apoptosis has also been linked to inflammation and especially to the effects of increased TNF- $\alpha$  levels in the AT.<sup>48</sup> The decrease in mRNA expression of TNF receptor superfamily observed in the present study might indicate the post inflammation state in weight maintenance. This is in line with the decrease found in the high-mobility group box 1-gene mRNA expression as well as the monocyte to macrophage differentiation-associated gene mRNA expression. Both of these genes have been suggested to be proinflammatory factors,<sup>49</sup> which may promote inflammatory effects on the tissue level and promote insulin resistance in their part.

Interestingly, we found a strong downregulation of the *TNMD* gene (fold change 0.67) along with weight reduction. Moreover, the expression of *TNMD* was associated with several characteristics of the metabolic syndrome. Tenomodulin has been suggested to mediate antiangiogenic activity, and its C-terminal extracellular domain modulates endothelial cell proliferation<sup>50–52</sup> indicating that it also might have a role in the function of ECM. It is also involved in collagen fibril maturation.<sup>53</sup> To our knowledge, this is the first study to show a link between *TNMD* and characteristics of metabolic syndrome including insulin resistance, but the mechanisms by which tenomodulin may be involved in insulin sensitivity remains to be elucidated. Weight reduction has downregulated the expression of *TNMD* also in healthy obese subjects.<sup>26</sup> Recently, our studies have shown an association between the *TNMD* genetic variation and measures of obesity and the risk of T2DM.<sup>54</sup>

In a few short-term studies dramatic weight reduction has produced downregulation in the gene expression of inflammation factors,<sup>27</sup> and genes related to desaturation of fatty acids in AT.<sup>26</sup> In cross-sectional studies comparing obese and lean subjects, upregulation of inflammation-related genes in

**Table 5** Up- or downregulated genes in the subjects selected for microarray analysis from the weight reduction group

| Gene  | Gene symbol   | Accession number | Weight reduction group<br>Fold change (range) <sup>a</sup> | Paired<br>P-value | Control group<br>Fold change (range)/NS |
|---|---------------|------------------|--|-------------------|---|
| <i>Extracellular matrix</i>   |               |                  |  |                   |   |
| Lysyl oxidase   | LOX           | NM_002317        | 0.85 (0.73–1.00)   | 0.007             | 1.09 (0.87–1.37)                        |
| Osteonectin   | SPARC         | AL575922         | 0.91 (0.84–1.04)   | 0.007             | 1.01 (0.91–1.11)                        |
| Microfibrillar-associated protein 5   | MFAP5         | AW665892         | 0.78 (0.64–1.03)   | 0.004             | 1.00 (0.76–1.54)                        |
|   |               | AW665892         | 0.79 (0.60–0.99)   | 0.005             | 1.00 (0.80–1.51)                        |
| Lysyl oxidase   | LOX           | L16895           | 0.83 (0.71–0.95)   | 0.003             | 1.09 (0.87–1.32)                        |
| Collagen, type V, $\alpha$ 1  | COL5A1        | CA430162         | 0.76 (0.62–0.89)   | 0.001             | absent                                  |
| Collagen, type I, $\alpha$ 1  | COL1A1        | BE221212         | 1.23 (0.96–1.57)   | 0.004             | 1.06 (0.84–1.72)                        |
| Osteoglycin (osteoinductive factor, mimecan)  | OGN           | NM_014057        | 1.43 (0.92–2.91)   | 0.007             | 1.14 (0.65–1.73)                        |
| <i>Cell death</i>   |               |                  |  |                   |   |
| Notch homolog 2   | NOTCH2        | AA291203         | 0.92 (0.83–0.99)   | 0.003             | 0.99 (0.80–1.11)                        |
|   | NOTCH2        | AU158495         | 0.93 (0.88–0.99)   | 0.008             | 0.98 (0.83–1.17)                        |
| High-mobility group box 1   | HMGB1         | NM_002128        | 0.92 (0.86–1.01)   | 0.005             | 1.02 (0.96–1.15)                        |
| B-cell translocation gene 1, antiproliferative  | BTG1          | AL535380         | 0.91 (0.83–0.98)   | 0.005             | 1.00 (0.83–1.09)                        |
| Prostaglandin E receptor 3 (subtype EP3)  | PTGER3        | AL031429         | 0.81 (0.66–0.98)   | 0.003             | 1.02 (0.80–1.52)                        |
| Tumor necrosis factor receptor superfamily, member 25                                       | TNFRSF25      | NM_003790        | 0.79 (0.51–1.03)   | 0.007             | 1.06 (0.82–1.26)                        |
| PERP, TP53 apoptosis effector   | PERP          | AJ251830         | 0.81 (0.71–1.07)   | 0.006             | 0.95 (0.79–1.13)                        |
| Rac/Cdc42 guanine nucleotide exchange factor 6  | ARHGGEF6      | D25304           | 0.82 (0.75–0.90)   | 0.000             | 0.97 (0.86–1.08)                        |
| Adhesion molecule with Ig-like domain 2   | AMIGO2        | AC004010         | 0.75 (0.59–0.92)   | 0.000             | 1.05 (0.77–1.38)                        |
| Monocyte to macrophage differentiation-associated   | MMD           | AW104453         | 1.28 (1.04–1.73)   | 0.006             | 1.08 (0.74–1.83)                        |
| BCL2-like 11 (apoptosis facilitator)  | BCL2L11       | NM_138626        | 1.10 (1.01–1.17)   | 0.008             | 0.96 (0.80–1.11)                        |
| <i>Others</i>   |               |                  |  |                   |   |
| <b>Cyclin D2</b>  | <b>CCND2</b>  | <b>NM_001759</b> | <b>0.84 (0.67–0.97)</b>                                    | <b>0.003</b>      | <b>0.98 (0.82–1.14)</b>                 |
|   | CCND2         | AW026491         | 0.69 (0.55–1.00)   | 0.006             | 1.07 (0.60–2.15)                        |
| Androgen receptor   | AR            | M73069           | 0.81 (0.61–1.02)   | 0.007             | 1.03 (0.74–1.28)                        |
| Integrin, $\beta$ 5   | ITGB5         | AL048423         | 0.83 (0.66–0.94)   | 0.001             | 1.01 (0.86–1.33)                        |
|   |               | NM_002213        | 0.85 (0.70–1.08)   | 0.005             | 1.00 (0.87–1.21)                        |
| Ubiquitin carboxyl-terminal esterase L1   | UCHL1         | NM_004181        | 0.82 (0.71–1.00)   | 0.001             | 1.04 (0.87–1.16)                        |
| Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2  | PLOD2         | AI754404         | 0.83 (0.71–1.05)   | 0.002             | 1.02 (0.87–1.32)                        |
| HMBA-inducible  | HIS1          | NM_006460        | 0.84 (0.67–1.05)   | 0.009             | 1.04 (0.84–1.29)                        |
| <b>Adenylate cyclase 7</b>  | <b>ADCY7</b>  | <b>NM_001114</b> | <b>0.84 (0.68–1.01)</b>                                    | <b>0.004</b>      | <b>1.00 (0.88–1.14)</b>                 |
| Neuronatin  | NNAT          | NM_005386        | 0.69 (0.60–0.98)   | 0.006             | 0.98 (0.64–1.36)                        |
| Cyclin-dependent kinase 8   |               | RS9697           | 0.87 (0.74–0.98)   | 0.010             | 0.97 (0.88–1.17)                        |
| SH3 domain binding glutamic acid-rich protein   | SH3BGR        | NM_007341        | 0.86 (0.71–1.05)   | 0.010             | 1.04 (0.69–1.37)                        |
| <b>Platelet-derived growth factor receptor-like</b>   | <b>PDGFRL</b> | <b>NM_006207</b> | <b>1.22 (1.00–1.56)</b>                                    | <b>0.005</b>      | <b>1.15 (0.92–1.50)</b>                 |
| <b>Phosphatidylinositol 4-kinase, catalytic, <math>\beta</math> polypeptide</b>             | <b>PIC4CB</b> | <b>NM_002651</b> | <b>1.13 (1.04–1.27)</b>                                    | <b>0.003</b>      | <b>1.02 (0.82–1.16)</b>                 |
| Colony stimulating factor 2 receptor, $\alpha$ , low-affinity                               | CSF2RA        | NM_006140        | 0.83 (0.72–0.99)   | 0.006             | 0.98 (0.59–1.68)                        |
| RAB2, member RAS oncogene family  | RAB2          | AI743756         | 0.88 (0.73–1.04)   | 0.010             | 0.99 (0.91–1.18)                        |
| RNA binding motif protein 25  | RBM25         | BE466128         | 0.85 (0.76–0.99)   | 0.010             | 0.98 (0.82–1.14)                        |
| ATPase, class II, type 9A   | ATP9A         | AB014511         | 0.90 (0.79–1.03)   | 0.009             | 0.98 (0.84–1.10)                        |
| PTPRF interacting protein, binding protein 2  | PPFIBP2       | AI692180         | 0.81 (0.68–1.07)   | 0.005             | 1.00 (0.82–1.55)                        |
| A disintegrin and metalloproteinase domain 22   | ADAM22        | AW242701         | 0.74 (0.54–0.95)   | 0.008             | 0.89 (0.54–1.15)                        |
| Phosphatidylinositol glycan, class B  | PIGB          | AU144243         | 0.86 (0.80–1.03)   | 0.005             | 1.08 (0.93–1.23)                        |
| Ferritin, heavy polypeptide 1   | FTH1          | AA083483         | 0.87 (0.73–1.03)   | 0.006             | 1.00 (0.84–1.31)                        |
| Phosphodiesterase 3B, cGMP-inhibited  | PDE3B         | NM_000753        | 0.88 (0.78–1.06)   | 0.009             | 0.98 (0.80–1.07)                        |
| Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, $\beta$ polypeptide | YWHAH         | NM_014052        | 0.94 (0.85–0.98)   | 0.010             | 1.00 (0.90–1.14)                        |
| UPF3 regulator of nonsense transcripts homolog B  | UPF3B         | NM_023010        | 0.85 (0.72–1.00)   | 0.007             | 1.01 (0.86–1.15)                        |
| Inter- $\alpha$ inhibitor H5  | ITIHS         | NM_030569        | 0.86 (0.79–0.99)   | 0.008             | 0.99 (0.93–1.08)                        |
| <b>Tenomodulin</b>  | <b>TNMD</b>   | <b>NM_022144</b> | <b>0.67 (0.44–1.08)</b>                                    | <b>0.000</b>      | <b>1.06 (0.62–1.95)</b>                 |
| Receptor-interacting factor 1   | RIF1          | NM_018372        | 0.81 (0.67–1.04)   | 0.007             | 0.96 (0.84–1.16)                        |
| Solute carrier family 7, member 10  | SLC7A10       | NM_017965        | 1.68 (0.49–2.27)   | 0.006             | 1.05 (0.79–1.37)                        |
| PHD finger protein 10   | PHF10         | BF431618         | 0.91 (0.86–1.00)   | 0.004             | 0.99 (0.89–1.20)                        |
| AER61 glycosyltransferase   | AER61         | AK023140         | 0.88 (0.72–0.98)   | 0.007             | 1.06 (0.82–1.31)                        |
| AP1 $\gamma$ subunit binding protein 1  | AP1GBP1       | AI472320         | 0.90 (0.81–0.99)   | 0.009             | 0.97 (0.86–1.16)                        |
| Mouse mammary Tumor virus receptor homolog 1  | MTVR1         | AF052151         | 1.12 (0.92–1.29)   | 0.008             | 0.96 (0.87–1.04)                        |
| Zinc-finger protein 337   | ZNF337        | AL049942         | 1.24 (0.98–1.56)   | 0.007             | 1.07 (0.86–1.33)                        |
| Glucocorticoid modulatory element binding protein 2   | GMEB2         | AA045183         | 1.13 (1.00–1.28)   | 0.009             | 0.96 (0.88–1.05)                        |
| <b>PR domain containing 4</b>   | <b>PRDM4</b>  | <b>W22625</b>    | <b>1.15 (1.02–1.30)</b>                                    | <b>0.002</b>      | <b>1.01 (0.94–1.14)</b>                 |
| Abhydrolase domain containing 2   | ABHD2         | AI832249         | 0.73 (0.61–0.87)   | 0.003             | 0.93 (0.67–1.33)                        |
| Shwachman–Bodian–Diamond syndrome   | SBDS          | AK001779         | 0.93 (0.82–1.00)   | 0.008             | 0.98 (0.90–1.09)                        |
| Myo-inositol monophosphatase A3   |               | BF674724         | 0.84 (0.61–0.97)   | 0.007             | 1.04 (0.95–1.17)                        |
| SM-11044 binding protein  | SMBP          | BE621524         | 0.89 (0.77–1.01)   | 0.009             | 0.95 (0.86–1.05)                        |

Table 5 (continued)

| Gene  | Gene symbol          | Accession number | Weight reduction group<br>Fold change (range) <sup>a</sup> | Paired<br>P-value | Control group<br>Fold change (range)/NS |
|---|----------------------|------------------|--|-------------------|---|
| Transcription factor Dp-2 (E2F dimerization partner 2)  | <i>TFDP2</i>         | AI569747         | 0.92 (0.88–1.04)   | 0.006             | 0.96 (0.76–1.12)                        |
| Cartilage-associated protein  | <i>CRTAP</i>         | AW024741         | 0.86 (0.77–0.94)   | 0.001             | 1.03 (0.83–1.17)                        |
| Sorting nexin-associated Golgi protein 1  | <i>SNAG1</i>         | AU146771         | 0.87 (0.79–0.99)   | 0.002             | 1.01 (0.86–1.29)                        |
| Small EDRK-rich factor 2  | <i>SERINC4</i>       | AI092931         | 0.87 (0.78–1.02)   | 0.006             | 1.04 (0.89–1.20)                        |
| <b>A disintegrin and metalloproteinase domain 12</b>  | <b><i>ADAM12</i></b> | <b>AA147933</b>  | <b>0.81 (0.66–1.04)</b>                                    | <b>0.004</b>      | <b>1.16 (0.81–1.75)</b>                 |
| Exportin, tRNA  | <i>XPT</i>           | AW242820         | 0.86 (0.77–0.95)   | 0.002             | 1.02 (0.94–1.15)                        |
| Chloride intracellular channel 6  | <i>CLIC6</i>         | AI638295         | 0.77 (0.52–0.94)   | 0.003             | 0.97 (0.81–1.18)                        |
| Phosphatase and actin regulator 3   | <i>PHACTR3</i>       | AL357503         | 0.68 (0.12–0.97)   | 0.003             | 0.85 (0.48–1.08)                        |
| Acyl-Coenzyme A oxidase 1, palmitoyl  | <i>ACOX1</i>         | BF435852         | 0.77 (0.66–0.87)   | 0.000             | 0.99 (0.68–1.16)                        |
| Dihydropyrimidine dehydrogenase (E3 component of pyruvate dehydrogenase complex, 2-oxo-glutarate complex, branched chain keto acid dehydrogenase complex) | <i>DLD</i>           | BF212846         | 0.88 (0.79–0.94)   | 0.007             | 1.01 (0.81–1.16)                        |
| NMDA receptor-regulated gene 2  | <i>NARG2</i>         | BE780502         | 1.16 (1.04–1.31)   | 0.004             | 1.02 (0.89–1.15)                        |
| ATP synthase, H <sup>+</sup> transporting, mitochondrial F0 complex, subunit s (factor B)   | <i>ATP5S</i>         | AI308101         | 0.81 (0.69–0.99)   | 0.005             | 1.08 (0.92–1.29)                        |
| Developmentally regulated RNA-binding protein 1   | <i>DRB1</i>          | AW451271         | 1.24 (1.09–1.50)   | 0.007             | 1.07 (0.78–1.70)                        |
| Pleckstrin homology domain containing, family H member 2  | <i>PLEKHH2</i>       | AI217992         | 0.78 (0.57–0.94)   | 0.009             | 0.96 (0.65–1.54)                        |
| Translocation protein 1   | <i>TLOC1</i>         | NM_153039        | 0.85 (0.75–0.99)   | 0.003             | 1.04 (0.94–1.21)                        |
| BCL2-like 11 (apoptosis facilitator)  | <i>BCL2L11</i>       | NM_138626        | 1.10 (1.01–1.17)   | 0.008             | 0.96 (0.80–1.11)                        |
| Inter- $\alpha$ (globulin) inhibitor H5   | <i>ITIH5</i>         | NM_032817        | 0.81 (0.71–0.91)   | 0.006             | 1.01 (0.88–1.25)                        |
| Methionine sulfoxide reductase B3 isoform 1   | <i>MSRB3</i>         | BC040053         | 0.84 (0.66–0.98)   | 0.006             | 1.03 (0.82–1.26)                        |
| <i>Unknown</i>  |                      |                  |  |                   |   |
| KIAA 1102 protein   |                      | AK027231         | 0.82 (0.59–1.00)   | 0.004             | 1.09 (0.86–1.37)                        |
|   |                      | AK026815         | 0.80 (0.63–1.06)   | 0.002             | 1.02 (0.91–1.23)                        |
|   |                      | AB029025         | 0.78 (0.68–1.00)   | 0.003             | 1.05 (0.86–1.31)                        |
| KIAA1908 protein  |                      | BC036405         | 0.84 (0.63–0.96)   | 0.010             | 1.04 (0.67–1.30)                        |
| FLJ20031  |                      | BF726849         | 0.79 (0.48–0.98)   | 0.005             | 1.06 (0.59–1.63)                        |
| KIAA0934  |                      | N42910           | 0.87 (0.67–1.01)   | 0.009             | 1.00 (0.79–1.25)                        |
| BC027461.1  |                      | BC027461         | 1.20 (0.98–1.46)   | 0.006             | 0.94 (0.63–1.38)                        |
| BE674583  |                      | BE674583         | 0.73 (0.53–0.92)   | 0.005             | 0.91 (0.55–1.19)                        |
| Chromosome 18 open reading frame 4  |                      | AK021539         | 0.80 (0.54–0.94)   | 0.006             | 1.11 (0.86–1.65)                        |
| FLJ31842 (transmembrane protein 56)   |                      | AI004375         | 0.81 (0.62–1.04)   | 0.004             | 1.05 (0.92–1.30)                        |
| KIAA1430  |                      | AA868380         | 0.82 (0.58–0.98)   | 0.005             | 1.07 (0.87–1.41)                        |
| KIAA1295  |                      | BG054798         | 1.20 (1.01–1.41)   | 0.001             | 1.01 (0.74–1.30)                        |
| MRNA full-length insert cDNA clone EUROIMAGE 138904   |                      | AI081590         | 0.86 (0.77–0.94)   | 0.005             | 1.11 (0.84–1.24)                        |
| AW024350 = tumor protein, translationally-controlled  |                      | AW024350         | 0.84 (0.69–1.01)   | 0.008             | 0.94 (0.80–1.14)                        |
| LOC285831   |                      | AA775731         | 0.81 (0.71–0.90)   | 0.002             | 1.09 (0.96–1.36)                        |
| FLJ36031  |                      | AA191741         | 0.88 (0.76–1.02)   | 0.007             | 1.11 (0.99–1.26)                        |
| AI150000  |                      | AI150000         | 0.86 (0.74–0.99)   | 0.004             | 0.98 (0.89–1.12)                        |
| Chromosome 16 open reading frame 9  |                      | AI688331         | 0.89 (0.80–0.96)   | 0.004             | 1.01 (0.89–1.22)                        |
| LOC253827   |                      | AL048386         | 0.87 (0.80–1.01)   | 0.006             | 1.07 (0.87–1.26)                        |
| Chromosome 10 open reading frame 45   |                      | AL136885         | 0.83 (0.69–0.90)   | 0.001             | 1.03 (0.92–1.15)                        |
| FLJ11151  |                      | NM_018340        | 0.81 (0.63–1.07)   | 0.008             | 0.90 (0.67–1.59)                        |
| AI669379  |                      | AI669379         | 1.14 (0.97–1.29)   | 0.006             | 1.05 (0.80–1.60)                        |
| Z82202  |                      | Z82202           | 1.18 (1.02–1.47)   | 0.005             | 1.02 (0.75–1.27)                        |
| Similar to 60S ribosomal protein L29  |                      | AL008627         | 1.25 (1.10–1.45)   | 0.002             | 1.04 (0.86–1.28)                        |
| KIAA0934  |                      | N31807           | 0.88 (0.78–1.01)   | 0.005             | 0.97 (0.81–1.28)                        |
| Chromosome 10 open reading frame 56   |                      | AA131324         | 0.92 (0.86–1.01)   | 0.003             | 0.94 (0.81–1.06)                        |
| AW576195  |                      | AW576195         | 0.84 (0.68–0.96)   | 0.002             | 1.08 (0.90–1.18)                        |
| Similar to FKSG27   |                      | AK096168         | 1.18 (1.00–1.30)   | 0.002             | 0.96 (0.80–1.17)                        |

Fold changes (non-significant) of the genes are given also in the control group. Mean(range). <sup>a</sup>Values are fold changes: intensity of gene expressed after experiment divided by the intensity at baseline. Genes confirmed by QPCR are marked in bold. The comparisons were made within the groups, paired sample *t*-test with 100 permutations.

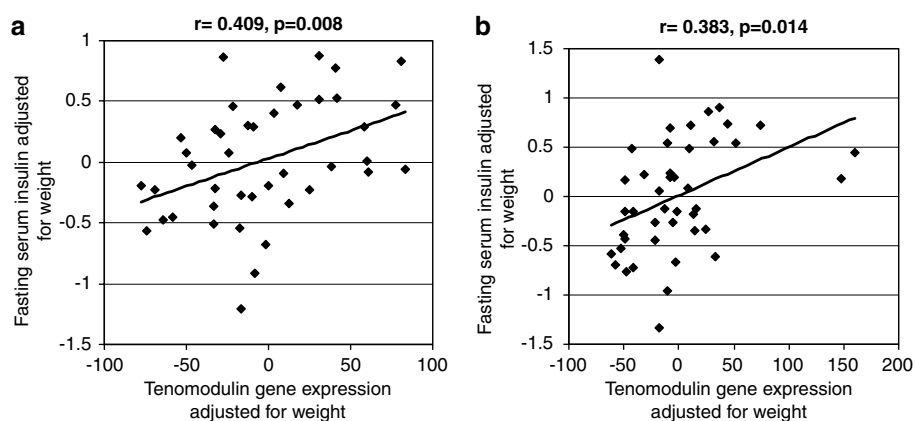
AT was found in the obese subjects.<sup>25</sup> Compared with previous studies, our study was long-term, with a follow-up lasting more than 8 months. In addition, the intervention was moderate, resulting in weight loss of about 8%. The long-term moderate weight reduction allows adaptation and

compensatory effects to take place. It might, therefore, not be surprising that we detected changes in different gene clusters than what has been reported previously. Our findings are more likely to describe stable long-term changes in AT at the molecular level after the acute changes in the

**Table 6** Gene expression in the weight reduction and control groups and in the subjects selected for microarray analysis before and after the intervention

|               | Study population             |                  | P <sup>a</sup> | Microarray analysis      |                  | P <sup>b</sup> |
|---------------|------------------------------|------------------|----------------|--------------------------|------------------|----------------|
|               | Weight reduction (n = 27/26) | Control (n = 18) |                | Weight reduction (n = 9) | Control (n = 10) |                |
| <i>TNMD</i>   |                              |                  |                |                          |                  |                |
| 0 week        | 104.9 ± 45.9                 | 77.9 ± 41.2      |                | 116.2 ± 36.2             | 72.1 ± 48.9      |                |
| 33 weeks      | 87.4 ± 55.9*                 | 76.0 ± 33.9      | ns             | 71.2 ± 27.6****          | 68.2 ± 34.8      | ns             |
| <i>ADAM12</i> |                              |                  |                |                          |                  |                |
| 0 week        | 75.7 ± 21.6                  | 100.3 ± 29.8     |                | 85.1 ± 18.9              | 90.8 ± 17.0      |                |
| 33 weeks      | 70.8 ± 24.3                  | 103.7 ± 24.0     | 0.0001         | 71.8 ± 19.5**            | 101.2 ± 24.6     | 0.051          |
| <i>PIC4CB</i> |                              |                  |                |                          |                  |                |
| 0 week        | 78.1 ± 14.5                  | 86.5 ± 16.7      |                | 73.9 ± 15.1              | 82.8 ± 18.9      |                |
| 33 weeks      | 79.2 ± 12.0                  | 89.1 ± 15.7      | 0.035          | 75.2 ± 9.5               | 83.6 ± 16.7      | ns             |
| <i>ADCY7</i>  |                              |                  |                |                          |                  |                |
| 0 week        | 87.6 ± 27.1                  | 82.1 ± 18.8      |                | 85.2 ± 29.7              | 79.6 ± 16.8      |                |
| 33 weeks      | 71.6 ± 16.4***               | 86.5 ± 22.2      | ns             | 72.2 ± 19.4              | 80.3 ± 15.5      | ns             |
| <i>CCND2</i>  |                              |                  |                |                          |                  |                |
| 0 week        | 78.9 ± 18.2                  | 83.1 ± 25.6      |                | 79.7 ± 15.6              | 84.1 ± 32.6      |                |
| 33 weeks      | 67.0 ± 24.8***               | 81.6 ± 25.0      | ns             | 59.9 ± 22.6**            | 86.6 ± 32.2      | ns             |
| <i>PRDM</i>   |                              |                  |                |                          |                  |                |
| 0 week        | 64.6 ± 25.5                  | 93.5 ± 36.4      |                | 74.8 ± 34.4              | 85.4 ± 35.5      |                |
| 33 weeks      | 67.8 ± 26.3                  | 90.3 ± 32.5      | 0.003          | 69.6 ± 30.8              | 88.3 ± 33.9      | ns             |
| <i>PDGFRL</i> |                              |                  |                |                          |                  |                |
| 0 week        | 64.8 ± 21.7                  | 105.7 ± 40.4     |                | 60.6 ± 14.4              | 99.5 ± 37.1      |                |
| 33 weeks      | 72.5 ± 23.5**                | 122.7 ± 39.4***  | 0.0001         | 68.0 ± 21.3              | 120.3 ± 41.4***  | 0.004          |

Mean ± s.d. Expression of target genes is related to the expression of cyclophilin A1-gene. Values are arbitrary units of the ratio between the expression of target gene to the expression of cyclophilin A1 multiplied by 100 are used. Abbreviations: TNMD = tenomodulin, ADAM12 = A disintegrin and metalloproteinase domain 12, PIC4CB = phosphatidylinositol 4-kinase, catalytic β polypeptide, ADCY7 = adenylate cyclase 7, CCND2 = cyclin D2, PRDM = pr domain containing 4, PDGFRL = platelet-derived growth factor receptor-like. Paired samples *t*-test for the change from baseline to the end of the study within the group: \**P* = 0.088, \*\**P* < 0.05, \*\*\**P* < 0.01, \*\*\*\**P* < 0.001. <sup>a</sup>General linear model for the interaction of time and group between the weight reduction and control group. <sup>b</sup>GLM, interaction of time and group between the subjects who were chosen for the microarray analysis from the weight reduction and control group.



**Figure 3** Correlations adjusted for baseline body weight between the tenomodulin gene expression in adipose tissue and fasting serum insulin concentration before (a) and after (b) the intervention. Weight reduction and control groups are combined. Fasting concentrations for serum insulin are logarithmic transformed.

gene expression that occur with short-term substantial weight reduction, and continuing negative energy balance. Previous findings<sup>27</sup> have also shown that different cell types contribute differently to the production or secretion of

adipokines and other molecules in AT. Function of different cell types in AT is, however, based on their mutual interaction, which determines the overall impact of AT on disease development.

Long-term moderate weight reduction and reduction of fat mass altered the gene expression of subcutaneous AT in humans. Genes involved in the function of the ECM and terminal differentiation of adipocytes—cell death showed a strong downregulation after weight reduction. This likely reflects a new stable state at the molecular level in AT. Further studies are warranted to elucidate the mechanisms of these genetic factors in human pathogenesis of insulin resistance and T2DM.

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