Weight loss improves the adipogenic capacity of human preadipocytes and modulates their secretory profile

Short running title: Weight loss affects adipocyte differentiation

Lenka Rossmeislová 1,2, Lucia Mališová 1,2, Jana Kračmerová 1,2, Michaela Tencerová 1,2, Zuzana Kováčová 1,2, Michal Koc 1,2, Michaela Šiklová-Vítková 1,2, Nathalie Viquerie 1,3,4, Dominique Langin 1,3,4,5 and Vladimír Štich 1,2

1Franco-Czech Laboratory for Clinical Research on Obesity, Third Faculty of Medicine, Prague, and Inserm, Toulouse

2Department of Sport Medicine, Third Faculty of Medicine, Charles University in Prague, CZ-100 00 Czech Republic;

3Inserm, UMR1048, Obesity Research Laboratory, Team 4, I2MC, Institute of Metabolic and Cardiovascular Diseases, F31432 Toulouse, France.

4University of Toulouse, UMR1048, Paul Sabatier University, F31432 Toulouse, France;

5CHU de Toulouse, Laboratory of Clinical Biochemistry, Toulouse, F-31000, France

Address correspondence and reprint requests to:

Lenka Rossmeislová
Department of Sport Medicine
Third Faculty of Medicine, Charles University in Prague,
Ruská 87, 100 00 Prague, Czech Republic.
Phone: +420 267 102 211. Fax: +420 267 102 263. E-mail: Lenka.Rossmeislova@lf3.cuni.cz

Number of words (text): 2265

Number of tables and figures: 1 Table, 3 Figures, 2 Supplemental Tables
Abstract

Calorie restriction-induced weight loss is accompanied by profound changes in adipose tissue characteristics. To determine the effect of weight loss on differentiation of preadipocytes and secretory capacity of in vitro differentiated adipocytes, we established cultures of these cells from paired subcutaneous adipose tissue biopsies obtained before and at the end of weight-reducing dietary intervention in 23 obese women. Based on lipid accumulation and the expression of differentiation markers, in vitro adipogenesis increased following weight loss and it was accompanied by enhanced expression of genes involved in de novo lipogenesis. This effect of weight loss was not driven by changes of PPARγ sensitivity to rosiglitazone. Weight loss also enhanced the expression of adiponectin and leptin while reducing that of MCP1 and IL8 by cultured adipocytes.

Thus, the weight-reducing dietary intervention increased adipogenic capacity of preadipocytes and shifted their secretion towards lower inflammatory profile. Reprogramming of preadipocytes could represent an adaptation to weight loss leading to partial restoration of pre-obese adipose tissue traits, and thus contribute to the improvement of metabolic status. However, enhanced adipogenesis could also contribute to the unwanted weight regain after initial weight loss.
Worsening of metabolic health in obesity is associated with the hypertrophy of adipocytes (1). Indeed, the recruitment of new and small adipocytes improves insulin sensitivity (2). These cells have a high potential to store lipids and therefore alleviate peripheral lipotoxicity associated with whole-body insulin resistance. However, adipose stroma-vascular cells derived from obese donors exhibit impaired adipogenic capacity (3) and the factors influencing sensitivity of human preadipocytes to adipogenic stimuli in vivo remain unknown. Weight reduction induced by hypocaloric diet is the key approach for treatment of obesity-related metabolic disturbances. A moderate loss of body weight induces an adaptation of human AT associated with improved whole-body metabolic status (4; 5). We hypothesized that cell cultures of preadipocytes established from subcutaneous AT collected before and after a weight loss-inducing dietary intervention (DI) correspond to two distinct metabolic and nutritional stages of the donor. The current knowledge on intrinsic adipogenic and endocrine potential of these cells is based on and limited to cross-sectional studies. Here, we show that DI-induced weight loss increased the differentiation capacity of preadipocytes and shifted their secretion towards less inflammatory profile. This reprogramming of preadipocytes by weight loss could represent a cellular mechanism leading to the restoration of pre-obese traits of AT and correction of inflammatory status.

**Materials and methods**

**Subjects**

Obese premenopausal women (n=23) were recruited at the Third Faculty of Medicine of Charles University (TFM) and University Hospital Kralovske Vinohrady, Czech Republic. Exclusion criteria were set as previously (6). The study was performed according to the Declaration of
Helsinki and was approved by the Ethical Committee of TFM. Volunteers signed informed consent before participation in the study.

**DI and clinical investigation**

The DI lasted 5-6 months. Participants reduced their calorie intake by 600 kcal/day in relation to the individually estimated energy requirement (initial resting metabolic rate multiplied by 1.3, the coefficient of correction for physical activity). Weight loss was achieved within the first 3 months, and then women were advised to keep the diet leading to the weight maintenance. Subjects consulted a dietitian once a week during the first 3 months and once a month during the weight maintenance phase.

Clinical investigation was performed after an overnight fast before and at the end of DI. Anthropometric measurements, blood sampling and needle biopsy of AT were performed as previously reported (6). Briefly, after administration of local anesthesia (1% Xylocaine), 1-2 mm incision was made 10 cm laterally from umbilicus and 12G needle coupled with syringe was used to aspirate fragments of superficial subcutaneous AT. On average 1.5 g of tissue was obtained (0.6g -2.5 g).

**Isolation and culture of preadipocytes**

AT was digested in 1.5 volume of collagenase I (300 U/ml, Biochrom, Berlin, Germany) for 60 minutes in 37°C shaking water bath and processed as described previously (7). Digested tissue was diluted with PBS/gentamycin and spun at 1300 rpm, 5 min. Cells were then shaken forcefully to complete the dissociation from mature adipocytes and centrifuged. Pellet containing cells from the stromavascular fraction was incubated in erythrocyte lysis buffer for 10 min at room temperature. Cells were centrifuged and, without any filtration step, they were resuspended in PM4 medium (8) with 132 nM insulin. PM4 was replaced every other day. Cells were subcultivated at 70% confluence; experiments were performed at passage 3. Differentiation of 2 day
post-confluent cells was induced by DMEM/F12 medium supplemented with 66 nM insulin, 1 
µM dexamethasone, 1 nM T3, 0.1 µg/ml transferrin, 0.25 mM IBMX, 1 µM rosiglitazone. After 6 
days rosiglitazone and IBMX were omitted and dexamethasone was replaced with 0.1 µM 
cortisol. The differentiation continued until day 12. Medium conditioned for 24 hours was then 
collected and cells were harvested for RNA and protein analysis. Protein concentration was 
determined by BCA assay (Pierce Biotechnology, Rockford, USA). For experiments focused on 
the effect of PPARγ activation, cells were induced to differentiate in the media containing either 
1µM rosiglitazone or DMSO. As non-differentiating controls, preadipocytes switched to serum free medium supplemented with transferrin and insulin were used.

Gene expression analysis

Total RNA was isolated using RNeasy Mini Kit (Qiagen, Hilden, Germany). mRNA levels were 
measured using reverse transcription quantitative PCR (Applied Biosystems, Carlsbad, 
California, USA) (9). GUSB was used as an endogenous control, results are expressed as ΔΔCt 
values.

Cytokine analysis

Cytokines were measured by ELISA (IL6 and MCP1, Ready-Go-sets, eBioscience, San Diego, 
USA; IL6 Quantikine HS, R&D Systems, Abingdon, UK, adiponectin DuoSet, R&D Systems, 
Minneapolis, USA). Detection of adiponectin isoforms was performed by native-PAGE and 
western blot (10). Chemiluminiscent signal was detected on Kodak Image Station 4000R and 
analyzed by associated software.

Oil Red O (ORO) and BODIPY staining

Cells were fixed, stained with ORO and analyzed as described previously (11) or stained with 
1µM BODIPY 493/503 (Life Technologies, Carlsbad, California, USA) and DAPI. Standard
curve from ORO stock was used to normalize data. The OD of eluates from cells reached values of 40% of ORO when 100% of cells were differentiated. Images for BODIPY analysis were acquired on fluorescent microscope DMI5000 coupled with CCD camera (Leica Microsystems, Wetzler, Germany).

Statistical analysis

Data were analyzed using GraphPad Prism 5.0 software using Wilcoxon matched-pair signed rank or Mann Whitney test, as appropriate. The level of significance was set at p<0.05.

Results

Clinical characteristics of obese subjects

The clinical data of subjects before and after DI are listed in Table 1. When compared to baseline values, the subjects’ body weight decreased by 9.7 % and insulin resistance assessed by HOMA-IR was reduced.

Preadipocytes derived from AT after weight loss exhibit increased adipogenesis.

The stroma-vascular cells from AT samples were isolated, expanded and differentiated into adipocytes. Cell cultures derived from the samples obtained after weight loss exhibited increased differentiation as evaluated by the ORO staining and mRNA expression of differentiation markers aP2 and PPARγ (Fig. 1A, B). Moreover, the expression of SCD1 (stearoyl-CoA desaturase), DGAT2 (diacylglycerol O-acyltransferase 2), FASN (fatty acid synthase), ACLY (ATP citrate lyase), ChREBPα (carbohydrate response element binding protein α) and GLUT4 was also upregulated, confirming higher capacity of cells for lipogenesis (Fig. 1B). Although mRNA levels of markers of mitochondrial biogenesis Nrf1 (nuclear respiratory factor 1) and
PGC1α (peroxisome proliferator-activated receptor gamma, coactivator 1 alpha) were not altered, UCP1 (uncoupling protein 1) expression was notably upregulated (Fig. 1B). Interestingly, cell differentiation into adipocytes was associated with an increase in total protein content that was more pronounced after weight loss (Fig. 1C). The ratio between protein content in adipocytes versus preadipocytes correlated with the degree of differentiation measured by ORO (Fig. 1D). Notably, there was a positive link between changes in protein content and mRNA levels of differentiation markers, lipogenic genes and UCP1 (Fig. 1E and Suppl. Table 1). The enhancement of adipogenesis was not caused by the alteration of proliferative capacity of preadipocytes, since there was no difference in the yield of the cells at passage 3 and length of cultivation period preceding the experiments (Suppl. Table 2).

To decipher the putative role of PPARγ in the reprogramming of preadipocytes induced by weight loss, preadipocytes were stimulated with differentiation medium supplemented either with DMSO (control) or 1 µM rosiglitazone (PPARγ ligand). As expected, rosiglitazone enhanced markedly the expression of FASN, SCD1 and aP2 as compared with control cells (Fig. 2A), however, the upregulation of lipogenic markers FASN and SCD1 in cells derived after the DI was more pronounced in the absence of rosiglitazone (Fig. 2A). Furthermore, the ratio of FASN and SCD1 expression under rosiglitazone vs. control treatment was not different between the cells obtained before and after the DI (Fig. 2B). In addition, the percentage of cells accumulating neutral lipids after 6 days of differentiation in the presence or absence of PPARγ ligand was in both cases higher after weight loss (Fig. 2C). Again, the ratio between the numbers of cells differentiated in the presence of rosiglitazone vs. DMSO was unchanged after the DI (not shown).

These data suggest that weight loss did not alter the sensitivity of cells to rosiglitazone and rather affected pathways upstream of PPARγ. Indeed, the expression of PPARγ itself as well as the...
expression of KLF9, the transcription factors that regulates PPARγ transcription, were not different in preadipocytes derived before or after weight loss (Fig. 2D). However, cells derived after weight loss exhibited a marked downregulation of expression of RUNX2, the transcription factor favoring osteogenic differentiation (12), both prior and during differentiation (Fig. 2D).

**The expression and secretion of cytokines by in vitro differentiated preadipocytes is altered after weight loss.**

To determine whether weight loss affects the intrinsic secretory potential of adipocytes, the secretion and mRNA expression of several cytokines was measured in *in vitro* differentiated preadipocytes derived from AT before and after weight loss. Both, expression and secretion of adiponectin and its high molecular weight (HMW) form were higher in adipocytes after DI compared to baseline (Fig. 3). However, when the secretion of total adiponectin was adjusted to the degree of differentiation assessed by ORO, the effect of DI was lost, suggesting a close relationship between adiponectin secretion and the differentiation state of adipocytes (not shown). Leptin mRNA levels were also elevated in adipocytes after weight loss (Fig. 3A), and this change was not related to the degree of differentiation. In contrast, MCP1 and IL8 mRNA levels in adipocytes obtained after DI were reduced compared to baseline (Fig. 3A). Secretion of MCP1 was lower (Fig. 3B) compared to baseline even after adjustments to the degree of differentiation (not shown), but no significant changes in secretion or expression of IL6 were observed (Fig. 3A, B).

**Discussion**

It has been hypothesized that worsening of metabolic health in obesity is related to dysfunction of hypertrophic adipocytes and/or diminished ability of AT to react to energetic surplus by the
enhanced adipogenesis from available precursors. The latter is evidenced by (i) insulin-resistant subjects exhibiting lower expression of adipogenic genes (13), and (ii) the insulin-sensitizing drugs thiazolidinediones alleviating insulin resistance by the recruitment of new adipocytes with a high potential to store lipids (14; 15).

In this study using cells derived from paired subcutaneous AT biopsies from obese women undergoing long-term DI, we showed that adipogenic potential of preadipocytes was increased by moderate weight loss. Obesity was shown to be associated with lower differentiation capacity of preadipocytes (3; 16). Our data obtained in the prospective study are therefore not only in agreement with the cross-sectional observations but provide evidence that the lowering of AT mass is associated with higher preadipocyte differentiation capacity and sensitivity to adipogenic stimuli. This implies that the effect of weight loss on AT function should not be ascribed only to changes in size and metabolism of mature adipocytes and in pro-inflammatory potential/numbers of infiltrated immune cells (6; 17), but also to reprogramming of preadipocytes. Lower RUNX2 expression in cells derived after weight loss suggests that weight loss inhibits alternative lineage programs (e.g osteogenesis), which in turn favors the adipogenic differentiation.

Nevertheless, the impact of in vivo changes of preadipocytes, which are important for the maintenance or development of AT (17), remains unknown. It is tempting to speculate that upon fat mass reduction, a higher sensitivity of precursor cells to adipogenic stimuli could enhance fatty acid storage and therefore indirectly lower lipotoxicity at the whole-body level while improving insulin sensitivity. On the other hand, increased adipogenesis following previous weight loss could compromise long term weight loss maintenance. Indeed, studies on obese and then calorie-restricted rats showed that short overfeeding after calorie restriction was accompanied with appearance of small adipocytes (18).
Development of mature adipocytes is dependent on active lipogenesis. In serum-free culture conditions, all accumulated lipids are synthesized de novo (19). De novo lipogenesis (DNL) in AT, possibly orchestrated by ChREBPβ (20), was downregulated in subjects with hypertrophied adipocytes who are more insulin resistant compared to subjects with smaller adipocytes (21). Since we observed that weight loss was accompanied with a higher expression of lipogenic genes FASN, DGAT2, SCD1, ACLY and ChREBPα (a regulator of ChREBPβ expression) in in vitro differentiated cells, it can be suggested that DNL capacity linked to higher insulin sensitivity represents intrinsic characteristics of adipocytes re-programmable by weight loss.

In obesity, adipocytes produce more pro-inflammatory cytokines and chemoattractants while secretion of insulin-sensitizing adiponectin is diminished (22). In our study, we show that weight loss altered the capacity of in vitro differentiated adipocytes to express IL8, MCP1, leptin and adiponectin. Lower secretion of MCP1 from adipocytes reprogrammed by weight loss could contribute to a lower infiltration of macrophages into AT. Selective increase of HMW adiponectin secretion might underlie beneficial effects of weight loss on insulin sensitivity.

Studies performed on cell culture models may be influenced by culture conditions. Although we cannot completely exclude possible effects of sub-cultivation on adipogenic and secretory potential of cells, it has been shown that in vitro conditions preserve the original phenotype of a donor as shown previously for preadipocytes and adipocytes (13; 23). Moreover, sub-cultivation of stromavascular cells eliminates contaminating cells like macrophages and results in more homogenous population than primary cells (3; 24). It is also unlikely that the observed differences were based on dissimilar starting numbers of cells as there was no difference in the length of cultivation or yield of cells before and at the end of DI.
In conclusion, our study shows that weight loss improves the adipogenic capacity of preadipocytes and alters their secretory potential. This effect may be associated with the improvement of metabolic status of obese as well as with an increased tendency for weight regain. We believe that the analysis of a distinct cellular population, such as preadipocytes subjected to uniform \textit{in vitro} conditions, can offer focused and unique image of an intrinsic adaptation of AT to weight loss.

\textbf{Acknowledgements}

L.R. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. L.R. designed the study, performed experiments and data analysis and wrote the manuscript, L.M, J.K, Z.K., M.K. and M.T. performed experiments and contributed to discussion, M.S, N.V. and D.L. contributed to discussion and writing of the manuscript, V.S. designed the study, organized clinical part of the study and contributed to discussion and writing of the manuscript. The study was supported by Collaborative Project ADAPT (www.adapt-eu.net), Contract No. HEALTH-F2-2008-2011 00), grant GACR 301/11/0748 of the Grant Agency of the Czech Republic, IGA NT 11450-3-2010 of Ministry of Health and UNCE 204015 of Charles University. We are indebted to Zuzana Pařízková, for technical expertise, Dr. Jan Polák for help with AT biopsies (both from Department of Sport Medicine, Third Faculty of Medicine, Charles University in Prague, Czech Republic) and Dr. Martin Rossmeisl (Institute of Physiology, Academy of Sciences of the Czech Republic, Czech Republic) for critical reading of the manuscript.

Authors declare no conflicts of interest.
References

1. Guilherme A, Virbasius JV, Puri V, Czech MP: Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. Nature Reviews Molecular Cell Biology 2008;9:367-377
2. Cristancho AG, Lazar MA: Forming functional fat: a growing understanding of adipocyte differentiation. Nat Rev Mol Cell Biol 2011;12:722-734
3. Isakson P, Hammarstedt A, Gustafson B, Smith U: Impaired preadipocyte differentiation in human abdominal obesity: role of Wnt, tumor necrosis factor-alpha, and inflammation. Diabetes 2009;58:1550-1557
4. Clement K, Viguierie N, Poitou C, Carette C, Pelloux V, Curat CA, Sicard A, Rome S, Benis A, Zucker JD, Vidal H, Laville M, Barsh GS, Basdevant A, Stich V, Cancello R, Langin D: Weight loss regulates inflammation-related genes in white adipose tissue of obese subjects. FASEB J 2004;18:1657-1669
5. Klimcakova E, Kovacikova M, Stich V, Langin D: Adipokines and dietary interventions in human obesity. Obes Rev 2010;11:446-456
6. Capel F, Klimcakova E, Viguierie N, Roussel B, Vitkova M, Kovacikova M, Polak J, Kovacova Z, Galitzky J, Maoret JJ, Hanacek J, Pers TH, Bouloumie A, Stich V, Langin D: Macrophages and adipocytes in human obesity: adipose tissue gene expression and insulin sensitivity during calorie restriction and weight stabilization. Diabetes 2009;58:1558-1567
7. Dubois SG, Floyd EZ, Zvonic S, Kilroy G, Wu X, Carling S, Halvorsen YD, Ravussin E, Gimble JM: Isolation of human adipose-derived stem cells from biopsies and liposuction specimens. Methods Mol Biol 2008;449:69-79
8. Skurk T, Ecklebe S, Hauner H: A novel technique to propagate primary human preadipocytes without loss of differentiation capacity. Obesity (Silver Spring) 2007;15:2925-2931
9. Siklova-Vitkova M, Klimcakova E, Polak J, Kovacova Z, Tencerova M, Rossmeislova L, Bajzova M, Langin D, Stich V: Adipose Tissue Secretion and Expression of Adipocyte-Produced and Stromavascular Fraction-Produced Adipokines Vary during Multiple Phases of Weight-Reducing Dietary Intervention in Obese Women. J Clin Endocrinol Metab 2012;
10. Kovacova Z, Tencerova M, Roussel B, Wedelova Z, Rossmeislova L, Langin D, Polak J, Stich V: The impact of obesity on secretion of adiponectin multimeric isoforms differs in visceral and subcutaneous adipose tissue. Int J Obes (Lond) 2011;
11. Janderova L, McNeil M, Murrell AN, Mynatt RL, Smith SR: Human mesenchymal stem cells as an in vitro model for human adipogenesis. Obesity Research 2003;11:65-74
12. Rosen ED, MacDougald OA: Adipocyte differentiation from the inside out. Nat Rev Mol Cell Biol 2006;7:885-896
13. van Tienen FH, van der Kallen CJ, Lindsey PJ, Wanders RJ, van Greevenbroek MM, Smeets HJ: Preadipocytes of type 2 diabetes subjects display an intrinsic gene expression profile of decreased differentiation capacity. Int J Obes (Lond) 2011;35:1154-1164
14. Tang W, Zeve D, Seo J, Jo AY, Graff JM: Thiazolidinediones regulate adipose lineage dynamics. Cell Metab 2011;14:116-122
15. McLaughlin TM, Liu T, Yee G, Abbasi F, Lamendola C, Reaven GM, Tsao P, Cushman SW, Sherman A: Pioglitazone increases the proportion of small cells in human abdominal subcutaneous adipose tissue. Obesity (Silver Spring) 2010;18:926-931
16. van Harmelen V, Skurk T, Rohrig K, Lee YM, Halbleib M, Aprath-Husmann I, Hauner H: Effect of BMI and age on adipose tissue cellularity and differentiation capacity in women. Int J Obes Relat Metab Disord 2003;27:889-895
17. Spalding KL, Arner E, Westermark PO, Bernard S, Buchholz BA, Bergmann O, Blomqvist L, Hoffstedt J, Naslund E, Britton T, Concha H, Hassan M, Ryden M, Frisen J, Arner P: Dynamics of fat cell turnover in humans. Nature 2008;453:783-787
18. Jackman MR, Steig A, Higgins JA, Johnson GC, Fleming-Elder BK, Bessesen DH, MacLean PS: Weight regain after sustained weight reduction is accompanied by suppressed oxidation of dietary fat and adipocyte hyperplasia. Am J Physiol Regul Integr Comp Physiol 2008;294:R1117-1129
19. Collins JM, Neville MJ, Pinnick KE, Hodson L, Ruyter B, van Dijk TH, Reijngoud DJ, Fielding MD, Frayn KN: De novo lipogenesis in the differentiating human adipocyte can provide all fatty acids necessary for maturation. J Lipid Res 2011;52:1683-1692
20. Herman MA, Peroni OD, Villoria J, Schon MR, Abumrad NA, Bluher M, Klein S, Kahn BB: A novel ChREBP isoform in adipose tissue regulates systemic glucose metabolism. Nature 2012;484:333-338
21. Roberts R, Hodson L, Dennis AL, Neville MJ, Humphreys SM, Harnden KE, Micklem KJ, Frayn KN: Markers of de novo lipogenesis in adipose tissue: associations with small adipocytes and insulin sensitivity in humans. Diabetologia 2009;52:882-890
22. Antuna-Puente B, Feve B, Fellahi S, Bastard JP: Adipokines: the missing link between insulin resistance and obesity. Diabetes Metab 2008;34:2-11
23. Tchkonia T, Giorgadze N, Pirtskhalava T, Thomou T, DePonte M, Koo A, Forse RA, Chinnappan D, Martin-Ruiz C, von Zglinicki T, Kirkland JL: Fat depot-specific characteristics are retained in strains derived from single human preadipocytes. Diabetes 2006;55:2571-2578
24. Mitchell JB, McIntosh K, Zvonic S, Garrett S, Floyd ZE, Kloster A, Di Halvorsen Y, Storms RW, Goh B, Kilroy G, Wu X, Gimble JM: Immunophenotype of Human Adipose-Derived Cells: Temporal Changes in Stromal-Associated and Stem Cell-Associated Markers. Stem Cells 2006;24:376-385
Table 1. Clinical characteristics of the subjects before and after dietary intervention

|                                | baseline     | DI            | P value |
|--------------------------------|--------------|---------------|---------|
| Age (years)                    | 40.7±1.79    | 82.5±1.93     | <0.001  |
| Weight (kg)                    | 91.49±2.12   | 82.5±1.93     | <0.001  |
| BMI (kg/m²)                    | 32.97±0.91   | 29.71±0.82    | <0.001  |
| FM (%)                         | 39.71±1.1    | 36.47±1.11    | <0.001  |
| Waist (cm)                     | 102.6±2.24   | 93.04±2.13    | <0.001  |
| WHR                            | 0.86±0.02    | 0.84±0.02     | 0.011   |
| Glucose (mmol/L)               | 5.42±0.11    | 5.06±0.13     | 0.012   |
| Insulin (mIU/L)                | 9.62±0.97    | 7.27±0.92     | 0.002   |
| HOMA-IR                        | 2.36±0.27    | 1.71±0.27     | 0.002   |
| Total cholesterol (mmol/L)     | 5.38±0.27    | 4.63±0.18     | 0.006   |
| HDL-C (mmol/L)                 | 1.66±0.09    | 1.44±0.07     | 0.007   |
| Triglycerides (mmol/L)         | 1.19±0.09    | 0.78±0.05     | <0.001  |
| IL-6 (pg/ml)                   | 0.86±0.1     | 0.82±0.09     | 0.381   |
| MCP-1 (pg/ml)                  | 25.61±2.72   | 23.73±2.82    | 0.075   |
| Adiponectin (µg/ml)            | 1.89±0.12    | 1.92±0.14     | 0.721   |
BMI, body mass index; DI, dietary intervention; FM, fat mass; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of the insulin resistance index; NEFA, non-esterified fatty acids; WHR, waist to hip ratio.

Values are presented as means ± SEM (n=23).
Figure legend

Fig.1 Weight loss improves in vitro adipogenesis. Cells were differentiated for 12 days and then accumulation of lipids, protein content or gene expression was analyzed. A. Effect of weight loss on lipid accumulation. Representative images of adipocytes from one donor before and after DI stained with ORO and quantification of neutral lipid accumulation expressed as a % of stock ORO (n=22). B. Effect of weight loss on gene expression. mRNA expression (AU) in adipocytes normalized to GUSB expression (n=15). C. Effect of weight loss on protein content. Ratio between total protein content in adipocytes versus preadipocytes (n=22). White bars = baseline, black bars = DI. Data are means ±SE, *p<0.05, ** p<0.01, *** p<0.001. D. Linear regression between ORO accumulation and protein content in adipocytes (A) versus preadipocytes (P) at baseline. E. Linear regression between relative delta protein (A v. P) and delta mRNA expression of aP2 and PPARγ.

Fig.2 Weight loss does not enhance the sensitivity to PPARγ ligand rosiglitazone but is connected with the suppression of RUNX2 expression. A and B. Cells were differentiated for 3 days in the presence or absence of 1mM rosiglitazone. Cells kept in serum free medium supplemented with transferrin and insulin that did not undergo adipogenesis were used controls. A. Relative mRNA levels of selected genes were detected by qRT-PCR (n=6). White bars = baseline, black bars = DI. Data are means ±SE, *p<0.05, B. The ratio between expression of selected genes in cells differentiated in the presence and absence of rosiglitazone was calculated in cells derived before and after the DI. C. Cells were differentiated for 6 days in the presence or absence of 1mM rosiglitazone (n=4). After staining with BODIPY, the 45 microscopy images encompassing on average 2700 cells were analyzed and numbers of BODIPY+ cells were counted. Chart represents the percentage of BODIPY+ cells within analyzed populations (each consisting on average from 2700 cells). Data are means ±SE, *p<0.05.

Fig.3 Weight loss alters the expression and secretion of cytokines in adipocytes differentiated in vitro. Cells were differentiated for 12 days, conditioned media and cells were collected following 24hrs of incubation in freshly added media for analysis of cytokine expression and secretion. A. Effect of weight loss on cytokines gene expression. qRT-PCR analysis of mRNA for selected cytokines, normalized to GUSB expression (n=15). B. Effect of weight loss on cytokines secretion. Fold change over the basal values for MCP1, IL6 and adiponectin, in conditioned
media measured by ELISA, normalized to protein content (n=22). C. Effect of weight loss on adiponectin isoforms secretion. Quantification of adiponectin isoforms by native-PAGE and Western blot analysis (n=21). White bars = baseline, black bars = DI. Data are means ±SE, *p<0.05, ** p<0.01, *** p<0.001.
**Figure 1**

**A**
Baseline, ORO 28.4%
Diabetes, ORO 39.7%

**B**

- **AU mRNA level**
  - Basal
  - DI

- mRNA levels for various genes:
  - aP2
  - PPARγ
  - GLUT4
  - ChREBPα
  - ChREBPβ
  - HSL
  - DGAT2
  - SCD1
  - FASN
  - ACLY
  - PGC1α
  - Nrf1
  - UCP1

**C**

- **Protein ratio A/P**
  - Basal
  - DI

**D**

- **Protein ratio A/P vs. ORO**
  - R² = 0.563, p < 0.0001

**E**

- **Relative delta protein A/P vs. delta aP2**
  - R² = 0.736, p < 0.0001

- **Relative delta protein A/P vs. delta PPARγ**
  - R² = 0.652, p = 0.0003

p-values:
- Basal vs. DI:
  - **p < 0.0001**
  - **p = 0.054**

The **p-values** indicate statistical significance between the basal and DI groups.
Figure 2
Figure 3

A) mRNA fold change

B) Protein fold change

C) % of total adiponectin

Diabetes analysis showing changes in expression levels of adiponectin, MCP1, IL6, IL8, and leptin under basal and DI conditions.
**Supplemental Table 1.** Spearman correlation of delta gene expression versus relative delta protein ratio of adipocytes to preadipocytes (n=15)

| Gene       | Coefficient | P value |
|------------|-------------|---------|
| aP2        | 0.764       | 0.0009  |
| PPARg      | 0.729       | 0.0021  |
| GLUT4      | 0.575       | 0.0249  |
| ChREBPa    | 0.059       | 0.840   |
| ChREBPb    | -0.152      | 0.605   |
| FASN       | 0.721       | 0.0024  |
| DGAT2      | 0.639       | 0.0103  |
| SCD1       | 0.729       | 0.0021  |
| ACLY       | 0.482       | 0.0688  |
| HSL        | 0.693       | 0.0042  |
| Adiponectin| 0.679       | 0.0054  |
| Leptin     | 0.039       | 0.8895  |
| MCP1       | -0.346      | 0.2059  |
| IL8        | 0.075       | 0.7903  |
| IL6        | -0.489      | 0.0641  |
| Nrf1       | -0.055      | 0.873   |
| PGC1a      | 0.244       | 0.401   |
| UCP1       | 0.754       | 0.002   |
**Supplemental Table 2.** Yield of cells and time necessary for their sub-cultivation.

|                                | Basal       | DI          | p-value  |
|--------------------------------|-------------|-------------|----------|
| Days in culture total          | 26.79±1.42  | 24.43±0.76  | 0.642    |
| Days from set up of cultures to passage 1 | 9.21±1.05   | 7.21±0.65   | 0.1358   |
| Total yield of cells in passage 3 (millions) | 12.64±0.73  | 14.63±0.84  | 0.09     |