Weight Loss Improves the Adipogenic Capacity of Human Preadipocytes and Modulates Their Secretory Profile

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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 (DI) in 23 obese women. Based on lipid accumulation and the expression of differentiation markers, in vitro adipogenesis increased after 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 peroxisome proliferator–activated receptor γ sensitivity to rosiglitazone. Weight loss also enhanced the expression of adiponectin and leptin while reducing that of monocyte chemoattractant protein 1 and interleukin-8 by cultured adipocytes. Thus, the weight-reducing (DI) increased adipogenic capacity of preadipocytes and shifted their secretion toward lower inflammatory profile. Reprogramming of preadipocytes could represent an adaptation to weight loss leading to partial restoration of preobese 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. *Diabetes* **62:1990–1995, 2013**

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 stromavascular 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 adipose tissue associated with improved whole-body metabolic status (4,5). We hypothesized that cell cultures of preadipocytes established from subcutaneous adipose tissue 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 toward less inflammatory profile. This reprogramming of preadipocytes by weight loss could represent a cellular mechanism leading to the restoration of preobese traits of adipose tissue and correction of inflammatory status.

RESEARCH DESIGN AND METHODS

Subjects. Obese premenopausal women (n = 23) were recruited at the Third Faculty of Medicine of Charles University and University Hospital Kralovske Vinohrady, Prague, Czech Republic. Exclusion criteria were set as previously described (6). The study was performed according to the Declaration of Helsinki and was approved by the ethics committee of the Third Faculty of Medicine of Charles University. 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 adipose tissue were performed as previously described (6). Briefly, after administration of local anesthesia (1% xylocaine), a 1- to 2-mm incision was made 10 cm laterally from umbilicus and a 12G needle coupled with syringe was used to aspirate fragments of superficial subcutaneous adipose tissue. On average, 1.5 g tissue was obtained (0.6−2.5 g).

Isolation and culture of preadipocytes. Adipose tissue was digested in 1.5 volume of collagenase I (300 units/mL; Biochrom, Berlin, Germany) for 60 min in 37°C shaking water bath and processed as previously described (7). Digested tissue was diluted with PBS/gentamicin and spun at 1,300 rpm for 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 nmol/L insulin. PM4 was replaced every other day. Cells were subcultivated at 70% confluence; experiments were performed at passage 3. Differentiation of 2-day postconfluent cells was induced by Dulbecco's modified Eagle's/F12 medium supplemented with 66 nmol/L insulin, 1 μmol/L dexamethasone, 1 nmol/L T3, 0.1 μg/mL transferrin, 0.25 mmol/L isobutylmethylxanthine, and 1 μmol/L rosiglitazone. After 6 days, rosiglitazone and isobutylmethylxanthine were omitted and dexamethasone was replaced with 0.1 μmol/L cortisol. The differentiation continued until day 12. Medium conditioned for 24 h was then collected, and cells were harvested for RNA and protein analysis. Protein concentration was determined by BCA assay (Pierce Biotechnology, Rockford, IL). For experiments focused on the

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effect of peroxisome proliferator–activated receptor (PPAR)γ activation, cells were induced to differentiate in the media containing either 1 μmol/L rosiglitazone or DMSO. As nondifferentiating controls, preadipocytes switched to serum-free medium supplemented with transferrin and insulin were used.

Gene expression analysis. Total RNA was isolated using an RNeasy Mini kit (Qiagen, Hilden, Germany). mRNA levels were measured using reverse transcription quantitative PCR (Applied Biosystems, Carlsbad, CA) (9). GUSB (glucuronidase beta) was used as an endogenous control. Results are expressed as $\Delta\Delta C_t$ (threshold cycle) values.

Cytokine analysis. Cytokines were measured by ELISA (interleukin [IL]-6 and monocyte chemoattractant protein [MCP]1, Ready-Go-sets, eBioscience, San Diego, CA; IL6 Quantikine HS, R&D Systems, Abingdon, U.K.; and adiponectin DuoSet, R&D Systems, Minneapolis, MN). Detection of adiponectin isoforms was performed by native polyacrylamide gel electrophoresis and Western blot (10). Chemiluminescent signal was detected on Kodak Image Station 4000R and analyzed by associated software.

Oil Red O and BODIPY staining. Cells were fixed, stained with Oil Red O (ORO), and analyzed as previously described (11) or stained with 1 μmol/L BODIPY 493/503 (Life Technologies) and DAPI. Standard curve from ORO stock was used to normalize data. The OD of eluates from 100% differentiated cells reached values of 40% of stock ORO. Images for BODIPY analysis were acquired on fluorescent microscope DMI6000 coupled with CCD camera (Leica Microsystems, Wetzler, Germany).

Statistical analysis. Data were analyzed using GraphPad Prism 5.0 software with Wilcoxon matched-pair signed rank or Mann-Whitney *U* 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. Compared with baseline values, the subjects' body weight decreased by 9.7% and insulin resistance assessed by homeostasis model assessment of insulin resistance was reduced.

Preadipocytes derived from adipose tissue after weight loss exhibit increased adipogenesis. The stromavascular cells from adipose tissue 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. 1*A* and *B*). Moreover, the expression of stearoyl-CoA desaturase 1 (*SCD1*), diacylglycerol *O*-acyltransferase 2 (*DGAT2*), fatty acid synthase (*FASN*), ATP citrate lyase (*ACLY*), *ChREBPα*, and *GLUT4* was also upregulated, confirming higher capacity of cells for lipogenesis (Fig. 1*B*). Although mRNA levels of markers of mitochondrial biogenesis nuclear respiratory factor 1 and PPARγ coactivator 1α were not altered, *UCP1* expression was notably upregulated (Fig. 1*B*). Interestingly, cell differentiation into adipocytes was associated with an increase in total protein content that was more pronounced after weight loss (Fig. 1*C*). The ratio between protein content in adipocytes versus preadipocytes correlated with the degree of differentiation measured by ORO (Fig. 1*D*). Notably, there was a positive link between changes in protein content and mRNA levels of differentiation markers, lipogenic genes, and *UCP1* (Fig. 1*E* and Supplementary 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 (Supplementary 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 μmol/L rosiglitazone (PPARγ ligand). As expected, rosiglitazone enhanced markedly the expression of *FASN*, *SCD1*, and *aP2* compared with control cells (Fig. 2*A*); however, the upregulation of lipogenic markers *FASN* and *SCD1* in cells derived after the DI was more pronounced in the absence of rosiglitazone (Fig. 2*A*). Furthermore, the ratio of FASN and SCD1 expression under rosiglitazone versus control treatment was not different between the cells obtained before and after the DI (Fig. 2*B*). 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. 2*C*). Again, the ratio between the numbers of cells differentiated in the presence of rosiglitazone versus 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* (Kruppel-like factor 9), the transcription factors that regulate PPARγ transcription, was not different in preadipocytes derived before or after weight loss (Fig. 2*D*). However, cells derived after weight loss exhibited a marked downregulation of expression of runt-related transcription factor 2 (*RUNX2*), the transcription factor favoring osteogenic differentiation (12), both prior to and during differentiation (Fig. 2*D*).

Expression and secretion of cytokines by in vitro differentiated preadipocytes are altered after weight loss. For determination of whether weight loss affects the intrinsic secretory potential of adipocytes, the secretion and mRNA expression of several cytokines were measured in in vitro differentiated preadipocytes derived from adipose tissue before and after weight loss. Both expression and secretion of adiponectin and its high–molecular weight form were higher in adipocytes after DI compared with 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. 3*A*), and this change was not related to the degree of differentiation. In contrast, *MCP1* and *IL-8* mRNA levels

TABLE 1
Clinical characteristics of the subjects before and after dietary intervention

	Baseline	DI	*P*
Age (years)	40.7 ± 1.79		
Weight (kg)	91.49 ± 2.12	82.5 ± 1.93	**<0.001**
BMI (kg/m^2)	32.97 ± 0.91	29.71 ± 0.82	**<0.001**
Fat mass (%)	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
MCP1 (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

Data are means ± SEM. *n* = 23. HDL-C, HDL cholesterol; HOMA-IR, homeostasis model assessment of the insulin resistance index; WHR, waist-to-hip ratio. *P* values in bold reached the level of significance.

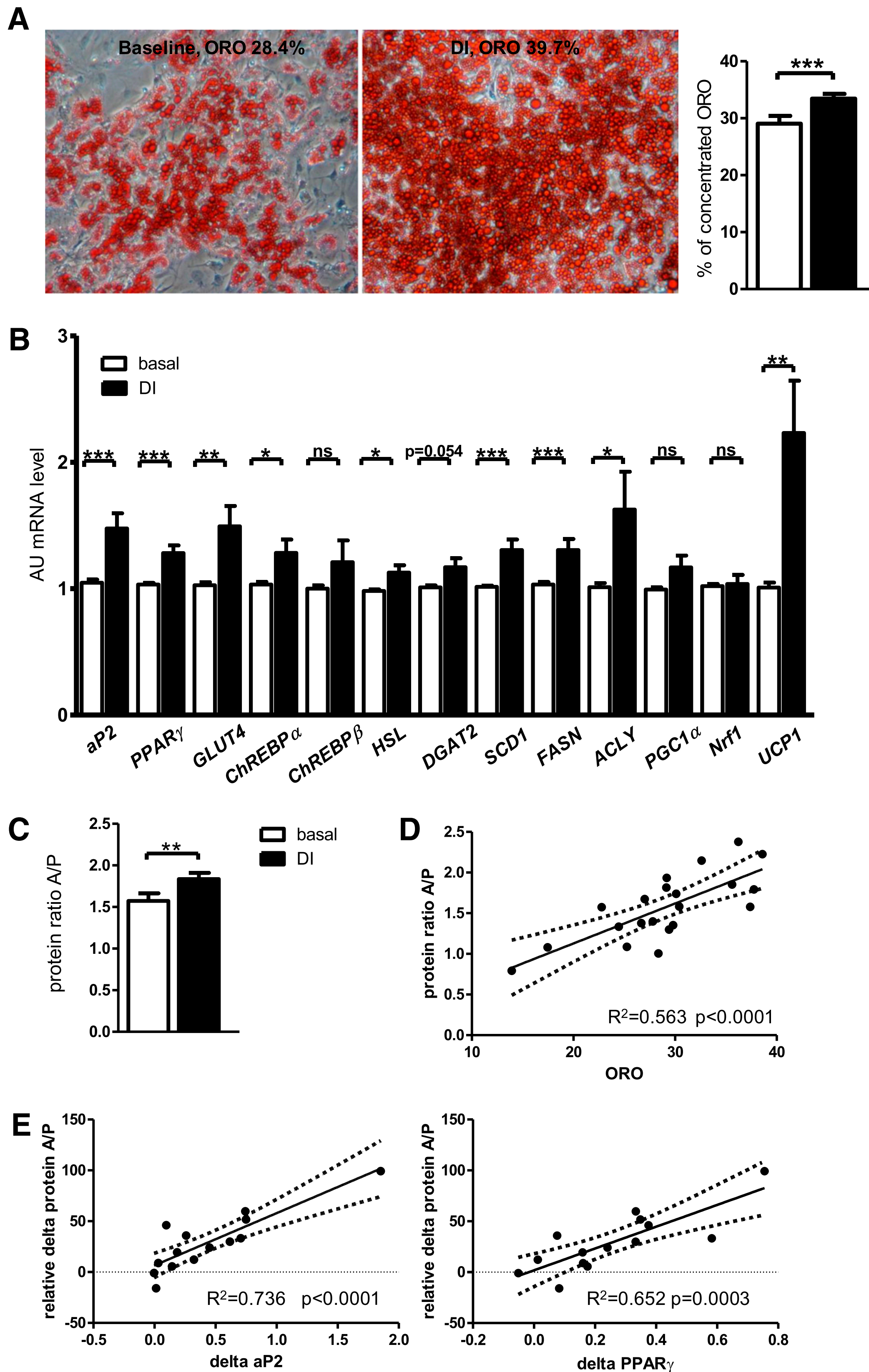

FIG. 1. Weight loss improves in vitro adipogenesis. Cells were differentiated for 12 days, and then accumulation of lipids, protein content, or gene expression was analyzed. *A*: Effect of weight loss on lipid accumulation. Representative images of adipocytes from one donor before and after DI stained with ORO and quantification of neutral lipid accumulation expressed as percent of stock ORO (n = 22). *B*: Effect of weight loss on gene expression. mRNA expression (arbitrary units [AU]) in adipocytes normalized to *GUSB* expression (n = 15). *C*: Effect of weight loss on protein content. Ratio between total protein content in adipocytes vs. preadipocytes (n = 22). 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) vs. preadipocytes (P) at baseline. *E*: Linear regression between relative Δprotein (adipocytes vs. protein) and ΔmRNA expression of *aP2* and *PPARγ*.

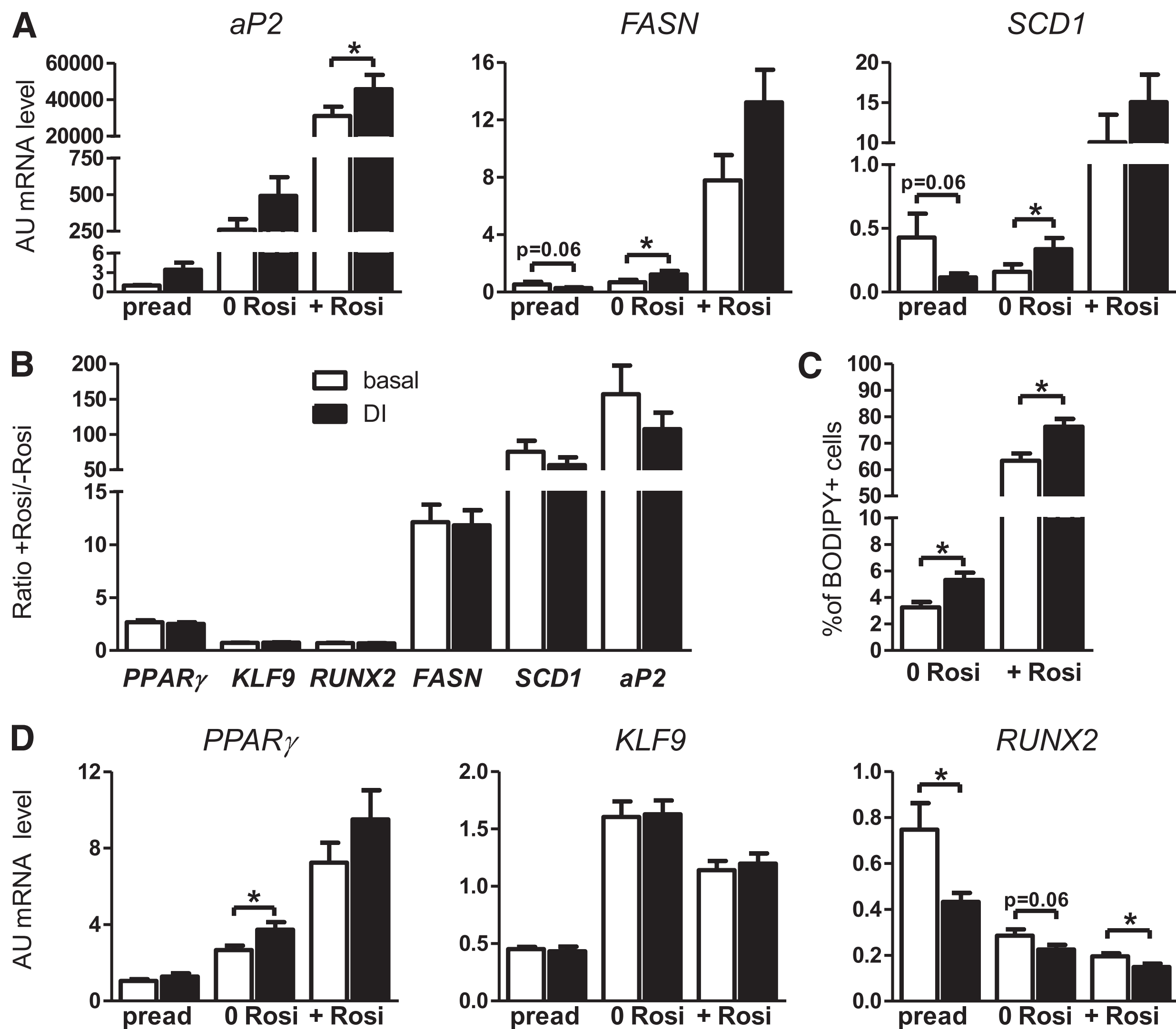

FIG. 2. Weight loss does not enhance the sensitivity to PPARγ ligand rosiglitazone (Rosi) but is connected with the suppression of *RUNX2* expression. *A* and *B*: Cells were differentiated for 3 days in the presence or absence of 1 mmol/L rosiglitazone. Cells kept in serum-free medium supplemented with transferrin and insulin that did not undergo adipogenesis were used as controls. *A*: Relative mRNA levels of selected genes were detected by quantitative RT-PCR (n = 6). □, Baseline; ■, 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 1 mmol/L rosiglitazone (n = 4). After staining with BODIPY, the 45 microscopy images encompassing an average of 2,700 cells were analyzed and numbers of BODIPY⁺ cells were counted. Chart represents the percentage of BODIPY⁺ cells within analyzed populations (each on average from 2,700 cells). Data are means ± SE; *P < 0.05. AU, arbitrary units; pread, preadipocytes.

in adipocytes obtained after DI were reduced compared with baseline (Fig. 3*A*). Secretion of MCP1 was lower (Fig. 3*B*) compared with baseline even after adjustments to the degree of differentiation (not shown), but no significant changes in secretion or expression of IL-6 were observed (Fig. 3*A* and *B*).

DISCUSSION

It has been hypothesized that worsening of metabolic health in obesity is related to dysfunction of hypertrophic adipocytes or diminished ability of adipose tissue to react to energetic surplus by the enhanced adipogenesis from available precursors. The latter is evidenced by *1*) insulin-resistant subjects exhibiting lower expression of adipogenic genes (13) and *2*) 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 adipose tissue 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 therefore not only are in agreement with the cross-sectional observations but also provide evidence that the lowering of adipose tissue mass is associated with higher preadipocyte differentiation capacity and sensitivity to adipogenic stimuli. This implies that the effect of weight loss on adipose tissue function should be ascribed not only to changes in size and metabolism of mature adipocytes and in proinflammatory 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

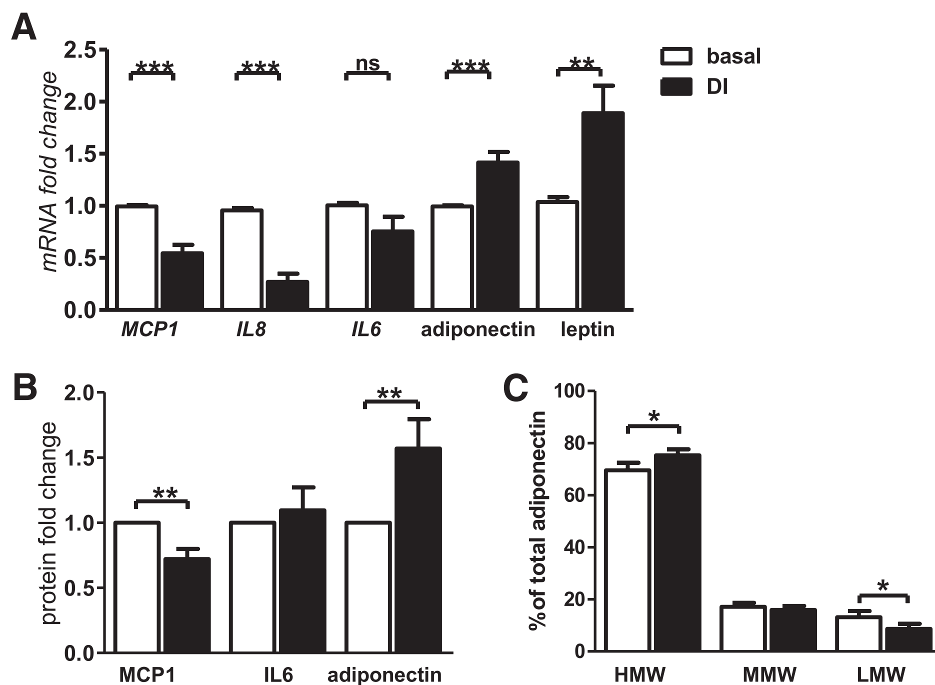

FIG. 3. Weight loss alters the expression and secretion of cytokines in adipocytes differentiated in vitro. Cells were differentiated for 12 days, and conditioned media and cells were collected after 24 h of incubation in freshly added media for analysis of cytokine expression and secretion. *A*: Effect of weight loss on cytokine gene expression. Quantitative RT-PCR analysis of mRNA for selected cytokines, normalized to *GUSB* expression ($n = 15$). *B*: Effect of weight loss on cytokine secretion. Fold change over the basal values for MCP1, IL-6, and adiponectin in conditioned media measured by ELISA, normalized to protein content ($n = 22$). *C*: Effect of weight loss on adiponectin isoform secretion. Quantification of adiponectin isoforms by native polyacrylamide gel electrophoresis and Western blot analysis ($n = 21$). Data are means ± SE; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. HMW, high molecular weight; LMW, low molecular weight, MMW, medium molecular weight.

(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 after 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 in adipose tissue, possibly orchestrated by ChREBPβ (20), was downregulated in subjects with hypertrophied adipocytes who are more insulin resistant compared with 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 de novo lipogenesis capacity linked to higher insulin sensitivity represents intrinsic characteristics of adipocytes reprogrammable by weight loss.

In obesity, adipocytes produce more proinflammatory 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 *IL-8*, *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 high–molecular weight 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 subcultivation on adipogenic and secretory potential of cells, it has been shown previously that in vitro conditions preserve the original phenotype of a donor in preadipocytes and adipocytes (13,23). Moreover, subcultivation of stromavascular cells eliminates contaminating cells like macrophages and results in a 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 the 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 in vitro conditions, can offer a focused and unique image of an intrinsic adaptation of AT to weight loss.

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No potential conflicts of interest relevant to this article were reported.

L.R. designed the study, performed experiments and data analysis, and wrote the manuscript. L.M., J.K., M.T., Z.K., and M.K. performed experiments and contributed to discussion. M.S.-V., N.V., and D.L. contributed to discussion and to the writing of the manuscript. V.S. designed the study, organized the clinical part of the study, and contributed to discussion and the writing of the manuscript. 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.

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