Lipedema: The Use of Cultured Adipocytes for Identification of Diagnostic Markers

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Background: Lipedema, diagnosed most often in women, is a progressive disease characterized by the disproportionate and symmetrical distribution of adipose tissue, primarily in the extremities. Although numerous results from in vitro and in vivo studies have been published, many questions regarding the pathology and genetic background of lipedema remain unanswered.
Methods: In this study, adipose tissue–derived stromal/stem cells were isolated from lipoaspirates derived from nonobese and obese donors with or without lipedema. Growth and morphology, metabolic activity, differentiation potential, and gene expression were evaluated using quantification of lipid accumulation, metabolic activity assay, live-cell imaging, reverse transcription polymerase chain reaction, quantitative polymerase chain reaction, and immunocytochemical staining.
Results: The adipogenic potential of lipedema and nonlipedema adipose tissue–derived stromal/stem cells did not rise in parallel with the donors' body mass index and did not differ significantly between groups. However, in vitro differentiated adipocytes from nonobese lipedema donors showed significant upregulation of adipogenic gene expression compared with nonobese controls. All other genes tested were expressed equally in lipedema and nonlipedema adipocytes. The adiponectin/leptin ratio was significantly reduced in adipocytes from obese lipedema donors compared with their nonobese lipedema counterparts. Increased stress fiber–integrated smooth muscle actin was visible in lipedema adipocytes compared with nonlipedema controls and appeared enhanced in adipocytes from obese lipedema donors.
Conclusions: Not only lipedema per se but also body mass index of donors affect adipogenic gene expression substantially in vitro. The significantly reduced adiponectin/leptin ratio and the increased occurrence of myofibroblast-like cells in obese lipedema adipocyte cultures underscores the importance of attention to the co-occurrence of lipedema and obesity. These are important findings toward accurate diagnosis of lipedema. *(Plast. Reconstr. Surg.* 152: 1036, 2023.)
Clinical Relevance Statement: Our study highlights not only the difficulty in lipedema diagnostics but also the tremendous need for further studies on lipedema tissue. Although lipedema might seem to be an underestimated field in plastic and reconstructive surgery, the power it holds to provide better treatment to future patients can not be promoted enough.

In recent years, medical research in lipedema has increased, largely because of the surprisingly high prevalence of this disease. Around 11% of postpubertal women worldwide have been estimated to have lipedema, a progressive disease that is characterized by the symmetrical accumulation of fat, primarily at extremities.[1–3] Lipedema progresses through stages, which are clearly distinguishable from one another but often appear

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as a transitional form between types and stages.[4,5] Despite considerable research undertaken to unravel the pathology of lipedema, the disease has remained enigmatic. No distinct genetic profile for the molecular diagnosis of lipedema is available to distinguish the disease from clinically overlapping conditions, but several lipedema-associated candidate genes have been identified (for a review, see Paolacci et al.[6]).

Experimental and medical evidence suggests that the progressive accumulation of adipose tissue at distinct regions of the body represents the visible end point of a process, which may start at the level of stem cell development and differentiation.[7–10] Thus, knowledge concerning the behavior and molecular alterations of adipose tissue–derived stromal/stem cells (ASCs) not only contributes to the understanding of adipose tissue–related pathologies, but also could be helpful in development of stem cell–based therapies for lipedema and other types of adipose-related diseases.[11–13]

Concerning lipedema, most of the data available so far have emerged from studies using cultured human ASCs, in vitro differentiated adipocytes, biopsied subcutaneous adipose tissue, and the stromal vascular fraction from donors with and without lipedema. Taken together, it may be concluded that lipedema manifests at various levels, including alterations of the blood and lymphatic microvasculature, adipocyte hypertrophy and hyperplasia, tissue fibrosis, inflammation, and pain development,[14–20] which adds a considerable level of complexity to its pathology.

The goal of this study was to further uncover the intricacies of this disease by focusing on body mass index (BMI)–related gene expression and morphological features of ASCs and in vitro differentiated adipocytes originating from lipedema and nonlipedema subcutaneous adipose tissue. We concentrated on markers related to adipogenesis, lipolysis, carbohydrate-related metabolism, and extracellular matrix/fibrogenesis. We found that the adipogenic potential of lipedema and control ASCs in culture is not in parallel with the donors' BMI. Expression of adipogenesis/lipogenesis-related genes is significantly increased in nonobese lipedema adipocytes compared with nonobese control adipocytes. The adiponectin/leptin ratio (ALR) was significantly reduced in obese lipedema adipocytes and stress fiber–integrated smooth muscle actin (SMA) was predominantly found in adipocyte cultures from lipedema donors. It is imperative to study lipedema-associated characteristics exclusively in nonobese tissue.

METHODS

Human Tissue Sampling

Lipoaspirates from 14 anonymized lipedema donors, categorized into obese (BMI 34.3 ± 4.0; $n = 5$) and nonobese (BMI 25.4 ± 1.9; $n = 9$), as well as nonlipedema donors (BMI 25.4 ± 1.9; $n = 4$ and BMI 31.2; $n = 1$), were used for isolation of ASCs. Donors had no known comorbidities. Average age was 32 ± 9 years at time of operation.

Written informed consent was obtained from all patients. All experimental protocols concerning human adipose tissues were approved by the ethics committee of Salzburg, Austria.

Cell Isolation

Isolation of the stromal vascular fraction from lipoaspirates was performed as described previously.[8] For cell isolation, 0.1% collagenase A (Sigma-Aldrich) was dissolved in phosphate-buffered saline (PBS). Collagenase solution and lipoaspirate were mixed at equal volumes and incubated for 30 minutes at 37°C in a water bath under constant agitation. Collagenase digestion was stopped by addition of StemMACS mesenchymal stem cell expansion medium (Miltenyi Biotech). After 10 minutes of centrifugation (340 *g*), supernatant was discarded, and the pellet was resuspended in PBS and filtered through a 100-μm cell strainer (Falcon). After a second centrifugation step (340 *g* for 10 minutes), the pellet was resuspended in culture medium before seeding in culture flasks.

Cell Culture

After isolation, the passage 0 ASCs were cultured in basic medium: high-glucose (4.5 g/L D-Glucose) DMEM with glutamine (Sigma), supplemented with 10% fetal bovine serum (Sigma) and 1% antibiotic/antimycotic solution (Sigma). The ASCs were fed once per week and passaged zero to three times before use. Experiments were performed in triplicate unless otherwise stated. Cells were divided into the following categories: LipOB, cells from obese lipedema donors; LipOW, cells from overweight lipedema donors; LipNW, cells from normal-weight lipedema donors; NonLipOB, cells from obese donors without lipedema; and NonLipNW/OW, cells from nonobese donors without lipedema.

Cell Morphological Analysis

To discover a potential morphological difference between lipedema and nonlipedema cells, photographs of each of the ASC cultures were taken at confluence (after 7 to 15 days) in the

25-cm^2 flasks. Five photographs per culture flask were taken at passages 0, 1, and 2 to determine whether the morphological appearance stayed stable across passaging. Photographs were taken using a Jenoptik Gryphax microscope camera, equipped with Gryphax software.

Proliferation Assay

Cell viability/proliferation of ASCs at passage 2/3 was measured using the PrestoBlue Cell Viability Reagent (Invitrogen). A total of 3000 cells/well (9600 cells/cm^2) were seeded into 96-well plates in quadruplets using phenol red-free basic medium. After 4 days, absorbance measurements were performed according to the manufacturer's protocol, using a Multiskan GO microplate reader (Thermo Scientific).

Adipogenic Differentiation and Lipid Load

ASCs were seeded in duplicates at a density of 40,000 cells/well (4200 cells/cm^2) into six-well plates. After 24 hours, the medium was replaced with adipogenic differentiation medium [high-glucose DMEM, 1 µmol/L dexamethasone, 0.5 mmol/L 3-isobutyl-1-methylxanthine (IBMX), 10 µg/mL insulin, and 100 µmol/L indomethacin]. After 4 days, the medium was exchanged with maintenance medium (high-glucose DMEM, 10 µg/mL insulin) for another 3 days. Medium changes were repeated two more times for a total of 21 days. DMEM, dexamethasone, IBMX, insulin, and indomethacin were purchased from Sigma. Adipogenic differentiation was evaluated by staining with Oil Red O solution (ORO-OD; Sigma) as described previously.[8] The dye was then eluted with 2-propanol, and absorption of the eluate was determined photometrically at 584 nm using a U-2000 Spectrophotometer (Hitachi). DNA yield was determined in parallel using the QIAamp DNA Mini Kit (Qiagen) according to the manufacture's protocol. Lipid load was expressed as the ratio of ORO-OD and DNA yield (µg).

Total RNA Extraction and Reverse Transcription

Cells were washed with sterile PBS and homogenized in TRIzol Reagent (Thermo Fisher Scientific). RNA extraction was performed according to the manufacturer's protocol. RNA pellets were solubilized in 20 to 30 µL of nuclease-free H_2O [Integrated DNA Technology (IDT)] with 1 µL (1:20 dilution) of RNAse inhibitor (Sigma). RNA quality was measured by analyzing the 260/280 nm ratios, and the RNA yield was determined with the NanoDrop 2000c Spectrophotometer (Thermo Fisher Scientific).

Reverse transcription was performed using the iScript cDNA synthesis kit (Bio-Rad) according to the manufacturer's protocol. A total of 1 µg of RNA was used for cDNA synthesis.

Reverse Transcription Polymerase Chain Reaction Analysis/Determination of ASC Markers

Semiquantitative reverse transcription polymerase chain reaction (RT-PCR) was performed using Q5 High-Fidelity DNA Polymerase (New England Biolabs), according to the manufacturer's protocol. Expression of CD14, CD31, CD34, CD44, CD45, CD73, CD90, CD105, CD146,[21] and desmin in lipedema and nonlipedema ASCs was monitored. Gene expression levels were compared against the housekeeping genes *PUM1* and *RPLP0*, which were stably expressed across all samples. The forward and reverse primers were designed and then selected with IDT software. [**See Table, Supplemental Digital Content 1**, which lists the forward and reverse primers used for RT-PCR (purchased after design from Integrated DNA Technologies), ***http://links.lww.com/PRS/G213***.] Amplicons were visualized with the ChemiDoc MP System and analyzed with Image Lab Software (Bio-Rad).

Quantitative PCR Analysis

Quantitative PCR (qPCR) was performed using the Luna Universal Probe qPCR Master Mix (New England Biolabs). A concentration of 5 ng/µL cDNA was analyzed per well. Predesigned qPCR assays were purchased from IDT using IDT software. [**See Table, Supplemental Digital Content 2**, which lists forward and reverse primers and probes used for qPCR (predesigned and purchased from Integrated DNA Technologies), ***http://links.lww.com/PRS/G214***.]

All samples were run in duplicate, and CQ values were analyzed using qBase+ v2.4 (Biogazelle). Data were normalized to the expression of three housekeeping genes (*TBP*, *PUM1*, and *RPL0*), which were stably expressed across sample conditions.

Immunofluorescence Staining

ASCs were seeded at 90% confluence on uncoated glass coverslips. After 21 days of adipogenic differentiation, cells were washed two times with PBS and fixed for 15 minutes with 4% paraformaldehyde. Cells were then washed three times with PBS and incubated in 0.2% Triton-X for 7 minutes. Unspecific staining was blocked

with 0.1% bovine serum albumin in PBS for 30 minutes. Mouse monoclonal anti-actin α–SMA antibody (Sigma), diluted to 1:100 in PBS, was used as the primary antibody (4°C overnight). As a secondary antibody, a donkey anti-mouse immunoglobulin G (488 Alexa Fluor Plus; Invitrogen) diluted to 1:1000 was used. F-actin staining was performed using Alexa Fluor 568 Phalloidin reagent (Molecular Probes). Nuclear counterstaining was carried out by a 5-minute incubation with the nuclear stain DAPI [1:500 of 500× (50 mg/mL) DAPI stock] (Sigma). Glycergel (Dako) was used as mounting medium.

Live Cell Imaging

Visualization of cell division of nonadipogenic fibroblasts was performed using live cell imaging (LCI). ASCs were plated in 35-mm Petri dishes designed for LCI analysis (Ibidi). After 21 days of differentiation, the dishes were transferred to a Nikon BioStation IM and kept at 37°C, 5% CO_2, and 95% relative humidity. High-resolution images were captured every 15 minutes over a total time interval of 48 hours to analyze the cells in predefined optical frames, specifically for adipogenic differentiation and mitotic activity.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism software (version 9.0.0). For data with normal distribution (assessed using the Shapiro-Wilk test), because of the uneven sample variances and sizes, statistical analysis was conducted using Welch one-way analysis of variance to compare the differences between all weight and diagnosis groups. For data without normal distribution, Kruskal-Wallis tests were applied. Dunn multiple comparison tests were performed afterward to compare two groups of data within the different groups. When comparing only two groups of data, Mann-Whitney tests were performed when data were not normally distributed.

RESULTS

Differentiation Potential and BMI

The adipogenic potential of the lipedema and nonlipedema adipose tissue cultures did not increase with the donors' rising BMI. A moderate but insignificant reduction in differentiation capacity was observed in the nonlipedema group, evident by the photometric evaluation of ORO staining and determination of DNA yield. A comparably high interdonor variability was visible within all groups of lipedema and nonlipedema adipocytes tested.

LCI studies showed the presence of proliferating nonadipogenic fibroblasts undergoing mitosis in lipedema and nonlipedema adipocyte cultures after 21 days of in vitro differentiation (Fig. 1).

Because the lipedema normal-weight and overweight categories were most similar in differentiation potential (and, as seen in the next section, in morphology), in the following figures and results, those two groups are combined (represented as LipNW/OW).

Morphological Heterogeneity and Growth Characteristics

Figure 2 shows representative examples of ASCs at early passages (p0 and p1). Although all ASCs showed fibroblastic morphology, considerable heterogeneity was apparent, even within single cultures. Lipedema and nonlipedema ASCs from NW/OW donors displayed both a cobblestone-like appearance and spindle-shaped morphology, and they tended to spread uniformly over the surface of the culture flasks, rarely staying packed together in small clusters during longer periods of cultivation. In Figure 2, representative examples of cultures from lipedema donors (LipNW, LipOW) are shown. In contrast, the ASCs from obese donors not only displayed a spindle-shaped morphology but also preferred to adhere to the culture flasks in tightly packed cell-cluster islands. Figure 2 also shows a representative example of a culture from an obese lipedema donor (LipOB). With this specific growth characteristic, ASCs from obese donors could be identified easily. This growth characteristic continued throughout early passaging. Thus, we conclude that pronounced clustered formation is more dependent on the donors' BMI than on the lipedema or nonlipedema nature of the ASCs.

Gene Expression of ASC Markers

ASC markers were equally expressed in lipedema and nonlipedema ASCs at the initial passage of 0. Analysis of semiquantitative RT-PCR showed strong expression of CD44, CD73, CD90, and CD105 and only minor expression of CD14, CD31, CD34, CD45, and CD146. Desmin was absent from both lipedema and nonlipedema ASC cultures (Fig. 2).

Viability/Proliferation

Lipedema and nonlipedema ASCs showed comparable cell viability after a 4-day incubation period (Fig. 3). Although ASCs derived from obese lipedema donors showed a moderately

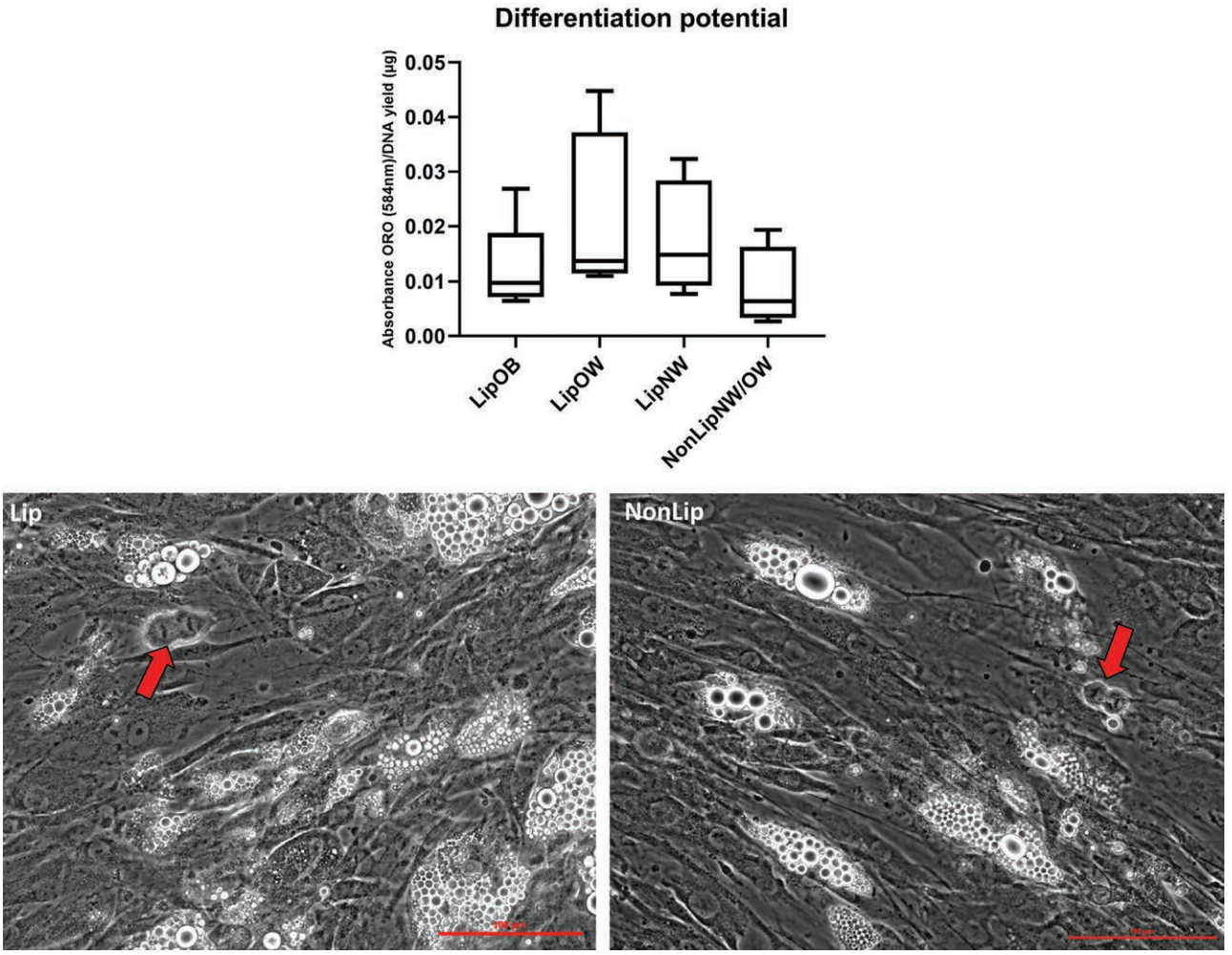

Fig. 1. (*Above*) Quantitative evaluation of the differentiation potential of lipedema and nonlipedema ASCs/adipocytes, as evidenced by Oil Red O solution (*ORO-OD*)/DNA-yield ratio. Cells were harvested from lipoaspirates derived from the lateral thighs of age-matched donors: normal-weight lipedema donors [*LipNW*; BMI, 22.2 to 24.9], $n = 5$; obese lipedema donors (*LipOB*; BMI, 30.2 to 39.9), $n = 5$; overweight lipedema donors (*LipOW*; BMI, 26.0–28.4), $n = 4$; and nonobese donors without lipedema (*NonLipNW/OW*; BMI, 23.9 to 28.6), $n = 4$. (*Below*) Live cell imaging: representative frames of lipedema (*left*) and nonlipedema (*right*) cultures. Predetermined areas were used. *Arrows* indicate fibroblastic cells undergoing mitosis (*scale bar*, 100 µm).

lower viability, this was not significant in the normally distributed data.

Gene Expression Studies (qPCR)

Relative expression of target genes is presented as box-whisker plots (created in GraphPad Prism, with data obtained from the qBase+ analyses). Medians were used to better represent the distribution of the relative gene expression in each sample group. Welch analysis of variance indicated a significant difference in the expression of three gene targets: adipogenesis-related genes (*CD36*, *PPARγ*, *FABP4*) were significantly upregulated in differentiated LipNW/OW adipocytes compared with NonLipNW/OW ($P < 0.05$ and $P < 0.01$). Furthermore, a significant suppression of *PPARγ* in LipOB compared with LipNW/OW adipocytes was found ($P < 0.05$) (Fig. 4, *above*).

Expression of SMA and connective tissue growth factor showed high interdonor variability within LipOB adipocytes. No significant alteration of SMA and connective tissue growth factor expression could be detected between LipOB/NW/OW and NonLipNW/OW adipocytes. Uncoupling protein 1 gene (*UCP1*) expression was present in all groups tested, but with considerable variability between donor groups (Fig. 4, *below*).

No significant differences could be observed concerning expression of interleukin-6 (*IL-6*), platelet-derived growth factor receptor α (*PDGFRα*), insulin receptor (*INSR*), or insulin-like growth factor receptor (*IGFR*) in differentiated

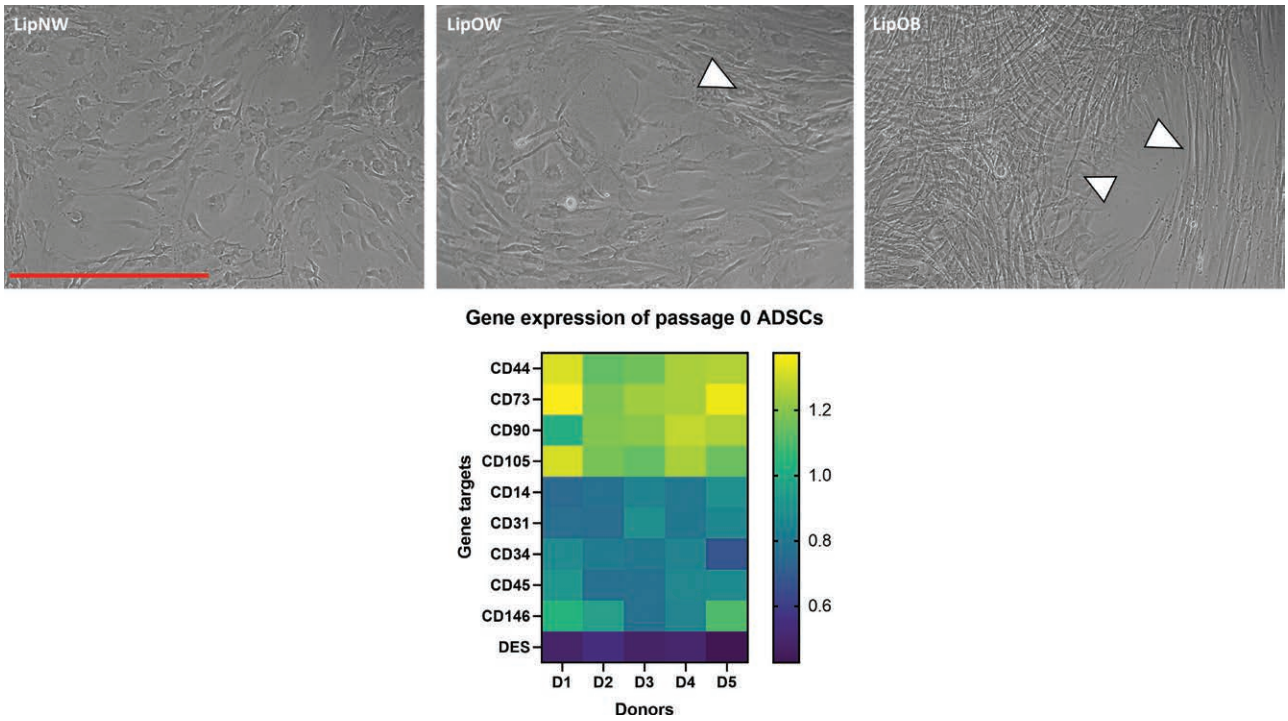

Fig. 2. (*Above*) Morphological heterogeneity and growth characteristics of lipedema and nonlipedema ASCs. BMI ranges of lipedema and nonlipedema groups were as follows: obese lipedema donors (*LipOB*), 30.2 to 39.9; overweight lipedema donors (*LipOW*), 26.0 to 28.6; and normal-weight lipedema donors (*LipNW*), 22.2 to 24.9. Representative pictures are shown. *Arrowheads* point to cell clustering. Pictures were taken with a Jenoptik Gryphax microscope camera using Gryphax software. Pictures were taken with the same magnification (*scale bar*, 500 μm). (*Below*) Expression of ASC/preadipocyte surface markers: representative examples of five donors (of all BMI categories) are shown after analysis of surface cell markers and desmin. Positive markers for ASCs are CD44, CD73, CD90, and CD105. Negative markers for ASCs are CD14, CD31, CD34, CD45, and CD146. The ASCs did not express desmin, a negative marker for myofibroblast-like cells.

lipedema and nonlipedema adipocytes,. The ASC marker CD44 was stable in the groups after differentiation (all $P > 0.05$).

Adipose triglyceride lipase (*ATGL*) was strongly expressed in LipNW/OW adipocytes, but because of the high interdonor variability, there was no significant difference compared with controls (all $P > 0.05$).

Adiponectin/Leptin Ratio

The adiponectin/leptin ratio (ALR), based on qPCR results of the target genes adiponectin (*ADIPOQ*) and leptin (*LEP*), was significantly reduced in LipOB compared with LipNW/OW adipocytes ($P < 0.01$) (Fig. 5). From BMI 22.2 up to BMI 30.1, positive ALR values were calculated (1.04 to 5.91), while a significant decrease was observed from BMI 31.1 onward (0.12 to 0.31).

Immunolocalization of SMA in Cultured Adipocytes

SMA immunoreactivity was seen in all adipocytes, either diffusely distributed in the cytoplasm of nonadipogenic fibroblasts or surrounding the lipid droplets in differentiated adipocytes (Fig. 6, *arrowheads*). SMA appeared integrated into stress fibers, as evidenced by the overlay of Phalloidin and SMA staining (merge) (Fig. 6, NonLipNW/OW, LipNW/OW, NonLipOB, LipOB; *arrows*). Increased stress fiber–associated SMA was found in adipocytes originating from obese lipedema donors (Fig. 6, LipOB).

DISCUSSION

ASCs have been shown to alter gene expression rapidly during prolonged cultivation, which strongly influences the adipogenic potential of these cells.[22] Therefore, in our experiments, only earliest passages of ASCs were used.

We have shown that the differentiation potential of lipedema and nonlipedema ASCs is not parallel with the donors' BMI. ORO-OD/DNA-yield ratio in cultures from obese donors was frequently found to be reduced compared with the ratio from cultures of nonobese donors. This is in line

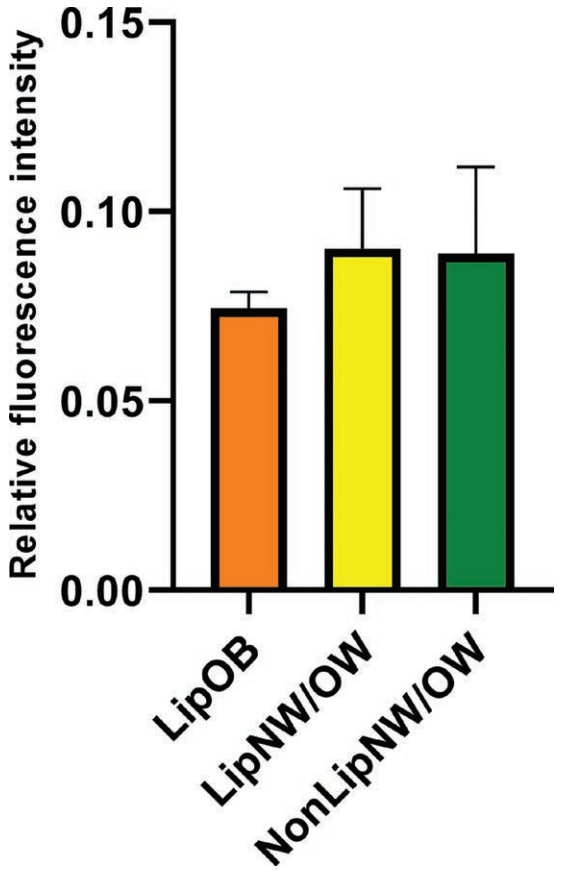

Fig. 3. Comparison of viability/proliferation ASCsfrom lipedema and nonlipedema donors with different BMIs. BMI ranges of lipedema and nonlipedema groups were as follows: obese lipedema donors (*LipOB*; $n = 4$), 31.0 to 38.2; nonobese lipedema donors (*LipNW/OW*; $n = 7$), 22.2 to 28.4; and nonobese donors without lipedema (*NonLipNW/OW*; $n = 4$), 23.9 to 28.6. Means and SDs are given.

with earlier reports showing that the differentiation capacity of human preadipocytes decreases with increasing BMI.[23,24]

The calculated differentiation potential of a given preadipocyte culture is dependent not only on the number of lipid-loaded adipogenic cells but also on the percentage of nonadipogenic fibroblasts present in the culture. The latter varies substantially among donors and does not necessarily depend on lipedema or nonlipedema state or BMI. Whereas adipogenic fibroblasts stop dividing upon differentiation, nonadipogenic fibroblasts do not cease proliferation, as demonstrated by our LCI studies. The function of nonadipogenic fibroblasts in adipogenic cultures or tissues is unclear. It cannot be excluded that these cells exhibit strongly delayed adipogenic differentiation or would differentiate along other than adipogenic pathways upon appropriate signals and stimuli.

We also have shown that the adipogenic potential of lipedema and nonlipedema preadipocytes did not differ significantly after 21 days of adipogenic induction in vitro. In an earlier report, we showed that lipedema preadipocytes show reduced lipid accumulation compared with nonlipedema control cells after 7 and 14 days of in vitro differentiation.[8] On the basis of our new results, we conclude that the adipogenic differentiation capacity of lipedema preadipocytes is capable of surpassing nonlipedema differentiation potential during prolonged periods of differentiation (>14 days). Inconsistent results concerning the differentiation potential and proliferative/metabolic activity of lipedema and nonlipedema preadipocytes have been reported.[7–10] This discrepancy of data may be attributed to different experimental setups, varying culture conditions, assays applied, and varying categories of donors in respect to weight or BMI used. Additional differences exist in the type of measurement, ranging from enzymatic assays (addressing metabolic endpoints) to true cell counting.

We also focused on growth characteristics and morphology of ASCs originating from stromal vascular fractions of lipoaspirates of donors with increasing BMI. The adipogenic potential could not be related to any of the morphology types observed. While heterogeneity in morphology was obvious in all lipedema and nonlipedema adipocyte cultures, a striking difference was observed concerning the growth characteristics of ASCs from obese donors. ASCs from obese donors were easily distinguishable by their strong formation of restricted clusters and never showed an even, homogeneous distribution of cells on the culture flask surface.

The ALR is an important biomarker that correlates with insulin resistance and other metabolic diseases better than ADIPOQ or LEP levels alone and is significantly reduced in patients with the metabolic syndrome.[25,26] Concerning the obesity-related cardiometabolic risk, an ALR of 1.0 or greater has been considered normal, a ratio of 0.5 to 1.0 indicates a moderately increased risk, and ratios less than 0.5 indicate a severe increase in cardiometabolic risk. We demonstrated that ALR values were consistently low in lipedema adipocytes from obese donors, never exceeding 0.31. This suggests that adiposity, but not lipedema per se, affects the ALR by strongly increasing LEP expression. From our results, it may also be concluded that ASCs are capable of retaining their obese or nonobese imprinting, even after several weeks of cultivation, passaging, and in vitro differentiation.

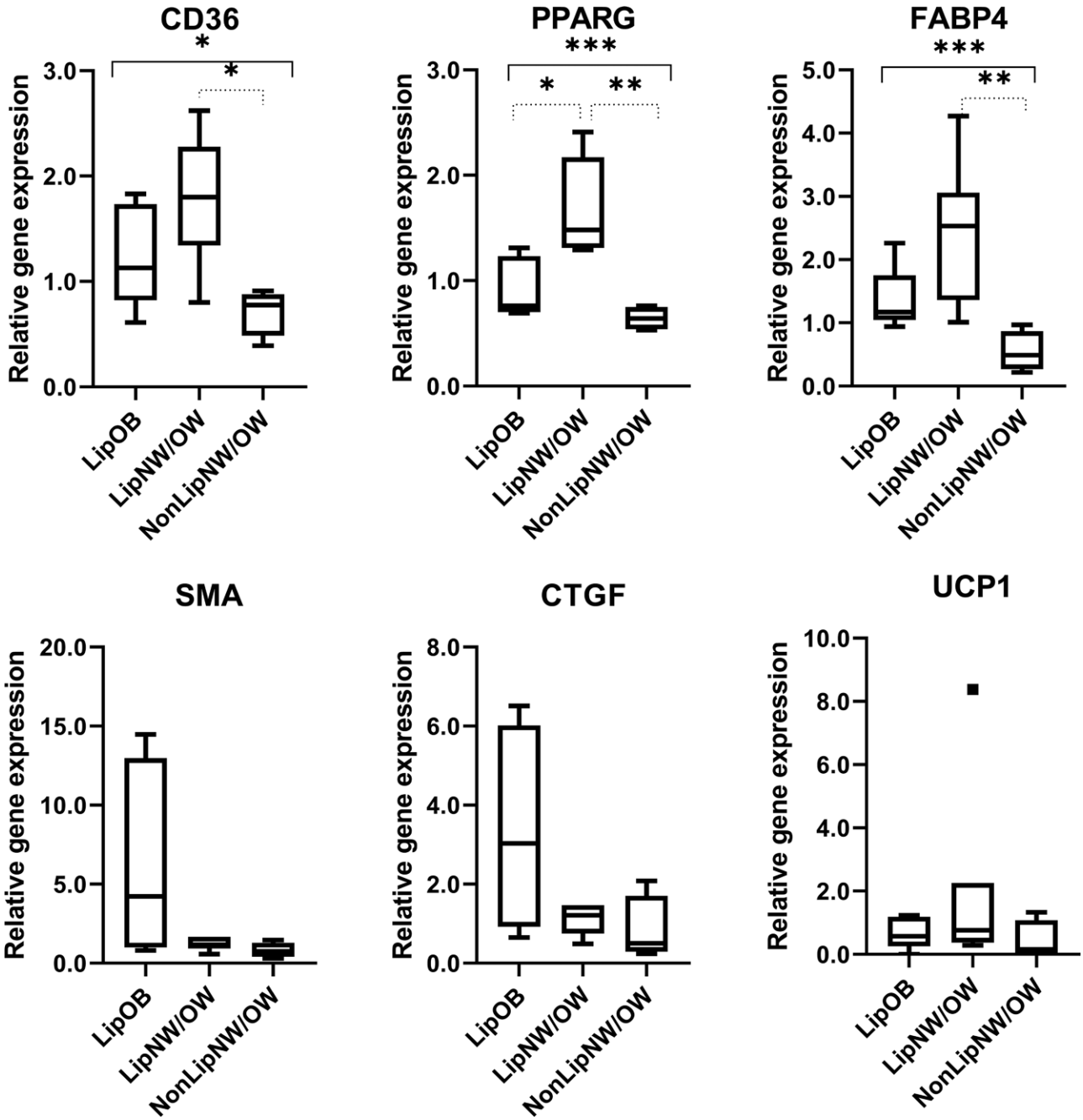

Fig. 4. Quantification of distinct gene expression in 21-day adipogenically differentiated cells from obese and nonobese lipedema and control donors. Obese lipedema donors (*LipOB*), $n = 5$; nonobese lipedema donors (*LipNW/OW*), $n = 7$; nonobese donors without lipedema (*NonLipNW/OW*), $n = 4$. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. *CD36*, cluster of differentiation 36; *CTGF*, connective tissue growth factor; *FABP4*, fatty acid binding protein 4; *PPARG*, peroxisome proliferator-activated receptor gamma; *SMA*, α-smooth muscle actin; *UCP1*, uncoupling protein 1.

We show that adipogenesis-related gene expression including *CD36*,[27] *PPARγ*,[28,29] and *FABP4*[30] was significantly upregulated in LipNW/OW adipocytes compared with BMI-matched controls. *FABP4* and *PPARγ* were also among the candidate genes found to be expressed at higher levels in lipedema stromal vascular fraction stromal cells compared with nonlipedema controls, as evidenced by a previously conducted single-cell RNA sequencing study (A. T. Lipp, D. Duscher, and M. Strunz, Technical University of Munich and Helmholtz

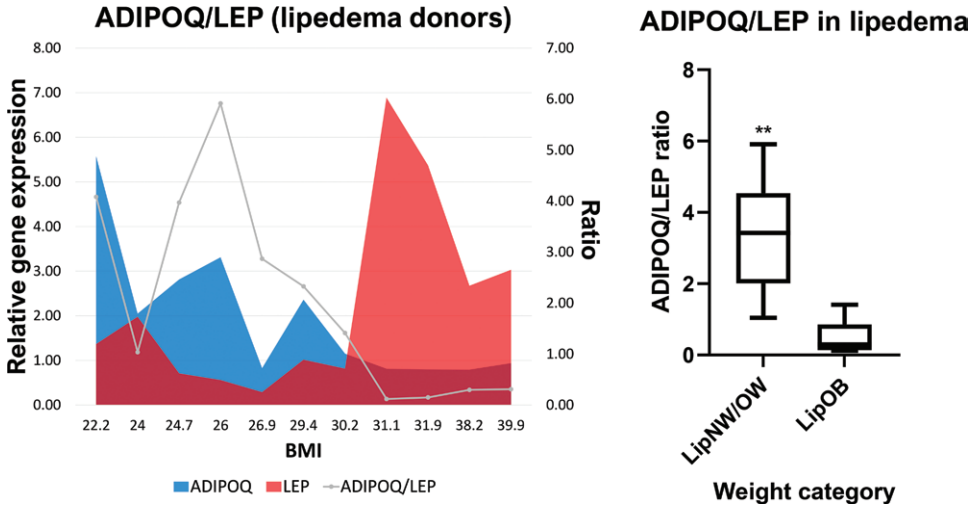

Fig. 5. (*Left*) Comparison of the adiponectin/leptin ratio (*ALR*) originating from obese lipedema donors (*LipOB*) ($n = 5$) compared with adipocytes from nonobese lipedema donors (*LipNW/OW*) ($n = 6$). The BMI range was 22.2 to 39.9. From BMI 31.1 onward, the ALR remained at a consistently low value (0.12 to 0.31). (*Right*) Mann-Whitney analysis of ALR (**$P < 0.01$). *ADIPOQ*, adiponectin; *LEP*, leptin.

Institute Munich, unpublished data). Within lipedema groups, adiposity appeared to suppress adipogenesis-related gene expression. Increased expression of *PPARγ* in lipedema adipocytes was previously reported by Al-Ghadban et al.,[9] using a two-dimensional (2D) culture model of lipedema and nonlipedema adipocytes. No such increase could be verified when three-dimensional (3D) cultures of the same cells were used for the studies.[10] It can be speculated that adipogenesis is strongly influenced by microenvironmental conditions. As has been shown recently, adipogenic gene expression levels in 3D models were higher compared with 2D models and were shown to resemble mature isogenic adipocytes more than 2D monolayer adipocytes do.[31] On the other hand, optimized 2D systems are easier to handle in daily routine laboratory work and have proven to deliver reproducible results throughout serial passaging. Nevertheless, data from preferably scaffold-free 3D models or organotypic culture systems could solidify findings from 2D studies and should be used more frequently in the future.

We also showed that *UCP1*, a marker of brown adipose tissue, is expressed in in vitro differentiated lipedema and nonlipedema adipocytes with high variability in the LipNW/OW group. So far, several lines of evidence have indicated that expression of brown adipose tissue–specific genes may be observed in human subcutaneous white adipose tissue.[32] In addition, in vitro differentiated adipocytes were shown to transiently express *UCP1*, which was attributed to the formation of cytoplasmic multilocular lipid droplets. It remains to be clarified whether *UCP1* expression in lipedema and nonlipedema adipocytes might be attributable to the occurrence of multilocular lipid droplets that inevitably form upon in vitro adipogenic induction.

Because the integration of SMA into stress fibers is, together with the absence of desmin, a major characteristic of myofibroblasts or myofibroblast-like cells,[33] we have followed intracellular localization of SMA in lipedema and nonlipedema adipocytes. We have shown that cytoplasmic SMA is diffusely distributed in nonadipogenic fibroblasts or surrounds the lipid droplets in adipocytes, which were differentiated from desmin-negative ASCs. The presence of SMA in adipose cells indicates that the cells originate from the perivascular niche (ie, from pericytic precursor cells).[34,35] Intense stress fiber–associated SMA expression was found in lipedema adipocyte cultures, particularly when originating from obese donors. Further studies are needed to discover whether the occurrence of myofibroblasts or myofibroblast-like cells accompany the progression of lipedema in advanced stages.

Taken together, our results show that lipedema and nonlipedema adipocytes differ significantly in their expression of adipogenic genes. We also demonstrated that obesity but not

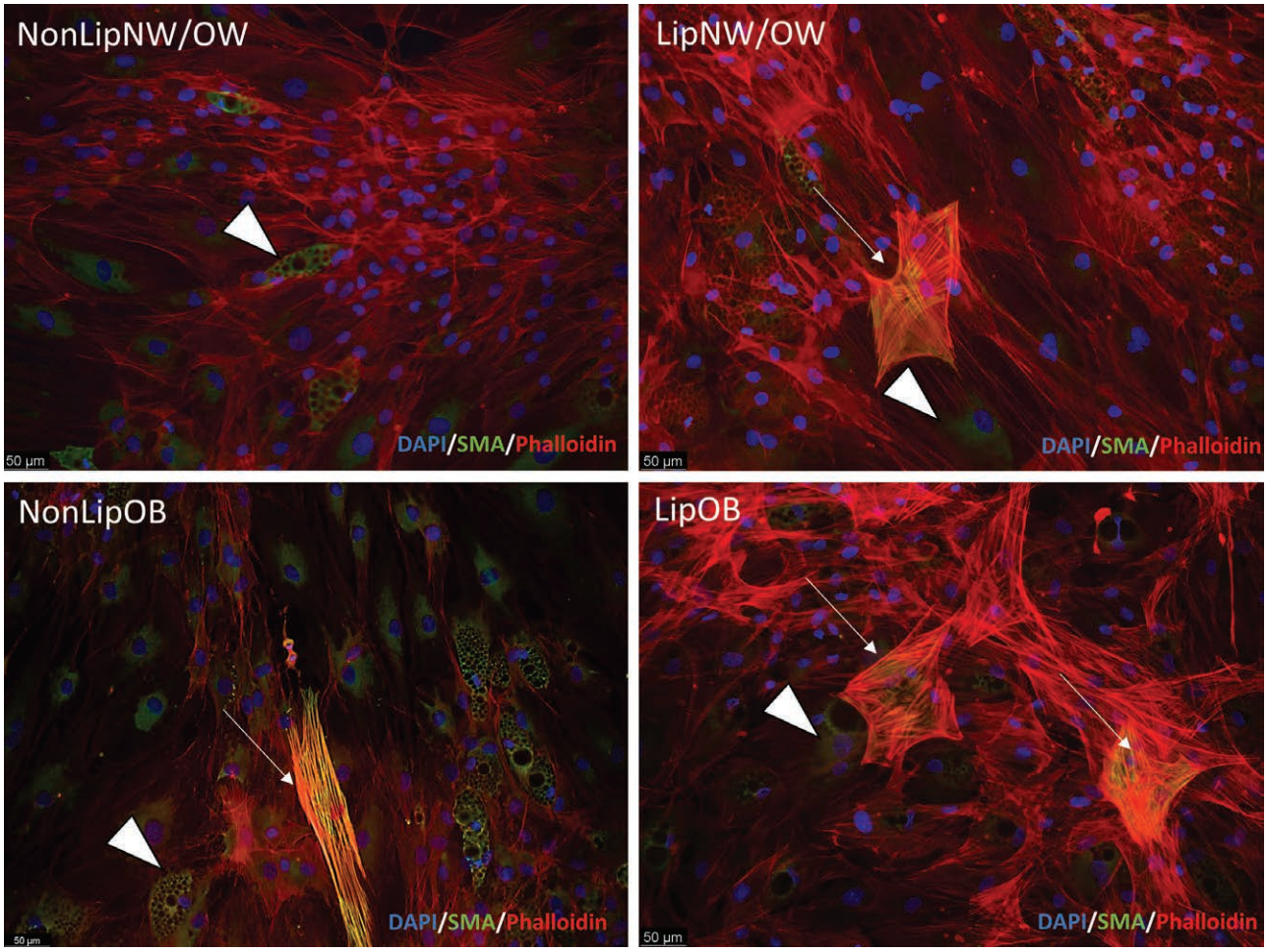

Fig. 6. Immunocytochemical staining of f-actin and SMA in cultured lipedema and nonlipedema adipocytes. The integration (colocalization) of SMA into stress fibers (f-actin) indicates the presence of myofibroblasts or myofibroblast-like cells in adipocyte cultures [nonobese donors without lipedema (*NonLipNW/OW*), nonobese lipedema donors (*LipNW/OW*), obese donors without lipedema (*NonLipOB*). Obese lipedema donors (*LipOB*), *arrows*]. The presence of SMA in adipocytes is retained in the cytoplasm (LipNW/OW, NonLipOB. LipOB, *arrowheads*). Phalloidin staining of f-actin is shown in *red*, SMA in *green*, and DAPI nuclear staining in *blue*. Representative pictures are shown (*scale bar*, 50 µm).

lipedema affects the ALR in lipedema adipose tissue. The potential role of myofibroblast-like cells in lipedema adipose tissue awaits further clarification, but would shed light on the progression of fibrosis, tension, and pain, particularly in obese patients with lipedema.

Limitations

A small number of obese donors without lipedema was used because of the difficulty in accessing age-matched donors without lipedema with no other fat distribution pathology. Another limitation of this study was the use of PCR to evaluate expression profiles rather than direct protein measurements. In the future, direct protein measurements will need to be performed on a similar patient cohort.

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DISCLOSURE

The authors have no financial interest in any of the products, devices, or drugs mentioned in this article.

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