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Supporting Information for
Leptin treatment has vasculo-protective effects in lipodystrophic mice.

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This PDF file includes:

Supporting text
Figures S1 to S2
Tables S1 to S5
Legends for Datasets S1 to S2
SI References

Supporting Information Text

Extended methods

RNA analysis.

RNA was isolated using the miRNeasy-Kit (#217004, Qiagen, USA). 1000 ng of isolated RNA was reverse-transcribed into cDNA using m-MLV Reverse Transcriptase (#28025013, Thermo Fisher Scientific, USA), 5 x First Strand Buffer (#18057018, Thermo Fisher Scientific, USA), 0,1 M DTT (# 18057018, Thermo Fisher Scientific, USA), Random Hexamers (#SO142, Thermo Fisher Scientific, USA), dNTPs (18427013, Thermo Fisher Scientific, USA), and RiboLock RNAse Inhibitor (#EO0382, Thermo Fisher Scientific, USA) for 10 min at 25 °C, 20 min at 37 °C, 5 min at 99 °C. cDNA was amplified in a qPCR using Power up SYBR Green Mastermix (#4385617, Thermo Fisher Scientific, USA) on a *Step One Plus Real-Time PCR System* (#4376600, Applied Biosystems, USA). The primer sequences used in qPCR are listed below. mRNA expression levels were normalized to RPLP0, which served as a reference gene, using the $2^{-\Delta Ct}$-method.

Table S1. Primer used for qPCR

Primer name	Primer sequence (5’ -> 3’)	Species	Company, Country
hRPLP0 - fw	TCGACAATGGCAGCATCTAC	human	Sigma-Aldrich, USA
hRPLP0 - rv	ATCCGTCTCCACAGACAAGG	human	Sigma-Aldrich, USA
hICAM1 - fw	TGATGGGCAGTCAACAGCTA	human	Sigma-Aldrich, USA
hICAM1 - rv	GGGTAAGGTTCTTGCCCACT	human	Sigma-Aldrich, USA
hE-Selectin - fw	AGTGTGACCCTGGCTTCAGT	human	Sigma-Aldrich, USA
hE-Selectin - rv	CTTCCATGCTCAGGGGATT	human	Sigma-Aldrich, USA
hSM22 - fw	AAGAATGATGGGCACTACCG	human	Sigma-Aldrich, USA
hSM22 - rv	ATGACATGCTTTCCCTCCTG	human	Sigma-Aldrich, USA
hCNN - fw	CTGGCTGCAGCTTATTGATG	human	Sigma-Aldrich, USA
hCNN - rv	CTGAGAGAGTGGATCGAGGG	human	Sigma-Aldrich, USA
hCMKLR1 - fw	CATTGGAGAGCTGCAGACAA	human	Sigma-Aldrich, USA

hCMKLR1 - rv	GTCCTCCAAAACCACAATGG	human	Sigma-Aldrich, USA
hGPR1 - fw	CACCACCCTCCATTTCATTC	human	Sigma-Aldrich, USA
hGPR1 - rv	CCTCCAAATCTTCCATGACC	human	Sigma-Aldrich, USA
hCCRL2 - fw	TGCCAGCTGATGAGACATTC	human	Sigma-Aldrich, USA
hCCRL2 - rv	ACCTCTGCTCCCTGAACCTT	human	Sigma-Aldrich, USA
hLEPR - fw	TGGAACATACAGTGCTGGCTA	human	Sigma-Aldrich, USA
hLEPR - rv	GGGGAACCAGAGGTGAATCT	human	Sigma-Aldrich, USA
hERBB4 - fw	CAGCCTCCCATCTGCACTAT	human	Sigma-Aldrich, USA
hERBB4 - rv	GCCATCCTTGAAAACTCAGC	human	Sigma-Aldrich, USA
hAdipoR1 - fw	GAAGCTGACACGGTGGAACT	human	Sigma-Aldrich, USA
hAdipoR1 - rv	GGGCATGTTTGCTCTTCTTC	human	Sigma-Aldrich, USA
hAdipoR2 - fw	ATGTTTGCCACCCCTCAGTA	human	Sigma-Aldrich, USA
hAdipoR2 - rv	TTAAGGAACCCCTCCGAGAT	human	Sigma-Aldrich, USA
hCDH13 - fw	ATACACACGCCCTGGTAAGC	human	Sigma-Aldrich, USA
hCDH13 - rv	CCCTGAATCTGTCACCATGA	human	Sigma-Aldrich, USA
hGDF15 - fw	CCTGCAGTCCGGATACTCAC	human	Sigma-Aldrich, USA
hGDF15 - rv	GAACAGAGCCCGGTGAAGG	human	Sigma-Aldrich, USA
hTGFBR2 - fw	GCCTGGTGAGACTTTCTTCA	human	Sigma-Aldrich, USA
hTGFBR2 - rv	ACAAGTCAGGATTGCTGGTGT	human	Sigma-Aldrich, USA
m36B4	AAGCGCGTCCTGGCATTGTCT	murine	Sigma-Aldrich, USA
m36B4	CCGCAGGGGCAGCAGTGGT	murine	Sigma-Aldrich, USA
GDF15	CAAGTCCTGACCCAGCTGTC	murine	Sigma-Aldrich, USA
GDF15	TCAGGGGCCTAGTGATGTCC	murine	Sigma-Aldrich, USA

Table S2. Primary Antibodies for Immunofluorescence

Antibody	Species	Concentration	Catalog No.	Company, Country
Anti-VE-Cadherin	rabbit	1:200	2500S	Cell signalling, USA
Anti-SM22	goat	1:100	ab10135	Abcam, UK

Table S3. Secondary Antibodies for Immunofluorescence

Antibody	Species	Concentration	Catalog No.	Company, Country
Anti-Goat-555	donkey	1:200	ab150130	Abcam, UK
Anti-Rabbit-488	donkey	1:200	A-21206	Thermo Fisher Scientific, USA

Cell culture of HUVECs.

Human umbilical vein endothelial cells (HUVECs) (#C12203, Lonza, Switzerland) were cultivated in EBM Plus media (#CC5036YY, Lonza, Switzerland), supplemented with 10% Fetal Calf Serum (10500064, Gibco, Thermo Fisher, USA), hydrocortisone (#CC-4547YY, Lonza, Switzerland), gentamycin + amphotericin B (#CC-4081YY, Lonza, Switzerland), ascorbic acid (#CC-4545YY, Lonza, Switzerland), recombinant human EGF (#CC-4546YY, Lonza, Switzerland), bovine brain extract (#CC-4549YY, Lonza, Switzerland,), L-glutamine (#CC-4543YY, Lonza, Switzerland,) and heparin (#CC4548YY, Lonza, Switzerland). Cells were seeded into cell culture plates 18 h prior to the experiment.

Table S4. Cell culture and differentiating media.

Media	Component	Catalog number	Company, Country
Endothelial full media (Control)	Endothelial Cell Basal Medium	CC-3121	Lonza, Switzerland
	50 ml Fetal Bovine Serum	10500064	Gibco, Thermo Fisher, USA
	Ascorbic Acid	CC-4116C	Lonza, Switzerland
	recombinant human Epidermal Growth Factor	CC-4017C	Lonza, Switzerland
	Hydrocortisone	CC-4035C	Lonza, Switzerland
	Bovine Brain Extract	CC-4092C	Lonza, Switzerland
	Gentamycin- Amphotericin B-1000	CC-4081	Lonza, Switzerland
Endothelial differentiating media	Endothelial Cell Basal Medium	CC-3121	Lonza, Switzerland
	50 ml Fetal Bovine Serum	10500064	Gibco, Thermo Fisher Scientific, USA
	Hydrocortisone	CC-4035C	Lonza, Switzerland
	Gentamycin- Amphotericin B-1000	CC-4081	Lonza, Switzerland

	Ascorbic Acid	CC-4116C	Lonza, Switzerland

Inflammation-Assay.

Prior to the experiment, HUVECs were seeded in media for 18 h. Cells were treated with IL-1β (2 ng/ml Sigma), leptin (200 ng/ml; #398-LP-01M, R&D Systems, USA) or IL-1β (2 ng/ml) and leptin for 6 h.

Endothelial-to-Mesenchymal-Transition-Assay.

4 x 10^5 HUVECs were seeded in either full or differentiation media 18 h prior to the experiment. Components of the media are listed in table S4. Cells were incubated with 10 ng/ml TGF-β2 (referred to as "EndMT", #302-B2-010, R&D Systems, USA) in differentiation media or received an additional treatment with 200 ng/ml leptin (#398-LP-01M, R&D Systems, USA). Control cells were incubated in full media. After 48 h of incubation, the treatment was repeated. After further 24 h, cells were analyzed as described.

Permeability assay.

Prior to the experiment, 2 x 10^5 HUVECs were seeded into inserts (#662610, Greiner, Austria) coated with 2 µg/ml fibronectin. After 18 h, cells were incubated with 10 ng/ml TGF-β2 (referred to as "EndMT", #302-B2-010, R&D Systems, USA) in differentiating media or received an additional treatment with 200 ng/ml leptin (#398-LP-01M, R&D Systems, USA). Full media served as control. Treatment of the cells was repeated after 24 h of incubation. After a total of 66 h, permeability was analyzed using fluorescein isothiocyanate (FITC)-dextran (#FD2000S-250MG, Sigma-Aldrich, USA), which was applied onto the cells after removal of the media. Additional thrombin-treatment (#T4648-1KU, Sigma-Aldrich, USA) served as a positive control. After 30 min of incubation, the fluorescent signal in the bottom well was measured using the *Varioskan Lux* (#VLB000D0, Thermo Scientific, USA).

Barrier function

Barrier function was assessed by resistance measurement after EndMT induction and additional leptin treatment. 96-well plates (#96W10idf, Applied BioPhysics Inc, USA) were coated with gelatin (#48723-500G-F, Sigma-Aldrich, USA) prior to experiment. 24 h after the coating, HUVECs were seeded. After 20 h, the cells were incubated with 10 ng/ml TGF-β2 with or without additional treatment with 200 ng/ml leptin in differentiating media. Full media served as a control. The treatment was repeated after additional 24 h. Monitoring of the resistance continued during the whole experiment using ECIS® ZΘ (Applied BioPhysics, Inc., USA).

For GDF15 treatment, 5.5 x 10^3 HUVECs were seeded into inserts (#83.3932.041, Sarstedt AG & Co., Germany). After 18 h, the cells were incubated with 50 ng/ml GDF15 (#957-GD-

025/CF, R&D Systems, USA) in differentiating media. Full media served as a control. The treatment was repeated after an additional 24 h. Monitoring of the impedance continued during the whole experiment using the cellZscope+ (nanoAnalytics GmbH, Germany).

siRNA transfection in HUVECs

4 x 10^5 HUVECs were seeded in full media 18 h prior to experiment. Transfection with siRNA was done using Lipofectamine™ RNAiMAX Transfection Reagent (#13778075, Thermo Fischer, USA) following the manufacturer's protocol. After 48 h of incubation the cells were either harvested or were used for further experiments.

Table S5. siRNA sequences used for transfection

Name	Sequence (5' -> 3')	Species	Company, Country
siScrG	UCUCUCACAACGGGCAUUU [dT][dT]	human	Sigma-Aldrich, USA
siGDF15	GCCAGUUCCGGGCGGCAAA [dT][dT]	human	Sigma-Aldrich, USA
siTGFBR2	GAAAUGACAUCUCGCUGUA [dT][dT]	human	Sigma-Aldrich, USA

Single Cell Sequencing

After treatment cells were harvested using trypsin (#25200-056, Gibco Life Technologies, USA). For optimal results the cells were diluted in PBS+0.04% BSA (#10010023, Thermo Fischer Scientific, USA and #15260-037, Gibco Life Technologies, USA) to an end concentration of 1200 cells/µl. Using the 10x Genomics Chromium Next GEM Single Cell 3' Reagent Kit v3.1 (#1000128, 10x Genomics, USA) the cells were transferred into oil droplets containing barcoded gel beads. Reverse transcription was performed in order to transfer the RNA into cDNA, containing cell-barcode and specific UMI (unique molecular identifier). The recovered cDNA was amplified and used for sequencing. The obtained raw data was processed to acquire count and read matrices. RStudio's package Seurat was used for downstream analysis of the matrices following the manufacturer's tutorial (satijalab.org). Possible dead cells (mitochondrial genes > 20%) were filtered out. The remaining data was normalized and scaled. For further joint analysis of different conditions (Control, EndMT and EndMT + Leptin) integration was performed using Seurat's functions FindIntegrationAnchors and IntegrateData. Seurat's function FeaturePlot was used for visualization of the average expression of *GDF15* or *SM22*.

Plasma proteomics

Plasma proteomics analysis was performed using a proximity extension assay (Olink CVD Panel III, Olink Proteomics, Uppsala, Sweden). In short, the panel includes 92 cardiovascular-

related proteins, primarily cytokines and chemokines. The assay utilizes epitope-specific binding and hybridization of a set of paired oligonucleotide antibody probes, which is subsequently, amplified using a quantitative PCR, resulting in log base-2 normalized protein expression (NPX) values**.**

Immunofluorescence of endothelial cells.

Nunc Lab-Tek Chamber Slides (#177402, Thermo Fisher Scientific, USA) were coated with 10 µg/ml Fibronectin (#PHE0023, Gibco, Thermo Fisher Scientific, USA). After 2 h, endothelial cells were seeded. After 24 h, cells were incubated with full media or received treatment with 10 ng/ml TGF-β2 or additional 200 ng/ml leptin. After additional 48 h, treatment was repeated. After a total of 96 h cells were fixated using 4% Paraformaldehyd (#P087.6, Carl Roth, Germany) for 10 min. After washing with DPBS (#17-512F, Lonza, Switzerland), cells were incubated with 0.1% Triton-X-100 (#T8787, Sigma-Aldrich, USA) for 10 min and washed again. 10% Donkey serum (#D9663, Sigma-Aldrich, USA) was used for 1 h for blocking. Cells were incubated with primary antibodies (listed in the table below) overnight. After a washing step with DBPS + 0.1% Tween (#P9416, Sigma-Aldrich, USA) cells were incubated with fluorescent secondary antibodies (listed in the table below) for 1 h. After a washing step and incubation with DAPI (#D9542-10MG, Merck, Germany) for 5 min, chambers were removed from the slide and mounted with mounting media (#S3023, DAKO, USA). The slide was stored at 4° C.

***In vitro* analysis of vascular gaps.**

After EndMT induction of endothelial cells growing on Nunc Lab-Tek Chamber Slides (#177402, Thermo Fisher Scientific, USA), cell were fixed and incubated with antibodies targeting CDH5 (1). Images of endothelial cells were acquired on a Zeiss microscope. One image of each condition from 3 individual experiments was analyzed. CDH5 gaps of the whole image were counted and the average of CDH5 gaps per square micrometer was calculated. The immunofluorescence staining was performed as described above.

Analysis of SM22 positive cells.

After EndMT induction in endothelial cells growing on Nunc Lab-Tek Chamber Slides (#177402, Thermo Fisher Scientific, USA) cell were fixed and incubated with antibodies targeting SM22 (TAGLN) (1). Images (Keyence microscope) were analyzed for expression of the mesenchymal marker *SM22*. One image of each condition from 3 individual experiments was analyzed. A distinction was made between SM22-negative, SM22-intermediate, and SM22-positive cells. The classification of SM22-negative, SM22-intermediate and SM22-positive cells was based on the intensity of the staining of the mesenchymal marker protein SM22 as well as the size of the cells. The immunofluorescence staining was performed as described above.

GDF15 treatment of cells

18 h prior to the experiment 4 x 10^5 HUVECs were seeded in full media. The cells were then incubated with 10 ng/ml TGF-β2 (#302-B2-010, R&D Systems, USA) in differentiation media as positive control of EndMT or with GDF15 (#957-GD-025/CF, R&D Systems, USA). Cells incubated in full media served as control. After 48 h of incubation, the treatment was repeated. After further 24 h, the cells were harvested using QiAzol Lysis Reagent (#1023537, Qiagen, USA) and the RNA was isolated using the miRNeasy-Kit (#217004, Qiagen, USA).

GDF15 inhibition using neutralizing antibodies in EndMT *in vitro*

18 h prior to starting the experiment, 4 x 10^5 HUVECs were seeded in control media. The cells were afterwards incubated with 10 ng/ml TGF-β2 (#302-B2-010, R&D Systems, USA) in differentiation media or received an additional treatment with 5 µg/ml GDF15 neutralizing antibodies (#MAB957-500, R&D Systems, USA). HUVECs incubated in full media were used as a control. After 48 h of incubation, the treatment was repeated. After further 24 h, the cells were harvested using QiAzol Lysis Reagent (#1023537, Qiagen, USA) and the RNA was isolated using the miRNeasy-Kit (#217004, Qiagen, USA).

Expression of adipokine receptors in a cell panel

RT-qPCR was performed using cDNA of different cell types (HUVEC = human umbilical vein endothelial cells, HEK = human embryonic kidney cells, HCAEC = human coronary artery endothelial cells, SMC = smooth muscle cells, HCM = human cardiomyocytes, HMVEC = human microvascular endothelial cells, PBMC = peripheral blood mononuclear cells) and adipokine receptor primers (listed the SYBR Green PCR primer table above). qPCR products were applied on an agarose gel.

Expression of adipokine receptors in a tissue panel

RT-qPCR was performed using cDNA of different tissue (R1234138-50) were purchased from Amsbio LLC, UK, quantified with the Denovix DS-11 spectrophotometer and 1 µg was transcribed into cDNA as described above. Generated cDNA was amplified with types and adipokine receptor primers (listed the SYBR Green PCR primer table above). qPCR products were applied on an agarose gel.

Animal care and animal experiments

Mouse breeding and experiments were performed in the Medical Experimental Center, University of Leipzig in compliance with animal welfare regulations. Animal protocols were approved by the local ethics committee (approval no. TVV37/12, 06/11/2012 and TVV 27/16,

22/09/2016). All mice were on a C57Bl/6 and low-density lipoprotein receptor knockout (*Ldlr*$^{-/-}$) background. They were maintained in a room under pathogen-free conditions with controlled 21 ± 1°C on a 12:12 h light/dark cycle (6 a.m./6 p.m.) and fed *ad libitum* with a modified, cholesterol (Chol)-enriched semisynthetic Clinton/Cybulsky diet (Sniff, Soest, Germany) starting at the age of 4 weeks. Eight-week-old male *Ldlr*$^{-/-}$; aP2-n*Srebp1c*-Tg mice, a transgenic murine model for congenital generalized LD were randomized into two groups, respectively (20). A dose of 3.0 mg/kg BW/d, leptin was shown to have physiological effects on body weight (12). Therefore, the groups were treated daily intraperitoneally with recombinant leptin (3.0 mg/kg body weight (BW); R&D Systems, Wiesbaden-Nordenstadt, Germany) or saline for 8 or 12 weeks, respectively. At 3.0 mg/kg BW/d, leptin has physiological effects, i.e., this dose is sufficient to normalize BW in male leptin-deficient *ob*/*ob* mice on the same background. Treatment was performed in the morning to mimic the dosing regimen used in LD patients. Non-LD littermates on a *Ldlr*$^{-/-}$ background served as control mice (12). At the end of the treatment period (at the total age of 16 weeks or 20 weeks for macrophage accumulation analysis), mice were fasted overnight and the last saline or leptin injection was performed 30 min before sacrifice. Cardiac blood was collected in tubes containing EDTA and plasma was separated by centrifugation. Intracapsular brown adipose tissue (iBAT), visceral adipose tissue (vAT), subcutaneous adipose tissue (sAT) were weighted and snap frozen. For metabolic characterization, cholesterol, triglyceride and transaminases were analyzed by standard laboratory methods in a certified laboratory. Plasma glucose was measured manually. Food intake is shown as the average over the entire treatment period in g/kg mouse/day. Quantification of food intake was done on a weekly basis.

Immunohistochemistry and atherosclerotic lesion quantification

10 µm cryosections were obtained of aortic roots and BCA. Lipids were detected with Oil- Red-O staining. For morphometric analysis of plaques, we performed modified Russell-Movat's staining (Movat Pentachrome Stain Kit, ScyTek Laboratories) on serial sections. Plaque area and vessel lumen were measured on images of the Movat's staining using Image J software. Picrosirius Red staining was performed to assess collagen and CD68 staining for macrophage content. At least three images from each of at least two spatially separated aortic root sections per mouse were evaluated. Data were averaged per animal and then used for statistical analyses. For immunofluorescence staining of endothelial and mesenchymal cells, thawed slides were fixed in acetone/methanol 1:1 for 5 min. After washing in PBS, the slides were blocked using 5 % donkey serum. Primary antibodies were applied overnight at 4 °C as follows: Anti-SM22 (#ab14106, Abcam, 1:100), Anti-CD31 (#AF3628, R&D Systems, 1:100), Anti-LepR (#ab216690, Abcam, 1:200). Slides were then washed and incubated with secondary antibodies (#ab6949: Donkey anti-goat IgG H&L (Cy3), Abcam; #A-21206: Anti-rabbit – 488

donkey, Thermo Fisher) for 1 h at room temperature. Dapi (1:200) was applied for 5 min. Sections were embedded in mounting media (#S3023, Anti Fade Dako) and examined using Keyence microscope. Images were analyzed by one member of our laboratory in a blinded manner using Image J software. In order to quantify the extent of EndMT, at least three images from each of at least two spatially separated aortic root sections per mouse at each time-point were evaluated. Data were averaged per animal and then used for statistical analyses. Plaque protrusion was defined as total plaque area per length of plaque-covered inner vessel wall determined in sections of vessel cross-section (Plaque protrusion= Plaque area (μm^2) / plaque covered vessel circumference (µm)).

Spatial transcriptomic

10 µm sections of OCT-embedded BCA of WT, Control, LD and LD mice treated with Leptin were placed within the capture area frames of a Visium gene expression slide (10x Genomics, #1000187). Tissue sections were fixed and stained according to the manufacturer's instructions for methanol fixation and HE staining. Stained tissues were imaged via a Keyence microscope (BZ-X800 series). Permeabilization duration was determined with a Visium tissue optimization slide (10x Genomics, #1000193). Tissue sections were permeabilized, cDNA was then synthesized and libraries were constructed according to the manufacturer's instructions for Visium spatial gene expression. Libraries were sequenced on a NovaSeq 6000 with an average sequencing depth of 54 M reads/ sample. Using Space Ranger, sequencing results were demultiplexed, converted to fastq format and aligned to the murine reference genome (mm10 Reference - 2020-A (June 23, 2020)) as well as the slide layout GPR file and the input slide image. Analysis of the Space Ranger outputs was done in Rstudio (R version 4.1.2). For clustering, dimensional reduction and enhancing the resolution of spatial gene expression we used the statistical tool BayesSpace (2020, Edward Zhao, bioRxiv), enhancing the resolution of the low-dimensional representation into "sub-spots", for which gene was imputed. Plaques areas were automatically identified by clustering and the expression values for genes were extracted.

GDF15 inhibition using neutralizing antibodies in EndMT *ex vivo*

4 x 10^5 HUVECs were seeded in full media 18 h prior to the experiment. Cells were then incubated with 500 µl serum from LD patients in differentiation media or received an additional treatment with 5 µg/ml GDF15-antibody (#MAB957-500, R&D Systems, USA). Cells incubated in full media served as a control, and cells incubated with 10 ng/ml TGF-β2 (#302-B2-010, R&D Systems, USA) in differentiation media as positive control for EndMT. After 48 h of incubation, the treatment was repeated. After further 24 h, the cells were harvested using QiAzol Lysis Reagent (#1023537, Qiagen, USA) and the RNA was isolated using the miRNeasy-Kit (#217004, Qiagen, USA).

Patients with lipodystrophy syndromes and control group

The LD cohort consisted of patients with non-human immunodeficiency virus-associated LD. LD was diagnosed according to Brown et al (2). Healthy control subjects without LD, diabetes mellitus and hypertriglyceridemia were matched based on age, gender and BMI. Recruitment of patients and controls was conducted at the Leipzig Lipodystrophy Centre at the Division of Endocrinology of the University of Leipzig. Patients gave informed consent for participation in the study. Blood samples were taken in the morning after a fasting period of at least 8 hours. Commercial Enzyme-linked immunoassays were used to measure concentrations of leptin and adiponectin (Mediagnost (Reutlingen, Germany)) as well as of GDF15 (R&D Systems (Minneapolis, MN)). Standard methods were carried out in a certified laboratory to measure routine laboratory parameters. HOMA-IR and eGFR were calculated according to Matthews et al. (3) and Levey et al. (4), respectively. The study was done in accordance with the declaration of Helsinki and approved by the Leipzig University Ethics Committee (135/13-ek, 08/07/2013).

Treatment of LD patients with recombinant leptin and leptin measurment

Sixteen patients (13 female / 3 male) with LD (14 partial LD / 2 generalized LD) were characterized before and at 1 week, 4 weeks of metreleptin treatment. Therapy with metrelepine (i.e. Amylin [San Diego, CA] / Bristol-Myers- Squibb [Munich, Germany] / Astra Zeneca [London, UK] / Aegerion [Cambridge, MA] was initiated if the inclusion criteria were met. The medication was provided by the manufacturer and application was carried out within the framework of a "compassionate use" program. In detail inclusion criteria included confirmed LD, insufficiently controlled diabetes mellitus and / or hypertriglyceridemia despite adequate antihyperglycemic and / or lipid-lowering treatment, age ≥ 5 years at baseline, and eGFR > 40 ml/min. Exclusion criteria were HIV infection infectious liver disease, primary hematologic abnormalities, active malignant tumor, pregnancy or lactation. The first 3 patients were treated subcutaneously with metreleptin at 0.04 mg/kg body weight for the first week and thereafter at 0.08 mg/kg body weight. After that dosing instructions changed. Therefore, in subjects 4-16 metreleptin was administered subcutaneously at 2.5 mg metreleptin per day in male patients and at 5 mg per day in women. From second week onwards dosage ranged from 2.5 to 7.8 mg per day (5). Data are presented as median and interquartile range (6).

Metreleptin was given subcutaneously one (in patient no. 4-16) or two (in patient no. 1-3) times a day. Blood was drawn in the morning right before the next injection of metreleptin. Thus, leptin thorough levels were measured. In pharmacokinetic analyses peak leptin serum concentration after subcutaneous injection of single metreleptin doses of 0.1 to 0.3 mg/kg bodyweight were reached after approx. 4 hours (7). However, we did not measure peak serum leptin levels after subcutaneous metreleptin application. Furthermore, Chan et al. showed that

leptin returned to levels similar to baseline values by 24 h after 0.01 mg/kg metreleptin in lean and obese men but was higher than baseline after 0.3 mg/kg metreleptin in lean and obese men (7). In our LD cohort, the first three patients (all female) administered metreleptin subcutaneously for the first week at 0.04 mg/kg body weight and thereafter at 0.08 mg/kg body weight. Since dosing instructions changed, patients 4 to 16 received 2.5 mg metreleptin per day (men) and 5 mg metreleptin per day (women). Applicated metreleptin doses in our LD cohort ranged between 0.02 mg/kg bodyweight per day (men) to 0.08 mg/kg bodyweight per day (8). The study was done in accordance with the declaration of Helsinki and approved by the Leipzig University Ethics Committee (135/13-ek, 08/07/2013).

Association analysis of plasma GDF15 and sub-clinical cardiovascular traits

Association analysis of plasma GDF15 and sub-clinical cardiovascular traits were performed in LIFE-Adult – a population-based study of 10,000 European residents of the city of Leipzig, Germany. Details of the study can be found elsewhere (9). LIFE-Adult meets the ethical standards of the Declaration of Helsinki and is approved by the Ethics Committee of the Medical Faculty of the University Leipzig, Germany (Reg. No 263-2009-14122009). Written informed consent including agreement with genetic analyses was obtained from all participants. *GDF15* was measured in plasma of 2,014 elderly participants using a proximity extension assay (Olink CVD Panel III, Olink Proteomics, Uppsala, Sweden). These participants comprised 1040 males / 974 females with a mean age of 62 years (SD=11 years). Carotid ultrasound and plaque assessment is described in detail in (10). ABI and baPWV assessment was performed using Vicorder device (Skidmore medical, Bristol, UK) as described in (11).

Enzyme-Linked Immunosorbent Assay

Plasma concentrations of GDF15 (MGD150, Mouse/Rat GDF15 Quantikine ELISA Kit R and D Systems) and leptin (#RD291001200R, Leptin Mouse/Rat ELISA, Biovendor) were measured by commercial ELISA systems according to the manufacturers' instructions.

S1

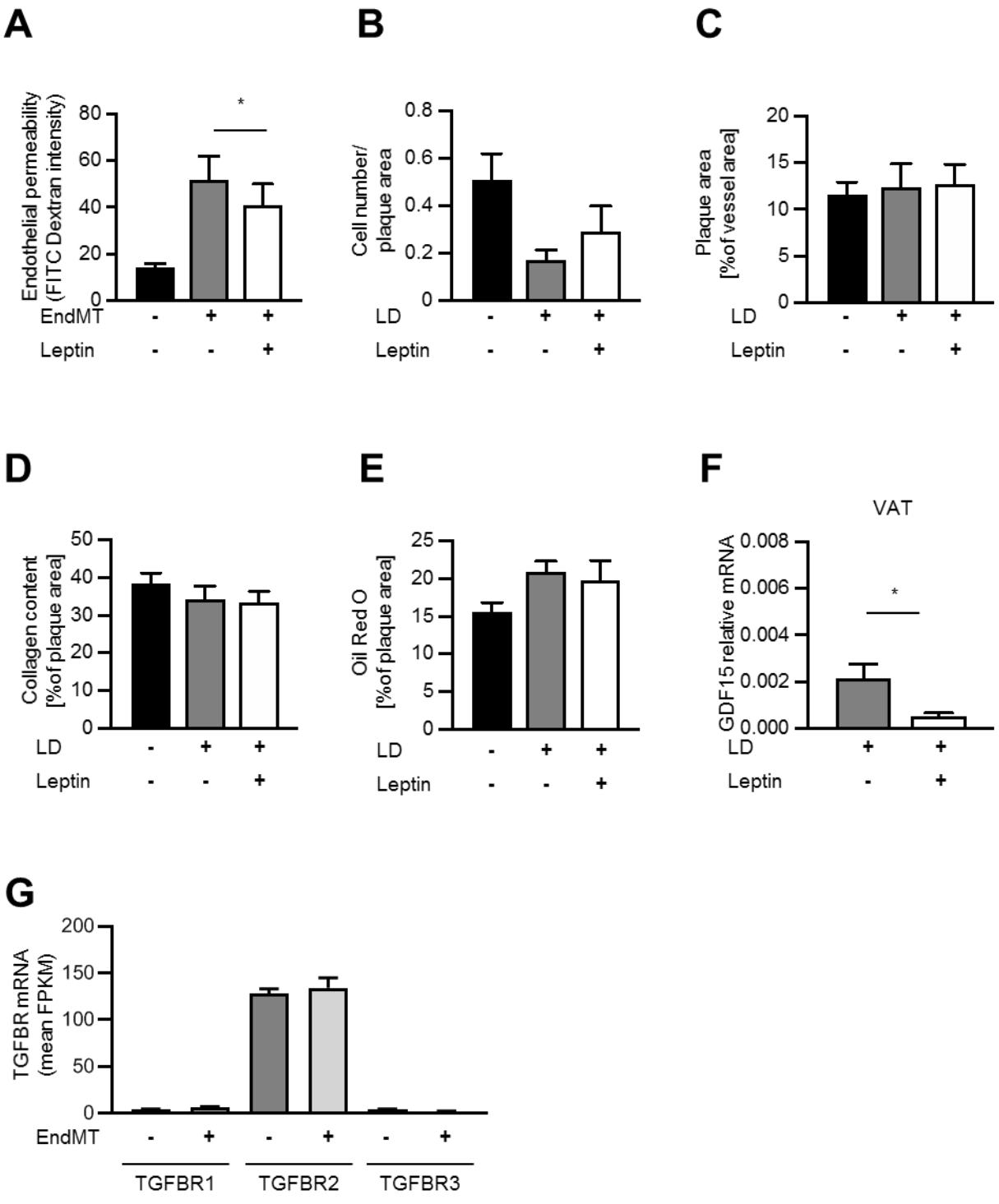
A
Endothelial permeability (FITC Dextran intensity)
80
60
40
20
0
*
EndMT - + +
Leptin - - +
B
Cell number/ plaque area
0.8
0.6
0.4
0.2
0
LD - + +
Leptin - - +
C
Plaque area [%of vessel area]
20
15
10
5
0
LD - + +
Leptin - - +
D
Collagen content [%of plaque area]
50
40
30
20
10
0
LD - + +
Leptin - - +
E
Oil Red O [%of plaque area]
25
20
15
10
5
0
LD - + +
Leptin - - +
F
VAT
GDF15 relative mRNA
0.008
0.006
0.004
0.002
0.000
*
LD + +
Leptin - +
G
TGFBR mRNA (mean FPKM)
200
150
100
50
0
EndMT - + - + - +
TGFBR1
TGFBR2
TGFBR3

Fig. S1: Leptin treatment reduces endothelial permeability. (*A*) Permeability as vascular leakage after EndMT with additional treatment with leptin (n = 3) (*P < 0.05) Student's t-test (EndMT vs. EndMT / leptin). (*B*) Number of cells per atherosclerotic plaque (n = 4-6). At least three images from each of at least two spatially separated aortic root sections per mouse were evaluated. Data were averaged per animal and then used for statistical analyses. Statistical analysis were done using ANOVA with Tukey post hoc test (*P < 0.05). Data are depicted as mean ± SEM. *(C*) The percentage of lesion area relative to whole vessel area was determined by modified Russel's Movat Pentachrome staining on aortic roots of control mice and LD mice with or without leptin treatment (n = 4-5). (*D*) Sirius Red staining was conducted for quantification of collagen content per lesion area (n = 4-6). (*D*) Quantification of lipid content per plaque area was implemented in Oil-Red-O staining (n = 5-6). (*E*) Relative mRNA expression of *GDF15* in visceral adipose tissue of LD mice treated with or without leptin (n = 3 per group) determined by qPCR, normalized to *m36b4* mRNA ($2^{-\Delta Ct}$). (*F*) mRNA levels of TGFB receptors (*TGFBR1-3*) in HUVECs after EndMT using RNA sequencing (n = 3). Data presented are mean FPKM ± SEM

S2

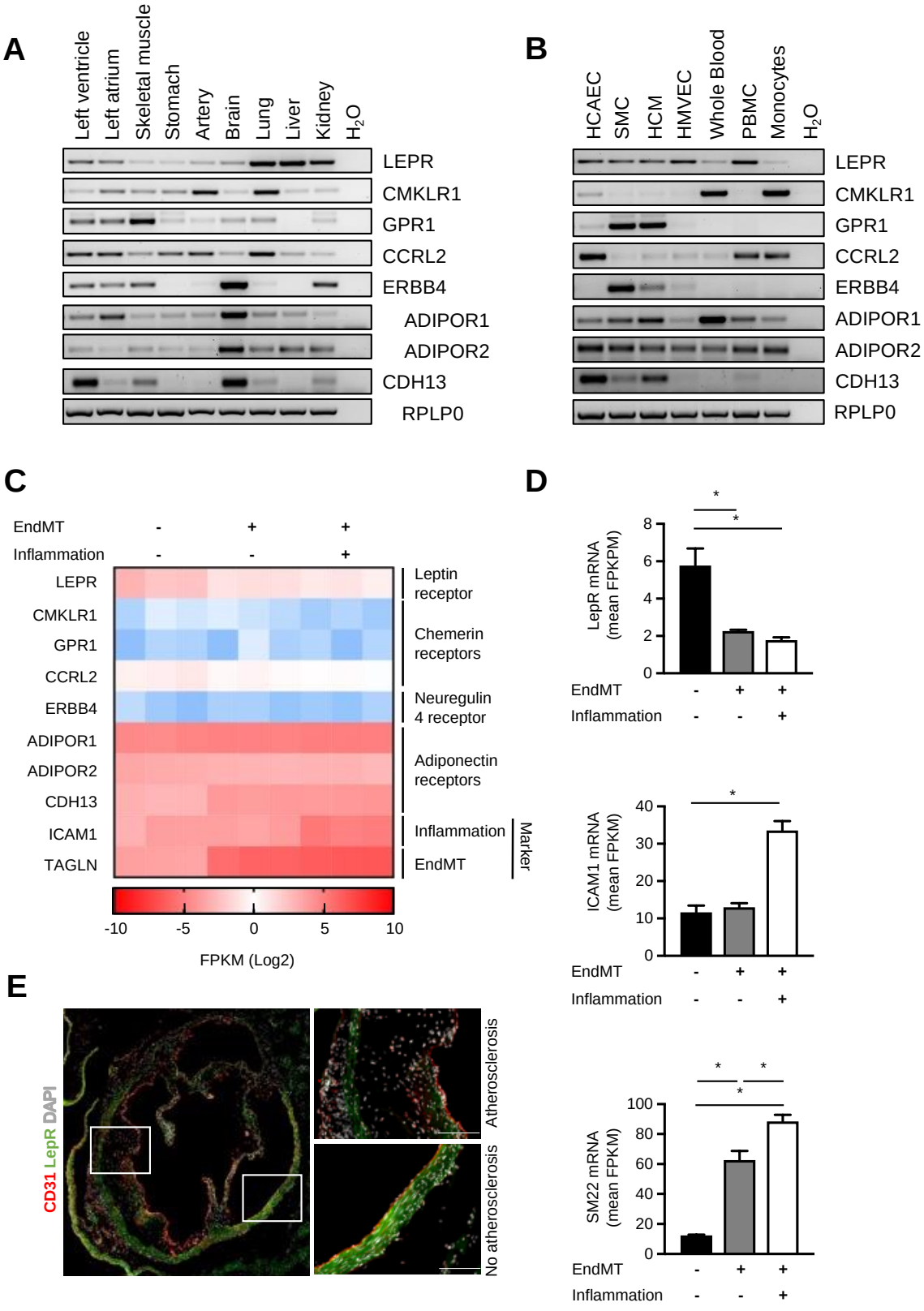
A
Left ventricle
Left atrium
Skeletal muscle
Stomach
Artery
Brain
Lung
Liver
Kidney
H2O
LEPR
CMKLR1
GPR1
CCRL2
ERBB4
ADIPOR1
ADIPOR2
CDH13
RPLP0
B
HCAEC
SMC
HCM
HMVEC
Whole Blood
PBMC
Monocytes
H2O
LEPR
CMKLR1
GPR1
CCRL2
ERBB4
ADIPOR1
ADIPOR2
CDH13
RPLP0
C
EndMT - + +
Inflammation - - +
LEPR
CMKLR1
GPR1
CCRL2
ERBB4
ADIPOR1
ADIPOR2
CDH13
ICAM1
TAGLN
Leptin receptor
Chemerin receptors
Neuregulin 4 receptor
Adiponectin receptors
Inflammation
EndMT
Marker
-10 -5 0 5 10
FPKM (Log2)
D
LepR mRNA (mean FPKM)
0 2 4 6 8
EndMT - + +
Inflammation - - +
ICAM1 mRNA (mean FPKM)
0 10 20 30 40
EndMT - + +
Inflammation - - +
SM22 mRNA (mean FPKM)
0 20 40 60 80 100
EndMT - + +
Inflammation - - +
E
CD31 LepR DAPI
Atherosclerosis
No atherosclerosis

Fig S2: The leptin receptor is reduced by inflammation and EndMT. (*A*) Expression of leptin and other adipokine receptors in human tissues analyzed on mRNA level by RT-PCR. (*B*) Expression of adipokine receptors in human cardiovascular cell types analyzed on mRNA level by RT-PCR. HUVEC = human umbilical vein endothelial cells, HEK = human embryonic kidney cells, HCAEC = human coronary artery endothelial cells, SMC = smooth muscle cells, CM = cardiomyocytes, HMVEC = human microvascular endothelial cells, PBMC = Peripheral Blood Mononuclear cells. (*C*) Heatmap of mRNA expression of adipokine receptors in human endothelial cells after inflammation (IL1-β-stimulation) or in combination with EndMT (TGF-β2-stimulation) using RNA sequencing (n =4). (*D*) mRNA levels of leptin receptor (*LepR*), *ICAM1* as inflammatory marker and *SM22* as EndMT marker in HUVECs after inflammation or inflammation + EndMT using RNA sequencing (n = 3). Data presented are means ± SEM. (*E*) Leptin receptor immunofluorescence staining of aortic roots of LD mice. Endothelial cell marker CD31 appears in red and LepR in green, DAPI in grey. Statistical analyses were done using ANOVA with Dunnett post hoc test (*P < 0.05).

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