1

1. Supplementary Methods

Systemic and AT inflammatory status

Adipose Tissue Samples

Biopsies were immediately transferred into sterile vials and cooled on ice until further processing under sterile laboratory conditions. Aliquots of approximately 100 mg each were instantly snap-frozen in liquid nitrogen and transferred to long-term storage at -80 °C. RNA extraction was performed using RNeasy Lipid Tissue Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions and RNA was transcribed into cDNA using SuperScript III (ThermoFisher Scientific, Waltham, MA). DNA was isolated using DNeasy Tissue Mini Kit (Qiagen), and nucleic acid concentrations were measured on a Quantus Fluorometer (Promega, Madison, WI).

Isolation of adipocytes/ SVF

Samples were digested with collagenase I, which was diluted in HBSS buffer containing 2% BSA and 1% Penicillin/Streptomycin. Tissues were cut within the buffer on ice and transferred to gentleMACS C tubes, and run on the gentleMACS Octo dissociator for 40 minutes at 37° C. Suspension was sifted over a cell strainer to separate SVF from adipocytes (Greiner, # 542070), and SVF fraction was washed with HBSS. After centrifugation (10 minutes, 400 g, room temperature), supernatant was removed carefully and pellet was resuspended in ery-lysis buffer for 7 minutes. After a second centrifugation SVF was frozen in 500 µl growth medium (DMEM with 4.5g/l glucose, 1%Penicillin/Strptomycin, 10% FCS, 0.05 mM ascorbic acid) and 50 µl DMSO.

Blood chemistry

Blood cell counts and routine metabolic markers including glucose, insulin, kidney function and lipids were measured at the central laboratory unit of the hospital using routine methods.

High-sensitivity C-reactive protein (hsCRP) was quantified using an Image Automatic Immunoassay System (Beckman Coulter, Brea, CA) according to the manufacturer's manual. Tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6) were measured by high-sensitive ELISA (R&D Systems, Minneapolis, MN; HSTA00E and HS600B) using undiluted serum, and lipopolysaccharide-binding protein (LBP) was measured by ELISA (Hycult Biotech, Uden, Netherlands, #HK315-02) using diluted plasma (1:1000) according to the manufacturer's protocol. In addition, circulating adipokines and apolipoproteins were measured by mass spectrometry as described previously[1] .

Immunohistochemistry

Sample aliquots for immunohistochemistry were incubated in 4 % paraformaldehyde in PBS for 24 h and embedded in paraffin. Slides of 6 µm each were used and treated with DAKO retrieval solution (pH=9, Agilent, Santa Clara, CA) for 30 minutes by applying hot steam. Adipocytes were stained with anti-perilipin-1 (goat, 1:200, Abcam, Cambridge, UK, #ab61682) and macrophages with anti-Iba1 (rabbit, 1:500, Fujifilm WAKO, Japan, #019-19741) at 4 °C overnight. Supervised automated analysis was performed using CellSens software (OLYMPUS Life Science, Shinjuku, Japan) to assess adipocyte counts and diameter as well as counts of macrophages.

MRI-scan based quantification approach

Image-based quantification of visceral and subcutaneouse adipose tissue (VAT and SAT) by MRI (magnetic resonance imaging) or CT (computed tomography) is a well-established method [2, 3]. The differentiation of adipose tissue compartments in imaging depends on visible anatomic bordes, such as muscle, peritoneum, or fascia, that serve as outer limitations for volumetry. In our MRI-based approach to quantify the epiploic adipose tissue volume we found the peritoneal covering too thin to be resolved with imaging. Hence a separation of the

epiploic adipose tissue from other VAT compartments – and by that its quantification - was not feasible (see Supplementary Figure S1C). Findings are in line with previous description based on CT-images [4].

Sample Preparation

Methylation studies

To assess the DNA methylation status of CpG sites, the human Illumina Infinium Methylation EPIC array was used according to the manufacturer's instructions (Illumina, San Diego, USA). 250 ng of DNA were bisulfite-converted using the EZ-96 DNA methylation kit (Zymo Research, Irvine, CA). After isothermal amplification and fragmentation, DNA was hybridized to the array at 48°C for 16 hours followed by washing, staining, and scanning using the Illumina HiScan system according to the manufacturer's specifications.

Transcriptomics

First, RNA integrity and concentration were examined on an Agilent Fragment Analyzer (Agilent Technologies, Palo Alto, CA) using the RNA Kit (Agilent Technologies) according to the manufacturer's instructions. 250 ng of total RNA per sample were ethanol-precipitated with GlycoBlue (Invitrogen) as a carrier and dissolved to a concentration of 100-150 ng/µl prior to probe synthesis using the TargetAmp™-Nano Labeling Kit for Illumina Expression BeadChip (Epicentre Biotechnologies, Madison, WI). 750 ng of cRNA were hybridized to Human HT-12 v4 Expression BeadChips (Illumina, San Diego, CA) and scanned using the Illumina HiScan instrument according to the manufacturer's specifications.

Proteomics

30 µg protein of each sample were precipitated using four volumes of ice-cold acetone. The mixtures were incubated at -20 °C overnight, then centrifuged at 4 °C for 10 min at 16000 × g,

and the protein pellet was dried under vacuum. Samples were reconstituted in Laemmli sample buffer and incubated at 95 °C for 5 min at 1000 rpm, followed by 10 min of sonication. Protein separation was performed by 1-D SDS-PAGE with 4% stacking gel and 12% separation gel (Biostep, Burkhardtsdorf, Germany). Gels were stained with Coomassie Brilliant Blue R-250 dye (Thermo Fisher Scientific, MA). In-gel proteolytic cleavage was conducted as described previously using trypsin and samples were reconstituted in formic acid (0.1%, v/v) [5].

Data analysis

Methylation analysis

Processing of methylation data included removing of all probes with low quality (detP>0.01, in more than 1 data set) and batch correction with R's sva package [6]. Differentially methylated regions (DMR) analysis in batch corrected betas was performed using metilene with minimum number of CpGs set to 3 and maximum distance to 1000 [7]·[8]. Betas were also assigned to genes by overlapping with the gencode version 32 mapped to GRCh37 annotation of the human genome [8]. All CpGs 1500 bp upstream and 500 bp downstream of the transcription start site were used as a promoter, all other probes intersecting the gene as gene body probes. DMR finding using metilene (minimum number of CpGs again 3) was performed on these CpGs, using the genes as pseudo chromosomes, and numbering the CpGs consecutively as pseudo positions. To be able to easily distinguish between promoter and gene body, 100 was added to the gene body positions. Heatmaps were created in R using the corrected Beta values. For IS vs. IR computations, two female and two male datasets for each were chosen to minimize sex effects.

Micro array analysis

Analysis of BeadChip data was performed within the R and differentially expressed genes were identified with limma v3.40.6 [9]. Transcriptome-based pathway analysis was performed

using the GSEA software [10] and the KEGG pathway database [11]. Expression of genes measured by qPCR was normalized to the expression of *GAPDH* and *B2M*, which were determined as optimal housekeeping genes after extensive testing [12]. Corrected expression results were calculated using the $\Delta\Delta C_t$ method [13].

Proteome analyses

Proteomics data were log2-transformed, and proteins were filtered for those, which were quantified in at least five subjects (Supplementary Table 4) before the calculation of average fold changes (Supplementary Table 5). For these steps and subsequent visualization of the results, several packages were used: readxl [14], qpcR [15], plyr [16], splitstackshape [17], tidyr [14], calibrate [18], circlize [19], gplots [20], ggplot2 [21], and ComplexHeatmap [22]. To address multiple testing errors, all p values were adjusted by Benjamini & Hochberg, and an adjusted $p< 0.05$ was considered significant.

Principal component analyses (PCAs) of the proteomes and transcriptomes were conducted using the package mixOmics [23]. To determine outliers, Mahalanobis distances and respective p-values were calculated using the package ClassDiscovery [24] based on the first two obtained principal components. A p-value threshold of 0.05 was used to define outliers (Supplementary Figure 1), which were removed for subsequent analyses.

IPA

Ingenuity Pathway Analysis (IPA; Qiagen, Germany) [25] was used for integrated pathway enrichment analysis with the definition of “human” as organism and selection of AT using significantly altered proteins and transcripts (adjusted p-value ≤ 0.05). Either proteins, transcripts, or a combination of both were investigated. Benjamini & Hochberg adjusted p-values and z-scores were extracted (Supplementary Table 6) and and used for visualizations analyte-based multi-omics integration was performed using the supervised DIABLO framework in mixOmics [23].

WGNCA

A Weighted Gene Correlation Network Analysis (WGCNA) [26] was performed on the integrated transcriptome and proteome data as described previously (Supplementary Table 7; Supplementary Figure 3) [27]. Results of the WGNCA key driver analysis were visualized using Cytoscape 3.7.2 [28] and stringApp [29].

The *Weighted Gene Correlation Network Analysis* (WGCNA) [26] was performed as follows: An Analysis of variance (ANOVA) was performed on the processed proteome and transcriptome data to identify proteins and transcripts, which significantly (p-value ≤ 0.05) differed between the tested conditions, resulting in 1511 proteins and 7610 transcripts. Next, data were filtered for analytes, which were quantified in at least half of the samples, yielding 1399 proteins and 7610 transcripts for WGCNA. Calculations were carried out as previously described [27] but with the following parameters: A signed network was created with a soft power threshold of 9 to arrive at network adjacency. The cut height was set to 0.25, minimum module size to 100, and maximum module size to 500. Fourteen modules were identified and assigned to different colors (Supplementary Figure 3A). A summary of the analyte-module-assignment was generated (Supplementary Table 7). For each of the modules, significantly enriched pathways were determined using IPA as described above, but without defining a p-value threshold (Supplementary Table 7). The obtained pathways were filtered based on significance, and the top two enriched pathways (based on adjusted p-values) were determined for each module (Supplementary Table 7). Furthermore, correlations with the different traits were investigated, and key drivers for epiAT of subjects with IS or IR were determined using following criteria: gene significance (GS) ≥ 0.4, module membership (MM) ≥ 0.4, connectivity ≥ 0.1, and not only connections to isoforms. Obtained results were visualized using Cytoscape 3.7.2 [28] and stringApp [29]. Key drivers for all investigated traits before connectivity filtering can be found in (Supplementary Table 7).

2. REFERENCES

1 Krieg L, Schaffert A, Kern M, et al. An MRM-Based Multiplexed Quantification Assay for Human Adipokines and Apolipoproteins. *Molecules* 2020;25(4).

2 Schaudinn A, Hudak A, Linder N, et al. Toward a Routine Assessment of Visceral Adipose Tissue Volume from Computed Tomographic Data. *Obesity (Silver Spring)* 2021;29(2):294–301. doi:10.1002/oby.23061 [published Online First: 25 December 2020].

3 Linder N, Schaudinn A, Garnov N, et al. Age and gender specific estimation of visceral adipose tissue amounts from radiological images in morbidly obese patients. *Sci Rep* 2016;6:22261. doi:10.1038/srep22261 [published Online First: 24 March 2016].

4 Eberhardt SC, Strickland CD, Epstein KN. Radiology of epiploic appendages: acute appendagitis, post-infarcted appendages, and imaging natural history. *Abdom Radiol (NY)* 2016;41(8):1653–65.

5 Kalkhof S, Förster Y, Schmidt J, et al. Proteomics and metabolomics for in situ monitoring of wound healing. *Biomed Res Int* 2014;2014:934848.

6 Leek JT, Johnson WE, Parker HS, et al. The sva package for removing batch effects and other unwanted variation in high-throughput experiments. *Bioinformatics* 2012;28(6):882–83.

7 Jühling F, Kretzmer H, Bernhart SH, et al. metilene: fast and sensitive calling of differentially methylated regions from bisulfite sequencing data. *Genome Res* 2016;26(2):256–62.

8 Frankish A, Diekhans M, Ferreira A-M, et al. GENCODE reference annotation for the human and mouse genomes. *Nucleic Acids Research* 2019;47(D1):D766-D773.

9 Ritchie ME, Phipson B, Di Wu, et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Research* 2015;43(7):e47.

10 Subramanian A, Tamayo P, Mootha VK, et al. Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. *PNAS* 2005;102(43):15545–50. https://www.pnas.org/content/pnas/102/43/15545.full.pdf.

11 Kanehisa M, Goto S. KEGG: kyoto encyclopedia of genes and genomes. *Nucleic Acids Research* 2000;28(1):27–30.

12 Massier L, Chakaroun R, Tabei S, et al. Adipose tissue derived bacteria are associated with inflammation in obesity and type 2 diabetes. *Gut* 2020. https://gut.bmj.com/content/early/2020/04/21/gutjnl-2019-320118.info.

13 Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Research* 2001;29(9):e45.

14 Hadley Wickham, Mara Averick, Jennifer Bryan, et al. Welcome to the tidyverse. *Journal of Open Source Software* 2019;4(43):1686.

15 Spiess A-N. qpcR: Modelling and Analysis of Real-Time PCR Data: Model fitting, optimal model selection and calculation of various features that are essential in the analysis of quantitative real-time polymerase chain reaction (qPCR) 2018.

16 Wickham H. The Split-Apply-Combine Strategy for Data Analysis. *J. Stat. Soft.* 2011;40(1).

17 Mahto A. splitstackshape: Stack and Reshape Datasets After Splitting Concatenated Values 2019.

18 Graffelman J. calibrate: Calibration of Scatterplot and Biplot Axes 2020.

19 Gu Z, Gu L, Eils R, et al. circlize Implements and enhances circular visualization in R. *Bioinformatics* 2014;30(19):2811–12.

20 Gregory R. Warnes, Ben Bolker, Lodewijk Bonebakker, et al. gplots: Various R Programming Tools for Plotting Data 2020.

21 Hadley Wickham. ggplot2: Elegant Graphics for Data Analysis 2016.

22 Gu Z, Eils R, Schlesner M. Complex heatmaps reveal patterns and correlations in multidimensional genomic data. *Bioinformatics* 2016;32(18):2847–49.

23 Rohart F, Gautier B, Singh A, et al. mixOmics: An R package for 'omics feature selection and multiple data integration. *PLoS Comput Biol* 2017;13(11). https://pubmed.ncbi.nlm.nih.gov/29099853/.

24 Coombes KR. ClassDiscovery 2019.

25 Kramer A, Green J, Pollard J, JR, et al. Causal analysis approaches in Ingenuity Pathway Analysis. *Bioinformatics* 2014;30(4):523–30.

26 Langfelder P, Horvath S. WGCNA: an R package for weighted correlation network analysis. *BMC Bioinformatics* 2008;9:559.

27 Isabel Karkossa, Anne Bannuscher, Bryan Hellack, et al. An in-depth multi-omics analysis in RLE-6TN rat alveolar epithelial cells allows for nanomaterial categorization. *Part Fibre Toxicol* 2019;16(1):1–19.

28 Shannon P, Markiel A, Ozier O, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res* 2003;13(11):2498–504.

29 Doncheva NT, Morris JH, Gorodkin J, et al. Cytoscape StringApp: Network Analysis and Visualization of Proteomics Data. *J Proteome Res* 2019;18(2):623–32.

3. Supplementary files information

Table S1: Body donors' cohort characteristics

Table S2: Characteristics of sub-cohort used for muti-omics analyses (N_{IR}=9, N_{IS}=9, matched for sex, age and BMI)

Table S3: Characteristics of sub-cohort used for transcriptomics (N_{IR}=15, N_{IS}=9, matched for sex, age and BMI)

Table S4: List of Taqman Probes

Table S5: Quantification of adipose tissue SVF cell populations via FACS ; cohort characteristics of subjects included in FACS characterization of EpiAT

Table S6: Gene expression data in the different adipose tissues

Table S7: identified proteins and genes in each patient.

and gene significances together with identified key drivers in the different adipose tissues.

Table S8: FCs and p-values obtained from proteomics and transcriptomics.

Table S9: Methylation data.

Table S10: Summary of enriched IPA pathways for proteome, transcriptome and of integrative analysis.

Table S11: characteristics of adipocytes donors

Table S12: Weighted Gene Correlation Network Analysis (WGCNA) results; Lists of analytes and their assignment to the obtained modules, as summary of enriched IPA pathways for each module and the calculated module memberships

Figure S1 (referring to Figure 2). (A) Gating strategy of FACS analysis, (B) FACS analysis of AT SVF (n=7) for immune cell populations (remaining clusters), shown are individual observations, median and upper and lower quartiles, paired Mann-Whitney-*U*-test, bars above boxplots indicate comparisons, p-values are listed above comparison-bars (C) MR-image (T1-weighted gradient echo inphase sequence) of a 40-year-old female study patient in transversal orientation at the level of lumbar vertebrae 3. Visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) are separated by muscle. Yet there is no adequate peritoneal coverage thick enough to be distinguished as borders of epiploic adipose tissue (arrows)

Figure S2 (referring to Figure 3). Outliers defined by Mahalonobis distance for transcriptome data (A) and proteome data (B), gene set enrichment analysis using KEGG for epiAT vs scAT (C) and epiAT vs mesAT (D), adipocyte specific expression of LEP in an independent cohort (E), cell type specific expression pattern of highlighted genes (F)

Figure S3 (referring to Figure 4). (A) Overlap between reliably quantified and significantly altered transcripts/proteins between epiAT and omAT, (B) heatmap of marker for brown and white AT based on Perdikari et al. and (C) heatmap for markers of beige AT based on Pilkington et al.

Figure S4 (referring to Figure 5). (A) KEGG pathways based on transcriptome data differentially enriched in patient with or without IR, shown as normalized enrichment score (NES), (B) FACS quantification of immune cell population between patients with and without T2D

Figure S5 (referring to Figure 6). Overlap between differentially regulated analytes between IS and IR in epiAT for chemokine signaling pathway (A), toll like receptor signaling pathway (B), B cell receptor pathway (C), leukocyte transendothelial migration (D), as well as generalized for all significantly altered KEGG pathways (E)

Figure S6 (referring to Figure 7). (A) WGCNA: cluster dendrogram, (B) WGCNA: module-trait correlations of cluster eigengenes, (C) key driver analysis of IS epiAT (yellow module

12

272