Glucose tolerance and insulin sensitivity define adipocyte transcriptional programs in human obesity

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ABSTRACT

Objective: Although debated, metabolic health characterizes 10–25% of obese individuals and reduces risk of developing life-threatening co-morbidities. Adipose tissue is a recognized endocrine organ important for the maintenance of whole-body metabolic health. Adipocyte transcriptional signatures of healthy and unhealthy obesity are largely unknown.

Methods: Here, we used a small cohort of highly characterized obese individuals discordant for metabolic health, characterized their adipocytes transcriptional signatures, and cross-referenced them to mouse phenotypic and human GWAs databases.

Results and conclusions: Our study showed that glucose intolerance and insulin resistance co-operate to remodel adipocyte transcriptome. We also identified the Nuclear Export Mediator Factor (NEMF) and the Ectoderm-Neural Cortex 1 (ENC1) as novel potential targets in the management of metabolic health in human obesity.

Keywords Obesity; Glucose tolerance; Insulin sensitivity; Transcriptomics; Mouse genetics; Systemic phenotyping

1. INTRODUCTION

Obesity, characterized by an excess of body fat, and global body fat dysfunction, has reached epidemic proportions worldwide. Almost 300 million people are clinically obese (BMI > 30 kg/m²) and, therefore, at high risk of developing life-threatening co-morbidities, such as diabetes, cardiovascular disease, stroke, and cancer. Of note, 10–25% of obese people stay healthy. These individuals remain glucose tolerant and insulin sensitive, with normal blood pressure and a favorable lipid profile [1]. A recent study demonstrated that the majority of obese individuals slip into poor health and chronic illness over time [2]. Conversely, other studies suggest that, while healthy obese individuals have higher risk of developing co-morbidities when compared to a lean population [3] they are still protected as the onset of co-morbidities is delayed compared to an unhealthy obese population [1]. Thus, maintaining metabolic health in obesity is important for long-term disease management. Despite the current clinical definition of healthy obesity [1] and the recently discovered metabolomic signatures [4], the molecular underpinnings of metabolic health in obesity is largely unknown. Here, we analyzed primary subcutaneous adipocytes from morbidly obese individuals (BMI > 40) [4] discordant for metabolic health (Tables 1 and S3). We discovered that instead of insulin resistance, impaired glucose homeostasis is a major determinant of adipocyte transcriptional response. Insulin resistance, instead, appears to be a modifier of this response. Interrogating systemic mouse phenotypic data [5]

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and human GWAS catalogs, we identified 19 genetic associations with phenotypes of glucose homeostasis and/or body weight and composition. In particular, we describe the Nuclear Export Mediator (NEMF) and the Ectoderm-Neural Cortex protein 1 (ENC1) as novel potential targets in the management of metabolic health in obese individuals.

2. RESULTS

2.1. Insulin sensitivity does not rewire adipocyte transcriptome in human obesity

Adipose tissue is a recognized endocrine organ critical for the maintenance of whole-body metabolic homeostasis [6]. We obtained subcutaneous adipose tissue biopsies during bariatric surgery of morbidly obese individuals discordant for metabolic health [7]. Accessible, frozen, primary pre-adipocytes were differentiated ex-vivo into mature adipocytes that were used for RNA-sequencing analyses. Total RNA was prepared from mature adipocytes and, after quality check, ribosomal RNA-depleted libraries were generated for single-end Illumina sequencing (see materials and methods section for more details). Although the use of ex-vivo differentiated adipocytes constitutes a limitation of the current study as the observed differences might be biased by intrinsic and individual differences in adipogenesis potential, we aimed to experimentally control for that. We therefore pulled out the 200 human adipogenesis-relevant genes from the Geneset Enrichment Analysis (GSEA) database (GSEA_Hallmark_human_Adpogenesis) and performed unsupervised clustering and principal component analysis to identify any association between metabolic status and adipogenesis efficiency. As shown in Supplementary Figure 1, less than 10 genes are differentially expressed between males and females (Fig. S1F), and the vast majority of them are associated to sex chromosomes, thus confirming that gender is not a determinant of adipocyte transcriptional response.

Once controlled for adipogenesis potential and gender effect, we studied the adipocyte transcriptional signatures of metabolically healthy and unhealthy obese individuals. Since metabolic health is primarily defined by maintenance of whole-body insulin sensitivity, we compared adipocytes from insulin sensitive and resistant individuals (Table 1). Interestingly and unexpected, loss of insulin sensitivity did not affect adipocytes transcriptional programs as both principal component (Figure 1A) and differential expression (Figure 1B) analyses revealed a complete overlap between adipocytes from insulin sensitive and resistant individuals. These findings are in line with previously published work [8] showing that adipose tissue transcriptional response to insulin is determined primarily by obesity rather than insulin sensitivity.

2.2. Glucose homeostasis rewires adipocyte transcriptome in human obesity

Together with insulin sensitivity, metabolic health is characterized by maintenance of whole body glucose homeostasis. Hyperglycemia, independent from insulin resistance, has a strong impact on adipose tissue function as it activates several pathways important for adipocyte cellular homeostasis (e. g., ER stress response, mRNA processing and protein translation) and is critical in directing adipose tissue growth towards the unhealthy hypertrophic state (reviewed in [9]). Mechanistically, glucose has been shown to activate several downstream signaling pathways, which culminate in rewiring of cellular transcriptional responses [10,11]. To test the effect of glucose intolerance on adipocyte transcriptional programs, we re-grouped the obese individuals based on their glucose tolerance (Table 2) and re-ran the bioinformatic analysis. Deterioration of glucose tolerance is stepwise and proceeds from Normal Glucose Tolerance (NGT — fasting glycaemia <5.5 mmol/L and 2hr after OGTT <7.7 mmol/L) to Impaired Fasting Glycaemia (IFG — fasting glycaemia 5.6—6.9 mmol/L and 2hr after OGTT <7.7 mmol/L), Impaired Glucose Tolerance (IGT - fasting glycaemia ≤5.5 mmol/L and 2hr after OGTT 7.8—11 mmol/L) and a more severe derangement of glucose homeostasis characterized by both IFG and IGT (IGF + IGT). Interestingly, the number of differentially expressed genes (DEGs) between adipocytes from tolerant and intolerant individuals substantially increased with decreasing glucose tolerance (Fig. S2A—C and Table S1) and, while enriched for lipid metabolism in both IFG and IGT samples, they clustered to pathways critical for proper metabolic control (e. g. Insulin, PI3K/Akt, FoxO) in IFG + IGT samples (Fig. S2D—F and Table S1). Also, a principal component analysis run on all-expressed genes (fpkm > 0.3) indicated that adipocytes from intolerant individuals cluster together and away from those of tolerant individuals (Figure 1C).

Thus, hyperglycemia rewires adipocyte transcription and, while different adipocyte transcriptional programs reflect the degree of glucose intolerance, intolerant donors constitute a “single” group whose health determinant is glucose homeostasis. Stemming from these findings, we grouped the adipocytes samples as coming from individuals with normal (NGH) or impaired glucose homeostasis (IGH — which includes IFG, IGT and IFG + IGT individuals — Table 1) and re-ran the bioinformatic pipeline reasoning that this would deepen our knowledge on the biological consequences of impaired glucose homeostasis in adipocytes. Indeed, both principal component (Figure 1D) and differential expression (Figure 1E) analyses showed...
that glucose homeostasis highlights two transcriptionally different groups defined by more than 1000 DEGs (Figure 1D–E), which cluster to pathways important for longevity and metabolic control, such as the Insulin, the MAPK and the AMPK signaling pathways (Figure 1F–G and Table S1). Critically, the NGH group was significantly younger than the IGH, and therefore we ran a control analysis to make sure that the observed differences in gene expression were not age-dependent. Briefly, we stratified the obese individuals by age and looked at the expression of the differentially expressed genes between NGH and IGH individuals. As shown in supplementary figure 3 (Fig. S3A), differential gene expression is age-independent as expression patterns of young IGH (Ad20 and Ad03) or old NGH (Ad26) individuals still reflect their metabolic status rather than the age group. Thus, impaired glucose homeostasis alters the transcription of critical metabolic pathways in ex-vivo differentiated adipocytes from morbidly obese individuals. Unfortunately, data on adipose tissue morphology and immune cell infiltration — important parameters to define a metabolically healthy
adipose tissue—are not are available from this cohort and the exact relationship between these parameters and the adipocyte transcriptional response remain a limitation of the study. Nevertheless, the same individuals have been previously characterized for adipose tissue insulin sensitivity and systemic inflammation [4]; here, we have deepened our adipocyte characterization by integrating individuals’ glucose homeostasis and insulin sensitivity in the analysis to understand the relationship between the two and their impact on adipocyte transcriptional response.

2.3. Insulin sensitivity cooperates with glucose intolerance to modify adipocytes transcriptional programs in human obesity

Glucose intolerance and insulin resistance are independent and additive risk factors for the development of diabetes, cardiovascular disease and premature death. Insulin resistance occurs independently from glucose tolerance also in the study cohort, with both NGH and IGH individuals being insulin sensitive or resistant (Figure 2A). Interestingly from glucose tolerance also in the study cohort, with both NGH and IGH disease and premature death. Insulin resistance occurs independently of the complex interplay between hyperglycemia and insulin resistance in the maintenance of metabolic health in human obesity. Highly divergent transcriptional regulatory mechanisms, although converging into the activation of common downstream pathways, indicate that different intracellular molecular machineries, aimed at fine-tuning cellular responses potentially critical for organismal health, integrate extracellular metabolic signals. Intriguingly, activation of downstream pathways which are commonly associated to loss of metabolic health in intact adipose tissue in vivo, such as those involved in inflammation and tissue remodeling, suggest—at one side—their adipocyte origin in vivo and—on the other side—that ex vivo differentiated adipocytes reflects, at least to a certain extent, in vivo physiology.

2.4. NEMF and ENC1 emerge as novel potential target in organismal metabolic homeostasis

In keeping with the divergence in the transcriptional regulatory mechanisms, the number of co-DEGs between the insulin sensitive and resistant sample sets is restricted to roughly 5% of all DEGs (Figure 3A). To further understand the role of these genes in metabolic control, we turned to mouse and human genetics and interrogated public databases for genetic associations with relevant phenotypes. We started by harnessing the resource of the International Mouse Phenotyping Consortium (IMPC) [5,13], which has already provided the community with several thousands disease models [15,16] and identified genes important for hearing [17], sexual dimorphism [18], and metabolic control [19]. To date, the IMPC has generated and phenotyped ~25% (96 genes) of the 401 genes differentially expressed in adipocytes from the different subgroups of insulin sensitive and resistant individuals, and ~20% of those (21 genes) have significant associations with Mouse Phenotype (MP) terms related to glucose homeostasis and/or body composition (Figure 3B,C and Table S2).

Supporting the robustness and sensitivity of our approach, we identified, among the 21 genes with significant MP term associations, two

| Table 2 — Characteristics of individuals with obesity regrouped based on their glucose homeostasis (NGH or IGH) and insulin sensitivity (IS or IR). |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | NGH_IS          | NGH_IR          | IGH_IS          | IGH_IR          | p               |
| N (women/men)   | 3/0             | 2/3             | 4/4             | 5/1             | 0.2            |
| Age (y)         | 39 ± 16         | 29 ± 6          | 44 ± 8          | 48 ± 11         | 0.014           |
| BMI (kg/m²)     | 52.6 ± 5.1      | 47.7 ± 3.1      | 52.4 ± 9.7      | 54.8 ± 7.4      | 0.4            |
| Body fat (%)    | 60.1 ± 2.1      | 48.2 ± 12.2     | 44.9 ± 12.0     | 54.1 ± 11.7     | 0.5            |
| Fasting glucose (mmol/L) | 5.37 ± 0.14 | 5.16 ± 0.17 | 5.54 ± 0.51 | 6.16 ± 0.39 | 0.007          |
| 2-h glucose (mmol/L) | 6.89 ± 0.39 | 5.92 ± 0.79 | 7.31 ± 1.45 | 9.05 ± 1.27 | 0.002          |
| HbA1c (%)       | 5.87 ± 0.23     | 6.00 ± 0.38     | 5.49 ± 0.42     | 5.90 ± 0.14     | 0.07           |
| IS-OFT (m²/m²)  | 6.62 ± 1.44     | 2.69 ± 0.48     | 7.28 ± 2.42     | 2.63 ± 0.90     | 0.0001         |
| Insulinogenic index (<10⁻⁸) | 214 ± 105 | 351 ± 83 | 195 ± 163    | 253 ± 150       | 0.6            |
| Leukocytes (μL⁻¹) | 7.540 ± 1.607 | 12.390 ± 3.378 | 6.875 ± 1.913 | 8.167 ± 1.230 | 0.06          |
| CRP (mg/dL)     | 0.65 ± 0.60     | 0.97 ± 0.93     | 0.60 ± 0.52     | 1.48 ± 1.10     | 0.7            |
| AST (U/L)       | 21 ± 4          | 25 ± 11         | 23 ± 6          | 25 ± 10         | 1.0            |
| ALT (U/L)       | 23 ± 9          | 46 ± 25         | 25 ± 10         | 32 ± 15         | 0.6            |
| Fetalin-A (μg/mL)| 281 ± 60        | 275 ± 55        | 275 ± 37        | 256 ± 38        | 0.7            |

Data represent numbers or means ± SD. ALT—alanine transaminase; AST—aspartate transaminase; BMI—body mass index; CRP—C-reactive protein; HbA1c—hemoglobin A1c; IGH—impaired glucose homeostasis; IS—insulin-resistant; IS—insulin-sensitive; NGH—normal glucose homeostasis; OGGT—oral glucose tolerance test.

a  χ² test.
b  Multiple linear regression (adjusted for gender).
c  Multiple linear regression (adjusted for age and BMI).
d  Multiple linear regression (adjusted for gender, age, and BMI).
e  Multiple linear regression (adjusted for gender, age, BMI, and insulin sensitivity).
with known function in metabolic control, namely the leptin gene (LEP) [20,21] and the Polycomb repressive complex 1 subunit BMI1 (B Lymphoma Mo-MLV Insertion Region 1) [14]. Among the remaining 19 genes with significant MP term associations, we focused on the 6 associated with phenotypes related to whole body glucose homeostasis. Of those, two are novel potential targets for the management of glucose homeostasis in obesity, namely the Nuclear Export Mediator Factor (NEMF) and the Ectodermal-Neural Cortex 1 (ENC1). NEMF and ENC1 are up-regulated in adipocytes from glucose intolerant donors either insulin sensitive (NEMF Figure 3B) or resistant (ENC1 Figure 3C) and, in both cases, genetic ablation in the mouse results in reduced non-fasted glycemia (Figure 3D,F). ENC1 knockout mice also show a favorable switch in body composition with reduced fat mass (Figure 3F). Similar phenotypes also result from the manipulation of the Engulfment and cell Motility 1 (ELMO1) gene, which is up-regulated in adipocytes from glucose intolerant donors either insulin sensitive (NEMF — Figure 3B) or resistant (ENC1 — Figure 3C) and, in both cases, genetic ablation in the mouse results in reduced non-fasted glycemia (Figure 3D,F). ENC1 knockout mice also show a favorable switch in body composition with reduced fat mass (Figure 3F).

The remaining three genes — the Aspartoacylase (ASPA), the Frizzled Receptor 3 (FZD3) and the Cytochrome P450 Family 7 Subfamily B Member 1 (CYP7B1) — are up-regulated in adipocytes from glucose intolerant donors either insulin sensitive (ASPA Figure 3B) or resistant (FZD3 and CYP7B1 Figure 3C) and lead to impaired glucose homeostasis when knocked-out in mice (Figure 3G-I), namely elevated fasting glycemia (ASPA Figure 3H) or impaired glucose tolerance (CYP7B1 and FZD3 Figure 3G,I). ASPA, FZD3 and CYP7B1 are all involved in different aspects of lipid metabolism and adipose tissue biology. CYP7B1, also known as Oxysterol 7-Alpha-Hydroxylase, functions in hepatic and extrahepatic tissues to metabolize cholesterol to oxysterols and bile acids, which are important for metabolic physiology [26]. ASPA and FZD3 are expressed in adipose tissue and function in adipogenesis, lipid metabolism and organismal development. ASPA deacetylates N-Acetyl-L-Aspartic Acid (NAA) into aspartate and acetate and is critical for the formation of myelin sheaths [27]. Loss-of-function mutations result in NAA accumulation and Canavan disease in humans [28]. The role of ASPA in adipocyte biology and metabolic health is unclear, although preliminary observations have linked ASPA to lipid turnover, histone acetylation and function of brown adipocytes [29,30].

Figure 2: Insulin sensitivity is a modifier of adipocyte transcriptional programs imposed by glucose tolerance. A. ISI Matsuda Index in NGH or IGH donors. Horizontal bars indicate the median. Significance explained in Table 1. B-C. Volcano plot representation of RNA-Seq data from adipocytes of NGH vs IGH donors featuring insulin sensitivity (B), or resistance (C). D-E. KEGG pathway analysis of DEGs in IS_IGH_NGH (D) and IR_IGH_NGH (E). F. Enrichr-based grid representation of transcriptional regulatory clusters. Left part: ChEA-based enriched transcription factors in DEGs from IS_IGH_NGH (top) or IR_IGH_NGH (bottom). p-value < 0.05 is considered statistically significant. Cluster 1 — RNF2, JARID2, PAX3; Cluster 2 — EZH2, p53, GATA2; Cluster 3 — SUZ12, SMAD, ZNF217, ESR1, CEBPD, NRF2. Right part: ENCODE-based enriched histone PTMs on DEGs from IS_IGH_NGH (top) and IR_IGH_NGH (bottom).
Figure 3: Combined mouse and human genetics identifies novel determinant of metabolic health in human obesity. A. Venn diagram representation of co-differentially expressed genes in IS_IGH_NGH and IR_IGH_NGH. B-C. Heatmap representation of IS_IGH_NGH (B) and IR_IGH_NGH (C) DEGs with significant MP term associations (red — glucose homeostasis; blue — body weight and composition). D-I. 5–95 percentile plots of representative genes and MP terms (* = p < 0.01 calculated with one-way ANOVA with multiple comparisons using GraphPad Prism 6). Plain colored boxes represent mutant animals; dotted colored boxes represent control animals. Males in blue, females in pink.
FZD3 is a member of the Frizzled gene family and encodes a seven-transmembrane domain protein, receptor of the Wnt family of signaling proteins. While most of the frizzled receptors activate the canonical wnt/beta-catenin signaling, FZD3 is a Wnt5A receptor and activator of non-canonical Wnt signaling pathway in inflammatory conditions [31], axon guidance, and neurogenesis [32]. In keeping with a critical function in neuronal development, FZD3 knockout mice show prenatal lethality (Table S2). Of note, the reported phenotypes are just the most relevant to this manuscript. Complete phenotypic information is available from the IMPC database and links to individual mouse lines are reported in the Supplementary Table S2.

SNP analysis did not reveal genome-wide significant associations with human metabolic syndrome for any of the 6 genes with significant annotations to MP terms (Table S2), indicating that the observed alteration in their expression results from alternative mechanisms, such as gene/environment interaction and epigenetic phenomena.

3. CONCLUSIONS

Taken together, our findings show that glucose intolerance and insulin resistance cooperatively regulate adipocyte transcription in human obesity, with glucose intolerance (and thus hyperglycemia) being — most likely — the most potent signal. Our data not only validates genes previously associated to metabolic health (e.g. LEP and BMI), but also identifies six novel candidates functionally validated using mouse genetics and systemic phenotyping. Among those, NEMF and ENC1 are upregulated in unhealthy obese individuals and, when knocked out in mice, show consistent amelioration of metabolic homeostasis. Therefore, NEMF and ENC1 appear as two potential novel targets for the management of glucose homeostasis in human obesity.

4. MATERIALS AND METHODS

4.1. Characteristics and stratification of adipose tissue donors

Twenty-two, age-matched Caucasian morbidly obese individuals, who underwent sleeve gastrectomy and concomitant abdominal subcutaneous fat biopsy, were matched for gender and BMI and grouped into two equally sized subgroups discordant for whole-body insulin sensitivity (Table 1). The reason for choosing morbidly obese individuals, although they do not fully represent the more common population of “mild” obese individuals is at least two fold: (1) “Mild” obese individuals unlikely undergo bariatric surgery and therefore, it would have been more complicated to get access to biopsies; and (2) A population of morbidly obese individuals is more homogeneous (in terms of obesity development) and we expected adipose tissue phenotypes to mostly reflect their metabolic health status, rather than their obesity stage. Overt diabetes, other severe diseases, and/or glucose-tolerance-affecting medication were exclusion criteria. From all 22 obese individuals, information about comorbidities is available (Table S3). To estimate whether these comorbidities could have affected our results, we tested whether they were unevenly distributed between the groups (between IS and IR as well as between NGH_IS, NGH_IR, IGH_IS, and IGH_IR). We only tested the most frequent comorbidities, i.e., musculoskeletal problems (N = 11) and arterial hypertension (N = 10), because tests with less frequent comorbidities, e.g., depression (N = 6) or sleep apnea (N = 5), would have been prone to false-positive results. Using \( \chi^2 \)-tests, we could not detect uneven distribution of musculoskeletal problems or arterial hypertension between the groups (\( p \geq 0.2 \)), all. All participants underwent physical examination and routine laboratory tests. For determination of glucose tolerance and insulin sensitivity, they underwent a 5-point 75-g oral glucose tolerance test with glucose, insulin, and C-peptide measurements, as reported earlier [33]. Whole-body insulin sensitivity was calculated according to Matsuda and DeFronzo [34]: insulin sensitivity (in \( 10^{-3} \text{ L}^2/\text{mol}^2 \)) = \( 10,000 \times \text{fasting glucose} \times \text{fasting insulin} / \text{mean glucose} \times \text{mean insulin}^{1/2} \). Body fat content (in %) was measured by bioelectrical impedance (BIA-101, RJL Systems, Detroit, MI, USA).

All participants gave written informed consent to the study. The study adhered to the ethical principles of the Declaration of Helsinki, and the ethics committee of the University of Tübingen approved the study protocol.

4.2. Preadipocyte isolation and differentiation

From all donors, paired samples of subcutaneous and visceral preadipocytes were prepared from adipose tissue biopsies [7] and properly frozen and biobanked. While frozen visceral preadipocytes are hard to differentiate ex-vivo, we could obtain good and homogeneous differentiation efficiency from subcutaneous preadipocytes. Briefly, isolated cells were grown in \( \alpha \)-MEM/Ham’s nutrient mixture F12 (1:1), 20% fetal calf serum, 1% chicken embryo extract (Sera Laboratories, Haywards Heath, UK), 100 IU/mL penicillin, 0.1 mg/mL streptomycin, 0.5 mg/mL fungizone, 2 mmol/L glutamine (= growth medium). Second-pass cells were stored in liquid nitrogen (in 90% fetal calf serum, 10% DMSO). Stored cells were thawed and grown to confluence in growth medium. Thereafter, adipocyte differentiation was started by shifting the cultures to DMEM/Ham’s nutrient mixture F12 (1:1) containing 5% fetal calf serum, 17 \( \mu \)mol/L pantothenate, 1 \( \mu \)mol/L biotin, 2 \( \mu \)g/mL apo-transferrin, 1 \( \mu \)mol/L human insulin, 1 \( \mu \)mol/L dexamethasone, 100 IU/mL penicillin, 0.1 mg/mL streptomycin, 0.5 mg/mL fungizone, 2 mmol/L glutamine (= differentiation medium) supplemented with 0.5 mmol/L 3-isobuty1-1-methyl-xanthine, 2 nmol/L triido-thyronine, 50 \( \mu \)mol/L indomethacin, 10 \( \mu \)mol/L Trogilitazine and maintaining them in these conditions for seven days. Finally, the cells were allowed to terminally differentiate for another 11 days by culturing them in differentiation medium alone. Culture media and supplements were purchased from Lonza (Basel, Switzerland) and Biochrom (Berlin, Germany).

4.3. RNA-sequencing and data analysis

Total RNA was prepared from differentiated adipocytes using the RNAspy mini kit (QIAGEN) according to the manufacturer’s instructions. RNA concentration and integrity was then controlled on a Bioanalyzer system (Agilent) and only RNA samples with RIN (RNA Integrity Number) values \( > 7 \) were used for downstream applications. Library construction and sequencing was outsourced to IGA Technology Services Srl. Libraries were constructed using the Nextera Library Prep Kit (Illumina) according to the manufacturer’s instructions and sequenced on an Illumina HiSeq 2500 at 75bp single-ended, with a minimum output of 40 million reads per sample. Read mapping and differential expression analysis was performed using the A.L.R (Artificial Intelligence RNA-Seq) software from Sequentia Biotech with the following pipeline: BBDuk (reads trimming — http://jgi.doe.gov/data-and-tools/ bbtools/bb-tools-user-guide/bbdurk-guide), STAR (reads mapping to the human genome GRCh38 [ENSEMBL] — https://github.com/ alexdobin/STAR), featureCounts (gene expression quantification — http://bioinf.wehi.edu.au/featureCounts/), and EdgeR (differential gene expression — https://www.bioconductor.org/packages/devel/bioc/html/edgeR.html). Heatmap and PCA analyses were performed with
the web-application ClustVis using default parameters [35]. Knowledge-based analysis, including KEGG, ChEA, and ENCODE Histone PTMs was performed using the web-based application Enrichr (http://amp.pharm.mssm.edu/Enrichr/) [36].

4.4. IMPC data extrapolation and analysis

Mouse phenotypic information was extrapolated from the database of the International Mouse Phenotyping Consortium (IMPC — http://www.mousephenotype.org) using the batch query tool on differentially expressed genes in adipocytes from NGH and IGH donors being either insulin sensitive or resistant. Raw data from mouse lines with relevant MP term associations were downloaded from the database, re-analyzed and re-plotted using GraphPad Prism 6.

4.5. SNP analysis

SNP analysis was performed as previously described [19] with slight modifications. Differentially expressed genes associated to significant MP terms were used for analysis. We searched for SNPs in the Type 2 Diabetes Knowledge Portal (http://www.type2diabetesgenetics.org) and SNiPA (https://snipa.helmholtz-muenchen.de/snipa3/index.php) databases and used the cross-phenotype meta-analysis (CPMA) method to evaluate their significance across 16 metabolic phenotype GWAs from various consortia, as described [19].

AUTHORS’ CONTRIBUTIONS

H. S. and R. T. conceived the study, analyzed the data and wrote the manuscript; R. G., L. B., J. D., M. L., F. S., A. K., A. B., and H. G. performed the experiments and analyzed the data; M. H. A., H. G., and H. H. read and commented on the manuscript.

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CONFLICT OF INTEREST

None declared.

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://doi.org/10.1016/j.molmet.2018.09.004.

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