UNIVERSITY OF COPENHAGEN

The expression signatures in liver and adipose tissue from obese Göttingen Minipigs reveal a predisposition for healthy fat accumulation

Cirera, Susanna; Taöz, Emirhan; Juul Jacobsen, Mette; Schumacher-Petersen, Camilla; Østergaard Christoffersen, Berit; Kaae Kirk, Rikke; Pagh Ludvigsen, Trine; Hvid, Henning; Duelund Pedersen, Henrik; Høier Olsen, Lisbeth; Fredholm, Merete

Published in: Nutrition and Diabetes

DOI: 10.1038/s41387-020-0112-y

Publication date: 2020

Document version Publisher's PDF, also known as Version of record

Document license: CC BY

Citation for published version (APA):

Cirera, S., Taöz, E., Juul Jacobsen, M., Schumacher-Petersen, C., Østergaard Christoffersen, B., Kaae Kirk, R., ... Fredholm, M. (2020). The expression signatures in liver and adipose tissue from obese Göttingen Minipigs reveal a predisposition for healthy fat accumulation. *Nutrition and Diabetes*, *10*(1), [9]. https://doi.org/10.1038/s41387-020-0112-y

ARTICLE

Open Access

The expression signatures in liver and adipose tissue from obese Göttingen Minipigs reveal a predisposition for healthy fat accumulation

Susanna Cirera¹, Emirhan Taşöz¹, Mette Juul Jacobsen¹, Camilla Schumacher-Petersen¹, Berit Østergaard Christoffersen², Rikke Kaae Kirk², Trine Pagh Ludvigsen², Henning Hvid², Henrik Duelund Pedersen^{1,3}, Lisbeth Høier Olsen¹ and Merete Fredholm¹

Abstract

Background: Model animals are valuable resources for dissecting basic aspects of the regulation of obesity and metabolism. The translatability of results relies on understanding comparative aspects of molecular pathophysiology. Several studies have shown that despite the presence of overt obesity and dyslipidemia in the pig key human pathological hepatic findings such as hepatocellular ballooning and abundant steatosis are lacking in the model.

Objectives: The aim of this study was to elucidate why these histopathological characteristics did not occur in a high fat, fructose and cholesterol (FFC) diet-induced obese Göttingen Minipig model.

Methods: High-throughput expression profiling of more than 90 metabolically relevant genes was performed in liver, subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) of male minipigs diet fed: standard chow (SD, n = 7); FFC diet (n = 14); FFC diet in streptozotocin-induced diabetic pigs (FFC_{DIA}, n = 8). Moreover, histopathological assessment of SAT and VAT was performed.

Results: 12, 4 and 1 genes were highly significantly differentially expressed in liver, SAT and VAT when comparing the FFC and SD groups whereas the corresponding numbers were 15, 2, and 1 when comparing the FFC_{DIA} and SD groups. Although the minipigs in both FFC groups developed sever obesity and dyslipidemia, the insulin-signaling pathways were not affected. Notably, four genes involved in lipid acquisition and removal, were highly deregulated in the liver: *PPARG, LPL, CD36 and FABP4*. These genes have been reported to play a major role in promoting hepatic steatosis in rodents and humans. Since very little macrophage-associated pro-inflammatory response was detected in the adipose tissues the expansion appears to have no adverse impact on adipose tissue metabolism.

Conclusion: The study shows that morbidly obese Göttingen Minipigs are protected against many of the metabolic and hepatic abnormalities associated with obesity due to a remarkable ability to expand the adipose compartments to accommodate excess calories.

Correspondence: Merete Fredholm (mf@sund.ku.dk)

¹Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, 1870 Frederiksberg, Denmark ²Global Drug Discovery, Novo Nordisk A/S, Novo Nordisk Park, Måløv, Denmark Full list of author information is available at the end of the article

© The Author(s) 2020

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

Introduction

Obesity has been associated with a strong predisposition to metabolic syndrome (MS) and Type 2 diabetes mellitus (T2DM). In turn, these diseases are strongly associated with non-alcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH). These conditions are characterized by a build-up of fat in the liver (hepatic steatosis), inflammation, fibrosis, and cell damage, and in extreme cases NASH can lead to liver cirrhosis with hepatic failure and/or hepatocellular carcinoma $(HCC)^1$. The molecular events underlying the development of NAFLD/NASH are poorly understood, however, it has been shown that there is a greater impact of metabolic health on the development of NAFLD, as compared to obesity per se². One determinant of metabolic health is the mechanism by which adipose tissue depots expand^{3,4}. This has led to the adipose tissue expandability hypothesis stating that the capacity of an individual to expand the fat mass to store lipid is a more important determinant of obesity-associated metabolic problems than the absolute amount of adipose tissue⁵. The expandability hypothesis predicts that some individuals tend to have a bigger capacity for adipose tissue storage and adaptation to excess energy while remaining metabolically healthy (metabolically healthy obese (MHO)). This capacity is most likely determined by genetic and epigenetic factors⁶.

Different breeds of minipigs, including Bama, Ossabaw and Göttingen have been used as models for MS and obesity, and some of them specifically as models for liver disease in humans. In these studies, different Western diets and atherogenic diets have been shown to have potential for producing liver fibrosis, systemic inflammation, insulin resistance and steatohepatitis^{7–13}. In general, however, these models only show very limited and primarily microvesicular hepatic steatosis as opposed to the more extensive macrovesicular type observed in humans. This indicates that inflammation and fibrosis are driven by other factors than steatosis in these porcine models.

We have previously provided evidence of genetic predisposition for an MHO-like phenotype in Göttingen Minipigs¹⁴. In this study, we have performed highthroughput qPCR on genes of relevance for metabolism in liver, subcutaneous adipose (SAT) and visceral adipose tissues (VAT) to characterize the transcriptional changes underlying the alterations observed in these tissues in Göttingen Minipigs fed chow, FFC diet, or FFC diet on a background of streptozotocin-induced diabetes for 13 months. The minipigs in the two groups fed the FFC diet were morbidly obese and dyslipidemic, however, key human pathological hepatic findings characterizing NAFLD/NASH were lacking. Our aim was to elucidate the molecular components underlying the histopathological changes observed in the model, and to explain the reason for the limited hepatic steatosis that characterizes the porcine models of metabolic syndrome and NASH.

Materials and methods

Animals

Castrated male Göttingen Minipigs (Ellegaard Gottingen Minipigs A/S, Dalmose, Denmark) (n = 84 in total) aged 6–7 months were weight stratified and distributed into six treatment groups and fed once daily for thirteen months. Of these, 29 pigs distributed in three groups were studied in this study. The included groups were: a lean control pigs (SD, N = 7) fed standard diet (Mini-pig, SDS, UK); a group fed high fat/fructose/cholesterol diet (FFC, N = 14) with (2%) cholesterol (5B4L) for the first five months and changed to a similar diet with 1% cholesterol (9G4U) for the next eight months (Test diet[®], Missouri, USA); a streptozotocin-induced diabetic group (FFC_{DIA}, N = 8) fed a high fat/fructose/cholesterol (1%) diet throughout the study (9G4U). Data from the same 29 pigs have been included in parallel studies focusing on histological changes in the liver tissue and myocardial changes^{12,15}. Basic phenotypic and metabolic characteristics, measured as described in¹² are reported in Table 1.

In the diabetic group (FFC_{DIA}) type 1-like diabetes was induced with streptozotocin (as described in ref. ¹²). Diabetic pigs were treated subcutaneously once daily with long acting insulin (Lantus[®], Sanofi A/S, Denmark) in order to maintain morning fasting blood glucose around 15 mM.

All animals were fasted overnight before euthanasia by exsanguination in deep general anesthesia (mixture of zolazepam, tiletamine, ketamine, xylazin and butorphanol). Samples from liver (left medial lobe), subcutaneous adipose (SAT) and visceral adipose (VAT) tissues were collected, snap frozen in liquid nitrogen and kept at -80 °C for expression studies.

RNA isolation

Fifty miligram of frozen tissue were used for RNA isolation using the Tri[®] Reagent protocol (MRC Gene, Cincinnati, OH 45212 USA). Briefly, the tissue was homogenized in 2 ml Tri[®] Reagent using M-tubes in a gentleMACS[™] Octo Dissociator machine (Miltenyi Biotec, Bergisch Gladbach, Germany) and processed according to the manufacturer's instructions. RNA samples were subsequently DNAse treated using RNeasy MinElute Cleanup kit (Qiagen, GmbH, Germany). RNA from adipose tissues were isolated from 20–180 mg tissue using the method described by ref. ¹⁶ including DNAse I treatment.

Concentration and purity of the RNA samples were measured on a Nanodrop ND-1000 spectrophotometer (NanoDrop technologies, Wilmington, USA). RNA integrity was assessed on an Experion machine using the RNA stdSens kit. All liver samples had an RNA-quality index (RQI) between 8.10 and 9.70 (average = 9.25 ± 0.36). Adipose tissue samples with an RQI between 6.5 and 10 were included for further processing (average = 8.4 ± 0.68 for SAT and average = 8.2 ± 0.92 for VAT). One SAT sample from the FFC_{DIA} group and three VAT samples (2 from the SD and 1 the FCC_{DIA} group) were excluded from the study due to low RQI.

Diet group	SD (<i>n</i> = 7)	FFC (<i>n</i> = 14)	FFC _{DIA} (<i>n</i> = 8)	Over all <i>p</i> -value
BW (kg)	39 (38; 41)	78 (69; 81) ^a	60 (54; 64) ^{ab}	<.0001
LW (g)	485 (458; 564)	1732 (1067; 2219) ^a	2077 (1478; 2439) ^a	<.0001
TBF%	28 (24; 31)	64 (61; 68) ^a	55 (53; 56) ^{ab}	<.0001
VAT (g)	510 (462–543)	2326 (1645–2946) ^a	1335(1032–1922) ^{a,b}	<0001^^^
TG in liver (mg/g)	12.6 (8.1–14.4)	15.0 (13.3–17.7)	19.6 (13.6–26.7) ^a	NS^
Plasma TC [#] (mmol/L)	1.70 (1.64; 2.18)	11.94 (11,00; 13.18) ^a	18.91 (16.91; 27.00) ^{ab}	<0.0001
Plasma TG [#] (mmol/L)	0.34 (0.29; 0.35)	0.63 (0.54; 0,88) ^a	1.45 (0.57; 1.72) ^{ab}	0.0002
Plasma GLU [#] (mmol/L)	3.48 (3.32; 3.67)	3.72 (3.60; 3.83)	15.1 (14.67; 15.45) ^{ab}	<.0001

Table 1Phenotypic and metabolic characteristics of the minipigs.

Results are presented as median and 25% and 75% quartiles; *n = 6 for SD, n = 13 for FFC, n = 6 for FFC_{DIA} due to catheter failure; BW body weight, LW liver weight, TBF Total body fat, VAT Visceral adipose tissue, TC total cholesterol, TG triglycerides, GLU glucose.

Transformed;

^{^^}Non-parametric test;

^aSignificantly different from SD

^bSignificantly different from FFC. Data except VAT have been presented previously (Andreasen et al., 2018; Schumacher-Petersen et al., 2019).

cDNA synthesis

Two replicates of cDNA were prepared from each RNA sample using 500 ng of DNAse treated RNA from liver tissue samples and 100 ng of DNAse treated RNA from adipose tissue samples. Briefly 0.5 μ l Improm-IITM reverse transcriptase (Promega, Madison, USA), 0.25 μ g 1:3 OligodT/Random primers, 2 μ l 5× ImProm-II buffer, 10 units RNasin Ribonuclease inhibitor (Promega, Madison, USA), 2.5 mM MgCl2 and 2 mM dNTP were mixed with RNA in a final reaction volume of 10 μ l. Reactions were incubated for 5 min at room temperature, 1 h at 42 °C and 15 min inactivation at 70 °C. Two negative controls were made for each tissue with no reverse transcriptase added (-RT control). The liver cDNA samples were diluted 1:16 and the adipose cDNA samples were diluted 1:8 prior to qPCR and stored at -80 °C until use.

Primer design

The genes included in this study were selected from an *in house* obesity panel used in other projects (e.g., ref. ¹⁷) supplemented by additional genes of importance to liver and adipose tissue metabolism (see Table S1). Four reference genes selected according to ref. ¹⁸ were included for normalization for each tissue. Primers were designed using the tool "Pick Primers" in PubMed to amplify a product in the range of 100 nucleotides, and if possible, were designed to span a large intron. Primer sequences, gene names and the respective tissues profiled are available in Table S1.

qPCR

High-throughput qPCR was performed on the Biomark HD system (Fluidigm Corporation, California, USA) using four 96.96 IFC chips. The diluted cDNA was used for preamplification (15 cycles for liver and SAT samples and 14 cycles for VAT samples) using TaqMan PreAmp Master Mix (Life Technologies, Nærum, Denmark). Subsequent cleanup with Exonuclease I (New England BioLabs, Herlev, Denmark) was performed according to the Fluidigm protocol (Fluidigm PN 100-5875 C1). A single modification to the standard protocol was made: we used 250 nM primer concentrations in the primer pool. Exonuclease cleaned liver and SAT cDNA were diluted 5x and VAT cDNA was diluted 10× before running the qPCR reactions using SsoFastTM EvaGreen[®] Supermix with Low ROX (Bio-Rad Laboratories, Copenhagen, Denmark) according to the manufacturer's instructions (PN 100–9792 B1) with the modification of using primer concentrations of 5 µM. A standard curve was established for each tissue using a dilution serial of a pool of preamplified cDNA samples. Data was obtained using the associated software. A few genes (TGFB1, LDAH, and BCL2 for liver and PPARG and CD36 for VAT) had several missing values in the Fluidigme qPCR study and were therfore re-run on the MxPro (Stratagene) platform. In addition, four of the deregulated genes (CD36, FABP4, LPL, and PPARG) were analyzed on the Mx3005P platform in all three tissues in order to make a direct comparison of expression level between tissues. In this study two additional primer sets were included for PPARG in order to amplify isoform 1 and isoform 2 separately (see Table S1). For all genes run on the Mx3005P platform, QuantiFast SYBRGreen master mix (Qiagen, GmbH, Germany) was used according to the manufacturer's protocol.

qPCR data processing and statistical analysis

qPCR raw data was pre-processed using Genex 6 Pro software (MultiD Analyses AB, Götteborg, Sweden).

Briefly, data was corrected according to PCR efficiency (PCR efficiencies between 80–110% were accepted), data was normalized to the two most stable reference genes (*TBP* and *ACTB* in liver and SAT; *TBP* and *YWHAZ* in VAT). Subsequently, technical cDNA replicates were averaged and relative expression was calculated by scaling data with the lowest expressed sample for each assay. Next, data was log2 transformed to achieve normal distribution before statistical analysis. Multiple test correction was applied to the analysis and statistical significance threshold was set at P < 0.0006 (according to the Dunn-Bonferroni correction for multiple testing). Comparisons between the different experimental groups were performed using *t*-test and fold-changes (FC) and *P* values under < 0.05 were reported (Tables 1, 2, 3).

For the metabolic and physical measurements, group differences were evaluated using ANOVA and post hoc *t*-test. Parameters were transformed if they did not meet model requirements. Kruskal-Wallis test with Wilcoxon Rank-sum post hoc test (non parametric) was used if transformed data did not meet model requirement even after transformation. Data and results are presented as median and 25% and 75% quartiles. Values of P < 0.05 were considered statistically significant.

Pearson correlation analyses were performed on gene expression data for *CD36*, *FABP4*, *LPL*, and *PPARG* in the liver tissue and relevant metabolic/physical measurements using Rstudio¹⁹. For the Pearson correlation analysis, log2 transformed expression values were further processed using R scripts, together with the Göttingen Minipig physiological measurements

Histology

VAT and SAT tissue samples were fixed in formalin and subsequently embedded in paraffin following standard procedures. Sections of $3 \mu m$ thickness were cut from SAT and VAT and routinely stained with hematoxylin and eosin (HE) as described previously¹².

Results

The expression profiles of 96 genes in liver, and 98 genes in SAT and VAT samples were assayed on the Fluidigm high-throughput qPCR platform and/or on the Mx3005P 96-format platform (see Table S1 for details). Since the *PPARG* transcript generates two isoforms (*PPARG1* and *PPARG2*) three primer pairs were used for the amplification of the transcripts. The *PPARG* primers amplify both isoforms whereas *PPARG1* and *PPARG2* amplify the respective isoforms. The total number of successful assays (including reference genes) was: 82 in liver, 86 in SAT and 90 in VAT. Fourteen assays in liver, 12 in SAT and eight in VAT failed due to one of the following reasons: unspecific amplification as evidenced by more than one peak in the melting curve; expression

levels under the limit of detection; qPCR efficiencies out of range (<80% or >110%) or no amplification. Raw Cq values for all successfully profiled genes in liver, SAT and VAT are presented in Tables S2, S3, and S4 respectively. All differentially expressed genes for which P < 0.05 are listed in Tables 2, 3, 4. In the following only differentially expressed genes with fold changes (FC) > 2 or <-1.5 and multiple testing-corrected P values < 0.0006 will be discussed together with a few genes of interest having differential expression with P < 0.05.

Differential expression in liver

As shown in Table 2, when looking at gene expression in the liver of animals subjected to the FFC diet vs. the SD diet 12 genes showed significant P values after correction for multiple testing with FC of >2 or < -1.5. Four of these genes were upregulated (LPL (FC = 172.23), CD68 (FC = 5.17), *PPARG* (FC = 19.08) and *CD36* (FC = 3.36), and eight were downregulated (*KLB* (FC = -22.24), *HMGCR* (FC = -13.36), FDFT1 (FC = -6.31), LDLR (FC = -5.92),SOD1 (FC = -1.76), STAT3 (FC = -1.92), PPARGC1A (FC = -2.38) and *MTTP* (FC = -1.73)). In addition, 33 genes were deregulated with P < 0.05 and of those, 21 genes had FC > 2 or > -1.5; among these FABP4 with FC = 2.56 (see Table 2). A total of 9 out of the 12 genes deregulated between the FFC and the SD groups were also deregulated between the FFC_{DIA} and the SD groups. The differential expression between these two groups was, however, much higher for some of the genes, i.e., FC =308.25 for LPL; FC = 35.56 for PPARG; FC = -16.30 for *FDT1*; FC = -36.89 for *KLB*; FC = -6.30 for *CD36*; and FC = 11.8 for *FABP4* indicating that induction of diabetes had additional impact on perturbation of liver metabolism compared to the FFC diet alone. This is further supported by the fact that additional genes obtained multiple testing corrected P values < 0.0006 (AGT, TLR4, FABP4, MMP9, GHR, and GCKR) (see Table 2). Generally, the insulinsignaling pathways were not affected neither in the FFC diet group nor in the FFC_{DIA} diet group as documented by an unchanged level of transcription of e.g., IDE, INSIG1, INSIG2, IGF1, IGF2, IGFBP2, and IRS1. Only IRS2 was slightly downregulated in both the FFC and the FFC_{DIA} groups (FC = -1.73 and -1.94, respectively) and INSR was slightly downregulated in the FFC group. (FC = -1.32).

Differential expression in adipose tissues

As shown in Table 3a, b the fold changes of the differentially expressed genes were much lower in the adipose tissues relative to the differential expression observed in liver. There was only four (*PN-1*, *EBF2*, *ABCG1*, *IL6*) and two (*ABCG1*, *IL6*) genes with FC > 2 or < -1.5 in SAT when comparing FFC vs. SD and FFD_{DiA} vs. SD, respectively with *P* values passing multiple testing.

Table 2 Differential expression in the liver.

Genes FC P value Genes FC P value FU 172.3 1.00E-08 LPL 380.25 1.00E-08 CD68 5.17 1.00E-08 CD68 6.30 1.00E-08 PARIG 133.6 2.224 7.66E-00 FDFT1 -16.30 5.96E-07 HMCCR -13.35 2.22E-05 KLB -36.89 3.74E-05 FDFT1 -6.31 3.76E-06 CD36 6.30 3.94E-05 SOD1 -1.76 5.02E-03 ACT 2.99 3.06E-05 SOD1 -1.76 5.02E-03 ACT 2.99 3.06E-05 STAT3 -1.92 0.0003/1.384 HMGCR -14.25 4.28E-05 PPARGCIA -3.38 0.00088323 GHR -2.66 0.000146258 ACDS -1.57 0.001397134 LDLR -4.38 0.00066271 TR4R4 2.56 0.00139734 LDLR -4.38 0.00160529 CD63 -1.57 0.001595	FFC vs. SD			FFC _{DIA} vs. SD		
LPL 172.23 1.00E08 LPL 308.25 1.00E08 CD68 5.17 1.00E08 CD68 6.30 1.00E08 PARIG 19.08 1.66F68 PARIG 3.556 1.70E07 KLB -22.24 7.66F05 KLB -36.99 2.74E05 FDFT1 -6.31 3.76E05 CD36 6.30 3.94E05 SOD1 -1.76 S.07E05 ACT -2.02 1.49E05 SOD1 -1.76 S.07E05 ACT -2.89 3.04E05 SOD3 3.36 8.79E05 ACT -2.80 2.44E05 STAT3 -1.92 D.000371384 HMGCR -14.36 4.28E05 PARGC1A -2.38 D.00038187 FABP4 1.181 9.91E-05 MTTP -1.73 D.0035223 GFA -2.65 0.000416758 AFO8 -1.57 D.01352823 GFA -2.265 0.000416759 MMF9 6.02 D.001562475 <th>Genes</th> <th>FC</th> <th>P value</th> <th>Genes</th> <th>FC</th> <th>P value</th>	Genes	FC	P value	Genes	FC	P value
CDGS 5.17 1.0E-08 CDGS 6.30 1.0E-08 PARG 1928 1.0E-08 PARG 53.56 1.7E-08 KLB -2224 7.69E-06 PDFT1 -16.30 5.04E-07 HMCCR -13.26 2.2E-05 KLB -56.69 3.7E-05 SD1 -7.07 5.02E-05 SO1 -2.02 1.4FE-05 CD36 3.36 8.7E-05 ACT -2.89 2.45E-05 CD36 3.36 8.7E-05 TLR 2.89 0.00016411 PARGCIA -2.28 0.000371384 HMGCR -1.45 0.00016411 FAR4 2.65 0.00130232 GHR -2.05 0.000164125 AF08 -1.57 0.001698259 TMS52 -1.33 0.0005621 MP9 6.02 0.001698259 TMS52 -1.33 0.000169825 MS72 -1.48 0.002197267 FLA7 -3.00 0.0020272 MMP9 6.02 0.003613261 LA7 </td <td>LPL</td> <td>172.23</td> <td>1.00E-08</td> <td>LPL</td> <td>308.25</td> <td>1.00E-08</td>	LPL	172.23	1.00E-08	LPL	308.25	1.00E-08
PPARG 19.08 1.06—08 PPARG 1.9.5.6 1.06—07 KLB -22.24 7.696—06 FDFT1 -16.30 3.746—06 FDFT1 -6.31 3.746—05 CD36 6.30 3.946—05 FDFT1 -6.31 3.746—05 CD36 6.30 3.946—05 SD01 -1.76 0.026—05 ACT -2.20 1.496—05 SD13 -1.92 0.000371344 HMGCR -1.41.6 4.286—05 STA3 -1.92 0.000371344 HMGCR -1.42.6 4.000406524 PARC1A -2.23 0.00016947 FABP4 -1.63 0.000016947 FABP4 2.50 0.001362476 GCKR -2.13 0.000016947 FABP4 1.50 0.001169476 GCKR -2.13 0.000016947 TL44 1.89 0.001169476 GCKR -2.13 0.000015959 CK17 -1.31 0.00214902 LIN1 -2.46 0.00213927 TL65 1.58	CD68	5.17	1.00E-08	CD68	6.30	1.00E-08
KLB -22/4 7.06 FDFT1 -16.30 5.04E-07 HMGCR -13.36 2.28E-05 KLB -36.89 3.74E-06 DFT1 -6.31 3.76E-05 CD36 6.30 3.94E-06 LDLR -5.92 3.87E-05 SD01 -2.02 1.48E-05 CD36 3.36 8.78E-05 TLR -2.02 1.48E-05 CD36 3.36 8.78E-05 TLR -2.02 1.48E-05 CD36 3.36 8.79E-05 TLR -2.02 1.48E-05 PARGCIA -2.33 0.00348187 FABP4 1.181 9.91E-05 MTTP -1.73 0.0035024 MMP9 7.80 0.00014628 APOS -1.157 0.00136263 TMSF2 -1.31 0.0005851 MMP9 6.02 0.0016856 TMSF2 -1.33 0.000179799 ACT -1.66 0.00214768 SREF1 3.65 0.00217929 MMS2 1.13 0.002518911 SCARB1	PPARG	19.08	1.60E-08	PPARG	35.56	1.70E-07
HMCCR -13.36 22805 KLB -3.689 3.74E05 FPTT1 -6.31 3.76E-05 SOD1 -2.02 1.47E-05 SOD1 -1.76 5.02E-05 AGT -2.80 2.45E-05 SOD1 -1.76 5.02E-05 AGT -2.80 2.45E-05 STAT3 -1.92 0.000371384 HMCCR -14.26 4.28E-05 PPARGCIA -2.33 0.000488187 FABP4 11.81 9.91E-05 MTP -1.73 0.00052021 GKR -2.65 0.00016407 FABPA 1.89 0.00156275 GCKR -2.15 0.00065221 MMP9 6.02 0.001688359 TM6572 -1.53 0.0001799769 TM4 1.89 0.0023751 TM572 -1.53 0.0001799769 TM552 -1.31 0.0023751 TM572 -1.53 0.0001799769 TM552 1.58 0.002417482 LCAT -3.60 0.0022747 SREF1 2.60 0.	KLB	-22.24	7.69E-06	FDFT1	-16.30	5.04E-07
FPFT1 -631 376-05 CD36 630 394E-05 LDLR -5.92 382E-05 SO01 -2.02 1.49E-05 CD36 -3.36 8.7E-05 ACT -2.80 2.82E-05 CD36 -3.36 8.7E-05 TLR4 2.89 3.0EE-05 PARGCTA -2.38 0.00048187 FABP4 1.181 9.91E-05 MTTP -1.73 0.00052084 MMP9 7.80 0.00016051 AP08 -1.57 0.001387134 LDLR -4.38 0.00048251 MMP9 6.62 0.001698359 TM65F2 -1.33 0.000129999 MMP9 6.62 0.00214768 SREF1 3.65 0.00212925 CL3 1.58 0.00214768 SREF1 3.65 0.0022325 CL3 1.58 0.00313726 LNN1 -2.84 0.0022325 CL3 1.58 0.003410192 LNN1 -2.84 0.00202327 RP4 -1.70 0.00330325	HMGCR	-13.36	2.28E-05	KLB	-36.89	3.74E-06
LDLR -592 322-05 SOD1 -202 1,49-05 SOD1 -1.76 5.02E-05 AGT -280 2,45E-05 SOD3 3.36 8.79E-05 TLR4 2.80 3.06E-05 STAT3 -1.92 0.000371384 HMGCR -14.26 4.28E-05 PARIGCIA -2.38 0.0004807 FABP4 11.81 9.91E-05 MTP -1.73 0.00052084 MMP9 7.80 0.000166417 FABP4 2.56 0.001362823 GHR -2.25 0.00046153 AP08 -1.57 0.001352476 GCKR -2.15 0.0005821 MMP9 6.02 0.0014768 SEEF1 3.65 0.001297969 AGT -1.66 0.00214768 SEEF1 3.60 0.001297267 TM652 -1.31 0.002397241 PNPLA3 -5.26 0.001391929 BC12 2.14 0.0024302 LCAT -3.00 0.00028525 CLS 1.58 0.0024302 LCAT -3.00 0.000289257 REP4 -1.70 0.00360576 IR52 -1.94 0.002132627 REP4 2.60 0.0044657 MBOA17 -1.44 0.002904274 <tr< td=""><td>FDFT1</td><td>-6.31</td><td>3.76E-05</td><td>CD36</td><td>6.30</td><td>3.94E-06</td></tr<>	FDFT1	-6.31	3.76E-05	CD36	6.30	3.94E-06
SOD1 -1.76 S02E-05 AGT -2.80 2.45E-05 CD36 3.36 8.79E-05 TLR4 2.89 3.06E-05 STAT3 -1.92 0.000371384 HMGCR -1.42 4.28E-05 PARGC1A -2.38 0.00052084 MMP9 7.80 0.000166147 FAR4 2.55 0.00136223 GHR -2.65 0.0006621 APO8 -1.57 0.001562476 GCKR -2.15 0.0005621 MMP9 6.02 0.00168359 TM65F2 -1.33 0.000259769 AGT -1.69 0.00214768 SEEF1 3.65 0.00129769 AGT -1.69 0.00214768 SEEF1 3.66 0.002132976 RGT -1.69 0.00214702 LCAT -3.00 0.0002525 CL2 2.14 0.002743402 LCAT -3.00 0.0002132027 RE4 -1.70 0.003613226 L18 -3.60 0.002132027 RE52 -1.73 0.0004697	LDLR	-5.92	3.82E-05	SOD1	-2.02	1.49E-05
CD36 3.36 8.78–05 TL4 2.89 3.06–05 STAT3 -1.92 0.00037134 HMGCR -1.42.6 4.28F–05 PPARGCIA -2.38 0.00048187 FABP4 1.181 9.91E–05 MTTP -1.73 0.00037034 MMP9 7.80 0.000461238 AP08 -1.57 0.00136223 GHR -2.65 0.00048521 MMP9 6.02 0.00168276 GCR -2.15 0.0005621 MMP9 6.02 0.0018939 TM657 -1.33 0.00015959 MCF -1.66 0.00214768 SREBF1 3.65 0.00157959 RCI2 2.14 0.00273402 LCAT -3.00 0.00203255 CCI5 1.58 0.003410192 LPN1 -2.84 0.00203255 CCI5 1.58 0.003406457 MD6AT -2.01 0.00203257 REPA -1.20 0.003906641 RDA -2.01 0.00303255 REBF1 2.60 0.00446	SOD1	-1.76	5.02E-05	AGT	-2.80	2.45E-05
STATS -1.92 0.00371384 HMGCR -1.426 4.28E-OS PPARGC1A -2.38 0.00048187 FABP4 11.81 9.91E-OS MTTP -1.73 0.00052084 MMP9 7.80 0.00016417 FABP4 2.56 0.001397134 LDR -4.38 0.00048531 APOB -1.57 0.001397134 LDR -4.38 0.00065221 MMP9 6.02 0.00198359 TMSF2 -1.53 0.000129766 MMS7 -1.66 0.00214768 SEBF1 3.65 0.00129769 SCL2 2.14 0.002518911 SCAR81 -1.59 0.000212826 BCL2 2.14 0.00235251 LIB -3.60 0.00229327 SEBF1 2.60 0.00448657 MBOAT7 -1.44 0.00029257 SEBF1 2.60 0.00448657 MBOAT7 -1.44 0.00029247 SEBF1 2.60 0.00448657 MBOAT7 -1.44 0.00039247 GLUT2 -1.81 <td>CD36</td> <td>3.36</td> <td>8.79E-05</td> <td>TLR4</td> <td>2.89</td> <td>3.06E-05</td>	CD36	3.36	8.79E-05	TLR4	2.89	3.06E-05
PPARGC1A -2.38 0.000488187 FABP4 11.81 9.91E-05 MTTP -1.73 0.00052084 MMP9 7.80 0.000164278 APOB -1.57 0.001397134 LDLR -4.38 0.0004628 APOB -1.57 0.001362476 GCCR -2.15 0.00056283 MMP9 6.62 0.0016359 TMSF2 -1.53 0.00056856 GCKR -1.66 0.00214768 SREBF1 3.65 0.00157959 AGT -1.69 0.002518911 SCARB1 -1.59 0.00028255 CLS 1.58 0.00316326 L18 -3.60 0.00213227 RS4 -1.70 0.0036641 RORA -2.01 0.000203255 CLS 1.58 0.003410192 LPN1 -2.84 0.000213227 RS4 -1.70 0.0036641 RORA -2.01 0.00302747 RS4 2.60 0.00448657 MBOAT7 -1.44 0.00302747 RS4 -1.73 <t< td=""><td>STAT3</td><td>-1.92</td><td>0.000371384</td><td>HMGCR</td><td>-14.26</td><td>4.28E-05</td></t<>	STAT3	-1.92	0.000371384	HMGCR	-14.26	4.28E-05
MTTP -173 0.00052084 MMP9 7.80 0.000166417 FABP4 2.56 0.000136223 GHR -2.65 0.00048511 TLR4 1.89 0.001562476 GCKR -2.15 0.00056221 MMP9 6.02 0.001698359 TM65F2 -1.53 0.00057559 CKR -1.66 0.00214768 SRBF1 3.55 0.00175959 CKR -1.69 0.002518911 SCARB1 -1.59 0.00075255 CL2 2.14 0.00237741 PNPLA3 -5.26 0.00075255 CL5 1.58 0.00361326 LPIN1 -2.84 0.00207255 CL5 1.58 0.00364132 LPIN1 -2.84 0.002132827 RP4 -1.70 0.0036641 RDA -2.01 0.00312325 SREF1 2.60 0.00444657 M60AT7 -1.44 0.0029274 SREF1 2.60 0.00497217 STAT3 -2.01 0.003124751 GUT2 -1.81 <	PPARGC1A	-2.38	0.000488187	FABP4	11.81	9.91E-05
FABP4 2.56 0.001362823 GHR -2.65 0.000416288 APOB -1.57 0.001397134 LDLR -4.38 0.00048851 MMP9 6.02 0.001698359 TM65F2 -1.53 0.00065221 MMP9 6.02 0.00129768 SREP1 3.65 0.00129759 MGK2 -1.61 0.00214768 SREP1 3.65 0.00129759 AGT -1.69 0.00218911 SCARB1 -1.59 0.00127959 AGT -1.69 0.00213827 LCAT -3.00 0.00205255 CL2 2.14 0.00274302 LCAT -3.00 0.00205255 SEEP1 2.56 0.003405261 LLB -3.60 0.002132827 RS2 -1.73 0.003405611 RDAAT -1.14 0.00299874 CS4 -2.57 0.004465716 IS2 -1.94 0.002247 NR12 -1.48 0.00497217 STAT3 -2.01 0.003147541 GL72 -1.73	MTTP	-1.73	0.00052084	MMP9	7.80	0.000166417
APOB -1.57 0.001397134 LDLR -4.38 0.000488511 TLR4 1.89 0.001562476 GCKR -2.15 0.00056221 MMP9 6.02 0.001698359 TM65F2 -1.53 0.000189759 GCKR -1.66 0.00214768 SREBF1 3.65 0.001297595 AGT -1.69 0.002297241 PNPLA3 -5.26 0.0018971992 BCL2 2.14 0.002297240 LCAT -3.00 0.00205255 CCL5 1.58 0.003410192 LPIN1 -2.84 0.002092867 RBP4 -1.70 0.003613226 IL18 -3.60 0.00212787 RS2 -1.73 0.003005041 RS2 -1.94 0.0022747 SREBF1 2.60 0.00448657 MBOAT7 -1.44 0.003012741 GUT2 -1.81 0.00505041 RS2 -1.94 0.003147441 GUT2 -1.81 0.00505041 RDA -1.73 0.004045161 GNAT -2.19	FABP4	2.56	0.001362823	GHR	-2.65	0.000416258
TLH 1.89 0.001562476 GCKR -2.15 0.00056221 MMP9 6.02 0.00159839 TM65F2 -1.53 0.0001865 CCKR -1.66 0.002397241 PNPLA3 -5.26 0.001597595 AGT -1.69 0.00231911 SCARB1 -1.59 0.00128275 CL2 2.14 0.002414268 CLAT -3.00 0.00205255 CL3 1.58 0.003410192 LPIN1 -2.24 0.00208226 R8P4 -1.70 0.003613266 L18 -3.60 0.002132827 RS52 -1.73 0.003906641 RORA -2.01 0.0030022447 PCS89 -2.57 0.004967516 IR52 -1.94 0.00302247 NR12 -1.48 0.004979217 STAT3 -2.01 0.004047541 GUT7 -1.81 0.006004771 RCL2 2.63 0.00421219 ACACA -1.61 0.00600471 TKF -2.63 0.00567015 GURAT -1.30 <td>APOB</td> <td>-1.57</td> <td>0.001397134</td> <td>LDLR</td> <td>-4.38</td> <td>0.000488511</td>	APOB	-1.57	0.001397134	LDLR	-4.38	0.000488511
MMP9 6.02 0.001698359 TM6SF2 -1.53 0.00061856 GCKR -1.66 0.00214768 SREBF1 3.65 0.00157959 AGT -1.69 0.002397241 PNPLA3 -5.26 0.001575959 AGT -1.69 0.002319211 SCARB1 -1.59 0.002087265 BCL2 2.14 0.00241402 LCAT -3.00 0.002087265 CCL5 1.58 0.003410192 LPIN1 -2.84 0.002087265 R84 -1.70 0.00346611 RORA -2.01 0.00208727 SRE5F1 2.60 0.00444657 MBOAT7 -1.44 0.00390277 SR12 -1.81 0.00505941 RS2 -1.93 0.0043257 GMT -2.19 0.003392574 DGAT2 -1.73 0.00446619 APCC3 -1.69 0.00604771 RCL2 2.63 0.00424219 ACCA -1.61 0.00889157 NR112 -1.49 0.00866271 IGFBP2 -1.80<	TLR4	1.89	0.001562476	GCKR	-2.15	0.00056221
GCKR -1.66 0.00214768 SREBF1 3.65 0.002199769 TM6SF2 -1.31 0.002397241 PNPLA3 -5.26 0.00157595 AGT -1.69 0.00218911 SCARB1 -1.59 0.00205255 CL1 2.14 0.002743402 LCAT -3.00 0.00205255 CCL5 1.58 0.003410192 LPIN1 -2.84 0.002093257 RSP4 -1.70 0.003613226 LIB -3.60 0.002093257 RSP4 -1.73 0.003906641 RORA -2.01 0.003022747 RSP4 -2.57 0.004967516 IRS2 -1.94 0.003022747 NR12 -1.48 0.00497917 STAT3 -2.01 0.003147541 GILT2 -1.13 0.0060050541 RBP4 -1.79 0.00434561 GINT -2.19 0.00505041 RBP4 -1.79 0.0064771 ACACA -1.61 0.006609175 NR112 -1.49 0.00664227 ISIG1 <td< td=""><td>MMP9</td><td>6.02</td><td>0.001698359</td><td>TM6SF2</td><td>-1.53</td><td>0.00061856</td></td<>	MMP9	6.02	0.001698359	TM6SF2	-1.53	0.00061856
TM6SF2 -1.31 0.002397241 PNPLA3 -526 0.001575959 AGT -1.69 0.002518911 SCARB1 -1.59 0.00207285 BCL2 2.14 0.00243402 LCAT -3.00 0.00205255 CL5 1.58 0.003410192 IPIN1 -2.84 0.002012827 RBP4 -1.70 0.003613226 IL18 -3.60 0.00213287 IR52 -1.73 0.003905641 RORA -2.01 0.00290887 PCSN9 -2.57 0.004967516 IR52 -1.94 0.003012747 RI12 -1.48 0.004979217 STAT3 -2.01 0.003147541 GUT2 -1.81 0.005005011 RBP4 -1.73 0.004045161 GNMT -2.19 0.00604771 BCL2 2.63 0.00421219 ACACA -1.61 0.006039175 PARGC1A -2.65 0.005578895 MBOAT7 -1.63 0.007224475 PARGC1A -2.65 0.00880278 ISFEP2	GCKR	-1.66	0.00214768	SREBF1	3.65	0.001299769
AGT -1.69 0.002518911 SCAR81 -1.59 0.001871992 BCL2 2.14 0.002743402 LCAT -3.00 0.00205255 CCL5 1.58 0.003410192 LPIN1 -2.84 0.002087286 BRP4 -1.70 0.0030613262 LI1B -3.60 0.00293257 IRS2 -1.73 0.003906641 RORA -2.01 0.002903257 SREBF1 2.60 0.004448657 MBOAT7 -1.44 0.002903274 PCSk9 -2.57 0.004979217 STAT3 -2.01 0.003022747 SRL1 -1.48 0.000505041 RBP4 -1.79 0.004036518 GUT2 -1.81 0.00505041 RBP4 -1.79 0.004036518 GNMT -2.19 0.005639175 RDR12 -1.49 0.00557895 MBOAT7 -1.30 0.007224475 PPARGC1A -2.63 0.00567015 IGFP2 -1.86 0.006639175 NR112 -1.49 0.00580714 INSIG1 -2.20 0.012447244 EPR_01 2.55 0.008870278 <tr< td=""><td>TM6SF2</td><td>-1.31</td><td>0.002397241</td><td>PNPLA3</td><td>-5.26</td><td>0.001575959</td></tr<>	TM6SF2	-1.31	0.002397241	PNPLA3	-5.26	0.001575959
BCL2 2.14 0.002743402 LCAT -3.00 0.00205255 CCL5 1.58 0.00310192 LPN1 -2.84 0.002087266 RBP4 -1.70 0.003613226 L1B -3.60 0.002132827 IRS2 -1.73 0.003906641 RORA -2.01 0.00290327 SREBF1 2.60 0.00446657 MBOAT7 -1.44 0.00299834 PCSK9 -2.57 0.004967516 IRS2 -1.94 0.00302747 NR12 -1.48 0.004979217 STAT3 -2.01 0.003147541 GLV12 -1.81 0.0050392574 DGAT2 -1.73 0.004063618 GNMT -2.19 0.005392574 DGAT2 -1.73 0.0046421219 ACACA -1.61 0.006004771 BCL2 2.63 0.005578895 MBOAT7 -1.30 0.007224475 PPARC1A -2.63 0.00592895 MBOAT7 -1.30 0.007224475 PAAC1A -1.65 0.008870278 ISFBP2 -1.86 0.001049444 LEPR_01 2.55 0.008111383 <t< td=""><td>AGT</td><td>-1.69</td><td>0.002518911</td><td>SCARB1</td><td>-1.59</td><td>0.001871992</td></t<>	AGT	-1.69	0.002518911	SCARB1	-1.59	0.001871992
CCL5 1.58 0.003410192 LPIN1 -2.84 0.00207286 RBP4 -1.70 0.003613226 LL1B -3.60 0.00213287 IRS2 -1.73 0.003906641 RDRA -2.01 0.00299287 SREBF1 2.60 0.00448657 MBOAT7 -1.14 0.003902747 NR12 -1.48 0.00497516 IRS2 -1.94 0.00302747 SNT1 -1.48 0.005392574 DRAT2 -1.73 0.00436518 GNMT -1.69 0.00509217 STAT3 -2.01 0.003147541 GL12 -1.81 0.0050392574 DRAT2 -1.73 0.00442121 APOC3 -1.69 0.00604771 BCL2 2.63 0.004578895 MBOAT7 -1.30 0.007224475 PPARC1A -2.63 0.00569715 IGFBP2 -1.86 0.0089145 TNF -2.63 0.007249724 ISIG1 -2.80 0.01049444 LEPR_011 2.55 0.00811383 MCM5	BCL2	2.14	0.002743402	LCAT	-3.00	0.00205255
RBP4 -1.70 0.003613226 L1B -3.60 0.002132827 IRS2 -1.73 0.003906641 RORA -2.01 0.002903257 SREBF1 2.60 0.004448657 MBOAT7 -1.44 0.002998874 PCSk9 -2.57 0.004967516 IRS2 -1.94 0.003022747 NRI12 -1.48 0.004979217 STAT3 -2.01 0.003147541 GLUT2 -1.81 0.005005011 RBP4 -1.79 0.004035618 GNMT -2.19 0.005392574 DGAT2 -1.73 0.004160409 ACACA -1.61 0.006809141 TNF -2.96 0.005578895 MBDAT7 -1.30 0.007224475 PPARGC1A -2.63 0.005607015 IGFBP2 -1.86 0.008639175 NR112 -1.49 0.006840227 LPIN1 -2.02 0.010494044 LEPR_01 2.55 0.008111383 MCM5 1.67 0.0108981469 APOA1 6.29 0.007329076 INSIG1 -2.80 0.01173678 FGFR4 -1.70 0.008870278 <td>CCL5</td> <td>1.58</td> <td>0.003410192</td> <td>LPIN1</td> <td>-2.84</td> <td>0.002087286</td>	CCL5	1.58	0.003410192	LPIN1	-2.84	0.002087286
IRS2 -1.73 0.003906641 RORA -2.01 0.002903257 SREBF1 2.60 0.004448657 MBOAT7 -1.44 0.00299874 PCSK9 -2.57 0.004967516 IRS2 -1.94 0.003022747 NR112 -1.48 0.004979217 STAT3 -2.01 0.003147541 GLUT2 -1.81 0.0050392574 DGAT2 -1.73 0.004160409 APOC3 -1.69 0.006004771 BCL2 2.63 0.005392578 MBOAT7 -1.30 0.00722475 PPARGC1A -2.63 0.005578895 MBOAT7 -1.36 0.0060839175 NR112 -1.49 0.00564227 LPIN1 -2.02 0.010294258 APOA4 6.29 0.007329076 INSG1 -2.80 0.010494044 LEPR_01 2.55 0.008111383 MCM5 1.67 0.018981469 APOA1 -1.65 0.00890278 SCAR1 -1.38 0.02173678 FGFR4 -1.70 0.009491741 SCAR1 -1.38 0.021396819 APOC3 -1.66 0.01454742 <td>RBP4</td> <td>-1.70</td> <td>0.003613226</td> <td>IL1B</td> <td>-3.60</td> <td>0.002132827</td>	RBP4	-1.70	0.003613226	IL1B	-3.60	0.002132827
SREBF1 2.60 0.00448657 MBOAT7 -1.44 0.00299874 PCSK9 -2.57 0.004967516 IRS2 -1.94 0.003022747 NR12 -1.48 0.004979217 STAT3 -2.01 0.003147541 GLUT2 -1.81 0.005005041 RBP4 -1.79 0.004035618 GNMT -2.19 0.005392574 DGAT2 -1.73 0.004160409 APOC3 -1.69 0.006004771 BCL2 2.63 0.005578895 MBOAT7 -1.30 0.00722475 PPARGC1A -2.63 0.005050715 IGFBP2 -1.86 0.008639175 NR112 -1.49 0.00684227 LPIN1 -2.02 0.010294258 APOA4 6.29 0.00722076 ISIG1 -2.80 0.010494044 LEPR_01 -1.55 0.008870278 RORA -1.63 0.021173678 FGFR4 -1.70 0.009941781 SCARB1 -1.38 0.021366174 TGFB1 1.72 0.016120599	IRS2	-1.73	0.003906641	RORA	-2.01	0.002903257
PCSK9 -257 0.004967516 IRS2 -1.94 0.003022747 NR112 -1.48 0.004979217 STAT3 -2.01 0.003147541 GLUT2 -1.81 0.00505041 RBP4 -1.79 0.004035618 GNMT -2.19 0.005392574 DGAT2 -1.73 0.004160409 APOC3 -1.69 0.006004771 BCL2 2.63 0.002578895 MBOAT7 -1.30 0.007224475 PARGC1A -2.63 0.00560715 IGFBP2 -1.86 0.00639175 NR112 -1.49 0.006804271 LFN1 -2.02 0.010294258 APOA4 6.29 0.007329076 INSIG1 -2.80 0.010494044 LEPR_01 2.55 0.008111383 MCM5 1.67 0.018981469 APOA1 -1.65 0.008870278 RORA -1.63 0.021173678 FGFH4 -1.70 0.0019941741 SCARB1 -1.38 0.022467243 PCSK9 -2.67 0.015028677 SCD 1.80 0.022467243 PCSK9 -2.67 0.015028677	SREBF1	2.60	0.004448657	MBOAT7	-1.44	0.002998874
NR112 -1.48 0.004979217 STAT3 -2.01 0.003147541 GLUT2 -1.81 0.00505041 RBP4 -1.79 0.004035618 GNMT -2.19 0.005392574 DGAT2 -1.73 0.004160409 APOC3 -1.69 0.006004771 BCL2 2.63 0.004241219 ACACA -1.61 0.006809141 TNF -2.63 0.0050578895 MBOAT7 -1.86 0.008639175 PPARGC1A -2.63 0.00507015 LFRP2 -1.86 0.008639175 NR112 -1.49 0.006646227 LPN1 -2.02 0.010294258 APOA4 6.29 0.007329076 INSIG1 -2.80 0.010494044 LEPR_01 2.55 0.00811383 MCM5 1.67 0.018981469 APOA1 -1.65 0.008870278 RORA -1.63 0.02173678 FGFF4 -1.70 0.009941781 SCAP -1.80 0.02467274 TGFFB1 1.72 0.016102656 SCAP<	PCSK9	-2.57	0.004967516	IRS2	-1.94	0.003022747
GLUT2 -1.81 0.005005041 RBP4 -1.79 0.004035618 GNMT -2.19 0.005392574 DGAT2 -1.73 0.004160409 APOC3 -1.69 0.006004771 BCL2 2.63 0.004241219 ACACA -1.61 0.006809141 TNF -2.96 0.005578895 MBOAT7 -1.30 0.007224475 PPARGC1A -2.63 0.005607015 ICFBP2 -1.86 0.008639175 NR112 -1.49 0.008846227 LPIN1 -2.02 0.010294258 APOA4 6.29 0.007329076 INSG1 -2.80 0.010494044 LEPR_01 2.55 0.0088111383 MCM5 1.67 0.018981469 APOA1 -1.65 0.008970278 SCAR81 -1.38 0.021173678 FGFR4 -1.70 0.00941781 SCAR81 -1.38 0.021396819 APOC3 -1.66 0.014547442 DGAT2 -1.70 0.02487243 PCSK9 -2.67 0.015028567 SCAP -1.28 0.03246191 APOB -1.40 0.02257703	NR112	-1.48	0.004979217	STAT3	-2.01	0.003147541
GNMT -2.19 0.005392574 DGAT2 -1.73 0.00416049 APOC3 -1.69 0.00604771 BCL2 2.63 0.004241219 ACACA -1.61 0.00689141 TNF -2.96 0.005578895 MBOAT7 -1.30 0.007224475 PPARGC1A -2.63 0.006846227 LPIN1 -2.02 0.010294258 APOA4 6.29 0.007329076 INSIG1 -2.80 0.01049044 LEPP_01 2.55 0.00811781 SCARB1 -1.63 0.021173678 FGFR4 -1.70 0.009941781 SCARB1 -1.38 0.021396819 APOC3 -1.66 0.014547442 DGAT2 -1.70 0.024872243 PCSK9 -2.67 0.015028567 SCD 1.80 0.022667474 TGFB1 1.72 0.017813835 SCAP -1.28 0.03246191 APOB -1.40 0.022577003 TIMP1 1.87 0.032793714 GLUT2 -1.84 0.024123063 GHR <td>GLUT2</td> <td>-1.81</td> <td>0.005005041</td> <td>RBP4</td> <td>-1.79</td> <td>0.004035618</td>	GLUT2	-1.81	0.005005041	RBP4	-1.79	0.004035618
APOC3 -1.69 0.006004771 BCL2 2.63 0.004241219 ACACA -1.61 0.006809141 TNF -2.96 0.005578895 MBOAT7 -1.30 0.007224475 PPARGC1A -2.63 0.005607015 IGFBP2 -1.86 0.008639175 NR112 -1.49 0.006846227 LPIN1 -2.02 0.010294258 APOA4 6.29 0.007329076 INSIG1 -2.80 0.010494044 LEPR_01 2.55 0.008111383 MCM5 1.67 0.018981469 APOA1 -1.65 0.00897028 RORA -1.63 0.021173678 FGFR4 -1.70 0.009941781 SCARB1 -1.38 0.021396819 APOC3 -1.66 0.014547442 DGAT2 -1.70 0.024872243 PCSK9 -2.67 0.015028567 SCD 1.80 0.025667474 TGFB1 1.72 0.016112059 COL1A1 2.49 0.032446191 APOB -1.40 0.022577003 TIMP1 1.87 0.032793714 GLUT2 -1.84 0.02412063	GNMT	-2.19	0.005392574	DGAT2	-1.73	0.004160409
ACACA -1.61 0.006809141 TNF -2.96 0.005578895 MBOAT7 -1.30 0.007224475 PPARGC1A -2.63 0.005607015 IGFBP2 -1.86 0.008639175 NR112 -1.49 0.006846227 LPIN1 -2.02 0.010294258 APOA4 6.29 0.007329076 INSIG1 -2.80 0.010494044 LEPR_01 2.55 0.008111383 MCM5 1.67 0.018981469 APOA1 -1.65 0.008970278 RORA -1.63 0.021173678 FGFR4 -1.70 0.009941781 SCARB1 -1.38 0.021396819 APOC3 -1.66 0.014547442 DGAT2 -1.70 0.024872243 PCSK9 -2.67 0.015028567 SCD 1.80 0.022667474 TGFB1 1.72 0.016112059 COL1A1 2.49 0.028417769 MTTP -1.47 0.012577003 SCAP -1.28 0.03246191 APOB -1.40 0.022577003 TIMP1 1.87 0.032793714 GLUT2 -1.84 0.02123063	APOC3	-1.69	0.006004771	BCL2	2.63	0.004241219
MBOAT7 -1.30 0.007224475 PPARGC1A -2.63 0.005607015 IGFBP2 -1.86 0.008639175 NR112 -1.49 0.006846227 LPIN1 -2.02 0.010294258 APOA4 6.29 0.007329076 INSIG1 -2.80 0.010494044 LEPR_01 2.55 0.008111383 MCM5 1.67 0.018981469 APOA1 -1.65 0.008870278 RORA -1.63 0.021173678 FGFR4 -1.70 0.009941781 SCARB1 -1.38 0.021396819 APOC3 -1.66 0.014547442 DGAT2 -1.70 0.024872243 PCSK9 -2.67 0.015028567 SCD 1.80 0.025667474 TGFB1 1.72 0.016112059 COL1A1 2.49 0.02841769 MTTP -1.47 0.017813835 SCAP -1.28 0.032446191 APOB -1.40 0.02257703 IIMP1 1.87 0.032793714 GLUT2 -1.84 0.024120603 G	ACACA	-1.61	0.006809141	TNF	-2.96	0.005578895
IGFBP2 -1.86 0.008639175 NR112 -1.49 0.006846227 LPIN1 -2.02 0.010294258 APOA4 6.29 0.007329076 INSIG1 -2.80 0.010494044 LEPR_01 2.55 0.008111383 MCM5 1.67 0.018981469 APOA1 -1.65 0.008870278 RORA -1.63 0.021173678 FGFR4 -1.70 0.009941781 SCARB1 -1.38 0.021396819 APOC3 -1.66 0.014547442 DGAT2 -1.70 0.024872243 PCSK9 -2.67 0.015028567 SCD 1.80 0.025667474 TGFB1 1.72 0.017813835 SCAP -1.28 0.03246191 APOB -1.40 0.022577003 TIMP1 1.87 0.032793714 GLUT2 -1.84 0.024123063 GHR -1.32 0.0356708148 FOX01 -1.49 0.043783298 LDAH -1.37 0.04920525 SCD 1.96 0.043783298 LDAH	MBOAT7	-1.30	0.007224475	PPARGC1A	-2.63	0.005607015
LPIN1 -2.02 0.010294258 APOA4 6.29 0.007329076 INSIG1 -2.80 0.010494044 LEPR_01 2.55 0.008111383 MCM5 1.67 0.018981469 APOA1 -1.65 0.008870278 RORA -1.63 0.021173678 FGFR4 -1.70 0.009941781 SCARB1 -1.38 0.021396819 APOC3 -1.66 0.014547442 DGAT2 -1.70 0.024872243 PCSK9 -2.67 0.015028567 SCD 1.80 0.025667474 TGFB1 1.72 0.017813835 SCAP -1.28 0.03246191 APOB -1.40 0.022577003 TIMP1 1.87 0.032793714 GLUT2 -1.84 0.024173693 GHR -1.82 0.034630017 CTGF 2.16 0.035424956 INSR -1.32 0.035708148 FOXO1 -1.49 0.043783298 LDAH -1.37 0.04920525 SCD 1.96 0.0433939123 COL1A1	IGFBP2	-1.86	0.008639175	NR112	-1.49	0.006846227
INSIG1 -2.80 0.010494044 LEPR_01 2.55 0.008111383 MCM5 1.67 0.018981469 APOA1 -1.65 0.008870278 RORA -1.63 0.021173678 FGFR4 -1.70 0.009941781 SCARB1 -1.38 0.021396819 APOC3 -1.66 0.014547442 DGAT2 -1.70 0.024872243 PCSK9 -2.67 0.015028567 SCD 1.80 0.025667474 TGFB1 1.72 0.016112059 COL1A1 2.49 0.032446191 APOB -1.40 0.022577003 TIMP1 1.87 0.032793714 GLUT2 -1.84 0.024173693 GHR -1.82 0.034630017 CTGF 2.16 0.035424956 INSR -1.32 0.035708148 FOXO1 -1.49 0.043783298 LDAH -1.37 0.04920525 SCD 1.96 0.04339123 COL1A1 2.56 0.045340498 COL1A1 2.56 0.045340498	LPIN1	-2.02	0.010294258	APOA4	6.29	0.007329076
MCM5 1.67 0.018981469 APOA1 -1.65 0.008870278 RORA -1.63 0.021173678 FGFR4 -1.70 0.009941781 SCARB1 -1.38 0.021396819 APOC3 -1.66 0.014547442 DGAT2 -1.70 0.024872243 PCSK9 -2.67 0.015028567 SCD 1.80 0.025667474 TGFB1 1.72 0.016112059 COL1A1 2.49 0.032446191 APOB -1.40 0.022577003 TIMP1 1.87 0.032793714 GLUT2 -1.84 0.024123063 GHR -1.32 0.035708148 FOXO1 -1.49 0.043783298 LDAH -1.37 0.04920525 SCD 1.96 0.043340498	INSIG1	-2.80	0.010494044	LEPR 01	2.55	0.008111383
RORA -1.63 0.021173678 FGFR4 -1.70 0.009941781 SCARB1 -1.38 0.021396819 APOC3 -1.66 0.014547442 DGAT2 -1.70 0.024872243 PCSK9 -2.67 0.015028567 SCD 1.80 0.025667474 TGFB1 1.72 0.016112059 COL1A1 2.49 0.032446191 APOB -1.40 0.022577033 TIMP1 1.87 0.032793714 GLUT2 -1.84 0.024123063 GHR -1.82 0.034630017 CTGF 2.16 0.035424956 INSR -1.32 0.035708148 FOXO1 -1.49 0.043783298 LDAH -1.37 0.04920525 SCD 1.96 0.04339123	MCM5	1.67	0.018981469	APOA1	-1.65	0.008870278
SCARB1 -1.38 0.021396819 APOC3 -1.66 0.014547442 DGAT2 -1.70 0.024872243 PCSK9 -2.67 0.015028567 SCD 1.80 0.025667474 TGFB1 1.72 0.016112059 COL1A1 2.49 0.028417769 MTTP -1.47 0.017813835 SCAP -1.28 0.032446191 APOB -1.40 0.022577003 TIMP1 1.87 0.032793714 GLUT2 -1.84 0.024123063 GHR -1.82 0.034630017 CTGF 2.16 0.035424956 INSR -1.32 0.035708148 FOXO1 -1.49 0.043783298 LDAH -1.37 0.04920525 SCD 1.96 0.04334048	RORA	-1.63	0.021173678	FGFR4	-1.70	0.009941781
DGAT2 -1.70 0.024872243 PCSK9 -2.67 0.015028567 SCD 1.80 0.025667474 TGFB1 1.72 0.016112059 COL1A1 2.49 0.028417769 MTTP -1.47 0.017813835 SCAP -1.28 0.03246191 APOB -1.40 0.022577003 TIMP1 1.87 0.032793714 GLUT2 -1.84 0.024123063 GHR -1.82 0.034630017 CTGF 2.16 0.035424956 INSR -1.32 0.035708148 FOXO1 -1.49 0.043783298 LDAH -1.37 0.04920525 SCD 1.96 0.04339123	SCARB1	-1.38	0.021396819	APOC3	-1.66	0.014547442
SCD 1.80 0.025667474 TGFB1 1.72 0.016112059 COL1A1 2.49 0.028417769 MTTP -1.47 0.017813835 SCAP -1.28 0.032446191 APOB -1.40 0.022577033 TIMP1 1.87 0.032793714 GLUT2 -1.84 0.024123063 GHR -1.82 0.034630017 CTGF 2.16 0.035424956 INSR -1.32 0.035708148 FOXO1 -1.49 0.043783298 LDAH -1.37 0.04920525 SCD 1.96 0.0433939123 COL1A1 2.56 0.045340498 0.045340498 0.045340498 0.045340498	DGAT2	-1.70	0.024872243	PCSK9	-2.67	0.015028567
COLIA1 2.49 0.028417769 MTTP -1.47 0.017813835 SCAP -1.28 0.032446191 APOB -1.40 0.022577003 TIMP1 1.87 0.032793714 GLUT2 -1.84 0.0324123063 GHR -1.82 0.034630017 CTGF 2.16 0.035424956 INSR -1.32 0.035708148 FOXO1 -1.49 0.043783298 LDAH -1.37 0.04920525 SCD 1.96 0.043340498	SCD	1.80	0.025667474	TGEB1	1.72	0.016112059
SCAP -1.28 0.032446191 APOB -1.40 0.022577003 TIMP1 1.87 0.032793714 GLUT2 -1.84 0.024123063 GHR -1.82 0.034630017 CTGF 2.16 0.035424956 INSR -1.32 0.035708148 FOXO1 -1.49 0.043783298 LDAH -1.37 0.04920525 SCD 1.96 0.043340498	COL1A1	2.49	0.028417769	MTTP	-1.47	0.017813835
TIMP1 1.87 0.032793714 GLUT2 -1.84 0.024123063 GHR -1.82 0.034630017 CTGF 2.16 0.035424956 INSR -1.32 0.035708148 FOXO1 -1.49 0.043783298 LDAH -1.37 0.04920525 SCD 1.96 0.0433939123 COL1A1 2.56 0.045340498	SCAP	-1.28	0.032446191	APOB	-140	0.022577003
GHR -1.82 0.034630017 CTGF 2.16 0.035424956 INSR -1.32 0.035708148 FOXO1 -1.49 0.043783298 LDAH -1.37 0.04920525 SCD 1.96 0.043939123 COLLAL 2.56 0.045340498	TIMP1	1.20	0.032793714	GLUT2	-1.84	0.024123063
INSR -1.32 0.035708148 FOXO1 -1.49 0.043783298 LDAH -1.37 0.04920525 SCD 1.96 0.043939123 COLIA1 2.56 0.045340498	GHR	-1.87	0.034630017	CTGE	216	0.035424956
LDAH -1.37 0.04920525 SCD 1.96 0.043939123 COL1A1 2.56 0.043340498	INSR	-1 32	0.035708148	FOXO1	-149	0.043783298
COLIA1 256 0.043340498	IDAH	-1 37	0.04920525	SCD	196	0.043939123
		1.57	0.0 10 200 20	COL 1A1	2.56	0.045340498

FC fold change; P-values: all genes with p < 0.05 are listed (ranked according to P-values): genes highlighted in bold have FC > 2 or < -1.5 and multiple testing-corrected P values <0.0006; A positive FC indicates that the gene is upregulated in the FFC and FFC_{DIA} groups, respectively, and a negative FC indicates that the gene is downregulated in the FFC and FFC_{DIA} groups, respectively.

In VAT only one gene with FC > 2 or < -1.5 and *P* values passing multiple testing was seen in the FFC vs. SD and FFD_{DIA} vs. SD comparisons (*LDLR* and *RPS29* respectively). SAT presented a higher number of differentially expressed genes compared to VAT in response to both the FFC and FFC_{DIA} diet. A common trend in the

Table 3 Differential expression in SAT and VAT.

(a) SAT					
FFC vs. SD			FFC _{DIA} vs. SD		
Genes	FC	P value	Genes	FC	P value
PN-1	2.05	3.20E-05	ABCG1	3.89	0.00011538
EBF2	-1.69	0.00010886	IL6	2.67	0.00035439
ABCG1	2.64	0.00022215	ABCA1	2.63	0.00067173
IL6	2.87	0.00043198	PN-1	2.77	0.00137394
GNAS	-1.57	0.00070595	MYC	1.52	0.0089967
SMPDL3A	3.03	0.00136871	SMPDL3A	2.98	0.01752013
FADS1	-2.18	0.00517238	ISLR	-1.82	0.02213799
ISLR	-2.23	0.00629454	ADCY5	1.74	0.02372845
ELOVL4	-2.00	0.01003605	GLUT4	-2.27	0.02893844
LEP	2.27	0.01139943	PON1	3.42	0.03258829
SP1	-1.59	0.0206303	SMAD6	1.48	0.03903247
DGAT2	-1.94	0.0210936	CD36	1.49	0.04384441
GLUT4	-2.57	0.02112195			
CXCR4	2.06	0.02381762			
MYC	1.62	0.02602224			
PELI2	-1.42	0.02939702			
HPRT1	6.24	0.03237427			
CD36	1.37	0.03572498			
DICER1	-1.78	0.03598225			
LDLR	-1.76	0.03737362			
LCN2 (NGAL)	-1.89	0.03992909			

(b) VAT

FFC vs. SD			FFC _{DIA} vs. SD		
Genes	FC	P value	Genes	FC	P value
LDLR	-2.17	0.00053665	RPS29	-2.28	5.88E-07
RPS29	-1.42	0.00282892	ABCA1	2.27	0.00151131
ABCA1	2.09	0.00375619	LDLR	-1.96	0.01065563
TLR4	1.62	0.00451224	ABCG1	2.44	0.01258091
ACTB	1.54	0.01270316	IRS1	1.52	0.02980169
LITAF	1.25	0.01489724	LEPR	-4.24	0.03295196
LSS	-1.81	0.02886919			
FAS	-1.53	0.03170324			
TGFB1	1.50	0.03959778			

Page 6 of 12

two adipose tissues is that genes involved in lipid homeostasis are upregulated. I.e., *ABCG1* in SAT (FC = 2.64 in FFC vs. SD; FC = 3.89 in FFC_{DIA} vs. SD); *ABCA1* in SAT (FC = 2.63 in FFC_{DIA} vs. SD), and in VAT (FC = 2.09 in FFC vs. SD; FC = 2.27 in FFC_{DIA} vs. SD). Furthermore, *IL6* is upregulated in SAT (FC = 2.87 and FC = 2.67 in FFC vs. SD and FFC_{DIA} vs. SD, respectively).

Relative expression of selected genes in liver, SAT, and VAT

The expression of PPARG and its target genes (FABP4, LPL, and CD36) were unchanged in SAT and VAT, but highly differentially expressed in the liver (see Tables 2, 3, 4 and Tables S2, S3, S4). To compare the relative expression levels between liver, SAT, and VAT the four genes were assayed using the Mx3005P 96-format platform. The expression levels of the genes in the liver from the SD group of pigs were used as baseline. All three primer pairs amplifying the isoforms of PPARG were included. Both PPARG isoforms (PPARG1 and PPARG2) were expressed in the three tissues. PPARG1 was slightly, but not significantly, higher expressed in the liver compared to PPARG2, whereas PPARG2 was slightly, but not significantly, higher expressed compared to PPARG1 in SAT and VAT (see Table S5). As illustrated in Fig. 1, CD36, FABP4, and LPL were, as expected, expressed at a much higher level in the adipose tissues whereas PPARG expression in the liver of the FFC and FFC_{DIA} groups were comparable to the expression in the adipose tissues.

Correlation between adipose tissue expansion and expression of selected genes in liver

A Pearson correlation analysis was performed to investigate the possible association between expression of *PPARG* and its target genes in the liver and increasing fat deposition.

As seen in Table 4, the expansion of body fat and visceral fat were significantly correlated (r = 0.81, p < 0.00001). Furthermore, expression levels of *PPARG*, *LPL*, and *CD36* were significantly correlated (p < 0.00001, p < 0.00001, and p < 0.0001, respectively) with total body fat (r = 0.76, 0.81, and 0.67, respectively), whereas, only *LPL* expression was moderately correlated with VAT (r = 0.6, P < 0.001). Expression of the four genes was correlated with highly significant correlation between *PPARG*, *LPL* and *CD36* (r > 0.9; P < 0.0001), and moderately significantly correlation with *FABP4* (r > 0.6, P < 0.001). As expected no correlation was found between any of the parameters and TG in liver.

Histopatholology and expression of pro-inflammatory cytokines

Histopathological examination of sections from SAT and VAT revealed no increase in macrophage infiltration in the adipose tissues from the pigs subjected to the FFC

Table 4 Correlation between adipose tissue expansion and expression of selected genes in the liver.

BF%	TBF%						
'AT (g)	0.81***	VAT (g)					
G in liver			TG in liver				
2D36	0.67**			CD36			
ABP4				0.69**	FABP4		
PL	0.81***	0,6*		0.95***	0.65*	ТЫТ	
PARG	0.76***			0.95***	0.64*	0.94***	PPARG
<i>BF</i> Total body fat, <i>V.</i> <i>P</i> < 0.001; * <i>P</i> < 0.0001; ** <i>P</i> < 0.00001.	47 Visceral adipose tissue, 7G	triglycerides.					



diet relative to the pigs on the SD diet and Crown-like structures were not observed (see Fig. 2). These findings are supported by the expression profiles of the tissues, that is, of the pro-inflammatory transcripts examined (*IL18, IL1B, IL6, TLR4,* and *TNF*) only *IL6,* was upregulated in SAT (FC = 2.86 and 2.67 in FFC vs. SD and FFC_{Dia} vs. SD, respectively) whereas none of them were upregulated in VAT.

Discussion

In this study we have profiled expression of genes of relevance for metabolism in liver, subcutaneous adipose (SAT) and visceral adipose tissues (VAT) to characterize a Göttingen Minipig model of metabolic syndrome and NASH.

As shown in Table 1, the FFC and FFC_{DIA} groups developed obesity with high body weight, high total body fat % and dyslipidemia (i.e., increased triglyceride (TG) and total cholesterol (TC) levels in plasma)¹². In the study by ref.¹², it was also shown that the FFC diet resulted in development of hepatomegaly with hepatic fibrosis, inflammation, cytoplasmic alterations, and increased content of cholesterol, whereas no difference in triglyceride content in the liver was found. Thus, hallmarks of human NAFLD/NASH like severe steatosis and hepatocellular ballooning were lacking. Inducing diabetes on top of the FFC diet did not exacerbate the histopathological findings compared to the FFC diet¹². Both the histopathological findings and the results of the expression studies clearly document that the FFC diet challenged the metabolism in the liver. That diet rather than obesity per se is the driving factor is supported by a previous study in which hepatic differential expression between Göttingen Minipigs fed standard minipig chow restrictively (lean controls) and Göttingen Minipigs fed the same diet ad libitum (obese) was studied²⁰. Although, these treatments resulted in an obese group obtaining roughly the same body weight as the FFC diet groups included in this study, liver metabolism was far from affected at the level documented here, indicating that the diet components play a more important role than obesity per se.

The increased inflammation observed in the liver in the FFC and FFC_{DIA} groups¹² was reflected in the expression study, i.e., both the *CD68* and *TLR4* transcripts were upregulated (the latter only in the FFC_{DIA} group). It is however noteworthy that staining with allograft inflammatory factor-1 (IBA1) only revealed moderate infiltration of macrophages in the liver of the animals subjected to the FFC diet¹² perhaps explaining why in this study none of the transcripts representing pro-inflammatory cytokines (e.g., *TNF, IL1B, IL18, IL6*) were upregulated in the liver of these animals. The increased content of collagen in liver detected in the precious study¹² is in concordance with the increased expression of the *MMP9* transcript which encodes a protein that can cleave different types of collagen.

Abundant hepatic steatosis, which is lacking in our model, arises from an imbalance between triglyceride acquisition and removal. In particular, four of the genes that were upregulated in the liver have been reported to play an important role in this context: PPARG, and three of its target genes, i.e., LPL and CD36, and FABP4. Under normal physiological conditions these genes are expressed almost exclusively in adipose tissues in human and mouse. In human studies the two isoforms of PPARG have been shown to have different tissue distribution, i.e., PPARG1 is expressed in a wide variety of tissues, while PPARG2 is mainly expressed in adipose tissues (reviewed by ref.²¹). In this study, we have shown that the two isoforms are expressed at almost the same level in the FFC and FFC_{DIA} groups of pigs in both liver, SAT and VAT. Both isoforms are lipogenic transcription factors that function as inducers of adipocyte differentiation and several lines of evidence suggest that PPARG activation causes insulin sensitization in adipocytes (e.g., ref.^{22,23}). The implications of an increased expression level of PPARG in liver are less well documented. It has, however, been shown that PPARG expression is elevated in the liver of mice that develop fatty liver²⁴, and PPARG has been



Fig. 2 Examples of adipocytes in SAT and VAT from representative pigs. a (SAT) and **b** (VAT) from a pig subjected to the SD diet; **c** (SAT) and **d** (VAT) from a pig subjected to the FFC_{Dia} diet; **e** (SAT) and **f** (VAT) from a pig subjected to the FFC diet. Scale bar 500 μm. Hematoxylin and eosin staining.

reported to play a major role in promoting hepatic steatosis in mice²⁵. Both *PPARG1* and *PPARG2* also appear to be upregulated in liver during the pathogenesis of NAFLD in humans²⁶. The increased expression of FABP4, CD36, and LPL suggests increased fatty acid uptake, transport, and metabolism in the livers of the FFC diet fed pigs. FABP4 expression in liver has been shown to be significantly elevated in mouse models of obesity-promoted hepatocellular carcinoma and in patients with underlying hepatic steatosis resulting from NAFLD²⁷. Also, increased expression of CD36 in the liver has been shown to occur in response to diets rich in fatty acids, and this appears to increase hepatic fatty acid uptake and exacerbates both hepatic storage and secretion of triglyceride²⁸. LPL plays a critical role in regulating lipid metabolism and tissuespecific effects are still being explored. Tissue specific

has been reported to increased cellular stores of triglycerides leading to insulin resistance²⁹. Contrasting, a more recent study in mice has shown that hepatic LPL is involved in the regulation of plasma LPL activity and lipid homeostasis³⁰. Our results show that Göttingen Minipigs do not develop abundant hepatic steatosis in spite of the significantly increased expression of *PPARG*, *FABP4*, *CD36*, and *LPL* in the liver. Thus, it might be speculated that the ectopic expression of these genes are consequences of high fat diet/obesity rather than the cause of development of steatosis in the liver. We cannot rule out that longer term FFC diet treatment/obesity might lead to adverse metabolic responses. Still, abundant steatosis is not an immediate outcome of the highly increased expression level of *PPARG*, *FABP4*, *CD36*, and *LPL* in the

overexpression of LPL in skeletal muscle and liver in mice

liver of the severly obese minipigs. Conversely, since *PPARG* and *LPL* expression in liver is significantly correlated with the amount of body fat (see Table 4) the ectopic expression of the genes might have an influence on the repartitioning of lipid from liver to adipose tissues. This is also in keeping with the fact that the liver in pigs, in contrast to humans and rodents, is not the primary site of de novo lipogenesis³¹.

The ability of the Göttingen Minipigs to sustain the diet challenges is also reflected in differential regulation of genes involved in cholesterol biosynthesis in the liver. I.e. *HMGCR* and *FDFT1* are deregulated with a highly negative FC in the liver. Both genes are key regulators of cholesterol biosynthesis and the observed downregulation can most likely be explained by the abundance of cholesterol in the diet. *KLB* is also deregulated with a highly negative FC. KLB contributes to repression of CYP7A1—a rate limiting enzyme in the bile acid biosynthesis pathway that converts cholesterol into bile acids³². Thus, the deregulation of the genes involved in cholesterol biosynthesis appears to assist in rectifying the diet-induced increase in cholesterol through conversion of cholesterol into bile acids.

As seen in Table 1, both body fat and visceral fat content were highly significantly increased in the FFC diet fed groups, nevertheless, metabolism in the adipose tissues was not affected to a great extent, although more so in SAT compared to VAT. It is generally accepted that metabolism differs between SAT and VAT, and that excess VAT is unhealthier than excess SAT (e.g., ref. ³³). The increased expression of IL6 in the fat tissues is an indication of low-grade inflammation; however, adipogenesis does not seem to be severely adversely affected by the FFC diet neither in SAT nor in VAT. I.e., none of the insulin sensitizing (e.g., ADIPOQ) or resistance genes (e.g., TNF) were perturbed. Rather, mainly genes involved in lipid and cholesterol homeostasis (ABCA1, ABCG1) were upregulated in these tissues. Lipid metabolism in the fat tissue is supported by the high unchanged expression level of PPARG, FABP4, CD36, and LPL in these tissues (se Tables S2, S3, S4 and Fig. 1). Although both adipose tissue compartments were substantially expanded in the groups on the FFC diet, the highly significant correlation between body fat and expression of PPARG, LPL, and CD36 in the liver indicates, as previously mentioned, that ectopic expression of these genes supports adipogenesis/ lipogenesis in body fat compartments rather than in the liver. In contrast, obese human subjects with a high degree of metabolic endotoxemia have been shown to have lower expression of key genes for adipose tissue function and lipogenesis (SREBP1, FABP4, FASN, and LEP), but higher expression of inflammatory genes in VAT and SAT³⁴. In contrast to this, the only proinflammatory cytokine upregulated in the obese Göttingen Minipigs was IL6, which was upregulated in SAT but not in VAT. This is supported by the histopathological examinations which did not reveal an increase in macrophage infiltration in SAT and VAT in the obese pigs (see Fig. 2). Also, in contrast to our findings, the expression of PPARG has been shown to be significantly downregulated in SAT in severly obese women³⁵. Thus, our study shows that Göttingen Minipigs are able to maintain fatty acid synthesis, and expand the fat compartments without compromising adipose tissue metabolism. Our results support the notion that the capacity to expand fat mass to store lipids is a more important determinant of obesity-associated metabolic problems than the absolute amount of adipose tissue, as has also been shown in humans (reviewed by ref. ³⁶). The importance of the adipose tissue expandability is further supported by studies that have used thiazolidinediones (TZDs) to treat NAFLD and NASH and reverse insulin resistance in target tissues. These studies have demonstrated good efficacy of TZDs to reduce lipid content in the liver concordant with adipose tissue expansion^{37,38}. TZDs are potent PPARG agonists³⁹ and thus, the proposed role for PPARG as an inducer of steatosis in hepatocytes appears conflicting with the efficacy of TDZs in terms of reducing hepatic lipid content. On the other hand, it is important to note that the main target tissues for TDZs are adipose tissues and, as also suggested by ref.⁵, the expandability of the adipose tissue explains how TDZs can be beneficiary for NASH since the increasing capacity of adipose tissue to store fat allows repartitioning of lipid from liver to adipose tissue. Since Göttingen Minipigs are able to expand the fat compartments without compromising adipose tissue metabolism it might be hypothesized that naturally occurring fatty acids activate PPARG in the liver of the FFC diet treated pigs, mimicking treatments with TDZs, resulting in repartitioning of lipid from liver to adipose tissues. Our findings are in agreement with a previous study showing that haplotypes segregating from Göttingen Minipigs can uphold a healthy lipid profile despite development of obesity indicating they have a phenotype comparable to the MHO phenotype in humans¹⁴.

In conclusion, our study shows that severly obese Göttingen Minipigs have a large capacity for adipose tissue expansion and are protected against many of the metabolic and hepatic abnormalities associated with obesity. The study lends support to the hypothesis that adipose tissue expandability and adaptation plays a crucial role in the maintenance of metabolic homeostasis and elucidates some of the molecular components underlying the MHO-like phenotype in Göttingen Minipigs. In contrast to what has been reported in human and mouse studies, the highly significant upregulation of *PPARG*, *CD36*, *LPL*, and *FABP4* in the liver of the minipigs do not result in development of abundant hepatic steatosis. The coordinated activation of lipid uptake and lipid biosynthesis by *PPARG* in the liver appears to be balanced by the ability of the adipose tissues to expand and store excessive calories. Although our study shed light on some of the mechanisms that disassociate obesity from metabolic complications a large number of questions, for instance, regarding how adipose tissue plasticity is regulated still remain. Identification of additional underlying factors associated with the metabolic healthy obese phenotype in Göttingen Minipigs can contribute to a better understanding of the factors that predispose, delay or protect obese individuals from metabolic disturbances.

Acknowledgements

The authors would like to thank laboratory technicians Tina Neergaard Mahler and Minna B. Jakobsen for excellent technical assistance. We would also like to thank Professor Susanne Mandrup, University of Southern Denmark for valuable discussions of the results. The project was supported by a grant from the Independent Research Fund Denmark (DFF-1335–00127).

Author details

¹Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, 1870 Frederiksberg, Denmark. ²Global Drug Discovery, Novo Nordisk A/S, Novo Nordisk Park, Måløv, Denmark. ³Ellegaard Gottingen Minipigs A/S, Sorø Landevej 302, 4261 Dalmose, Denmark

Conflict of interest

B.Ø.C., R.K.K., T.L.P., and H.H. are full time employed at Novo Nordisk A/S. H.D.P. is full time employed at Ellegaard Göttingen Minipigs A/S. The remaining authors declare that they have no conflict of interest.

Publisher's note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Supplementary Information accompanies this paper at (https://doi.org/ 10.1038/s41387-020-0112-y).

Received: 15 July 2019 Revised: 14 January 2020 Accepted: 20 January 2020 Published online: 23 March 2020

References

- Hazlehurst, J. M., Woods, C., Marjot, T., Cobbold, J. F. & Tomlinson, J. W. Nonalcoholic fatty liver disease and diabetes. *Metabolism* 65, 1096–1108 (2016).
- Lee, M.-K. et al. Metabolic health is more important than obesity in the development of nonalcoholic fatty liver disease: a 4-year retrospective study. *Endocrinol. Metab.* **30**, 522–530 (2015).
- Gustafson, B., Hedjazifar, S., Gogg, S., Hammarstedt, A. & Smith, U. Insulin resistance and impaired adipogenesis. *Trends Endocrinol. Metab.* 26, 193–200 (2015).
- Hardy, O. T. et al. Body mass index-independent inflammation in omental adipose tissue associated with insulin resistance in morbid obesity. *Surg. Obes. Relat. Dis.* 7, 60–67 (2011).
- Virtue, S. & Vidal-Puig, A. It's not how fat you are, it's what you do with it that counts. *PLoS Biol.* 6, e237 (2008).
- Loos, R. J. F. & Kilpeläinen, T. O. Genes that make you fat, but keep you healthy. J. Intern Med. 284, 450–463 (2018).
- Li, S. J. et al. A nutritional nonalcoholic steatohepatitis minipig model. J. Nutr. Biochem. 28, 51–60 (2016).
- Xia, J. et al. Transcriptome analysis on the inflammatory cell infiltration of nonalcoholic Steatohepatitis in Bama Minipigs induced by a long-term highfat, high-sucrose diet. *PLoS ONE* 9, e113724 (2014).
- 9. Lee, L et al. Nutritional model of steatohepatitis and metabolic syndrome in the Ossabaw miniature swine. *Hepatology* **50**, 56–67 (2009).

- Bell, L. N. et al. Serum proteomic analysis of diet-induced steatohepatitis and metabolic syndrome in the Ossabaw miniature swine. Arn. J. Physiol. Gastrointest. Liver Physiol. https://doi.org/10.1152/ajpgi.00485.2009 (2010).
- 11. Liang, T. et al. Liver injury and fibrosis induced by dietary challenge in the Ossabaw miniature Swine. *PLoS ONE* **10**, e0124173 (2015).
- Schumacher-Petersen, C. et al. Experimental non-alcoholic Steatohepatitis in Göttingen Minipigs: consequences of high fat-fructose-cholesterol diet and diabetes. J. Transl. Med. https://doi.org/10.1186/s12967-019-1854-y (2019).
- Yang, S. L. et al. Hyperinsulinemia shifted energy supply from glucose to ketone bodies in early nonalcoholic steatohepatitis from high-fat high-sucrose diet induced Bama minipigs. *Sci. Rep.* 5, 13980 (2015).
- Frederiksen, S. D. et al. Haplotypes on pig chromosome 3 distinguish metabolically healthy from unhealthy obese individuals. *Plos ONE* **12**, e0178828 (2017).
- Andreasen, L. J. et al. Dietary normalization from a fat, fructose and cholesterolrich diet to chow limits the amount of myocardial collagen in a Göttingen Minipig model of obesity. *Nutr. Metab.* **15**, 64 (2018).
- 16. Cirera, S. Highly efficient method for isolation of total RNA from adipose tissue. *BMC Res. Notes* **6**, 472 (2013).
- Mentzel, C. M. J. et al. Deregulation of obesity-relevant genes as a result of progression in BMI and amount of adipose tissue in pigs. *Mol. Genet. Genomics.* 293, 129–136 (2018).
- Nygard, A. B., Jørgensen, C. B., Cirera, S. & Fredholm, M. Selection of reference genes for gene expression studies in pig tissues using SYBR green qPCR. *BMC Mol. Biol.* 8, 67 (2007).
- RStudio Team. RStudio: Integrated Development for R. RStudio, Inc. Boston http://www.rstudio.com/ (2015).
- Mentzel, C. M. et al. Joint profiling of miRNAs and mRNAs reveals miRNA mediated gene regulation in the Göttingen Minipig obesity model. *PLoS ONE* 11, e0167285 (2016).
- 21. Lehrke, M. & Lazar, M. A. The many faces of PPARG. Cell 123, 993-999 (2005).
- Rangwala, S. M. & Lazar, M. A. Peroxisome proliferator-activated receptor gamma in diabetes and metabolism. *Trends Pharmacol. Sci.* 25, 331–336 (2004).
- He, W. et al. Adipose-specific peroxisome proliferator-activated receptor gamma knockout causes insulin resistance in fat and liver but not in muscle. *Proc. Natl Acad. Sci. USA* **100**, 15712–15717 (2003).
- Schadinger, S. E., Bucher, N. L., Schreiber, B. M. & Farmer, S. R. PPARgamma2 regulates lipogenesis and lipid accumulation in steatotic hepatocytes. *Am. J. Physiol. Endocrinol. Metab.* 288, E1195–E1205 (2005).
- Morán-Salvador, E. et al. Role for PPARγ in obesity-induced hepatic steatosis as determined by hepatocyte- and macrophage-specific conditional knockouts. *FASEB J.* 25, 2538–2550 (2011).
- Pettinelli, P. & Videla, L. A. Up-regulation of PPAR-gamma mRNA expression in the liver of obese patients: an additional reinforcing lipogenic mechanism to SREBP-1c induction. J. Clin. Endocrinol. Metab. 96, 1424–1430 (2011).
- Thompson, K. J. et al. Altered fatty acid-binding protein 4 (FABP4) expression and function in human and animal models of hepatocellular carcinoma. *Liver Int.* 38, 1074–1083 (2018).
- Koonen, D. P. et al. Increased hepatic CD36 expression contributes to dyslipidemia associated with diet-induced obesity. *Diabetes* 56, 2863–2871 (2007).
- Kim, J. K. et al. Tissue-specific overexpression of lipoprotein lipase causes tissue-specific insulin resistance. PNAS 98, 7522–7527 (2001).
- Liu, G. et al. Regulation of plasma lipid homeostasis by hepatic lipoprotein lipase in adult mice. J. Lipid Res. 57, 1155–1161 (2016).
- Berger, W. G. & Mersmann, H. J. Comparative aspects of lipid metabolism: Impact on contemporary research and use of animal models. J. Nutr. 135, 2499–2502 (2005).
- Li, T. et al. Transgenic expression of cholesterol 7 alpha-hydroxylase in the liver prevents high-fat diet-induced obesity and insulin resistance. *Hepatology* 52, 678–690 (2010).
- Neeland, I. J. et al. Associations of visceral and abdominal subcutaneous adipose tissue with markers of cardiac and metabolic risk in obese adults. *Obesity* 21, E439–E447 (2013).
- Clemente-Postigo, M. et al. Metabolic endotoxemia promotes adipocyte dysfunction and inflammation in human obesity. *Am. J. Physiol. Endocrinol. Metab.* https://doi.org/10.1152/ajpendo.00277 (2018).
- Auguet, T. et al. Downregulation of lipogenesis and fatty acid oxidation in the subcutaneous adipose tissue of morbidly obese women. *Obesity* 22, 2032–2038 (2014).

- Balas, B. et al. Pioglitazone treatment increases whole body fat but not total body water in patients with non-alcoholic steatohepatitis. J. Hepatol. 47, 565–570 (2007).
- Belfort, R. et al. A placebo-controlled trial of pioglitazone in subjects with nonalcoholic steatohepatitis. N. Engl. J. Med. 355, 2297–2307 (2006).
- Lehmann, J. M. et al. An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor gamma (PPAR gamma). J. Biol. Chem. 270, 12953–12956 (1995).