SRT1720 ATTENUATES OBESITY AND INSULIN RESISTANCE BUT NOT LIVER DAMAGE IN THE OFFSPRING DUE TO MATERNAL AND POSTNATAL HIGH-FAT DIET CONSUMPTION

Long The Nguyen[1], Hui Chen[1, 2], Crystal Mak[2], Amgad Zaky[1], Carol Pollock[1] and Sonia Saad[1, 2]

[1] Renal medicine, Kolling Institute, Royal North Shore Hospital, University of Sydney, Sydney, New South Wales, Australia

[2] School of Life Sciences, Faculty of Science, University of Technology Sydney, Sydney, New South Wales, Australia

Corresponding author: Long The Nguyen

Address: Renal medicine, Kolling Building level 9, Royal North Shore Hospital, St. Leonard, NSW, Australia

Phone: +61 2 99264782

Fax: +61 0299265715

E-mail: long_t_nguyen@outlook.com

Running Title: SIRT1 attenuates fetal programming by maternal obesity

Word count: 3,027

ABSTRACT

Recent studies indicate that SIRT1, an important metabolic sensor and regulator of lifespan, plays a mechanistic role in maternal obesity-induced programming of metabolic disorders in the offspring. In this study we investigate whether SIRT1 activation in early childhood can mitigate metabolic disorders due to maternal and postnatal high-fat feeding in mice. Male offspring born to chow-fed (MC) or high-fat diet-fed dams (MHF) were weaned onto postnatal chow or high-fat diet and treated with SRT1720 (SRT, 25mg/kg/2days i.p) or vehicle control (VEH) for 6 weeks and examined for metabolic disorders. MHF exacerbated offspring body weight and insulin resistance in the offspring exposed to postnatal HFD (OHF). These metabolic changes were associated with reduced hepatic lipid droplet accumulation but increased plasma levels of alanine aminotransferase (ALT), a marker of liver damage. SRT1720 significantly decreased offspring body weight, adiposity, glucose intolerance, hyperleptinemia due to OHF, and reversed hyperinsulinemia and adipocyte hypertrophy due to the additive effects of MHF. Although SRT1720 suppresses liver lipogenesis, inflammation and oxidative stress markers, it also reduces antioxidants and increased liver collagen deposition in OHF offspring independent of MHF. Hepatic steatosis was attenuated only in MC/OHF offspring in association with elevated plasma ALT levels. The study suggests postnatal SRT1720 administration can mitigate obesity and insulin resistance in the offspring due to maternal and postnatal HFD exposure. However, the possibility of liver toxicity needs to be further examined.

INTRODUCTION

It is well-established that unhealthy parental lifestyles and related diseases such as obesity and diabetes can increase the risk of metabolic disorders in the offspring (13, 18). Specifically, an unbalanced diet such as high fat and high simple carbohydrate before and during pregnancy can predispose the offspring to childhood obesity and metabolic disorders such as diabetes and non-alcoholic fatty liver disease (NAFLD) through epigenetic and metabolic programming due to various factors such as overnutrition, oxidative stress and inflammation (2, 5, 16, 29). Importantly, the effects cannot be reversed by conventional approaches such as weight gain management during pregnancy (4). As such, investigation of alternative approaches has become imperative.

Sirtuin (SIRT)1 is an essential metabolic and lifespan regulating factor (12). Its expression and activity are dependent on the cellular availability of NAD^+, an energy carrier whose level is depleted upon feeding and throughout senescence. Its activation by Sirtuin-activating compounds

(STACs) or $NAD^{=}$-precursors has been shown to mimic the effects of caloric restriction on obesity and lifespan in animals (6, 14, 24). At the molecular level, SIRT1 activation leads to suppression of de novo lipogenesis (DNL) regulators including peroxisome proliferator-activated receptor gamma (PPARγ), sterol regulatory element-binding protein (SREBP-1c), fatty acid synthase (FASN), while boosting lipolysis markers such as peroxisome PPARγ-coactivator α (PGC-1α) and PPARα (12, 23). SIRT1 also positively regulates antioxidant enzymes and prevents oxidative stress (1). However, the effects of SIRT1 therapeutics in the context of maternal obesity are yet to be elucidated (23).

Multiple studies have demonstrated reduced SIRT1 expression in the placenta (27), oocyte (7), fetus (30), and neonatal tissue (22) due to maternal aging or high-fat diet (HFD) consumption, suggesting the relevance of SIRT1 in fetal programming (23). Particularly, our recent unpublished data demonstrated that SIRT1-transgenic mice born to obese dams have decreased body weight, adiposity, glucose intolerance and insulin resistance compared to Wild-type littermates at weaning, providing a proof of concept for SIRT1 therapeutics during early developmental periods to counteract the transgenerational effects of maternal obesity. The study is to further examine the hypothesis by administration of SRT1720, a potent STAC with well-established anti-diabetic effects in animals (21), in the offspring exposed to maternal and postnatal HFD in adolescence.

MATERIALS AND METHODS

Animals

The study was approved by the Animal Care and Ethics Committee of the University of Sydney (RESP/15/22). All procedures were performed in accordance with the relevant guidelines and regulations in the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. All mice were housed at the Kearns Animal Facility at the Kolling Institute, Royal North Shore Hospital. The animals were maintained at 22 ± 1°C with a 12/12 hour light to dark cycle, and monitored at least once per fortnight. Female C57BL/6 mice (8 weeks) were fed a high-fat diet (HFD, 20 kJ/g, 43.5% calorie as fat, Specialty Feed, WA, Australia) or standard rodent chow (11 kJ/g, 14% calorie as fat, Gordon's Speciality Stockfeeds, NSW, Australia) for 6 weeks before mating, throughout gestation and lactation (11, 22). On postnatal day (P) 1, female mice were culled and male mice were adjusted to 4-6 pups/ litter. At weaning (P20), the offspring were weaned on Chow or HFD and treated with SRT1720 (25mg/kg/2days i.p) for 6 weeks. At week 9, the offspring were sacrificed. Blood was collected via cardiac puncture after anaesthesia (Pentothal, 0.1mg/g, i.p., Abbott Australasia Pty Ltd, NSW, Australia). Phosphate-buffered saline (PBS, 1%)

was used for whole body perfusion. Tissues were snapped frozen and stored at −80 °C or fixed in Neutral buffered formalin (10%) for approximately 36h for later analyses.

Intraperitoneal glucose tolerance test (IPGTT)

At week 9, the animals were weighed and fasted for 5h prior to IPGTT, then a glucose solution (50%) was injected (2g/kg, i.p.). Tail blood glucose level was recorded prior to glucose injection at 15, 30, 60 and 90min post injection using a glucometer (Accu-Chek glucose meter; Roche Diagnostics) (13). The area under the curve (AUC) was calculated for each animal.

Protein and lipid extraction from tissues

The tissues were homogenized in Triton X-100 lysis buffer (pH 7.4, 150 mM NaOH, 50 mM Tris-HCl, 1% Triton X-100, Roche protease inhibitor) using TissueRuptor (Qiagen, Hilden, Germany). Lipid and protein was extracted and measured for concentrations according to our previously published protocol (14) using Roche triglyceride reagent GPO-PAP (Roche Life Science, NSW, Australia) and Pierce BCA Protein Assay Kit (Thermo Scientific, VIC, Australia) according to the manufacturer's instructions. Lipid concentrations were normalised to the protein concentration.

Quantitative RT-PCR

Total RNA of liver tissues was isolated using RNeasy Plus Mini Kit (Qiagen Pty Ltd, CA, USA) according to the manufacturer's instructions, while RNA of fat tissues was extracted using Trizol Reagent (Sigma-Aldrich). The purified total RNA was used as a template to generate first-strand cDNA using the First Strand cDNA Synthesis Kit (Roche Life Science, NSW, Australia). The amplicons of target genes were amplified with SYBR Green probes. Primer sequences were summarised in Table 1. Gene expression was standardized to β-actin mRNA.

Histology

Tissues were fixed in 10% formalin for 36-h and embedded in paraffin or frozen-embedded in OCT solution (Tissue-Tek). Paraffin sections were prepared at 4 μm thickness and mounted on microscope slides. The sections were stained with hematoxylin and eosin (H&E) for general structural visualisation. For adipocyte frequency analysis, H&E-stained paraffin sections were observed using bright-field microscope (Leica Microsystems, Germany) and 6 random non-overlapping fields were captured at 200X magnification. Adipocyte size analysis was done by Adiposoft software (9).

Hepatic steatosis was assessed by both H&E and Oil Red O (ORO) staining. In ORO staining, frozen tissues were sectioned at 12 μm thickness, briefly fixed in Neutral buffered formalin (10%), stained with Oil Red O (ORO, Sigma-Aldrich, MO, USA) working solution (3g in 60% isopropanol) for 15 mins, differentiated in 60% isopropanol and distilled water, and counter-stained with Mayer's haematoxylin for 15s. For liver collagen staining, paraffin sections were stained with Fast Green for 30 mins followed by rinsing in water and incubation in Picro-Sirius Red (PSR) for another 30 mins.

Alanine aminotransferase assay

The Alanine Transaminase Colorimetric Activity Assay was performed as per instructions by the manufacturer (Cayman, USA).

Statistical analysis

The data were analysed by two-way ANOVA followed by Bonferroni post hoc tests. $P<0.05$ is considered significant.

RESULTS

SRT1720 attenuated body weight and adiposity in offspring born to HFD-fed dams

Consistent with previous studies, offspring fed a postnatal HFD showed increased body weight ($P<0.001$, Figure 1B). Caloric intake showed a trend to increase in this group but was not significantly different from the control after adjustment to body weight (Figure 1D). In association, epididymal and retroperitoneal fat mass, as well as the plasma levels of triglyceride, non-esterified fatty acid and leptin were also augmented ($P<0.01$) (Figure 1C and 1E). Maternal HFD consumption (MHF) significantly increased body weight of HFD-fed offspring (OHF) at week 9 ($P<0.01$, Figure 1B), and further increased the offspring's caloric intake to a significant level ($P < 0.01$ vs MC/OC/V, Figure 1D). Plasma leptin levels also showed a trend of increase ($P=0.06$). No additive effects of MHF on OHF offspring adiposity and hyperlipidemia were seen.

SRT1720 administration significantly reduced body weight, white adipose tissue (WAT) mass and leptin levels in OHF offspring born to either chow or HFD-fed mothers (Figure 1B, 1C and 1E). Together with slightly increased caloric intake (Figure 1D), the results suggest improved energy expenditure. The treatment had no effect on hyperlipidemia due to HFD exposure (Figure 1E).

SRT1720 attenuated glucose intolerance and insulin resistance in offspring born to HFD-fed dams

Glucose tolerance was significantly impaired in MC/OHF offspring ($P<0.001$, Figure 2A and 2B). However, plasma insulin levels and the insulin resistance index (HOMA-IR) remained unchanged in this group. Hyperinsulinemia and insulin resistance was significantly increased only in those OHF offspring pre-exposed to MHF ($P<0.01$ and $P<0.05$ respectively in comparison to MC/OC) (Figure 2C and 2D).

SRT1720 administration significantly improved glucose tolerance in HFD-exposed offspring ($P<0.01$), and normalised hyperinsulinemia and insulin resistance in those pre-exposed to MHF ($P<0.05$ and $P=0.07$ respectively). Further mRNA expression analysis in offspring RpWAT revealed a similar trend in the regulation of insulin receptors to plasma insulin.

SRT1720 suppressed adipocyte hypertrophy and lipogenesis

Despite having no additive effects on offspring WAT mass, MHF significantly amplified adipocyte size ($P<0.05$ vs MC/OHF, Figure 3B). The mRNA expression of SREBP-1c was slightly but not significantly suppressed by postnatal HFD ($P=0.054$), whilst UCP1 showed a trend to increase (Figure 3C). The mRNA expression of uncoupling protein UCP2 was also significantly upregulated by OHF ($P<0.01$). MHF had no additive effects on the regulation of these markers (Figure 3C).

SRT1720 administration significantly suppressed the expression of the lipogenesis markers PPARγ in both MC/OHF and MHF/OHF offspring ($P<0.05$ and $P<0.01$ respectively, Figure 3C). Similarly, FASN was downregulated by SRT1720 in MC/OHF offspring only ($P<0.05$) and SREBP-1c showed a tendency of suppression in MHF/OHF/S group ($P=0.064$). No significant alternations in UCP1 mRNA expression were found between vehicle and SRT1720-treated groups despite a trend of normalisation in MC/OHF offspring. UCP2 was significantly increased by the treatment but in MC/OC offspring only ($P<0.01$).

The effects of MHF and SRT1720 on lipid deposition in offspring liver

Liver triglyceride contents were significantly increased in OHF offspring independent of MHF ($P<0.01$, Figure 4A). Lipid droplet accumulation, reflected by H&E and ORO staining, was also increased in OHF offspring ($P<0.001$, Figure 4B, 4C). Surprisingly, the increase was significantly suppressed in those pre-exposed to MHF ($P<0.01$, Figure 4B, 4C). The expression of SREBP-1c, a marker of liver lipotoxicity, was similarly upregulated in both HFD-exposed groups ($P<0.01$ and $P<0.05$ respectively, Figure 4D). In contrast, the cholesterol regulator Liver X receptor β (LXRβ)

showed a trend to downregulation by HFD. MHF slightly exacerbated the reduction of LXRβ in the offspring (P<0.05 vs MC/OC). In contrast, the protein expression of phosphorylated AMP-activated protein kinase (AMPK) tended to decrease in the presence of maternal HFD (Figure 4E). No change in the expression of total AMPK and PGC-1α was found.

SRT1720 decreased the level of lipid accumulation in MC/OHF offspring only, as reflected by liver TG (n.s), H&E staining, and ORO staining (P<0.001). These changes were associated with reduced mRNA expression of SREBP-1c in all groups (all P<0.01), FASN and carbohydrate-responsive element-binding protein (ChREBP) in MC/OHF and MHF/OHF offspring (all P<0.05). Interestingly, PPARγ expression was suppressed by SRT1720 only in MC/OHF offspring (P<0.05) (Figure 4D). In association, there was a trend of increase in the protein expression of pAMPK (Figure 4E).

SRT1720 regulated liver oxidative stress, inflammation, and liver damage in a diet-dependent manner

The expression of NADPH oxidase (NOX)2 mRNA was slightly but not significantly increased (P=0.09) in MHF/OHF offspring (Figure 5A), suggesting increased reactive oxygen species (ROS) production and potential oxidative damage. Antioxidant enzymes Glutathione Peroxidase (GPx)-1 and catalase (CAT) showed similar trends of reduction in OHF offspring (Figure 5A). Inflammatory marker tumour necrosis factor (TNF)α, macrophage and microglial marker F4/80, and pro-apoptotic/endoplasmic reticulum stress marker C/EBP homologous protein (CHOP) were not significantly regulated by postnatal or maternal HFD exposure (Figure 5A). Postnatal and maternal HFD also did not alter mRNA expression of collagen type IV (COL4) and fibronectin (FN) (Figure 5B) but led to a trend to increase in collagen protein expression as reflected by PSR staining (Figure 5C). The plasma level of alanine aminotransferase (ALT), a marker of liver damage, was increased only in the MHF/OHF group (P<0.05 vs MC/OC, Figure 5D).

SRT1720 administration consistently improved the levels of TNFα, SOD1, SOD2, GPx-1, CAT and NOX2 in control offspring, but suppressed the mRNA expression of these markers in those exposed to HFD (Figure 5A, 5B). The data suggests that the effects of SRT1720 on inflammation and oxidative stress are diet-dependent. SRT1720 significantly increased mRNA expression of COL4 and FN (P<0.05) and PSR staining (P<0.05) in both offspring groups exposed to HFD (Figure 5B, 5C) but significantly increased ALT levels only in MC/OHF cohort (P<0.05) (Figure 5D).

Discussion

Here we show that maternal high-fat diet consumption can increase body weight, adipocyte hypertrophy and insulin resistance in the offspring postnatally exposed to high-fat diet. MHF is also associated with a lower level of hepatic lipid droplet accumulation but higher level of liver damage. SRT1720 administration after weaning in the offspring suppressed their weight gain, adipocyte hypertrophy and hyperinsulinemia due to postnatal and maternal HFD.

It is noteworthy that maternal HFD did not exacerbate the effects of postnatal HFD feeding on offspring adiposity at 9 weeks of age, which is at variance with our previous data in a rat model at the same age (10), suggesting that mice are less susceptible to maternal HFD-induced developmental programming of abnormal fat deposition. This is potentially due to the more active lifestyle of mice compared with rats. Despite the lack of difference in fat mass due to MHF, which has been shown to persist till week 32 of age (11), MHF induced adipocyte hypertrophy in the offspring, which has also been regarded as an important contributor to insulin resistance (32). Indeed, in this study, MHF is associated with hyperinsulinemia and increased HOMA-IR index in the offspring at week 9. Glucose tolerance is not exacerbated by maternal HFD at this time point but later in adulthood (11). These results suggest MHF predisposes OHF offspring to insulin resistance and exacerbates glucose intolerance later in life.

In association with increased insulin, MHF also led to increased plasma leptin levels in OHF offspring. As leptin is primarily produced by adipocytes, the increased adipocyte size due to MHF is likely to underline this effect. The inhibition effect of SRT1720 on offspring hyperleptinaemia is consistent with the reduced fat mass and adipocyte hypertrophy. SRT1720 suppressed the expression of lipogenesis markers PPARγ, SREBP-1c and FASN, particularly in HFD-exposed offspring WAT, which supports reduced fat mass and smaller adipocyte size. The increases of UCP1 and UCP2 are consistent with previous studies in HFD-fed animals (8, 28), and are likely to reflect compensatory pathways for energy homeostasis.

With respect to liver outcomes, offspring of chow-fed dams that are exposed to HFD after weaning have increased liver lipogenesis and steatosis but normal plasma ALT levels. In comparison, HFD-fed offspring of HFD-fed dams have the same level of liver triglyceride but reduced steatosis and elevated ALT. The mechanism for the discrepancy between triglyceride and steatosis levels is unknown; however, we can postulate that not all triglycerides molecules were packed in the form of lipid droplets in the liver of these offspring. This may imply a disorder in hepatic lipid

mobilization due to maternal HFD. The result also suggests that it is not only the amount of lipid but also the form of lipid can contribute to liver injury. Indeed, it has been recently shown that free fatty acids, not triglycerides, are associated with progression of NAFLD in diet-induced obese rats (19). Moreover, the inhibition of triglyceride synthesis may improve hepatic steatosis but exacerbate liver oxidative damage and remodelling in obese mice (33). This is likely to explain why in our study, SRT1720-induced suppression of liver lipogenesis markers (PPARγ, FASN, ChREBP) and lipid droplet accumulation in HFD-fed offspring is associated with reduced levels of antioxidants and increased levels of liver fibrosis and injury markers. Consistent with the study by Yamaguchi (33), these negative effects occur despite reduced expression of ROS production and inflammation markers and improved systemic insulin sensitivity. It is also noteworthy that the negative effects of SRT1720 on fibrogenesis have been reported in several studies in different tissues (15, 26, 34) alongside with the benefits on metabolism.

High levels of suppression of de novo lipogenesis pathways in WAT can also lead to liver injuries in SRT1720-administrated offspring. SRT1720 suppresses WAT expression of PPARγ, which has been shown to disrupt adipogenesis-associated lipid storage and lead to lipotoxicity in peripheral tissues including liver (20). Activation of PPARγ, on the other hand, has been shown to result in lower body weight, visceral WAT and plasma triglyceride in the offspring born to HFD-fed mothers (17), which is likely to be associated with improved liver outcomes.

In contrast to other studies (3), the SIRT1-AMPK-PGC-1α pathway appear to play a limited role in the current model. Therefore, we believe that the effects of SRT1720 are mainly mediated via the regulation of lipogenesis markers including SREBP-1c and PPARγ. Supporting this hypothesis, it has been shown that SIRT1 can repress the expression of SREBP-1c and PPARγ as well as their downstream markers such as FASN (25, 31).

In conclusion, the study supports the use of SIRT1 agonists in the offspring at early ages to ameliorate the transgenerational effects of maternal obesity on systemic metabolism homeostasis of lipid and glucose. It is also clinically important that SRT1720 administration overall does not result in significant metabolic and liver changes in the control offspring. However, the side effects of the therapy on the liver in HFD-exposed offspring need to be further examined.

ACKNOWLEDGMENTS

L.T.N. was supported by Sydney Medical School's ECR PhD Scholarship and Amgen research scholarship.

AUTHOR CONTRIBUTION

L.T.N. designed and conducted all main experiments and data analyses. L.T.N. prepared figures and the manuscript. A.Z. assisted with tissue processing for histology. C.M. assisted with literature review, microscopy and histological analyses. H.C, C.P., and S.S. coordinated the execution of the project and involved in experiment design. H.C, C.P., and S.S reviewed data analysis and the writing of the manuscript.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s)

REFERENCES

1. **Alcendor RR, Gao S, Zhai P, Zablocki D, Holle E, Yu X, Tian B, Wagner T, Vatner SF, and Sadoshima J.** Sirt1 regulates aging and resistance to oxidative stress in the heart. *Circulation research* 100: 1512-1521, 2007.
2. **Alfaradhi MZ, Fernandez-Twinn DS, Martin-Gronert MS, Musial B, Fowden A, and Ozanne SE.** Oxidative stress and altered lipid homeostasis in the programming of offspring fatty liver by maternal obesity. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 307: R26-R34, 2014.
3. **Cantó C and Auwerx J.** PGC-1alpha, SIRT1 and AMPK, an energy sensing network that controls energy expenditure. *Current opinion in lipidology* 20: 98, 2009.
4. **Catalano P.** Maternal obesity and metabolic risk to the offspring: why lifestyle interventions may have not achieved the desired outcomes. *International Journal of Obesity* 39: 642-649, 2015.
5. **CHEN Xuan LH-p.** Effects of high fat diet on metabolism and inflammatory and oxidative stress responses in placenta of pregnant mice. 33: 1079-, 2013.
6. **Cohen HY, Miller C, Bitterman KJ, Wall NR, Hekking B, Kessler B, Howitz KT, Gorospe M, de Cabo R, and Sinclair DA.** Calorie Restriction Promotes Mammalian Cell Survival by Inducing the SIRT1 Deacetylase. *Science* 305: 390, 2004.
7. **Di Emidio G, Falone S, Vitti M, D'Alessandro AM, Vento M, Di Pietro C, Amicarelli F, and Tatone C.** SIRT1 signalling protects mouse oocytes against oxidative stress and is deregulated during aging. *Human Reproduction* 29: 2006-2017, 2014.
8. **Fleury C, Neverova M, Collins S, Raimbault S, Champigny O, Levi-Meyrueis C, Bouillaud F, Seldin MF, Surwit RS, and Ricquier D.** Uncoupling protein-2: a novel gene linked to obesity and hyperinsulinemia. *Nature genetics* 15: 269-272, 1997.
9. **Galarraga M, Campión J, Muñoz-Barrutia A, Boqué N, Moreno H, Martínez JA, Milagro F, and Ortiz-de-Solórzano C.** Adiposoft: automated software for the analysis of white adipose tissue cellularity in histological sections. *Journal of lipid research* 53: 2791-2796, 2012.
10. **Glastras SJ, Chen H, McGrath RT, Zaky AA, Gill AJ, Pollock CA, and Saad S.** Effect of GLP-1 Receptor Activation on Offspring Kidney Health in a Rat Model of Maternal Obesity. *Scientific Reports* 6: 23525, 2016.
11. **Glastras SJ, Chen H, Tsang M, Teh R, McGrath RT, Zaky A, Chen J, Wong MG, Pollock CA, and Saad S.** The renal consequences of maternal obesity in offspring are overwhelmed by postnatal high fat diet. *PloS one* 12: e0172644, 2017.

12. **Houtkooper RH, Pirinen E, and Auwerx J.** Sirtuins as regulators of metabolism and healthspan. *Nature reviews Molecular cell biology* 13: 225-238, 2012.
13. **Howie G, Sloboda D, Kamal T, and Vickers M.** Maternal nutritional history predicts obesity in adult offspring independent of postnatal diet. *The Journal of physiology* 587: 905-915, 2009.
14. **Hubbard BP and Sinclair DA.** Small molecule SIRT1 activators for the treatment of aging and age-related diseases. *Trends in pharmacological sciences* 35: 146-154, 2014.
15. **Imanishi S, Hayashi R, Ichikawa T, Suzuki K, Sasahara M, Kondo T, Ogawa H, and Tobe K.** SRT1720, a SIRT1 activator, aggravates bleomycin-induced lung injury in mice. *Food and Nutrition Sciences* 3: 157, 2012.
16. **Jones HN, Woollett LA, Barbour N, Prasad PD, Powell TL, and Jansson T.** High-fat diet before and during pregnancy causes marked up-regulation of placental nutrient transport and fetal overgrowth in C57/BL6 mice. *The FASEB Journal* 23: 271-278, 2009.
17. **Kalanderian A, Abate N, Patrikeev I, Wei J, Vincent KL, Motamedi M, Saade GR, and Bytautiene E.** Pioglitazone therapy in mouse offspring exposed to maternal obesity. *American journal of obstetrics and gynecology* 208: 308. e301-308. e307, 2013.
18. **Leddy MA, Power ML, and Schulkin J.** The impact of maternal obesity on maternal and fetal health. *Reviews in obstetrics and gynecology* 1: 170, 2008.
19. **Liu J, Han L, Zhu L, and Yu Y.** Free fatty acids, not triglycerides, are associated with non-alcoholic liver injury progression in high fat diet induced obese rats. *Lipids in health and disease* 15: 27, 2016.
20. **Medina-Gomez G, Gray SL, Yetukuri L, Shimomura K, Virtue S, Campbell M, Curtis RK, Jimenez-Linan M, Blount M, and Yeo GS.** PPAR gamma 2 prevents lipotoxicity by controlling adipose tissue expandability and peripheral lipid metabolism. *PLoS genetics* 3: e64, 2007.
21. **Milne JC, Lambert PD, Schenk S, Carney DP, Smith JJ, Gagne DJ, Jin L, Boss O, Perni RB, and Vu CB.** Small molecule activators of SIRT1 as therapeutics for the treatment of type 2 diabetes. *Nature* 450: 712-716, 2007.
22. **Nguyen LT, Chen H, Pollock C, and Saad S.** SIRT1 reduction is associated with sex-specific dysregulation of renal lipid metabolism and stress responses in offspring by maternal high-fat diet. *Scientific Reports* 7, 2017.
23. **Nguyen LT, Chen H, Pollock CA, and Saad S.** Sirtuins—mediators of maternal obesity-induced complications in offspring? *The FASEB Journal* 30: 1383-1390, 2016.
24. **Pfluger PT, Herranz D, Velasco-Miguel S, Serrano M, and Tschöp MH.** Sirt1 protects against high-fat diet-induced metabolic damage. *Proceedings of the National Academy of Sciences* 105: 9793-9798, 2008.
25. **Picard F, Kurtev M, Chung N, Topark-Ngarm A, Senawong T, Machado de Oliveira R, Leid M, McBurney MW, and Guarente L.** Sirt1 promotes fat mobilization in white adipocytes by repressing PPAR-γ. *Nature* 429: 771, 2004.
26. **Ponnusamy M, Zhuang MA, Zhou X, Tolbert E, Bayliss G, Zhao TC, and Zhuang S.** Activation of Sirtuin-1 promotes renal fibroblast activation and aggravates renal fibrogenesis. *Journal of Pharmacology and Experimental Therapeutics* 354: 142-151, 2015.
27. **Qiao L, Guo Z, Bosco C, Guidotti S, Wang Y, Wang M, Parast M, Schaack J, Hay WW, and Moore TR.** Maternal High Fat Feeding Increases Placenta Lipoprotein Lipase Activity by Reducing Sirt1 Expression in Mice. *Diabetes*: db141627, 2015.
28. **Rippe C, Berger K, Böiers C, Ricquier D, and Erlanson-Albertsson C.** Effect of high-fat diet, surrounding temperature, and enterostatin on uncoupling protein gene expression. *American Journal of Physiology-Endocrinology And Metabolism* 279: E293-E300, 2000.
29. **Saben J, Lindsey F, Zhong Y, Thakali K, Badger TM, Andres A, Gomez-Acevedo H, and Shankar K.** Maternal obesity is associated with a lipotoxic placental environment. *Placenta* 35: 171-177, 2014.

30. **Suter MA, Chen A, Burdine MS, Choudhury M, Harris RA, Lane RH, Friedman JE, Grove KL, Tackett AJ, and Aagaard KM.** A maternal high-fat diet modulates fetal SIRT1 histone and protein deacetylase activity in nonhuman primates. *The FASEB Journal* 26: 5106-5114, 2012.
31. **Walker AK, Yang F, Jiang K, Ji JY, Watts JL, Purushotham A, Boss O, Hirsch ML, Ribich S, Smith JJ, Israelian K, Westphal CH, Rodgers JT, Shioda T, Elson SL, Mulligan P, Najafi-Shoushtari H, Black JC, Thakur JK, Kadyk LC, Whetstine JR, Mostoslavsky R, Puigserver P, Li X, Dyson NJ, Hart AC, and Naar AM.** Conserved role of SIRT1 orthologs in fasting-dependent inhibition of the lipid/cholesterol regulator SREBP. *Genes Dev* 24: 1403-1417, 2010.
32. **Weyer C, Foley J, Bogardus C, Tataranni P, and Pratley R.** Enlarged subcutaneous abdominal adipocyte size, but not obesity itself, predicts type II diabetes independent of insulin resistance. *Diabetologia* 43: 1498-1506, 2000.
33. **Yamaguchi K, Yang L, McCall S, Huang J, Yu XX, Pandey SK, Bhanot S, Monia BP, Li YX, and Diehl AM.** Inhibiting triglyceride synthesis improves hepatic steatosis but exacerbates liver damage and fibrosis in obese mice with nonalcoholic steatohepatitis. *Hepatology* 45: 1366-1374, 2007.
34. **Zerr P, Palumbo-Zerr K, Huang J, Tomcik M, Sumova B, Distler O, Schett G, and Distler JH.** Sirt1 regulates canonical TGF-β signalling to control fibroblast activation and tissue fibrosis. *Annals of the rheumatic diseases* 75: 226-233, 2016.

413 **Table 1. RT-PCR primer sequences.**

414

No.	Gene	Forward primer sequence	Reverse primer sequence
1	Actin	CTAAGGCCAACCGTGAAAAG	ACCAGAGGCATACAGGGACA
2	CAT	CTCCATCAGGTTTGTTTCTTG	CAACAGGCAAGTTTTTGATG
3	CHOP	CCACCACACCTGAAAGCAGA	AGGCGAAAGGCAGAGACTCA
4	ChREBP	ATATCTCCGACACACTCTTC	CAACATAAGCATCTTCTGGG
5	COL1A	CATGTTCAGCTTTGTGGACCT	GCAGCTGACTTCAGGGATGT
6	COL3A	TCCCCTGGAATCTGTGAATC	TGAGTCGAATTGGGGAGAAT
7	COL4	AAGGACTCCAGGGACCAC	CCCACTGAGCCTGTCACAC
8	F4/80	CCTGGACGAATCCTGTGAAG	GGTGGGACCACAGAGAGTTG
9	FASN	TGCTCCCAGCTGCAGGC	GCCCGGTAGCTCTGGGTGTA
10	FN	CGGAGAGAGTGCCCCTACTA	CGATATTGGTGAATCGCAGA
11	GPX1	GGACAATGGCAAGAATGAAG	TTCGCACTTCTCAAACAATG
12	InsR	TTTGTCATGGATGGAGGCTA	CCTCATCTTGGGGTTGAACT
13	LXRB	TCACCCACTATTAAGGAAGAG	TCTAAGATGACCACGATGTAG
14	NOX2	CTACCTAAGATAGCAGTTGATG	TACCAGACAGACTTGAGAATG
15	PGC1A	AAACTTGCTAGCGGTCCTCA	TGGCTGGTGCCAGTAAGAG
16	PPARG	ATCTACACGATGCTGGC	GGATGTCCTCGATGGG
17	SIRT1	GCAGGTTGCGGGAATCCAA	GGCAAGATGCTGTTGCAAA
18	SOD1	CACTCTAAGAAACATGGTGG	GATCACACGATCTTCAATGG
19	SOD2	GGCCTACGTGAACAACCTGAA	CTGTAACATCTCCCTTGGCCA
20	SREBP-1c	GGAGCCATGGATTGCACATT	GCTTCCAGAGAGGAGGCCAG
21	TNF-a	CTGTAGCCCACGTCGTAGC	TTGAGATCCATGCCGTTG
22	UCP1	CTTTTTCAAAGGGTTTGTGG	CTTATGTGGTACAATCCACTG
23	UCP2	ACCTTTAGAGAAGCTTGACC	TTCTGATTTCCTGCTACCTC

415

FIGURE LEGENDS

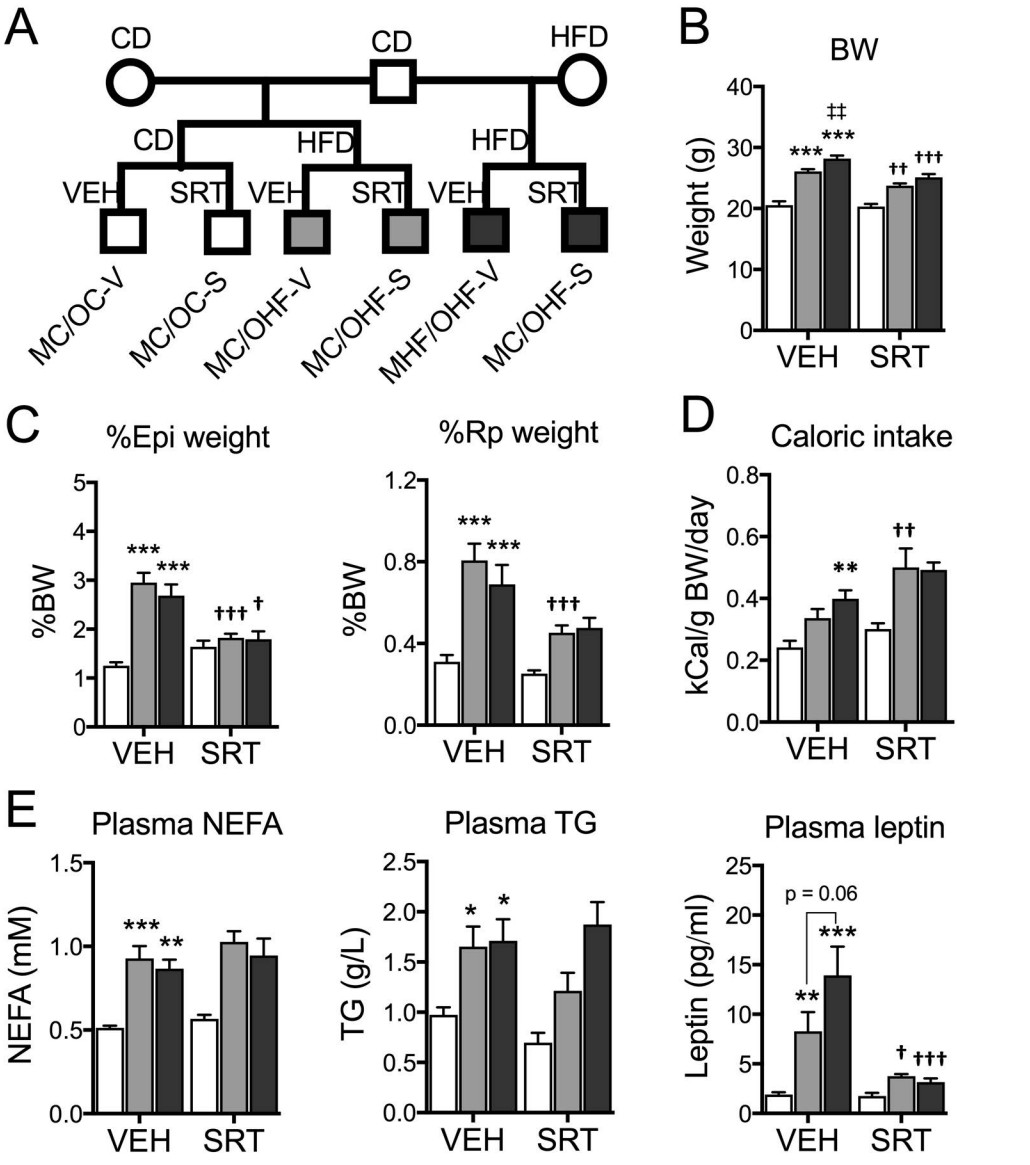
Figure 1. Experiment design, anthropometric data and blood analysis. A. Experiment design; B. Offspring body weight (n = 9 – 17); C. Fat mass (n = 9 – 17); D. Caloric intake (n = 9 – 15); E. Plasma levels of Non-Esterified Fatty Acid, Triglyceride and leptin. (n = 7 – 8). Data are presented as mean ± SEM. Vs MC/OC/V (*p < 0.05, **p < 0.01, ***p < 0.001); vs MC/OHF/V (‡‡p < 0.01); SRT1720-treatment group vs the corresponding VEH-treated controls (†p < 0.05††p < 0.01, †††p < 0.01)

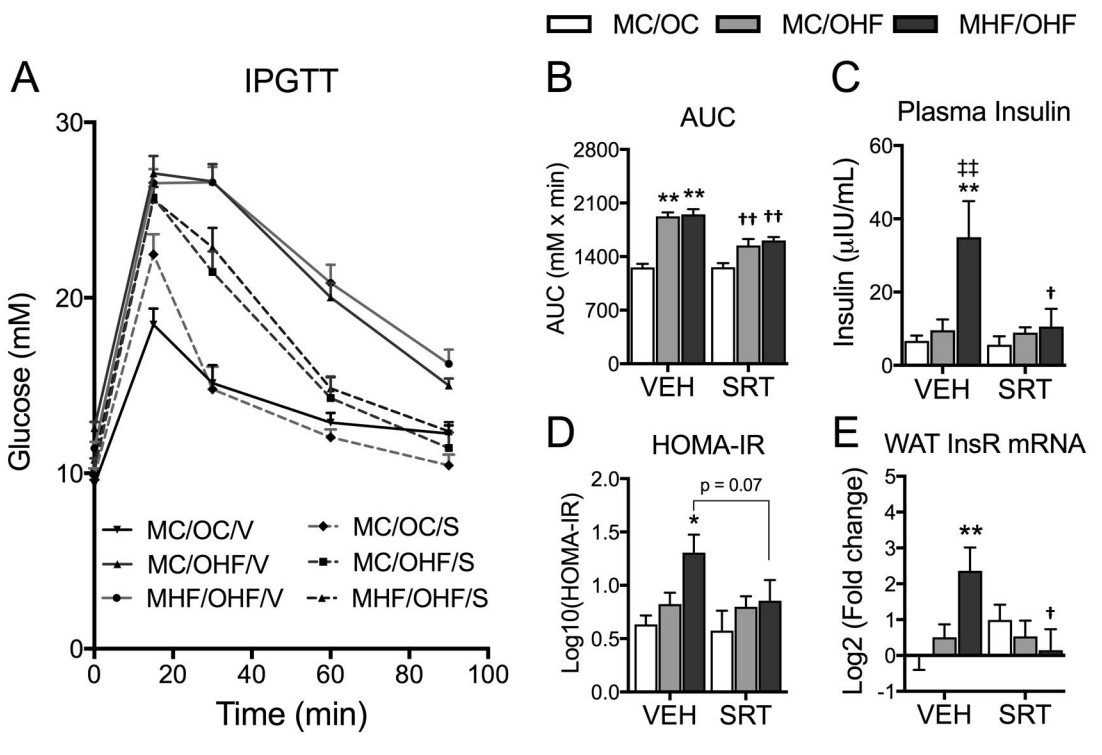
Figure 2. Offspring glucose tolerance and insulin sensitivity. A. Intraperitoneal glucose tolerance test and B. Area under the curve (n = 7 – 15). C. Plasma insulin level and D. Homeostatic model assessment of insulin resistance (n = 3 - 5). E. Insulin receptor mRNA expression in White adipose tissue. (n = 7 – 15). Data are presented as mean ± SEM. Vs MC/OC/V (*p < 0.05, **p < 0.01, ***p < 0.001); vs MC/OHF/V (‡‡p < 0.01); SRT1720-treatment group vs the corresponding VEH controls (†p < 0.05, ††p < 0.01, †††p < 0.01). V (VEH), S (SRT1720)

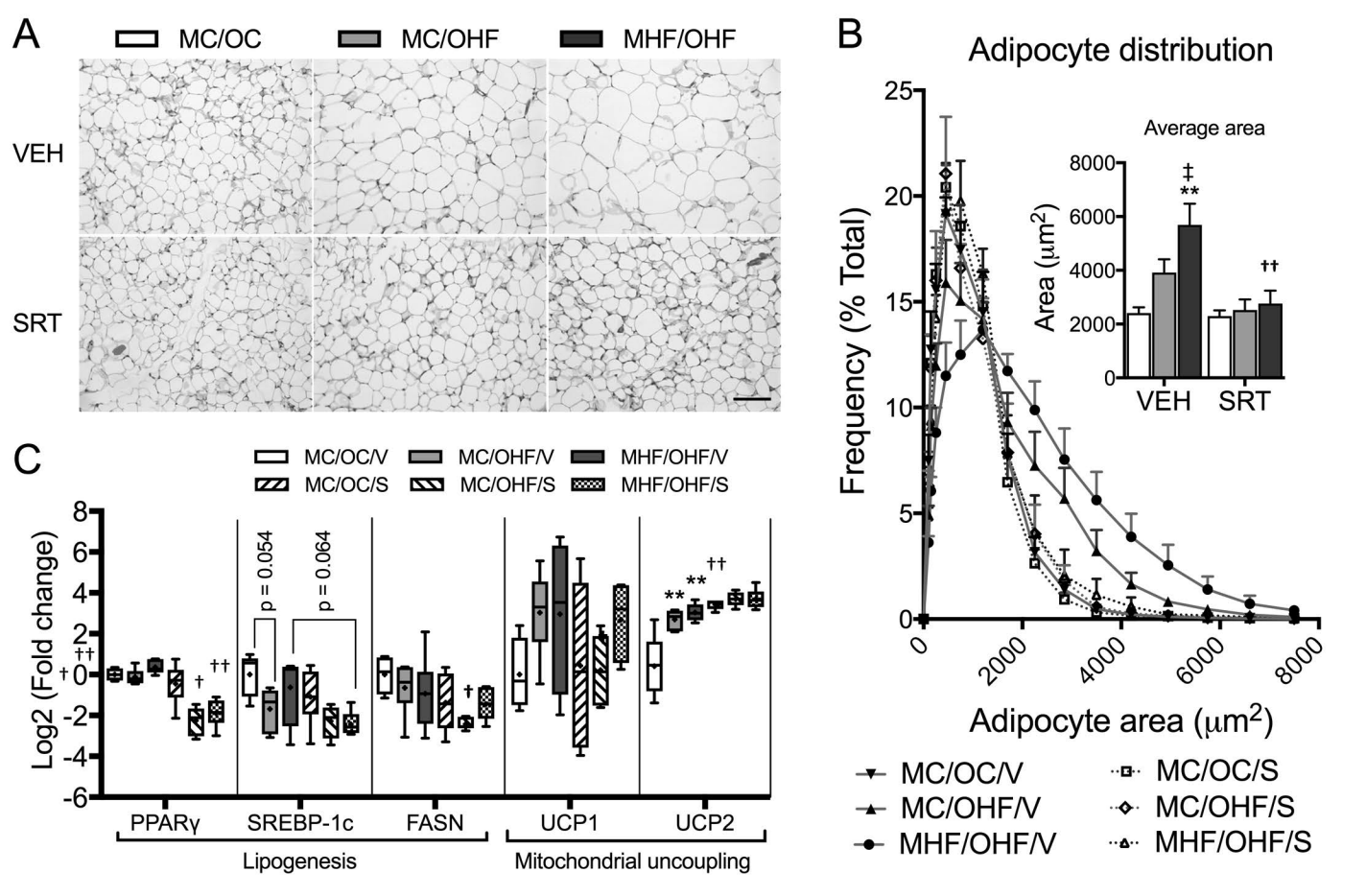
Figure 3. Adipose tissue analysis. A. H&E staining; B. Cell size analysis; C. mRNA expression. (n = 6). Data are presented as mean ± SEM. Vs MC/OC/V (**p < 0.01); vs MC/OHF/V (‡p < 0.05); SRT1720-treatment group vs the corresponding VEH controls (†p < 0.05, ††p < 0.01). V (VEH), S (SRT1720).

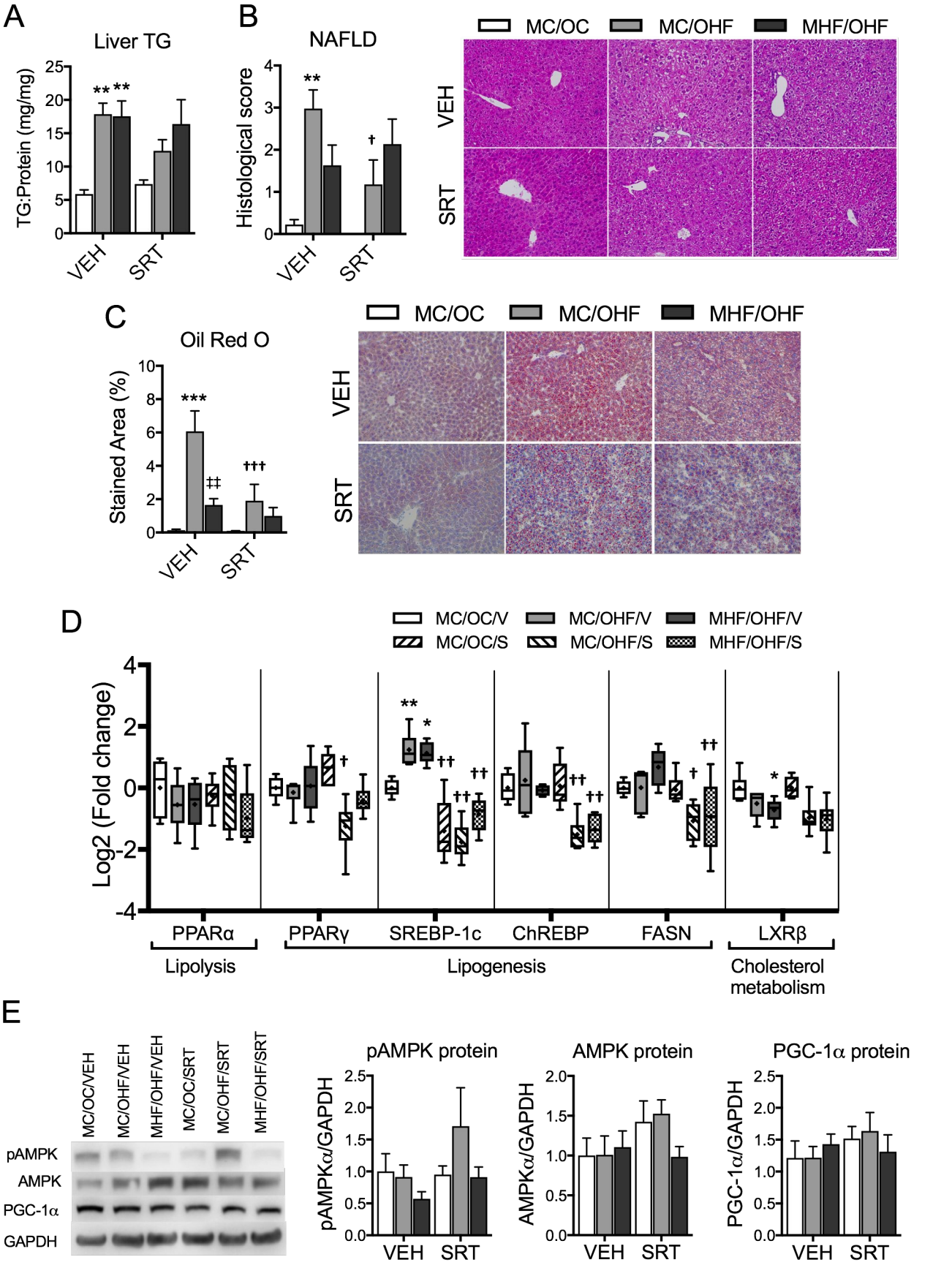
Figure 4. Liver lipid metabolism. A. Liver Triglyceride level; B. H&E staining; C. Oil Red O staining and quantitation of lipid droplets; D. Liver mRNA expression of lipid metabolism regulators. (n = 6). Data are presented as mean ± SEM. Vs MC/OC/V (*p<0.05, **p < 0.01, ***p < 0.001); vs MC/OHF/V (‡‡p < 0.01); SRT1720-treatment group vs the corresponding VEH controls (†p < 0.05, ††p < 0.01, †††p < 0.01). V (VEH), S (SRT1720). Scale bar = 100 μm.

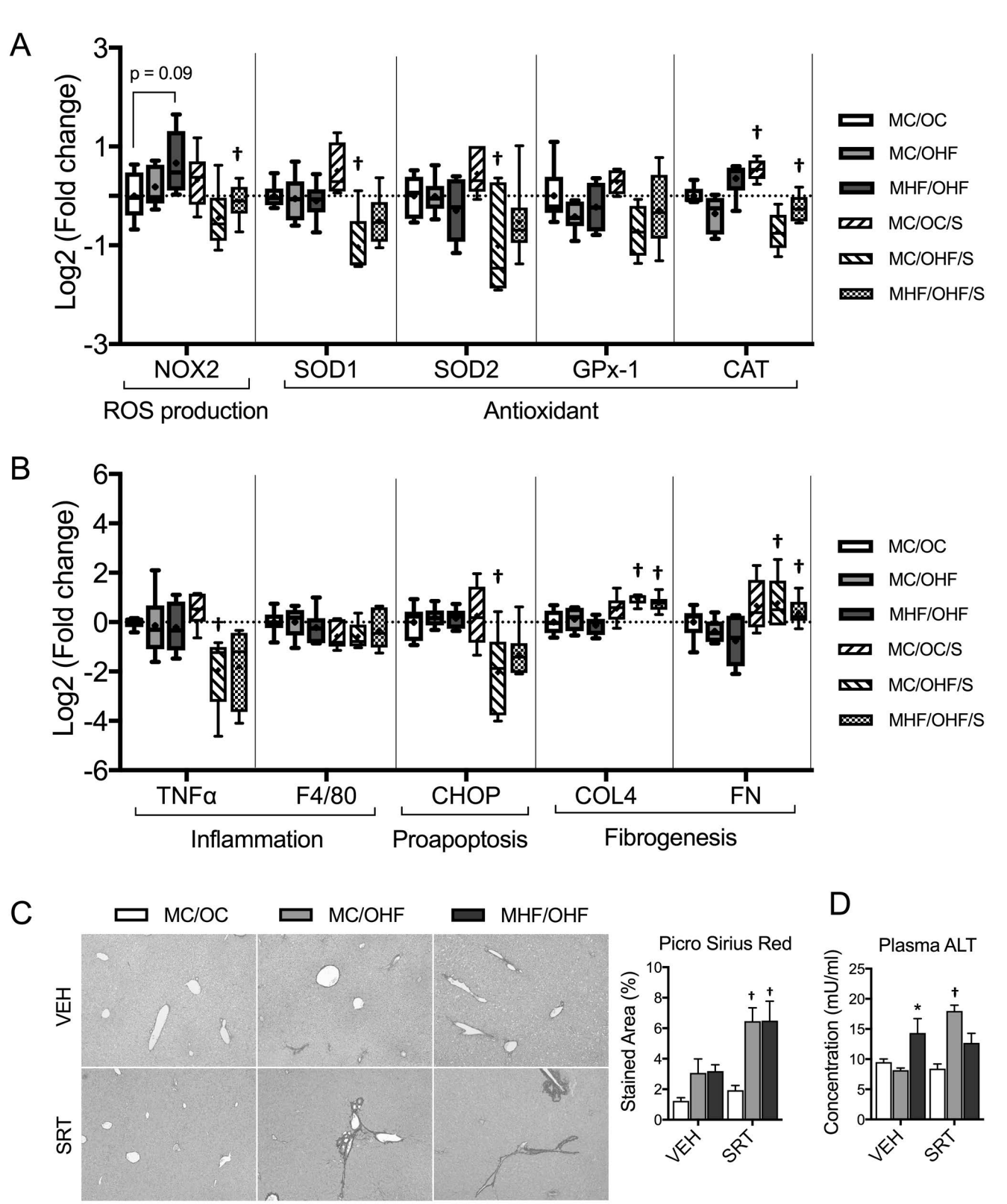
Figure 5. Offspring liver oxidative stress, inflammation, fibrogenesis and injury. A. Oxidative stress markers (RNS: reactive nitrogen species); B. Inflammation, apoptosis and fibrogenesis; C. Picro-Sirius Red staining; D. Plasma alanine Aminotransferase level. (n = 6). Data are presented as mean ± SEM. Vs MC/OC/V (*p < 0.05); SRT1720-treatment group vs the corresponding VEH controls (†p < 0.05). V (VEH), S (SRT1720)


A
CD
CD
HFD
CD
HFD
HFD
VEH
SRT
VEH
SRT
VEH
SRT
MC/OC-V
MC/OC-S
MC/OHF-V
MC/OHF-S
MHF/OHF-V
MC/OHF-S
B
BW
Weight (g)
VEH
SRT
C
%Epi weight
%BW
%Rp weight
%BW
D
Caloric intake
kCal/g BW/day
E
Plasma NEFA
NEFA (mM)
Plasma TG
TG (g/L)
Plasma leptin
Leptin (pg/ml)
p = 0.06


MC/OC
MC/OHF
MHF/OHF
A
IPGTT
Glucose (mM)
Time (min)
MC/OC/V
MC/OHF/V
MHF/OHF/V
MC/OC/S
MC/OHF/S
MHF/OHF/S
B
AUC
AUC (mM x min)
VEH
SRT
C
Plasma Insulin
Insulin (μIU/mL)
D
HOMA-IR
Log10(HOMA-IR)
p = 0.07
E
WAT InsR mRNA
Log2 (Fold change)


A
MC/OC
MC/OHF
MHF/OHF
VEH
SRT
B
Adipocyte distribution
Frequency (% Total)
Adipocyte area (μm2)
Average area
Area (μm2)
VEH
SRT
MC/OC/V
MC/OHF/V
MHF/OHF/V
MC/OC/S
MC/OHF/S
MHF/OHF/S
C
MC/OC/V
MC/OHF/V
MHF/OHF/V
MC/OC/S
MC/OHF/S
MHF/OHF/S
Log2 (Fold change)
p = 0.054
p = 0.064
PPARγ
SREBP-1c
FASN
UCP1
UCP2
Lipogenesis
Mitochondrial uncoupling


A
Liver TG
TG:Protein (mg/mg)
VEH
SRT
B
NAFLD
Histological score
MC/OC
MC/OHF
MHF/OHF
C
Oil Red O
Stained Area (%)
D
MC/OC/V
MC/OHF/V
MHF/OHF/V
MC/OC/S
MC/OHF/S
MHF/OHF/S
Log2 (Fold change)
PPARα
PPARγ
SREBP-1c
ChREBP
FASN
LXRβ
Lipolysis
Lipogenesis
Cholesterol metabolism
E
MC/OC/VEH
MC/OHF/VEH
MHF/OHF/VEH
MC/OC/SRT
MC/OHF/SRT
MHF/OHF/SRT
pAMPK
AMPK
PGC-1α
GAPDH
pAMPK protein
AMPK protein
PGC-1α protein
pAMPKα/GAPDH
AMPKα/GAPDH
PGC-1α/GAPDH


A
p = 0.09
Log2 (Fold change)
3
1
-1
-3
NOX2
SOD1
SOD2
GPx-1
CAT
ROS production
Antioxidant
MC/OC
MC/OHF
MHF/OHF
MC/OC/S
MC/OHF/S
MHF/OHF/S
B
Log2 (Fold change)
6
4
2
0
-2
-4
-6
TNFα
F4/80
CHOP
COL4
FN
Inflammation
Proapoptosis
Fibrogenesis
MC/OC
MC/OHF
MHF/OHF
MC/OC/S
MC/OHF/S
MHF/OHF/S
C
MC/OC
MC/OHF
MHF/OHF
VEH
SRT
Picro Sirius Red
Stained Area (%)
10
8
6
4
2
0
VEH
SRT
D
Plasma ALT
Concentration (mU/ml)
25
20
15
10
5
0
VEH
SRT