Contents lists available at ScienceDirect

Biochemistry and Biophysics Reports

Paternal high-fat diet alters triglyceride metabolism-related gene expression in liver and white adipose tissue of male mouse offspring

Shu Aizawa [*], Ai Tochihara, Yutaka Yamamuro

Laboratory of Animal Genetics and Physiology, Department of Animal Science, College of Bioresource Sciences, Nihon University, Japan

A B S T R A C T

Obesity is a major public health problem, and its prevalence is progressively increasing worldwide. In addition, accumulating evidence suggests that diverse nutritional and metabolic disturbances including obesity can be transmitted from parents to offspring via transgenerational epigenetic inheritance. The previous reports have shown that paternal obesity has profound impacts on the development and metabolic health of their progeny. However, little information is available concerning the effects of paternal high-fat diet (HFD) exposure on triglyceride metabolism in the offspring. Therefore, we investigated the effects of paternal HFD on triglyceride metabolism and related gene expression in male mouse offspring. We found that paternal HFD exposure significantly increased the body weight, liver and epididymal white adipose tissue (eWAT) weights, and liver triglyceride content in male offspring, despite consuming control diet. In addition, paternal HFD exposure had induced changes in the mRNA expression of genes involved in lipid and triglyceride metabolism in the liver and eWAT. These findings indicate transgenerational inheritance from the paternal metabolic disturbance of triglyceride and support the effects of paternal lifestyle choices on offspring development and health later in life.

1. Introduction

Obesity is currently a major public health problem, and its prevalence is progressively increasing worldwide [1]. Obesity is defined as abnormal or excess accumulation of adipose tissue, and a risk factor for the onset of metabolic disorders, including type 2 diabetes, hyperlipidemia, and cardiovascular disease. Although the causes of obesity are complex and have a genetic component, environmental factors also play an important role in the development of obesity and related metabolic disorders [2]. The chronic imbalance between energy intake and expenditure is the major environmental factor that can lead to the development of obesity in human and animal models.

Accumulating evidence suggests that diverse nutritional and metabolic conditions including obesity can be transmitted from parents to offspring via transgenerational inheritance. Epidemiological studies, including the Dutch Famine Birth Cohort study, demonstrated how maternal malnutrition during a specific gestational time window might affect the offspring's metabolic phenotype and health later in life [3–6]. In addition, a relationship between the parental body mass index and offspring obesity has been reported [7–11]. Although it is difficult in epidemiological studies to interpret the data due to the time scale

involved and the influence of genetic, social, and cultural factors, animal studies have similarly reported that the nutritional and metabolic status of the parents can impact the metabolic phenotype of the offspring.

Most animal studies have focused on the maternal dietary effects on the metabolic phenotype of the offspring. It has been demonstrated that maternal obesity predisposes the offspring to obesity, impaired glucose tolerance, hypertension, and other metabolic disorders [12–15]. More recently, increasing evidence has shown that paternal nutritional and metabolic status also has profound impacts on the development and metabolic health of their progeny. For instance, a paternal high-fat diet (HFD) impairs pancreatic β-cell function in female offspring [16] and exacerbates metabolic and reproductive disturbances in male offspring [17,18]. Collectively, these reports suggest that paternal obesity at conception has a marked effect on the offspring's metabolic health. However, little information is available about the effects of paternal HFD exposure on triglyceride metabolism in the offspring.

In the present study, we investigated whether paternal exposure to HFD influences triglyceride metabolism and related gene expression in male C57BL/6N mice offspring. We observed that male offspring from obese fathers exhibited increases in body weight, liver and epididymal white adipose tissue (eWAT) weights, and liver triglyceride content in

---

adulthood, despite offspring consuming control diet. Furthermore, we found altered expression of genes involved in triglyceride metabolism in the liver and eWAT of male offspring from HFD-fed fathers. Our findings indicate transgenerational inheritance of diet-induced metabolic disturbance of triglyceride in the offspring, at least the next generation.

2. Materials and methods

2.1. Animals

All animal experiments were approved by the Nihon University Animal Care and Use Committees and performed under the Guidelines for Animal Experiments, College of Bioresource Sciences, Nihon University. C57BL/6N male mice, 4 weeks of age, were purchased from Japan SLC, Inc. (Shizuoka, Japan) and housed in an animal experimental facility with a 12 h-light/12 h-dark cycle (lights on at 09:00) at 23 ± 1 °C. Mice were randomly assigned to receive either a control diet (CD: 10% kcal from fat, 3.85 kcal/g, D12450H, Research Diets, Inc., New Brunswick, NJ, USA) or a high-fat diet (HFD: 45% kcal from fat, 4.73 kcal/g, D12451, Research Diets, Inc.) beginning at 5 weeks of age and continuing for 10 weeks. At 15 weeks of age, CD- and HFD-fed males were individually mated with 11- to 13-week-old CD-fed C57BL/6N female mice for 4 days to generate F1 offspring. During mating, all mice were maintained with CD and water *ad libitum*. Litters were standardized to 6 pups on postnatal day 1 (the day of parturition was defined as postnatal day 0) to avoid postnatal nutritional imbalance. The pups were weaned at postnatal day (PND) 26. Male offspring were studied at 10 weeks of age for all experiments, and a maximum of two male offspring were taken from each litter to mitigate inter-litter effects. All animals except for the paternal mice were maintained on the CD throughout the experiment. Mice were sacrificed by CO_2 at the appropriate time points, and blood, liver, and eWAT were collected for the following experiments.

2.2. Histology

Tissues were fixed with Bouin solution (FUJIFILM Wako Pure Chemical Corp., Osaka, Japan) by immersion for 24 h at 4 °C and embedded in paraffin wax. Tissue sections were cut at a thickness of 5 μm and stained with hematoxylin and eosin (H&E). For H&E staining, Mayer's Hematoxylin Solution (FUJIFILM Wako Pure Chemical Corp.) and 1% Eosin Y Solution (FUJIFILM Wako Pure Chemical Corp.) were used. For Oil Red O staining, liver tissues were embedded in Tissue-Tek OCT Compound (Sakura Finetek Japan Co., Ltd., Tokyo, Japan) and frozen with carbon dioxide ice. Five-micrometer cryosections were postfixed with 4% paraformaldehyde in phosphate-buffered saline and stained with Oil Red O solution (Sigma-Aldrich, St. Louis, MO, USA). After rinsing in 60% isopropyl alcohol and H_2O, the sections were counterstained with Mayer's Hematoxylin Solution and mounted. The sections were observed using a light microscope (BX41, Olympus, Tokyo, Japan).

2.3. Triglyceride and cholesterol measurements

Blood samples were separated by centrifugation at $1,200 \times g$ for 15 min to collect serum samples. Lipid extraction from the liver was performed using the Bligh-Dyer method [19]. Briefly, 200 μl of liver homogenate was added into a mixture containing 200 μl of chloroform and 400 μl of methanol and vortexed. Then, 200 μl of ultrapure H_2O and 200 μl of chloroform were added to the mixture and centrifuged at $5,000 \times g$ for 10 min. After centrifugation, the organic layer was collected and dried in a heat block at 45 °C. The obtained lipid extract was redissolved in 150 μl of 5% Triton X-100/95% isopropyl alcohol solution. The triglyceride and total cholesterol levels were measured using a LabAssay Triglyceride Kit (FUJIFILM Wako Pure Chemical Corp.) and LabAssay Cholesterol Kit (FUJIFILM Wako Pure Chemical Corp.), respectively.

The absorbance at 600 nm was measured using a Tecan Spark 10 M microplate reader (Tecan Trading AG, Männedorf, Switzerland), and the data were analyzed by SparkControl Magellan software version 1.2 (Tecan Trading AG).

2.4. Real-time quantitative PCR analysis

Total RNAs from the liver and eWAT were isolated using TRIzol Reagent (Thermo Fisher Scientific, Waltham, MA, USA) and QIAzol Lysis Reagent (Qiagen, Hilden, Germany), respectively, and were reverse transcribed using the iScript cDNA Synthesis Kit (Bio-Rad Laboratories, Hercules, CA, USA). Real-time quantitative PCR was performed using the Rotor-Gene Q (Qiagen) and PowerUp SYBR Green Master Mix (Thermo Fisher Scientific). The primer sequences are listed in Supplemental Table 1. The PCR reaction consisted of 2 min of denaturation at 95 °C, followed by 40 cycles at 95 °C for 15 s, then 60 °C for 1 min. Following amplification, a melting curve analysis was performed to verify the authenticity of the amplified product based on its specific melting temperature. The relative amounts of target cDNA were normalized with reference to the *glyceraldehyde-3-phosphate dehydrogenase* (*Gapdh*) cDNA.

2.5. Statistical analysis

Data are presented as the mean ± standard error of the mean (SEM). The significance of the differences between groups was determined using Student's *t*-test via GraphPad Prism version 6.0 (GraphPad Software, Inc., San Diego, CA, USA). The criterion for significance was $P < 0.05$ in all cases.

3. Results

3.1. Effect of HFD on the body weight and fat deposits in male mice

To investigate the effects of paternal diet-induced obesity on the triglyceride metabolism and related gene expression in offspring, we first created diet-induced obese male mice. Five-week-old male mice were fed either a CD or HFD for 10 weeks. As shown in Fig. 1A, HFD feeding resulted in significantly increased body weights. The increase was first significant at 3 weeks after feeding, and the body weight of HFD-fed mice remained significantly elevated after 3 weeks. Ten weeks after HFD feeding, relative weights of eWAT, but not liver, to body weights of HFD-fed mice were significantly increased compared with those of CD-fed mice (Fig. 1B). Histological analysis of the liver revealed few vacuoles in the CD-fed mice sections, whereas numerous vacuoles were found in HFD mice (Fig. 1C, left). Oil red O staining of frozen liver sections also showed neutral fat accumulation of these vacuoles as lipid droplets (Fig. 1C, center). The histological analysis of eWAT showed that HFD-fed mice showed increased adipocyte size relative to CD-fed mice (Fig. 1C, right). The hepatic triglyceride concentrations were 7.6-fold higher in HFD-fed mice than CD-fed mice (Fig. 1D), although no obvious differences were observed in serum triglyceride levels (Fig. 1E). In HFD-fed mice, the liver (Fig. 1F) and serum (Fig. 1G) cholesterol contents were significantly elevated compared with those of CD-fed mice. These results demonstrated that HFD-fed male mice developed obesity.

3.2. Paternal HFD feeding impacts the body weight and fat deposits in male offspring

Next, we investigated the potential effects of paternal obesity on offspring. CD- and HFD-fed male founders were mated with CD-fed female mice and obtained F1 offspring. F1 male offspring were weaned at PND 26 from their mothers, fed with a CD, and the body weight and fat deposits were examined at 10 weeks of age (Fig. 2A). The mean body weights of F1 male offspring from HFD-fed males (HFD-F1) were
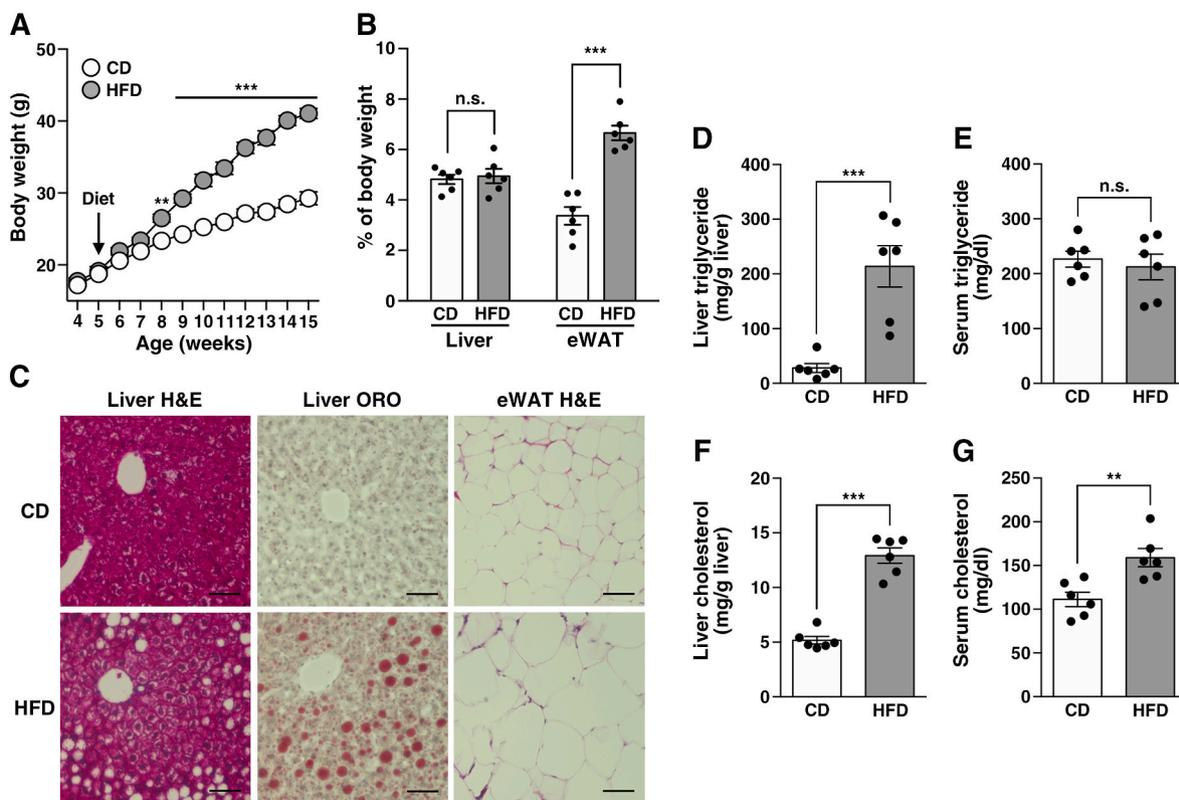
Fig. 1. Metabolic phenotypes of CD- or HFD-fed male founders. (A) Body weight changes for male mice fed with CD or HFD from 5 to 15 weeks of age. The data represent the mean $\pm$ SEM (n = 6). **$P < 0.01$, and ***$P < 0.001$ (Student's t-test). (B) Relative weights of liver and eWAT to body weights of CD- or HFD-fed male mice at 15 weeks of age. The data represent the mean $\pm$ SEM (n = 6). ***$P < 0.001$ (Student's t-test). (C) Representative H&E staining of the liver (left) and eWAT (right), and ORO staining of the liver (center). Scale bars indicate 50 µm. (D–G) Measurement of liver triglyceride, serum triglyceride, liver cholesterol, and serum cholesterol, respectively. The data represent the mean $\pm$ SEM (n = 6). **$P < 0.01$, and ***$P < 0.001$ (Student's t-test).

significantly elevated compared with the value of F1 male offspring from CD-fed males (CD-F1) ($P < 0.05$, Fig. 2B). As shown in Fig. 2C, relative weights of liver and eWAT to body weights of HFD-F1 mice were significantly increased compared with those of CD-F1 mice ($P < 0.01$). The hepatic triglyceride contents were significantly higher in HFD-F1 mice than in CD-F1 mice ($P < 0.01$, Fig. 2D). No obvious differences were observed in the serum triglyceride (Fig. 2E), liver cholesterol (Fig. 2F), or serum cholesterol (Fig. 2G). These results suggest that paternal HFD feeding affects lipid metabolism, especially triglyceride metabolism, in male offspring.

3.3. Transcriptional changes of lipid and triglyceride metabolism-related genes in liver and eWAT in male offspring

To examine the effects of paternal HFD exposure on triglyceride metabolism in male offspring at molecular levels, we measured the mRNA expression levels of genes involved in lipid and triglyceride metabolism in the offspring's liver and eWAT. The targets were genes encoding lipogenic transcription factors [*sterol regulatory element-binding transcription factor 1* (*Srebf1*, also known as *Srebp1c*), *peroxisome proliferator-activated receptor alpha* (*Ppaa*) for liver, and *peroxisome proliferator-activated receptor gamma* (*Pparg*) for eWAT, and *MLX interacting protein-like* (*Mlxipl*, also known as *Chrebp*)] and genes involved in *de novo* fatty acid synthesis [*acetyl-Coenzyme A carboxylase alpha* (*Acaca*), *fatty acid synthase* (*Fasn*), and *stearoyl-Coenzyme A desaturase 1* (*Scd1*)]. The genes involved in fatty acid transport [*solute carrier family 27 member 1* (*Slc27a1*), *CD36 molecule* (*Cd36*, also known as *Fat*), *fatty acid-binding protein 1* (*Fabp1*) for liver, and *fatty acid-binding protein 4* (*Fabp4*) for eWAT] and triglyceride synthesis [*acyl-CoA synthetase long-chain family member 1* (*Acsl1*), *glycerol-3-phosphate acyltransferase, mitochondrial* (*Gpam*), and *diacylglycerol O-acyltransferase 2* (*Dgat2*)] were

also investigated. In the liver, gene expressions of *Mlxipl* ($P < 0.05$), *Fasn* ($P < 0.01$), *Scd1* ($P < 0.05$), *Cd36* ($P < 0.05$), *Fabp1* ($P < 0.05$), and *Gpam* ($P < 0.05$) were significantly elevated in HFD-F1 mice compared with those of CD-F1 mice (Fig. 3A). Additionally, gene expressions of *Srebf1* ($P < 0.001$), *Fasn* ($P < 0.01$), *Cd36* ($P < 0.05$), *Acsl1* ($P < 0.05$), and *Dgat2* ($P < 0.01$) in the eWAT of HFD-F1 mice were significantly increased relative to those of CD-F1 mice (Fig. 3B). These findings suggest that paternal HFD feeding induced the changes in mRNA expression of genes involved in lipid and triglyceride metabolism in the liver and eWAT, resulting in the alternation of metabolic phenotypes in male offspring.

4. Discussion

Parents influence the development and health of their offspring through many routes, including the transmission of genetic information and associated epigenetic marks, the latter of which is known as trans-generational epigenetic inheritance. Although the concept of trans-generational inheritance is relatively new in biology, there is a growing body of evidence shows that environmental experiences in parents can be transmitted to the next generation [20,21]. The present study demonstrated that paternal mice with HFD exposure exhibited abnormal triglyceride metabolism and altered expression of the genes involved in triglyceride metabolism in the liver and eWAT in the offspring. These findings provide insight into the transgenerational inheritance of paternal nutritional and metabolic status, especially triglyceride metabolism, in the offspring.

In the present study, we found the altered gene expression in the liver and eWAT of male offspring from HFD-fed fathers. Several of the differentially expressed genes play an important role in lipid and triglyceride metabolism in the liver and adipose tissue. Although there is
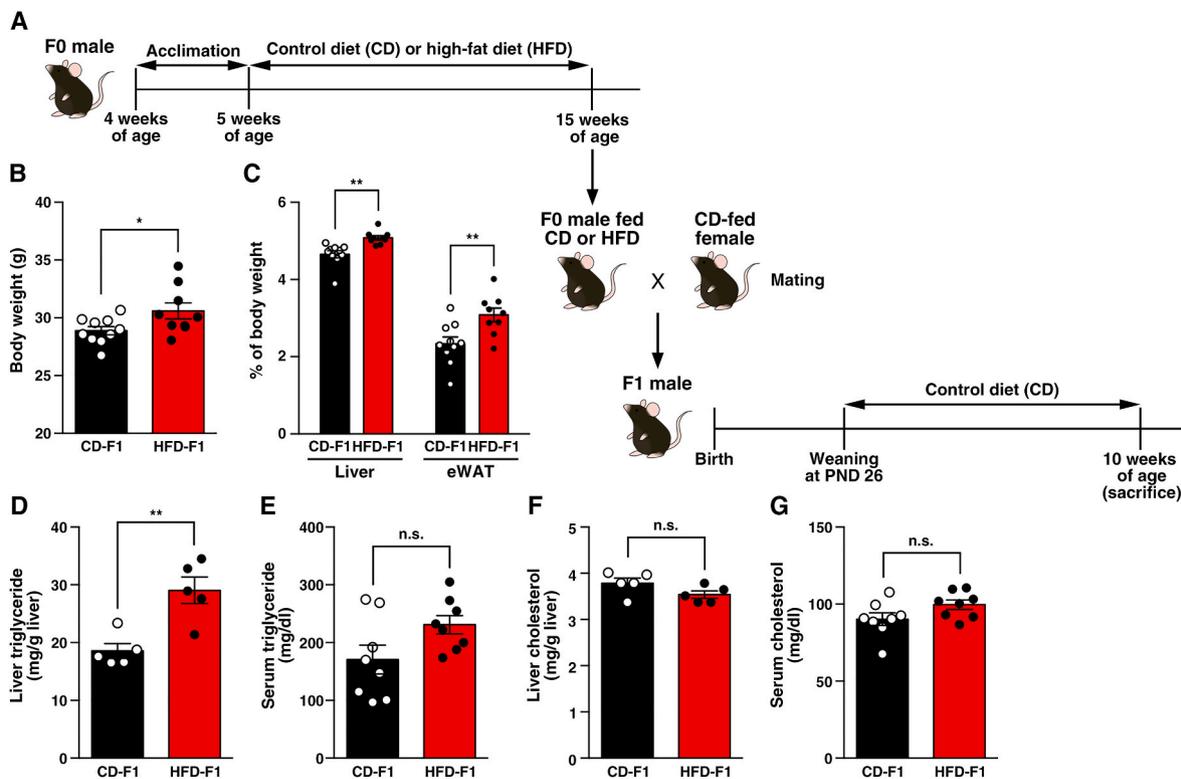
Fig. 2. Metabolic phenotypes of male offspring obtained from CD- or HFD-fed male mice. (A) Schematic diagram of the experimental design. (B) Mean body weights of offspring from CD-fed male (CD-F1) or HFD-fed male (HFD-F1) at 10 weeks of age. The data represent the mean ± SEM (n = 9–10). **$P < 0.01$, and ***$P < 0.001$ (Student's t-test). (C) Relative weights of liver and eWAT to body weights of CD- and HFD-F1 male mice at 10 weeks of age. The data represent the mean ± SEM (n = 9–10). **$P < 0.01$ (Student's t-test). (D–G) Measurement of liver triglyceride, serum triglyceride, liver cholesterol, and serum cholesterol, respectively. The data represent the mean ± SEM (n = 5–8). **$P < 0.01$ (Student's t-test).

no experimental evidence that up-regulation of these genes could be involved in observed triglyceride metabolism disturbance, these genes are known to be involved in the pathophysiology of metabolic disease. Mlxipl, also known as Chrebp, is a glucose mediated transcription factor that involved in glycolytic and lipogenic pathway, and controls lipid metabolism via regulating the expression of lipogenic genes such as *Fasn* and *Scd1* [22], suggesting the Mlxipl as key determinant of lipogenic pathway under physiological and pathophysiological conditions. Indeed, we observed that paternal HFD exposure induced increased expression of *Mlxipl* gene accompanied by increased gene expression of *Fasn* and *Scd1* in the liver of offspring. We also found the increased expression of *Cd36* gene in the liver of HFD-F1 mice. Recently, it has been reported that increased hepatic CD36 expression is involved in insulin resistance, hyperinsulinemia, and increased steatosis in patients with non-alcoholic steatohepatitis [23]. Other gene involved in triglyceride synthesis, such as *Gpam*, which catalyzes the first step of triglyceride synthesis, was also elevated in the liver of HFD-F1 mice, suggesting that increased expression of *Gpam* also involved in hepatic triglyceride accumulation in HFD-F1 mice. Similarly, in the eWAT of HFD-F1 mice, lipogenic transcription factor *Srebf1*, and several lipogenic genes such as *Fasn*, *Cd36*, *Acsl1* and *Dgat2* were also elevated. These findings suggest that paternal HFD exposure programs abnormal lipogenesis pathway in the liver and eWAT of offspring. However, in the present study, we merely observed the effects of paternal HFD exposure on mRNA levels, thus further analysis for protein levels are needed to clarify the paternal nutritional disturbance on offspring metabolism. In addition, there is no information concerning histological observation in the offspring. The relationship between paternal nutritional disturbance and histological abnormality in the liver and eWAT of offspring is currently unknown and requires further investigation.

To date, studies based on nutritional interventions in fathers have

demonstrated profound effects on the metabolic phenotypes in the offspring. Although mechanisms explaining how the paternal nutritional and metabolic status affects the development of offspring are still under debate, epigenetic changes in sperm have been hypothesized and shown to mediate paternal transgenerational inheritance [21,24]. In mammals, epigenetic information is mediated by three different, but closely interacting, molecular mechanisms consisting of DNA methylation, histone modification, and small non-coding RNAs, and is responsible for regulating gene expression during embryonic development and throughout life. Animal studies on various nutritional models, including HFD feeding, suggest that the paternal diet differentially methylated a small number of sperm loci and a limited subset of them can be maintained in offspring tissues [16,25–27]. However, several reports showed that somatic DNA methylation in the offspring failed to correlate with methylation patterns in the sperm of the fathers [28,29]. Additionally, although histones, the core structural elements of chromatin, are replaced by transition proteins, and then by protamines during spermatogenesis, a small fraction of histones in sperms escape this remodeling [30,31], and studies have suggested that retained histones may be involved in the intergenerational transmission of phenotypes [32]. For example, Terashima et al. [17] reported that HFD feeding changed the histone distribution and methylation of the sperm genome and altered hepatic gene expression in the offspring. Similar to these epigenetic marks, it has been recognized that small non-coding RNAs in sperm are the molecules enabling the important mediator for a paternal transgenerational inheritance to the offspring. In mammals, mature sperm contain a significant population of small non-coding RNAs including microRNAs, PIWI-interacting RNAs, and transfer RNA-derived fragments [33–35]. In this context, several studies have shown that the HFD alters the small non-coding RNAs content in sperm [33,36] and suggested that alternations of small non-coding RNAs might play an
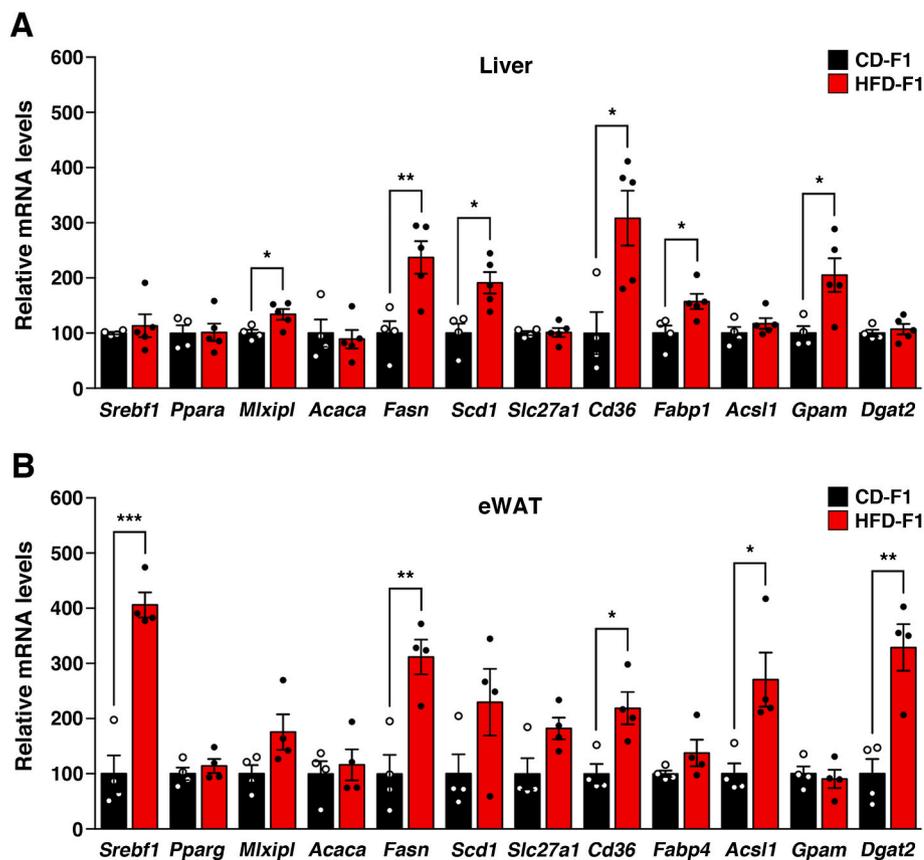
Fig. 3. Effect of paternal HFD on mRNA expression levels of genes involved in lipid and triglyceride metabolism. mRNA levels in (A) liver and (B) eWAT from CD- or HFD-F1 male mice. The amounts of mRNA including *Srebf1*, *Ppara* (liver), *Pparg* (eWAT), *Mlxipl*, *Acaca*, *Fasn*, *Scd1*, *Slc27a1*, *Cd36*, *Fabp1* (liver), *Fabp4* (eWAT), *Acsl1*, *Gpam*, and *Dgat2* were normalized with reference to those of *Gapdh* mRNA. The data represent the mean $\pm$ SEM (n = 4–5). *$P <$ 0.05, **$P <$ 0.01, and ***$P <$ 0.001 (Student's *t*-test).

important role in transgenerational inheritance. Although experimental evidence for epigenetic changes in sperm still needs to be investigated in our model, these reports suggest that epigenetic modifications in sperm may be transmitted to the offspring and induce gene expression changes of the lipid and triglyceride metabolism-related genes in the liver and eWAT. Thus, further studies are necessary to elucidate these changes that impact gene expression and triglyceride metabolism in the offspring.

HFD exposure affects the quality of seminal fluid in human and animal models [37,38]. It has been shown that seminal fluid contributes to placental growth and results in changes in the metabolic parameters in the offspring [39]. Thus, whether seminal fluid can contribute to the changes in the triglyceride metabolism of offspring also requires further investigation.

Notably, there are differential effects of paternal HFD exposure on offspring across different animal models. For instance, paternal consumption of a 40% fat diet resulted in increased body weight and induced glucose intolerance in male offspring from 8 weeks of age [25]. On the other hand, there was no difference in body weight from 4 to 16 weeks of age, followed by increased body weight and impaired insulin responses at 24 weeks of age in male offspring from males exposed to a 62% fat diet [40]. In the present study, we observed increased body weight and impaired triglyceride metabolism in male offspring at 10 weeks of age from males exposed to a 45% fat diet. These discrepancies may be due to the varying dietary fat compositions, feeding duration of the fathers, and ages of the examined offspring. It would be of great interest to investigate these issues and determine the metabolic consequences in offspring.

In conclusion, our findings demonstrated that paternal obesity significantly impacts triglyceride metabolism and related gene expression in their progeny, at least the next generation. These results indicate the transgenerational inheritance of diet-induced metabolic disturbance of triglyceride and highlight the importance of paternal lifestyle choices

and their effects on offspring development and health.

Conflicts of interest

The authors have no conflicts of interest to declare.

Declaration of competing interest

The authors have no conflicts of interest to declare.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrep.2022.101330.

References

[1] GBD 2015 Obesity Collaborators, Health effects of overweight and obesity in 195 countries over 25 years, N. Engl. J. Med. 377 (2017) 13–27, https://doi.org/10.1056/nejmoa1614362.
[2] R.J.F. Loos, G.S.H. Yeo, The genetics of obesity: from discovery to biology, Nat. Rev. Genet. 23 (2022) 120–133, https://doi.org/10.1038/s41576-021-00414-z.
[3] R.C. Painter, T.J. Roseboom, O.P. Bleker, Prenatal exposure to the Dutch famine and disease in later life: an overview, Reprod. Toxicol. 20 (2005) 345–352, https://doi.org/10.1016/j.reprotox.2005.04.005.

[4] R.C. Painter, C. Osmond, P.D. Guckman, M.A. Hanson, D.I.P. Phillips, T. J. Roseboom, Transgenerational effects of prenatal exposure to the Dutch famine on neonatal adiposity and health in later life, BJOG 115 (2008) 1243–1249, https://doi.org/10.1111/j.1471-0528.2008.01822.x.

[5] L.H. Lumey, A.D. Stein, E. Susser, Prenatal famine and adult health, Annu. Rev. Publ. Health 32 (2011) 237–262, https://doi.org/10.1146/annurev-publhealth-031210-101230.

[6] M.V.E. Veenendaal, R.C. Painter, S.R. de Rooji, P.M.M. Bossuyt, J.A.M. van der Post, P.D. Gluckman, M.A. Hanson, T.J. Roseboom, Transgenerational effects of prenatal exposure to the 1944-45 Dutch famine, BJOG 120 (2013) 548–553, https://doi.org/10.1111/1471-0528.12136.

[7] S. Danielzik, K. Langnase, M. Mast, C. Spethmann, M.J. Muller, Impact of parental BMI on the manifestation of overweight 5-7 year old children, Eur. J. Nutr. 41 (2002) 132–138, https://doi.org/10.1007/s00394-002-0367-1.

[8] L. Li, C. Law, R. Lo Conte, C. Power, Intergenerational influences on childhood body mass index: the effect of parental body mass index trajectories, Am. J. Clin. Nutr. 89 (2009) 551–557, https://doi.org/10.3945/ajcn.2008.26759.

[9] R. Cooper, E. Hypponen, D. Berry, C. Power, Associations between parental and offspring adiposity up to midlife: the contribution of adult lifestyle factors in the 1958 British birth cohort study, Am. J. Clin. Nutr. 92 (2010) 946–953, https://doi.org/10.3945/ajcn.2010.29477.

[10] K.L. Whitaker, M.J. Jarvis, R.J. Beeken, D. Boniface, J. Wardle, Comparing maternal and paternal intergenerational transmission of obesity risk in a large population-based sample, Am. J. Clin. Nutr. 91 (2010) 1560–1567, https://doi.org/10.3945/ajcn.2009.28838.

[11] C.M. Murrin, G.E. Kelly, R.E. Tremblay, C.C. Kelleher, Body mass index and height over three generations: evidence from the Lifeways crossgenerational cohort study, BMC Publ. Health 12 (2012) 81, https://doi.org/10.1186/1471-2458-12-81.

[12] G.A. Dunn, T.L. Bale, Maternal high-fat diet promotes body length increases and insulin insensitivity in second-generation mice, Endocrinology 150 (2009) 4999–5009, https://doi.org/10.1210/en.2009-0500.

[13] G.A. Dunn, T.L. Bale, Maternal high-fat diet effects on third-generation female body size via the paternal lineage, Endocrinology 152 (2011) 2228–2236, https://doi.org/10.1210/en.2010-1461.

[14] C.H.D. Fall, Evidence for the intra-uterine programming of adiposity in later life, Ann. Hum. Biol. 38 (2011) 410–428, https://doi.org/10.3109/03014460.2011.592513.

[15] H. Masuyama, Y. Hiramatsu, Effects of a high-fat diet exposure in utero on the metabolic syndrome-like phenomenon in mouse offspring through epigenetic changes in adipocytokine gene expression, Endocrinology 153 (2012) 2823–2830, https://doi.org/10.1210/en.2011-2161.

[16] S.F. Ng, R.C. Lin, D.R. Laybutt, R. Barres, J.A. Owens, M.J. Morris, Chronic high-fat diet in fathers programs β-cell dysfunction in female rat offspring, Nature 467 (2010) 963–966, https://doi.org/10.1038/nature09491.

[17] M. Terashima, S. Barbour, J. Ren, W. Yu, Y. Han, K. Muegge, Effect of high fat diet on paternal sperm histone distribution and male offspring liver gene expression, Epigenetics 10 (2015) 861–871, https://doi.org/10.1080/15592294.2015.1075691.

[18] M.A. Sanchez-Garrido, F. Ruiz-Pino, I. Velasco, A. Barroso, D. Fernandois, V. Heras, M. Manfredi-Lozano, M.J. Vazquez, J.M. Castellano, J. Roa, L. Pinilla, M. Tena-Sempere, Intergenerational influence of paternal obesity on metabolic and reproductive health parameters of the offspring: male-preferential impact and involvement of kiss1-mediated pathways, Endocrinology 159 (2018) 1005–1018, https://doi.org/10.1210/en.2017-00705.

[19] E.G. Bligh, W.J. Dyer, A rapid method of total lipid extraction and purification, Can. J. Biochem. Physiol. 37 (1959) 911–917, https://doi.org/10.1139/o59-099.

[20] J.P. Curley, R. Mashoodh, F.A. Champagne, Epigenetics and the origins of paternal effects, Horm. Behav. 59 (2010) 306–314, https://doi.org/10.1016/j.yhbeh.2010.06.018.

[21] F.A. Baxter, A.J. Drake, Non-genetic inheritance via the male germline in mammals, Philos. Trans. R. Soc. Lond. B Biol. Sci. 374 (2019), 20180118, https://doi.org/10.1098/rstb.2018.0118.

[22] S. Ishii, K. Iizuka, B.C. Miller, K. Uyeda, Carbohydrate response element binding protein directly promotes lipogenic enzyme gene transcription, Proc. Natl. Acad. Sci. U.S.A. 101 (2004) 15597–15602, https://doi.org/10.1073/pnas.0405238101.

[23] L. Zhao, C. Zhang, X. Luo, P. Wang, W. Zhou, S. Zhong, Y. Xie, Y. Jiang, P. Yang, R. Tang, Q. Pan, A.R. Hall, T.V. Luong, J. Fan, Z. Varghese, J.F. Moorhead, M. Pinzani, Y. Chen, X.Z. Ruan, CD36 palmitoylation disrupts free fatty acid metabolism and promotes tissue inflammation in non-alcoholic steatohepatitis, J. Hepatol. 69 (2018) 705–717, https://doi.org/10.1016/j.jhep.2018.04.006.

[24] I. Donkin, R. Barrès, Sperm epigenetics and influence of environmental factors, Mol. Metabol. 14 (2018) 1–11, https://doi.org/10.1016/j.molmet.2018.02.006.

[25] T. Fullston, E.M. Ohlsson-Teague, N.O. Palmer, M.J. DeBlasio, M. Mitchell, M. Corbett, C.G. Print, J.A. Owens, M. Lane, Paternal obesity initiates metabolic disturbances in two generations of mice with incomplete penetrance to the F2 generation and alters the transcriptional profile of testis and sperm microRNA content, Faseb. J. 27 (2013) 4226–4243, https://doi.org/10.1096/fj.12-224048.

[26] R. Lambrot, C. Xu, S. Saintphar, G. Chountalos, T. Cohen, M. Paquet, M. Suderman, M. Hallett, S. Kimmins, Low paternal dietary folate alters the mouse sperm epigenome and is associated with negative pregnancy outcomes, Nat. Commun. 4 (2013) 2889, https://doi.org/10.1038/ncomms3889.

[27] U. Sharma, O.J. Rando, Metabolic inputs into the epigenome, Cell Metabol. 25 (2017) 544–558, https://doi.org/10.1016/j.cmet.2017.02.003.

[28] B.R. Carone, L. Fauquier, N. Habib, J.M. Shea, C.E. Hart, R. Li, C. Bock, C. Li, H. Gu, P.D. Zamore, A. Meissner, Z. Weng, H.A. Hofmann, N. Friedman, O.J. Rando, Paternally induced transgenerational environmental reprogramming of metabolic gene expression in mammals, Cell 143 (2010) 1084–1096, https://doi.org/10.1016/j.cell.2010.12.008.

[29] J.G. Knezovich, M. Ramsay, The effect of preconception paternal alcohol exposure on epigenetic remodeling of the h19 and rasgrf1 imprinting control regions in mouse offspring, Front. Genet. 3 (2012) 10, https://doi.org/10.3389/fgene.2012.00010.

[30] J.M. Gatewood, G.R. Cook, R. Balhorn, E.M. Bradbury, C.W. Schmid, Sequence-specific packaging of DNA in human sperm chromatin, Science 236 (1987) 962–964, https://doi.org/10.1126/science.3576213.

[31] S. Erkek, M. Hisano, C.Y. Liang, M. Gill, R. Murr, J. Dieker, D. Schubeler, J. van der Vlag, M.B. Stadler, A.H.F.M. Peters, Molecular determinants of nucleosome retention at CpG-rich sequences in mouse spermatozoa, Nat. Struct. Mol. Biol. 20 (2013) 868–875, https://doi.org/10.1038/nsmb.2599.

[32] K. Siklenka, S. Erkek, M. Godmann, R. Lambrot, S. McGraw, C. Lafleur, T. Cohen, J. Xia, M. Suderman, M. Hallett, J. Trasler, S.A.H.F.M. Peters, S. Kimmins, Disruption of histone methylation in developing sperm impairs offspring health transgenerationally, Science 350 (2015), aab2006, https://doi.org/10.1126/science.aab2006.

[33] V. Grandjean, S. Fourré, D.A. De Abreu, M.A. Derieppe, J.J. Remy, M. Rassoulzadegan, RNA-mediated paternal heredity of diet-induced obesity and metabolic disorders, Sci. Rep. 5 (2016), 18193, https://doi.org/10.1038/srep18193.

[34] Q. Chen, M. Yan, Z. Cao, X. Li, Y. Zhang, J. Shi, G.H. Feng, H. Peng, X. Zhang, Y. Zhang, J. Qian, Sperm tsRNAs contribute to intergenerational inheritance of an acquired metabolic disorder, Science 351 (2016) 397–400, https://doi.org/10.1126/science.aad7977.

[35] U. Sharma, C. Conine, J. Shea, A. Boskovic, A. Derr, X. Bing, C. Belleannee, A. Kucukural, R. Serra, F. Sun, L. Song, B.R. Carone, E.P. Ricci, X.Z. Li, L. Fauquier, M.J. Moore, R. Sullivan, C.C. Mello, M. Garber, O.J. Rando, Biogenesis and function of tRNA fragments during sperm maturation and fertilization in mammals, Science 351 (2016) 391–396, https://doi.org/10.1126/science.aad6780.

[36] T. Fullston, E.M. Ohlsson-Teague, C.G. Print, L.Y. Sandeman, M. Lane, Sperm microRNA content is altered in a mouse model of male obesity, but the same suite of microRNAs are not altered in offspring's sperm, PLoS One 11 (2016), e0166076, https://doi.org/10.1371/journal.pone.0166076.

[37] K. Leisegang, P.J. Bouic, R. Menkveld, R.R. Henkel, Obesity is associated with increased seminal insulin and leptin alongside reduced fertility parameters in a controlled male cohort, Reprod. Biol. Endocrinol. 12 (2014) 34, https://doi.org/10.1186/1477-7827-12-34.

[38] N.K. Binder, J.R. Sheedy, N.J. Hannan, D.K. Garder, Male obesity is associated with changed spermatozoa Cox4i1 mRNA level and altered seminal vesicle fluid composition in a mouse model, Mol. Hum. Reprod. 21 (2015) 424–434, https://doi.org/10.1093/molehr/gav010.

[39] J.J. Bromfield, J.E. Schjenken, P.Y. Chin, A.S. Care, M.J. Jasper, S.A. Robertson, Maternal tract factors contribute to paternal seminal fluid impact on metabolic phenotype in offspring, Proc. Natl. Acad. Sci. U.S.A. 111 (2014) 2200–2205, https://doi.org/10.1073/pnas.130560911.

[40] H. Masuyama, T. Mitsui, T. Eguchi, S. Tamada, Y. Hiramatsu, The effects of paternal high-fat diet exposure on offspring metabolism with epigenetic changes in the mouse adiponectin and leptin gene promoters, Am. J. Physiol. Endocrinol. 311 (2016) E236–E245, https://doi.org/10.1152/ajpendo.00095.2016.