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

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ABSTRACT

Obesity is 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 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.

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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°C. Male mice were individually mated with 11- to 13-week-old CD-fed C57BL/6N females 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 CO2 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 H2O, 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 × 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 H2O and 200 μl of chloroform were added to the mixture and centrifuged at 5,000 × 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 Triacylglyceride 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, Manneford, 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 QiAzo I 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 significantly increased compared with those of CD-fed mice (Fig. 2B). The data were analyzed by 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.
significant differences were observed between CD- and HFD-fed male offspring. The hepatic triglyceride contents were significantly higher in HFD-F1 mice than in CD-F1 mice (Fig. 3B). Additionally, gene expressions of Scd1, Fasn, Gpam, and Dgat2 were significantly increased compared with those of CD-F1 mice (Fig. 3A). Furthermore, gene expressions of Srebf1, Cd36, and Slc27a1 were significantly increased compared with those of CD-F1 mice (Fig. 3E). These results suggest that paternal HFD feeding affected 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, we measured the mRNA expression levels of genes involved in lipid and triglyceride metabolism in the 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 (Ppara) for liver, and peroxisome proliferator-activated receptor gamma (Pparg) for eWAT, and MLX interacting protein-like (Mlxipl) for liver, and fatty acid transport (solute carrier family 27 member 1 (Slc27a1), Cd36 molecule (Cd36), also known as Fat), 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 transgenerational epigenetic inheritance. Although the concept of transgenerational 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 parental 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
HFD-F1 mice, lipogenic transcription factor genes such as Triglyceride accumulation in HFD-F1 mice. Similarly, in the eWAT of findings suggest that paternal HFD exposure programs abnormal lipo insulin resistance, hyperinsulinemia, and increased steatosis in patients expression of expression of Indeed, we observed that paternal HFD exposure induced increased 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, suggesting the Mlxipl as key determinant of lipogenic pathway 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, Acox1, 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 sperm 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
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.

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

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