Journal of Functional Foods 116 (2024) 106137

Contents lists available at ScienceDirect

Journal of Functional Foods

journal homepage: www.elsevier.com/locate/jff

Preconceptional capsaicin intervention mitigates negative effects of paternal obesity on metabolic characteristics in male offspring upon high-fat diet challenge

Jiamiao Hu [a,b,*], Zhongjing Lin [a], Yang Yang [c], Mark Christian [d], Shiyang Li [a], Baodong Zheng [a], Bee K. Tan [b], Shaoling Lin [a,*]

[a] College of Food Science, Fujian Agriculture and Forestry University, Fuzhou, PR China
[b] College of Life Sciences, University of Leicester, Leicester, LE1 7RH, United Kingdom
[c] PolyU Shenzhen Research Institute, The Hong Kong Polytechnic University, Shenzhen, PR China
[d] School of Science and Technology, Nottingham Trent University, Clifton, Nottingham, NG11 8NS, United Kingdom

ARTICLE INFO

Keywords:
Paternal obesity
Diet-induced obesity
Capsaicin
Offspring obesity
Preconceptional intervention

ABSTRACT

The activation of brown adipose tissue by cold exposure in paternal mice has been reported to improve the metabolic health of offspring. Notably, dietary components for activating BAT have drawn considerable attention owing to their high feasibility for human trials. Therefore, here the potential metabolic improving effects of dietary capsaicin intervention on diet-induced obese paternal mice and their offspring were measured. The results support that paternal preconceptional capsaicin intervention not only elicited the anticipated enhancement of brown fat activity in obese paternal mice, but also improved the metabolic profiles in male offspring evidenced by their elevated resistance to high-fat diet challenge. Moreover, changes in sperm DNA methylation and small non-coding RNA profile were noted in paternal mice with capsaicin intervention, though additional investigation is needed to further delve into the mechanisms. In summary, paternal dietary intervention activating BAT could represent a promising strategy for enhancing the metabolic health of offspring.

1. Introduction

In recent years, the number of overweight and obese adults has increased rapidly (Chooi, Ding, & Magkos, 2019; Ma et al., 2021). Moreover, evidence also suggests overweight and obese parents increase the risk of obesity and metabolic disease of their offspring (Weihrauch-Blüher, Schwarz, & Klusmann, 2019). In a population-based study, Jääskeläinen, A. *et al.* showed that pre-pregnancy obesity and overweight in parents increased genetic susceptibility to obesity of their offspring (Jääskeläinen et al., 2011). Furthermore, a number of studies have revealed the profound impact of maternal obesity on the offspring. The risk of early childhood obesity is significantly higher in the children of obese pregnancies than normal weight women (Adane, Dobson, Tooth, & Mishra, 2018; Hu et al., 2019; Josey, McCullough, Hoyo, & Williams-DeVane, 2019). A similar observation was reported in dietary-induced obesity (DIO) female mice (Kang, Kurti, Fair, & Fryer, 2014). Notably, although the influence of maternal obesity on offspring has drawn attention in recent decades, a growing number of studies have revealed the profound impact of paternal obesity. For instance, a *meta*-analysis performed by Campbell Jared M. *et al.* showed paternal obesity or overweight was significantly associated with augmented body weight growth of their children (Campbell & McPherson, 2019). Freeman E. *et al.* also reported offspring of obese fathers (with healthy mothers) had a higher obesity risk in a population-based study (Freeman et al., 2012). The animal study by Fullston Tod *et al.* also revealed that high-fat diet-induced obesity in paternal mice had a negative effect on the metabolic health of its F1 offspring, which even persisted in the subsequent F2 generation (Fullston et al., 2013). Similarly, paternal Western-style high-fat and high-sugar diet was associated with striking intergenerational impacts on the offspring including higher body weights in the first three weeks, increased *Actinobacteria* abundance in the gut microbiota and a food preference for Western-style diet in C57BL/6J mice (Bodden et al., 2022). These findings further challenge the traditional idea that sperm only transfers genetic information of genomic DNA sequences to offspring. Epigenetic changes have become an important underlying mechanism to explain the paternal inheritance of obesity. For example,

* Corresponding authors.
E-mail addresses: jiamiao.hu@fafu.edu.cn, jh921@leicester.ac.uk (J. Hu), shaoling.lin@fafu.edu.cn (S. Lin).

https://doi.org/10.1016/j.jff.2024.106137
Received 31 October 2023; Received in revised form 9 March 2024; Accepted 13 March 2024
Available online 16 March 2024

Grandjean Valérie *et al.* showed that high-fat diet-induced obesity in paternal mice affected the metabolic phenotype (including increased body weight, altered glucose tolerance and impaired insulin sensitivity) in the offspring by RNA-mediated epigenetic changes (Grandjean et al., 2015). Barbosa *et al.* also showed that a high-fat diet altered the DNA methylation profile and the expression pattern of small-noncoding RNA (sncRNA) in the paternal rat, inducing metabolic changes in the offspring (de Castro Barbosa et al., 2016).

Curbing paternal pre-pregnancy obesity presents a promising strategy to improve metabolic phenotype in the offspring. Indeed, many trials have been performed and identified exercise (Krout et al., 2018; McPherson, Lane, Sandeman, Owens, & Fullston, 2017; Stanford et al., 2018), and dietary intervention (McPherson, Owens, Fullston, & Lane, 2015) before pregnancy could improve metabolic health of offspring of obese parents. Notably, recent research further revealed that pre-pregnancy cold exposure could enhance metabolic function *via* promoting fat browning in obese paternal mice and protected male offspring from diet-induced obesity, which was mediated by changing sperm DNA methylation pattern in paternal mice (Sun et al., 2018). However, although cold exposure could effectively activate brown adipose tissue and enhance white adipose tissue browning (Ng et al., 2017; Z. Xu et al., 2019), the practical limitations to the implementation of cold exposure in humans greatly hinder the applicability of this strategy (Fudge, Bennett, Simanis, & Roberts, 2015). Therefore, understanding whether dietary browning agents have similar alleviating effects on paternal inheritance of obesity as an alternative to cold exposure represent a key research need.

Capsaicin, as the main bioactive component of *Capsicum annuum*, is a natural alkaloid (S. K. Sharma, Vij, & Sharma, 2013). The anti-obesity activity of capsaicin has been well documented by previous studies. The underlying mechanisms of capsaicin action mainly include increases in fat oxidation and energy expenditure, decreases in lipogenesis and lipid accumulation, enhancement of lipid metabolism, as well as remodeling of the intestinal microbiome (Zheng, Zheng, Feng, Zhang, & Xiao, 2017). Moreover, capsaicin has been identified as the agonist of TRPV1, a key regulator of brown adipose tissue activation. Indeed, capsaicin has been suggested to induce the activation of brown adipose tissue *via* multiple mechanisms (eg. stimulation of sympathetic nerves; induction of brown preadipocyte differentiation; activation of mitochondria biogenesis in adipocytes, etc.). For instance, Baskaran Padmamalini *et al.* showed that capsaicin treatment ranging from 0.399 to 3.99 mg/kg body weight reduced high-fat-induced obesity and metabolic stress by enhancing the expression of thermogenic genes (SIRT1, UCP-1 and TRPV1) in mice, which were highly associated with fat browning (Baskaran et al., 2019). Furthermore, Yuan *et al.* showed that capsaicin intervention improved metabolic disorders in pregnant women with gestational diabetes mellitus and reduced the incidence of large-for-gestational-age newborns, which may further lower the risk of metabolic disease of offspring with maternal lipid metabolism disorder in adulthood (Schaefer-Graf et al., 2008; L.-J. Yuan et al., 2016). The evidence derived from these human clinical trials also highlights that capsaicin intervention holds promise as a strategy to enhance the metabolic phenotype of offspring due to its good safety profile (Schaefer-Graf et al., 2008; L.-J. Yuan et al., 2016). Therefore, in the current study, the potential effects of pre-pregnancy capsaicin intervention on paternal inheritance of obesity as well as the possible underlying mechanisms were investigated.

2. Materials and methods

2.1. Materials and reagents

Four-week-old specific pathogen-free (SPF) C57BL/6J male and female mice were purchased from Wus Animal Center (Fujian, China). Capsaicin (≥95 %) was purchased from Shanghai Aladdin Bio-Chem Technology Co., LTD (Shanghai, China). H&E Staining Kit (Hematoxylin and Eosin), DAB Chromogenic Kit, Hematoxylin Solution, and normal rabbit serum were all purchased from Servicebio Technology Co., Ltd. (Hubei, China). The primary antibody used for immunohistochemistry was as UCP-1 antibody (GB112174, 1:200) and the secondary antibody was as HRP conjugated Goat Anti-Rabbit IgG antibody (GB 23303, 1:200), which were both purchased from Servicebio Technology Co., Ltd. (Hubei, China). The other chemical reagents used in experiments were all analytically pure grade.

2.2. Animal experiments

All mice were maintained in cages with wood-chips bedding, and the animal laboratory was controlled at 25 ± 1 °C temperature, 55 ± 5 % humidity, and 12/12 h light/dark cycle. After one week of acclimatization, the male mice in normal diet (NDp, n = 6) group and all female mice were fed with normal diet (12.11 kcal% fat, low fat). To establish the obese male mice model, the other male mice were fed with a high-fat diet (45.0 kcal% fat, high fat) for 4 weeks (Speakman, 2019). The diet-induced obesity model was considered to be established successfully when the body weight of mice with high-fat diet was over 20 % heavier than that with normal diet (C. Zhang et al., 2019). Then the obese male mice were randomly divided into high-fat diet group (HFDp, n = 6), capsaicin intervention group (CAPp, n = 6, HFD plus intragastrically administrated with 2.5 mg/kg/d capsaicin (Hosseini et al., 2020),(Baskaran, Krishnan, Ren, & Thyagarajan, 2016)) and cold exposure group (CEp, n = 6, HFD plus 8 °C exposure for 8 h each day (Sun et al., 2018)). All male mice were measured for respiratory metabolism, oral glucose tolerance test and body surface temperature after 6 weeks of treatment. The body weight of paternal mice was recorded upon arrival (week 0), end of week 1 (after acclimatization), end of week 5 (after DIO model establishment), and end of week 11 (after intervention). Each male mouse was mated with two female mice in separate cages to ensure successful pregnancy. When the vaginal plug was observed, the female mice were assumed to be pregnant (Sugiyama et al., 2021) and then housed separately with normal diet until delivery. The paternal mice were sacrificed by cervical dislocation and collected blood, sperm, liver, subcutaneous white adipose tissue (sWAT) and interscapular brown adipose tissue (iBAT).

To avoid differences in the nutritional acquisition of newborn offspring mice, two male and two female offspring mice (if possible) were raised by each maternal mice until weaning. The maternal mice were subsequently euthanized using the cervical dislocation method. The offspring mice were fed with normal diet until 8 weeks of age. After 8 weeks of age, the offspring mice were further divided into NDo group (continued to be fed with normal diet) and HFDo group (challenged by high-fat diet), with n = 5 in each group. Oral glucose tolerance test and body surface temperature were measured at 30 weeks of age before the offspring were sacrificed by cervical dislocation to collect blood, sWAT and iBAT samples.

All animal experiments were approved by the Animal Management and Use Committee of Fujian Agriculture and Forestry University (No. PZCASFAFU22037) and adhered to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.3. Respiratory metabolic measurements

After adaptation in the metabolism monitoring system for 12 h, carbon dioxide production and oxygen consumption of mice was measured in ZH-DX animal metabolism monitoring system (Anhui Zhenghua Biologic Apparatus Facilities Co., Ltd, China) by sampling every 30 s for 24 h (Liang et al., 2016). The mice had free access to food and water during the measurement at 25 °C with 12 h light/dark cycle. The data of carbon dioxide production and oxygen consumption was plotted with 1-h intervals and the respiratory exchange ratio (RER, VCO_2/VO_2) was calculated at each interval.

2.4. Oral glucose tolerance test (OGTT)

Blood was taken from tail-end vein of mice and glucose was measured by OneTouch Ultra blood glucose meter (Sinocare Inc., China). All mice were fasted overnight and the fasting blood glucose (0 min) was measured. Each mouse was administered orally with 10 % glucose solution at 10 μL/g·bw, and blood glucose was then measured at 15, 30, 60 and 120 min (Z. Yuan, He, Cui, & Takeuchi, 1998). Then the glucose tolerance curve was created and the area under the curve was analyzed.

2.5. Body surface temperature measurement

The body surface temperature of mice was measured by infrared camera (FLIR ONE Pro LT, FLIR Systems UK, Kent, UK) and analyzed by FLIR Tools according to previous literature (Sun et al., 2018).

2.6. Serum biochemical analysis

Blood was collected by eyeball removal before cervical dislocation. After resting for 30 min, the serum was separated by centrifugation at 3000 rpm for 10 min (Cao et al., 2014). The levels of triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were measured by automatic biochemical analyzer (7080, Hitachi Co., Japan).

2.7. Hematoxylin-eosin (H&E) and UCP-1 immunohistochemical staining

Liver and adipose tissues were collected and fixed in 4 % paraformaldehyde, embedded in paraffin and cut into sections (~5 μm). For H&E staining, the tissue sections were stained with hematoxylin and eosin after dewaxing and rehydration, and then sealed with neutral gum. For immunohistochemical staining, antigen of the tissue sections was retrieved with citric acid (pH 6.0) after dewaxing and rehydration. After washing with PBS (pH 7.4), the tissue sections were incubated with 3 % hydrogen peroxide in darkness for 25 min. The tissue sections were then washed with PBS again and blocked with rabbit serum for 30 min. Then the tissues sections were incubated at 4 °C overnight with the UCP-1 primary antibody followed by incubation with secondary antibody (HRP labeled) for 20 min at 37 °C. Enhanced DAB Chromogenic Kit (Servicebio, G1211) was used to visualize the localization of an HRP-conjugated antibody and the nuclei were counterstained with hematoxylin. Finally, the adipose tissue sections were sealed with neutral gum. The images were observed with microscope (NIKON ECLIPSE E100) and analysis with imaging system (NIKON DS-U3). The NAFLD activity score was determined according to a previous report (Kleiner et al., 2005). To avoid the potential subjectivity inherent in the interpretation (such as the degree of inflammation or ballooning), the NAFLD activity scores underwent independent assessment by S. Lin and Z. Lin before the mean values were obtained. The IHC staining were quantified using Image J software and reported as a percentage for each individual image (X. Q. Li et al., 2015). The adipocytes areas were also quantified using Image J and the distribution of adipocyte area was presented as violin plots (Lin et al., 2019).

2.8. qPCR

Total RNA was extracted from brown adipose tissue using QIAGEN RNeasy Plus Universal Mini Kit (QIAGEN, Hilden, Germany). In brief, the brown adipose tissue was weighed (≤100 mg) before homogenized in QIAzol Lysis Reagent (QIAGEN, Hilden, Germany). After addition of gDNA Eliminator Solution and chloroform with a vigorous shake, the homogenate was then separated into aqueous and organic phases by centrifugation at 12,000 g for 15 min at 4 °C. The upper aqueous phase was transferred into a new centrifuge tube and mixed with equal volume of 70 % ethanol, which was further purified using RNeasy spin columns. The RNA was eluted from the column using RNase-free water (40 μL) and quantified by NanoDrop 2000 UV–Vis spectrophotometers (Thermo Scientific, Massachusetts, United States), before 1 μg of total RNA was reverse-transcribed into cDNA using RevertAid First strand cDNA Synthesis kit. Real Time-PCR was performed using the SYBR Green Abstract PCR Mix (Sanggon Biotech Co., Ltd., Shanghai, China) and run on LightCycler 480 II system (Roche, Basel, Switzerland). The mRNA levels of target genes were normalized to β-actin. Primer sequences used are as shown in Supplementary Table S1.

2.9. Collection of sperm samples

After the paternal mice were sacrificed, the epididymis and vas deferens were detached from each testis and minced into small pieces (1–2 mm) in a 35-mm culture dish containing pre-warmed PBS buffer and placed in 6 % CO_2 and 5 % O_2 at 37 °C for 15 min (McPherson, Shehadeh, Fullston, Zander-Fox, & Lane, 2019). After filtering out tissue fragments, the suspension was centrifuged at 1500 rpm for 5 min to collect sperm precipitation.

2.10. DNA methylation analysis

DNA methylation analysis was performed according to a previous study (Sai et al., 2020). In brief, genomic DNA of paternal mice sperm was extracted by the phenol–chloroform method and purified by ethanol precipitation. Extracted DNA was fragmented (to 100–500 bp) with Bioruptor (Diagenode) ultrasonication, end repaired, A tailed, and ligated to adapters by using NEBNext® Ultra™ DNA Library Prep Kit (NEB, USA). According to the standard manufacturer's protocol (Active Motif, USA), methylated DNA immunoprecipitation (MeDIP) was performed with a monoclonal antibody against 5-methylcytosine, and MeDIP DNA libraries were quantified by using Quant-iT PicoGreen dsDNA Kits (Life Technologies, USA). Then samples were paired-end sequenced on Illumina NovaSeq 6000 sequencer to generated reads. Reads were quality controlled using Q30, with low quality reads filtered using cutadapt (v1.9.3) and high-quality reads aligned to the reference genome using bowtie2 (v2.2.4). Methylated peaks were called by using MACS software (v2.0). Differentially methylated regions (DMRs) were identified by diffReps software (v1.55.4) and annotated with the latest UCSC RefSeq database. The differentially methylated regions (DMRs) were identified with fold changes $\geq$ 2.0 and P-value $\leq$ 0.0001 in genome-wide methylation regions. Differentially methylated regions-associated genes were analyzed by Gene Ontology (GO, https://www.geneontology.org) and KEGG (https://www.genome.jp/kegg/) pathway.

2.11. Small-noncoding RNAs analysis

Sequencing libraries of sncRNAs (including miRNA and piRNA) were prepared by using the total RNA. Briefly, after ligating the 3′- and 5′-adaptor, the synthesized cDNA was used for PCR amplification and ~ 150 bp PCR amplicon was selected. The libraries were denatured as single-stranded DNA molecules, captured on Illumina flow cells, amplified *in situ* as clusters and finally sequenced for 50 cycles on Illumina Novaseq 6000 sequencer following the manufacturer's instructions.

The clean reads were generated by adaptor trimming (cutadapt software 1.9.3) and quality filtering. For miRNA and piRNA, clean reads were aligned to the merged Mouse pre-miRNA databases and merged Mouse piRNA databases using Novoalign software (v3.02.12) with at most one mismatch). The read counts were normalized by TPM (tag counts per million aligned miRNAs) approach. Differentially expressed miRNAs and piRNAs between two groups were filtered by fold change and p-value.

2.12. Statistical analysis

The data are expressed as mean ± standard deviation (SD), and statistical analysis was performed using GraphPad Prism 9. The Shapiro-Wilk test was used to confirm the normality of the data. For normally distributed data, comparison of the means among groups was evaluated using one-way analysis of variance with Tukey's post-hoc tests. A p-value < 0.05 was considered statistically significant.

3. Results

3.1. Capsaicin supplementation improved metabolic profiles and activated iBAT in paternal mice

As shown in Table 1, the paternal mice in all groups had similar initial body weight at the beginning of experiments. After high-fat diet intervention for 4 weeks, the body weight of mice in HFDp, CAPp and CEp groups was higher (>20 %) than that of the NDp group. Notably, compared to HFDp group, the body weight of mice with capsaicin supplementation (CAPp) or cold exposure (CEp) was decreased by 16.96 % and 14.73 %, respectively ($p < 0.05$) before mating. In addition, consistent with a previous study (Nie, Yan, Yan, Xia, & Zhang, 2015), the mice in CAPp and CEp groups also showed decreased fasting blood glucose level and serum TG levels compared to the mice in HFDp group, though no significant changes in TC, HDL-C and LDL-C levels were achieved ($p > 0.05$) (Table 1). Furthermore, as shown in Fig. 1A, high-fat diet-induced obesity also led to significantly elevated NAFLD activity score and increased adipocyte size in paternal mice, which were effectively ameliorated by capsaicin treatment and cold exposure.

Capsaicin is a well-documented browning agent that can also induce brown adipose tissue activation. Here, it was noted that capsaicin treatment significantly elevated the transcriptional levels of a brown adipogenic gene (PPARγ) and thermogenic gene (UCP-1) in iBAT as determined by real-time PCR (Fig. 1B), which was also supported by the results of immunohistochemical stain (Fig. 1C). In addition, the body surface temperature of mice photographed by thermal camera and the respiratory rate measurement also suggested an increased body surface temperature in the interscapular region (Fig. 1D) of the CAPp group and respiratory exchange ratio (RER) showed more similarity to the level of mice with normal diet (Fig. 1E). These findings may also suggest the enhanced iBAT activation upon capsaicin treatment.

Table 1
Effects of capsaicin supplementation on metabolic profiles in paternal mice.

Group		NDp	HFDp	CAPp	CEp
Body weight	B.W week 1	17.80 ± 1.03	18.10 ± 1.21	17.83 ± 1.01	18.13 ± 0.60
	B.W week 5	24.88 ± 0.77*	32.12 ± 1.29	29.97 ± 0.73	30.33 ± 0.46
	B.W week 11	26.55 ± 1.01*	37.55 ± 1.24	31.18 ± 0.88*, #	32.02 ± 0.61*, #
Blood glucose	FBG (mmol/L)	6.73 ± 0.62*	7.88 ± 0.26	6.57 ± 0.58*	6.07 ± 0.83*
Lipid profile	TG (mmol/L)	0.98 ± 0.05*	1.25 ± 0.14	0.92 ± 0.20*	0.74 ± 0.11*
	TC (mmol/L)	2.90 ± 0.34*	4.96 ± 0.46	4.88 ± 0.52	4.99 ± 0.13
	HDL-C (mmol/L)	1.59 ± 0.14*	2.21 ± 0.36	2.23 ± 0.24	2.23 ± 0.05
	LDL-C (mmol/L)	0.37 ± 0.05*	0.78 ± 0.04	0.71 ± 0.10	0.76 ± 0.05

All data in the table are shown as mean ± SD. *statistically significant difference ($p < 0.05$) obtained by Tukey post-hoc compared to HFDp group; # statistically significant difference ($p < 0.05$) obtained by Tukey post-hoc compared to NDp group.

3.2. Paternal capsaicin supplementation improved metabolic phenotype in male offspring

As shown in Fig. 2, upon normal diet feeding, both male and female offspring in all groups showed a similar trend on body weight gain. Interestingly, when the offspring were exposed to high-fat diet challenge, paternal capsaicin supplementation and cold exposure marginally decelerated their body weight gain, especially in male offspring. In addition, the blood lipids analysis also revealed the potential beneficial effects of paternal capsaicin supplementation on male offspring (Compared with the male HFDp-HFDo group, a significant increase in HDL-C level was observed in male CAPp-HFDo groups) (Table 2), though we failed to observe significant changes in OGTT tests in offspring when fed with normal diet or upon high-fat diet challenge (Supplementary Fig. S1). Additionally, the H&E staining images also illustrated that significant improvements in high-fat diet-induced hepatic steatosis and adipocyte enlargement were observed in the F1 male mice of CAPp group (CAPp-HFDo) when compared to the offspring with paternal high-fat diet exposure only (HFDp-HFDo) (Fig. 3A). However, this beneficial effect was less pronounced in F1 female mice (Fig. 3B). Notably, it seems the male offspring with paternal diet-induced exposure exhibited more steatosis than their female counterparts. This disparity may be a reason why capsaicin has a more obvious beneficial effect on male offspring.

Previous study showed activating paternal brown adipose tissue may influence thermogenic activity of brown adipose tissue in offspring (Sun et al., 2018). Therefore, to further elucidate the association between paternal capsaicin supplementation and changes in metabolic phenotype in male offspring fed with high-fat diet, we next evaluated the thermogenic potential of offspring and quantified the expression levels of UCP1 in iBAT. As shown in Fig. 4A, the thermal images demonstrated that upon high-fat diet challenge, male offspring of obese paternal mice showed impaired thermogenesis at the interscapular region, while paternal capsaicin intervention resulted in offspring with higher temperature captured at the interscapular region. Real-time PCR and immunohistochemical stain also showed that key the thermogenic effector (UCP-1) in brown adipose tissue was elevated in high-fat diet fed offspring with paternal capsaicin supplementation (Fig. 4B and 4C). These findings suggest that paternal capsaicin intervention induces brown adipocyte function in male offspring.

3.3. Paternal capsaicin supplementation induced alterations in DNA methylation patterns in paternal sperm

To further explore the possible mechanisms of paternal capsaicin intervention influencing offspring metabolic phenotypes upon high-fat diet challenge, global DNA methylation of sperm isolated from paternal mice was determined by MeDIP-5mC. The methylation peaks of sperm DNA of NDp, HFDp, CAPp and CEp groups were identified as approximately 6.93×10^4, 5.02×10^4, 3.42×10^4 and 6.79×10^4, respectively. Particularly, the major methylation regions of the peaks overlapping genomic annotations located in the intronic and intergenic regions in all tested groups (Fig. 5A). Pairwise comparation between groups further revealed that both cold exposure and capsaicin intervention in paternal obese mice resulted in widespread changes in DNA methylation on the genome, though the changing patterns displayed noticeable differences (Fig. 5B).

As DNA methylation changes in promoter regions can dramatically alter the gene expression profiles, Gene Ontology (GO) and KEGG pathways analysis were used to further analyze the biological function of differentially methylated related-genes (DMGs) in the promoter region (TSS −2000 ~ TSS + 2000) between the CAPp and HFDp groups. Interestingly, the GO analysis results showed promoter-related DMGs were significantly enriched in metabolic energy regulation-related pathways, such as "glycogen biosynthetic process (GO:0005978)", "negative regulation of lipid metabolic process (GO:0045833)" and

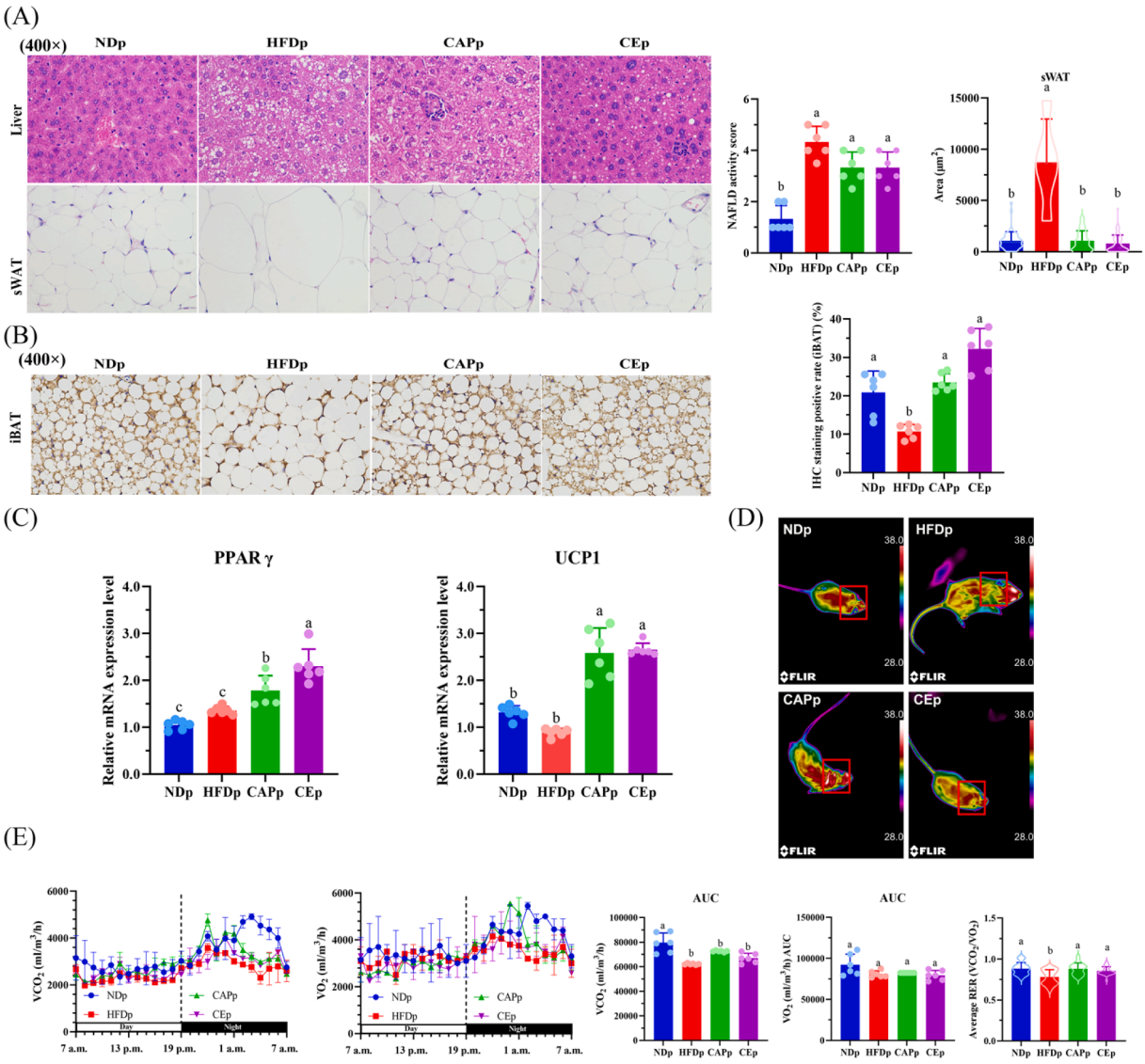

Fig. 1. Effects of preconceptional capsaicin intervention on histological features of liver and adipose tissue and metabolic characteristics. (A) H&E staining of liver and sWAT tissues of paternal mice. (B) The transcriptional levels of brown adipogenic gene (PPARγ) and thermogenic gene (UCP-1) in iBAT tissue normalized to β-actin. (C) UCP-1 immunohistochemical stain of iBAT of paternal mice. (D) Infrared imaging of paternal mice surface body heat. (E) Respiratory metabolic measurements of paternal mice. Data are presented as mean ± SD. Different lowercase letter indicates significant difference, $p < 0.05$, (n = 6).

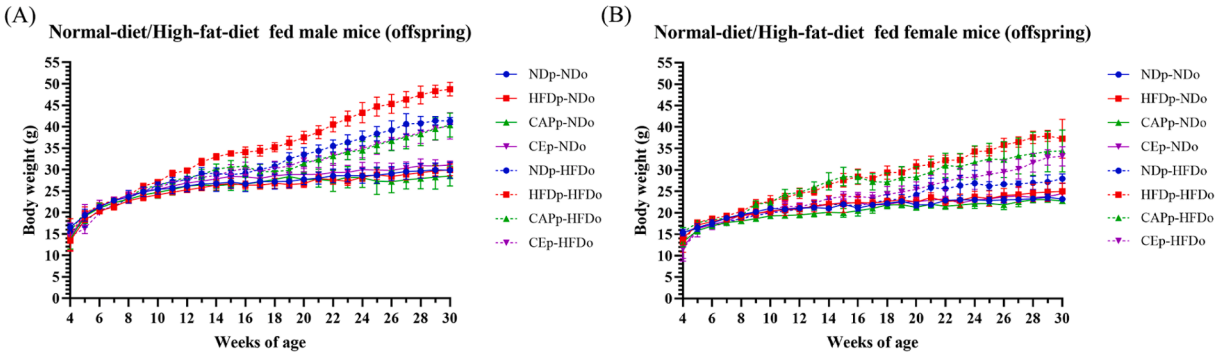

Fig. 2. Effects of paternal capsaicin intervention on body weight gain in offspring. (A) Body weight curves of male offspring with ND diet (solid line) or HFD diet (dashed line); (B) body weight curves of female offspring with ND diet (solid line) or HFD diet (dashed line). Data are presented as mean ± SD, (n = 5).

"energy reserve metabolic process (GO:0006112)" etc.; other terms were strongly associated with fatty acid and lipid regulation, such as "fatty acid transport (GO:0015908)", "medium-chain fatty acid metabolic process (GO:0051791)", "negative regulation of lipid storage (GO:0010888)" and "regulation of lipid catabolic process (GO:0050994)" etc. (Supplementary Table S2). Similarly, KEGG

Table 2
Effects of paternal capsaicin supplementation on blood lipids in offspring mice.

Group	TG (mmol/L)	TC (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)
Male offspring mice				
NDp-NDo	0.94 ± 0.25	2.88 ± 0.52	1.52 ± 0.32	0.44 ± 0.06
HFDp-NDo	0.76 ± 0.26	2.49 ± 0.55	1.52 ± 0.38	0.41 ± 0.04
CAPp-NDo	0.86 ± 0.23	2.82 ± 0.30	1.63 ± 0.21	0.34 ± 0.03
CEp-NDo	1.08 ± 0.14	2.64 ± 0.16	1.61 ± 0.11	0.35 ± 0.04
NDp-HFDo	1.30 ± 0.27	4.63 ± 0.76	2.16 ± 0.34	1.00 ± 0.17
HFDp-HFDo	1.43 ± 0.10	5.30 ± 1.05	1.84 ± 0.38	1.15 ± 0.27
CAPp-HFDo	1.23 ± 0.25	5.12 ± 0.64	2.38 ± 0.09$^{\#}$	0.99 ± 0.07
CEp-HFDo	1.10 ± 0.06	3.71 ± 0.57$^{\#}$	2.27 ± 0.26	0.95 ± 0.34
Female offspring mice				
NDp-NDo	0.93 ± 0.20	2.09 ± 0.15*	1.32 ± 0.08*	0.33 ± 0.04
HFDp-NDo	0.87 ± 0.19	2.37 ± 0.15	1.11 ± 0.19	0.29 ± 0.03
CAPp-NDo	0.97 ± 0.16	2.00 ± 0.09*	1.11 ± 0.09	0.28 ± 0.03
CEp-NDo	0.89 ± 0.06	2.24 ± 0.19	1.29 ± 0.12	0.32 ± 0.03
NDp-HFDo	1.10 ± 0.12	2.97 ± 0.43	1.41 ± 0.23	0.51 ± 0.04
HFDp-HFDo	1.32 ± 0.37	3.82 ± 0.52	1.66 ± 0.05	0.62 ± 0.09
CAPp-HFDo	1.42 ± 0.22	3.82 ± 0.71	1.67 ± 0.24	0.62 ± 0.12
CEp-HFDo	1.03 ± 0.18	3.09 ± 0.75	1.46 ± 0.32	0.66 ± 0.09

All data in the table are shown as mean ± SD. *statistically significant difference ($p < 0.05$) obtained by Tukey post-hoc compared to HFDp-NDo group; $^{\#}$ statistically significant difference ($p < 0.05$) obtained by Tukey post-hoc compared to HFDp-HFDo group.

pathway analysis also revealed 25 upregulated-methylated pathways and 17 downregulated-methylated pathways (Supplementary Table S3), of which a number of enriched pathways were found to be closely related to metabolism and obesity (Fig. 5C). For instance, enriched KEGG pathways including "Hedgehog signaling pathway (Gu et al., 2021)", "Hippo signaling pathway (Ardestani, Lupse, & Maedler, 2018)", "JAK-STAT signaling pathway (D. Xu, Yin, Wang, & Xiao, 2013)" were closely associated with the lipid metabolism; while enriched KEGG pathways of "Parathyroid hormone synthesis, secretion and action (Rendina-Ruedy & Rosen, 2022)" and "cAMP signaling pathway (Ravnskjaer, Madiraju, & Montminy, 2016)" were reported to be associated with the white adipose tissue browning.

3.4. Paternal capsaicin supplementation regulated the sncRNAs in paternal sperm

sncRNAs (include microRNA (miRNA) and Piwi-interacting RNA (piRNA)) also play crucial roles in mediating paternal intergenerational epigenetic inheritance (Klastrup, Bak, & Nielsen, 2019). Therefore, we compared the profiles of miRNA and piRNA of sperm samples among the paternal mice with different treatments. Specifically, 47 up-regulated miRNAs and 41 down-regulated miRNAs, 157 up-regulated piRNAs and 139 down-regulated piRNAs were identified in the HFDp group compared to the NDp group (Supplementary Fig. 2). Meanwhile, 95 up-regulated miRNAs and 37 down-regulated miRNAs, 274 up-regulated piRNAs and 46 down-regulated piRNAs were identified in the CAP group compared to the HFDp group (Fig. 6). Similarly, 80 up-regulated miRNAs and 64 down-regulated miRNAs in the CEp group compared to the HFDp group; 242 up-regulated piRNAs and 68 down-regulated piRNAs in the CEp group compared to the HFDp group (Supplementary Fig. 3, Supplementary Tables S4 and S5). The top 10 significantly differentially expressed miRNAs and piRNAs between samples from CAPp group and HFDp group are listed in Table 3. Among these top 10 differentially expressed miRNAs, it is worthy of note that mmu-miR-378a-5p and mmu-miR-223-3p were significantly down-regulated in the CAPp group compared to the HFDp group. Studies have already highlighted that miR-378a is strongly associated with adipogenesis and lipid storage. miR-378a-5p (also identified as miR-378*) as a mature strand of miR-378a is an important regulator of metabolism (Machado, Teodoro, Palmeira, & Rolo, 2020),(Krist, Florczyk, Pietraszek-Gremplewicz, Józkowicz, & Dulak, 2015). Michele Carrer *et al.* showed that miR-378* knockout mice were resistant to high-fat diet-induced obesity, which exhibited smaller white adipocyte size and lower liver fat deposition than wild type mice (Carrer et al., 2012). This result was coincident with ours. Similarly, our results showed significant down-regulation of miR-378a-5p in paternal capsaicin-supplemented sperm. Indeed, miR-223-3p was also reported to play important roles in lipid metabolism, Julia Sánchez-Ceinos *et al.* unveiled that both pre-adipocytes and adipocytes are miR-223-3p secreting cells. miR-223-3p accumulation and over-expression in adipocytes would cause metabolic disturbances (Sánchez-Ceinos et al., 2021). Another study demonstrated that obesity up-regulated miR-223-3p in subcutaneous adipose tissue; while miR-223-3p was down-regulated in both omental and subcutaneous adipose tissues of patients after weight loss (Macartney-Coxson et al., 2020). This might also suggest that the regulation of paternal and offspring sWAT phenotypes after paternal capsaicin supplementation was associated with miR-223-3p.

4. Discussion

Obesity has become a worldwide health problem. In the last decades, numerous bioactive compounds in foods (such as caffeine, catechins, gallic acid, anthocyanins, ascorbic acid, polyphenols, oleuropein, quercetin, and capsaicin) have been well-documented for their contribution to the prevention of obesity and its-related metabolic disorders (Konstantinidi & Koutelidakis, 2019). For example, Li *et al.* (W. Li, Yang, & Lu, 2019) reported capsaicin reduced both serum TG and TC levels in high beef fat-fed mice. Similarly, Kawada Teruo *et al.* (Kawada, Hagihara, & Iwai, 1986) demonstrated the TG was significantly lowered by capsaicin supplementation in 30 % lard-fed rats. Furthermore, it is reported that capsaicin supplementation could reverse the high-fat diet-induced decrease in RER and improved the metabolic activity of the mice (Baskaran et al., 2017). Here, as expected, our results also demonstrated that capsaicin supplementation could reduce high-fat diet induced excessive body weight gain, dysglycaemia and dyslipidemia, adipocyte enlargement and hepatic lipid droplet accumulation in paternal mice. Furthermore, consistent with previous studies, as the agonist of transient receptor potential vanilloid 1, capsaicin was also capable to activate brown adipose tissue in paternal mice evidenced by enhanced UCP1 expression and increased energy consumption rates.

Diet-induced paternal obesity had been reported to cause negative outcomes in the metabolism of offspring (McPherson, Fullston, Aitken, & Lane, 2014). Earlier studies revealed that preconceptional paternal obesity could increase the susceptibility to obesity in the offspring. Our study also found that the offspring of paternal mice with diet-induced obesity were more prone to developing an obese phenotype when exposed to a high-fat diet, including increased body weight, enlarged adipocyte size, and accumulated liver lipid droplets.

Recent evidence supports that brown adipose tissue could serve as a potential therapeutic target for preventing the intergenerational transmission of obesity and related metabolic diseases. For instance, activation of paternal brown adipose tissue by cold exposure alleviated susceptibility to metabolic challenge and enhanced the thermogenesis activity in male offspring (Sun et al., 2018). We found that paternal capsaicin supplementation could exert a similar beneficial effect to paternal cold exposure regarding the alleviation of metabolic disorders in obese paternal mice and improvement of metabolic phenotypes in their male offspring.

It has been widely accepted that sperm cells carry not only DNA sequence information but also epigenetic information at fertilization,

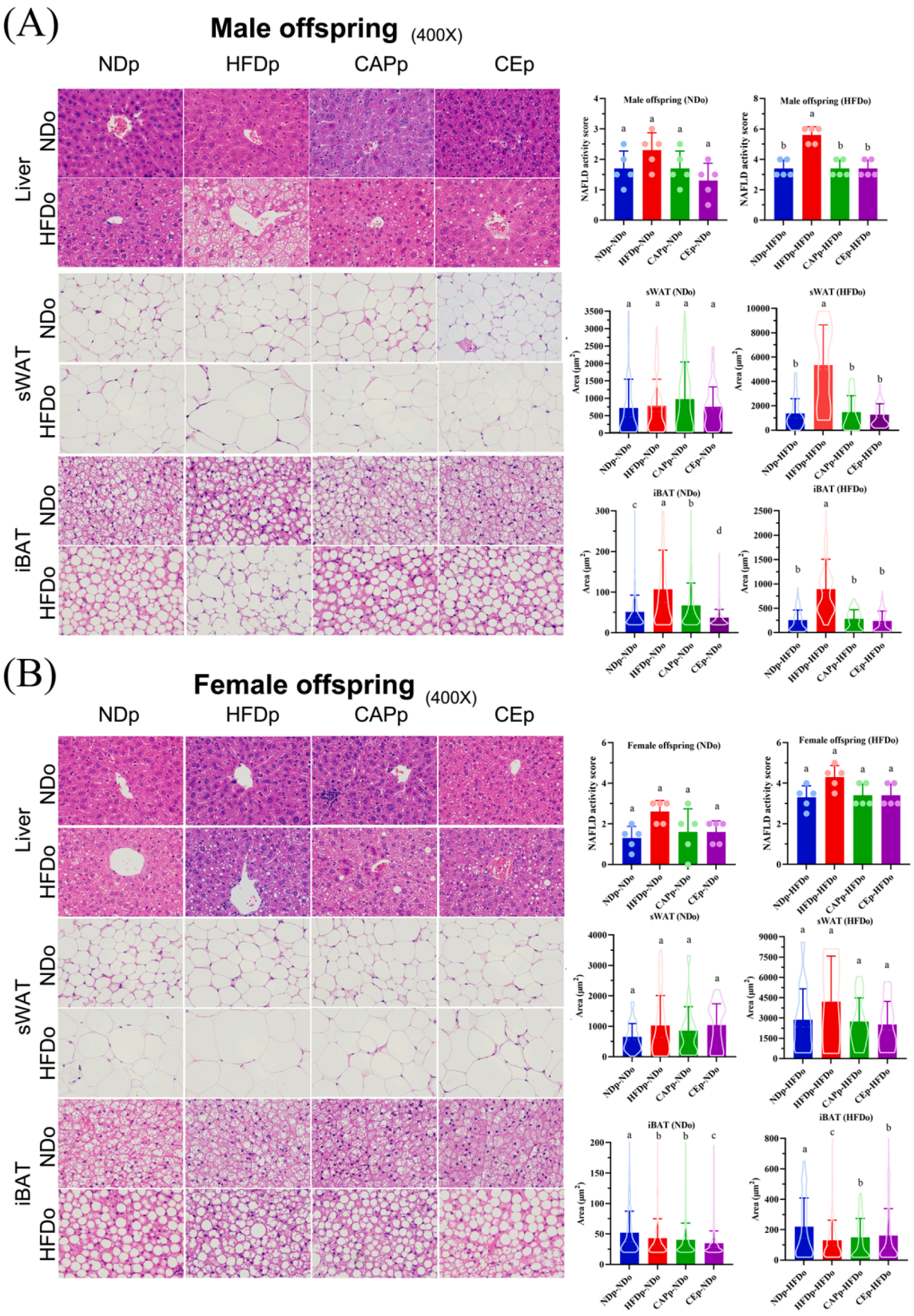

Fig. 3. Effects of paternal capsaicin intervention on histological features of liver and adipose tissue in offspring. Representative H&E staining of liver, sWAT and iBAT of male offspring (A) and female offspring (B). Data are presented as mean ± SD. Different lowercase letter indicates significant difference, $p < 0.05$, (n = 5).

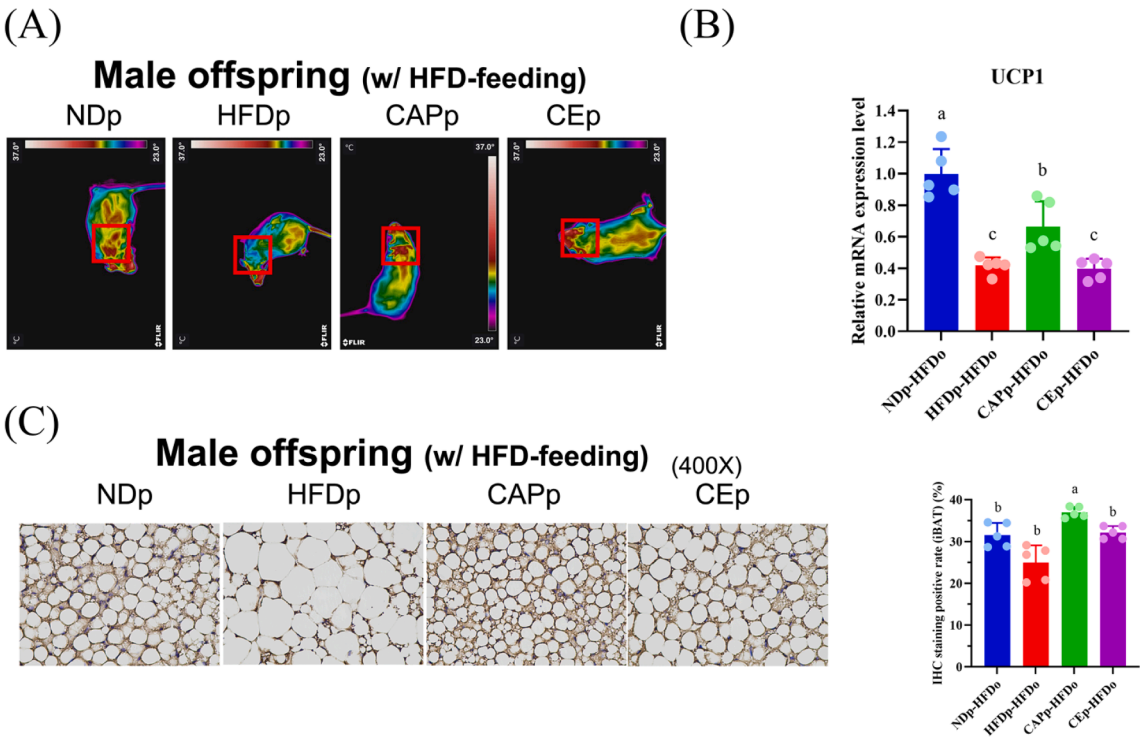

Fig. 4. Effects of paternal capsaicin intervention on brown adipose tissue in male offspring mice with HFD feeding. (A) Infrared imaging of surface body heat of male offspring mice with HFD feeding. (B) The transcriptional levels thermogenic gene (UCP-1) in iBAT tissue of male offspring mice with HFD feeding normalized to β-actin. (C) UCP-1 immunohistochemical stain of iBAT of male offspring mice with HFD feeding. Data are presented as mean ± SD. Different lowercase letter indicates significant difference, $p < 0.05$, (n = 5).

which could be influenced by environmental factors such as diet and stress (Güneş & Kulaç, 2013; Short et al., 2016). These epigenetic modifications have been recognized for their substantial impact on the lifelong health of offspring although the precise mechanisms remain largely unclear (Stuppia, Franzago, Ballerini, Gatta, & Antonucci, 2015; Y. Zhou, Wu, & Huang, 2018). For instance, a number of preconceptional interventions (such as diet or exercise interventions, etc.) have been demonstrated to improve the metabolic phenotype of offspring. Denham *et al.,* found exercise training for 3 months could significantly improve sperm motility and morphology with changes in the overall and genome-wide DNA methylation in human (Denham, O'Brien, Harvey, & Charchar, 2015). Donkin *et al.* also reported that bariatric surgery-induced weight loss could remodel DNA methylation in sperm of morbidly obese men. Furthermore, among differentially methylated genes, a number of genes were identified to be associated with the regulation of appetite control (Donkin et al., 2016). Here, our study showed that paternal capsaicin treatment could alter the sperm DNA methylation, which may be involved in the alleviation of intergenerational transmission of obesity. Although it is widely acknowledged that sperm DNA methylation is of paramount importance for mammalian embryonic development, how the intricate mechanisms through which sperm DNA methylation influences the metabolic profiles of offspring are still exceedingly complex (R. Zhou et al., 2022). During the subsequent stages of zygote formation, fetal development, and child development, DNA methylation patterns may undergo significant reprogramming. For instance, it has been found that a wave of global demethylation occurs in the zygote until it reaches the blastocyst stage. Therefore, it not possible to conclusively attribute the observed alterations in sperm DNA methylation patterns are causally linked to the phenotype improvement of offspring. However, the evidence still suggested that beneficial effects of paternal capsaicin intervention on offspring might be attributed to, at least partially, the changes in methylation in sperm DNA.

Small RNAs are also associated with a broad range of epigenetic phenomena. Indeed, a number of sncRNAs (eg. miRNA, piRNA (Kleeman et al., 2024; Short et al., 2017; Tyebji, Hannan, & Tonkin, 2020), tRNA-derived small RNAs (Chen et al., 2016; U. Sharma et al., 2016; Zeng et al., 2022; Y. Zhang et al., 2021), and mitochondria-derived small RNA (Rompala et al., 2018) identified in mature sperm were found to regulate paternal transgenerational inheritance *via* non-genomic signals derived from the sperm. Evidence from human studies also suggest that sperm sncRNAs are involved in the paternal intergenerational metabolic responses and profoundly influence offspring metabolic health (Daniel Nätt et al., 2019; D. Nätt & Öst, 2020). Therefore, the observed changes in sperm sncRNA profiles upon paternal capsaicin treatment may also contribute to the improvement of paternal intergenerational metabolic responses.

Although capsaicin treatment and cold exposure, as two well-known approaches for activating brown adipose tissue, showed comparable beneficial influences on the metabolic profiles of offspring, discernible differences in sperm DNA methylation and sncRNAs profiles were observed. Such findings also underscored the complexity of intergenerational inheritance from fathers to offspring. A previous human study uncovered a noteworthy observation that even short-term (6 weeks) dietary intervention has the potential to alter the sncRNA landscape in human sperm (Candida et al., 2021). This implies that the male germline can "rapidly" respond to physiological changes (such as dietary treatment, exercise, stress, etc) and alter epigenetic profiles accordingly.

Therefore, additional research is imperative to gain a more comprehensive understanding of the underlying mechanisms including exploring the impact of time point and duration of dietary treatment around conception on the epigenetic profiles in the male germline.

Compared to male offspring, our study revealed that the beneficial effects of paternal capsaicin supplementation were less obvious in female offspring. This might be because female mice already had a relative strong resilience to diet-induced obesity, which may retard the development of the obese phenotype and metabolic disorders (Arcones, Cruces-Sande, Ramos, Mayor Jr, & Murga, 2019). Salinero, A.E. *et al.*

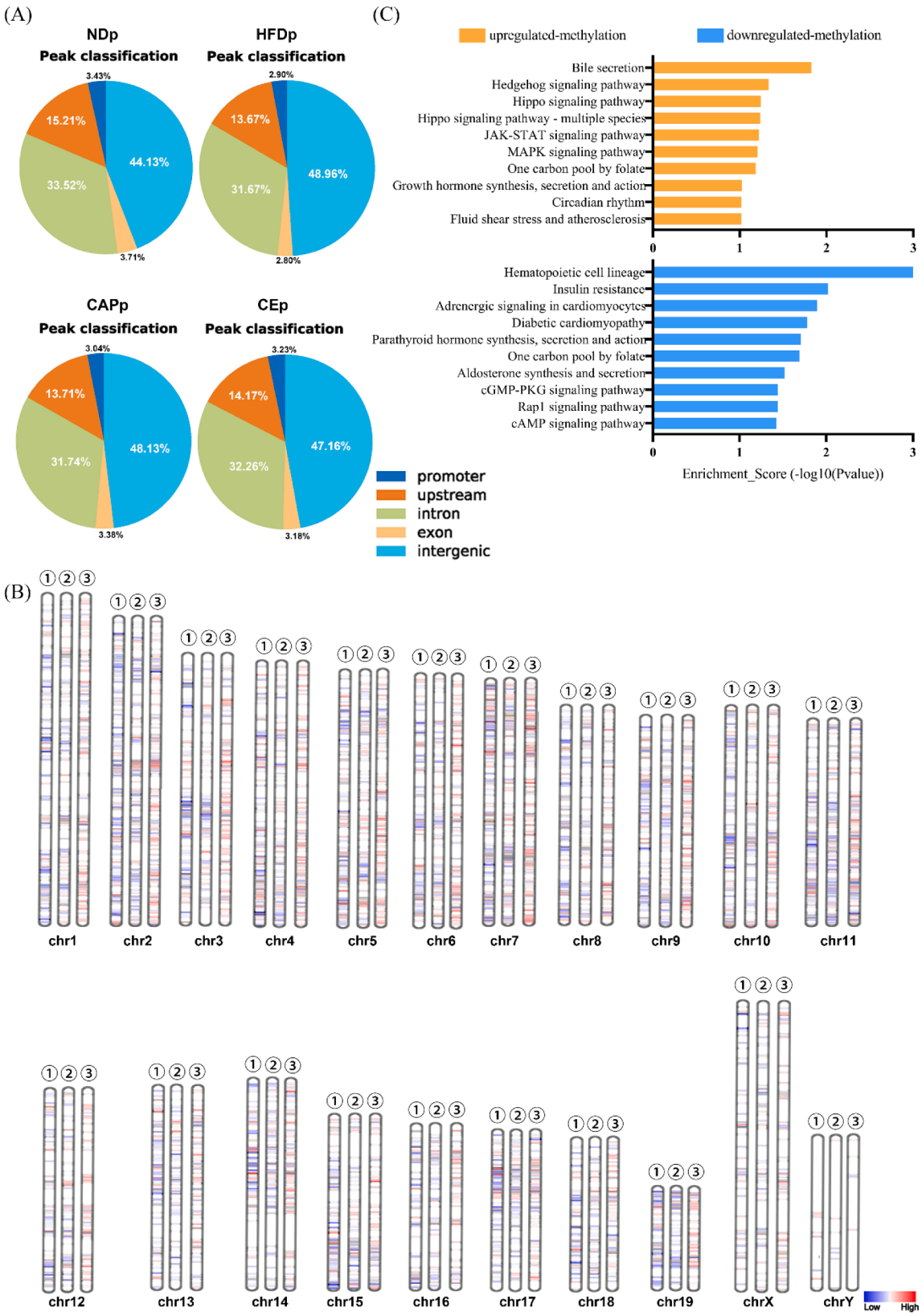

Fig. 5. Effects of preconceptional capsaicin intervention on sperm DNA methylation in paternal mice. (A) Methylation peak classification, (B) Differentially methylated regions (DMRs) distribution on genome. (C) The significant KEGG pathway of upregulated-methylated and down-methylated related-genes between HFDp group and CAPp group, (n = 3).

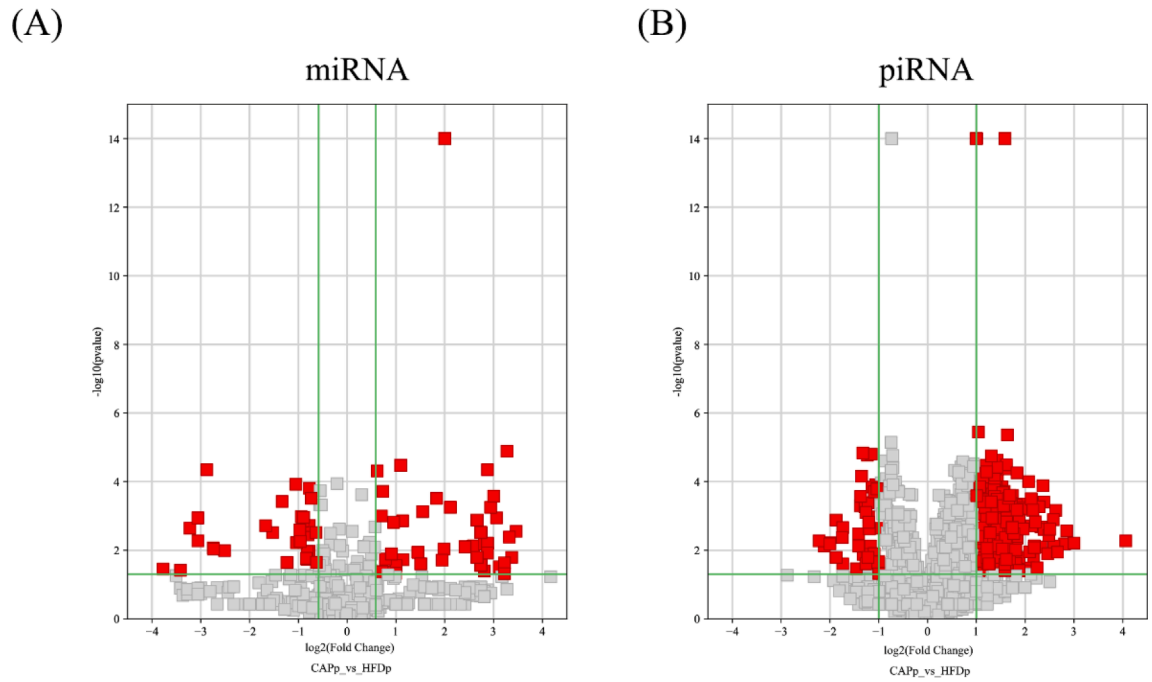

Fig. 6. Volcano plot of the differentially expressed miRNAs (A) and piRNA (B) in sperm of high-fat diet fed paternal with or without preconceptional capsaicin intervention. The y-axis indicates the -log10 of P-values and the x-axis is the log2 of fold-change, (n = 3).

Table 3
Differentially expressed sncRNAs in paternal sperm from CAP group compared to HFD group (excluding the novel sncRNAs).

miRNA		piRNA	
Mature name	Fold change	Name	Fold change
Up-regulated			
mmu-miR-152-5p	7.33	mmu-piR-008241	16.67
mmu-miR-27a-3p	7.00	mmu-piR-011324	6.00
mmu-miR-466c-5p	6.67	mmu-piR-009081	5.14
mmu-miR-704	6.00	mmu-piR-025682	5.00
mmu-miR-466b-3p	5.33	mmu-piR-001570	4.73
mmu-miR-466c-3p	5.33	mmu-piR-005879	4.20
mmu-miR-466p-3p	5.33	mmu-piR-021290	4.00
mmu-miR-92a-3p	4.33	mmu-piR-006982	3.67
mmu-miR-465a-3p	4.00	mmu-piR-011336	3.67
mmu-miR-465b-3p	4.00	mmu-piR-012086	3.67
Down-regulated			
mmu-miR-5114	−9.33	mmu-piR-032847	−4.67
mmu-miR-378a-5p	−7.33	mmu-piR-014579	−4.33
mmu-miR-1843a-3p	−6.70	mmu-piR-011546	−4.00
mmu-miR-223-3p	−5.70	mmu-piR-013448	−4.00
mmu-miR-598-3p	−2.88	mmu-piR-010111	−3.67
mmu-miR-434-5p	−2.52	mmu-piR-013627	−3.67
mmu-miR-128-3p	−2.07	mmu-piR-021967	−3.67
mmu-miR-195a-3p	−2.05	mmu-piR-037073	−3.67
mmu-miR-203-3p	−1.96	mmu-piR-000604	−3.33
mmu-miR-1a-3p	−1.95	mmu-piR-019151	−3.33

showed the effects of diet induced obesity (DIO) had significant sex differences. The juvenile males were more susceptible to obesity and worse glucose homeostasis of the high-fat diet than females (Salinero, Anderson, & Zuloaga, 2018). The experimental results from Hwang *et al.* also showed that compared to female mice, male mice were more susceptible to long-term high-fat diet-induced metabolic alterations such as high bodyweight, hyperglycemia, hyperinsulinemia, hypercholesterolemia, and hyperleptinemia (Hwang et al., 2010). This might be the reason for the limited effects on female offspring in our study. Indeed, similar findings were also reported by a number of studies where paternal intervention showed more obvious beneficial effects on male offspring. For example, Batista R.O. *et al.* reported that paternal exercise could reduce weight gain, improve lipid metabolism in liver and adipose tissue, and relieve hepatic steatosis in the male offspring upon high-fat diet feeding, but no significant improvements were observed in the female offspring (Batista et al., 2020). Similarly, Sun *et al.*, also showed paternal cold exposure resulted in more obvious protective effects on intergenerational transmission of obesity in male offspring (Sun et al., 2018).

5. Conclusion

In summary, our study demonstrated that preconceptional capsaicin intervention in paternal high-fat diet-induced obesity could significantly improve the metabolic disorders and induce the activation of brown adipose tissue. Furthermore, the preconceptional capsaicin intervention was also found to alleviate the high-fat diet-induced intergenerational inheritance of acquired obesity in male offspring. The changes in sperm DNA methylation and sncRNA profiles may contribute to these observations although additional research is warranted to further delve into the mechanisms. In conclusion, this study showed that preconceptional diet intervention targeting brown adipose tissue represents a promising strategy to improve offspring metabolic health.

6. Ethics statement

Hereby, the authors consciously assure that all animal experiments reported in the current study were approved by the Animal Management and Use Committee of Fujian Agriculture and Forestry University (No. PZCASFAFU22037) and adhered to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

CRediT authorship contribution statement

Jiamiao Hu: Supervision, Funding acquisition. **Zhongjing Lin:** Writing – review & editing, Visualization, Formal analysis. **Yang Yang:** Writing – original draft, Methodology, Investigation, Formal analysis. **Mark Christian:** Writing – review & editing, Visualization. **Shiyang Li:** Validation. **Baodong Zheng:** Supervision. **Bee K. Tan:** Conceptualization. **Shaoling Lin:** Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

The authors acknowledge that this work was supported by the following funds: National Key Research and Development Program of China (2023 YFF1104304); China Postdoctoral Science Foundation (2019 T120551); Research Fund for Taiwan-Straits Postdoctoral Exchange Program (2018B003); and Special Funds for Science and Technology Innovation of Fujian Agriculture and Forestry University (2022-82).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2024.106137.

References

Adane, A. A., Dobson, A., Tooth, L., & Mishra, G. D. (2018). Maternal preconception weight trajectories are associated with offsprings' childhood obesity. *International Journal of Obesity, 42*(7), 1265–1274.

Arcones, A. C., Cruces-Sande, M., Ramos, P., Mayor, F., Jr, & Murga, C. (2019). Sex differences in high fat diet-induced metabolic alterations correlate with changes in the modulation of GRK2 levels. *Cells, 8*(11), 1464.

Ardestani, A., Lupse, B., & Maedler, K. (2018). Hippo signaling: Key emerging pathway in cellular and whole-body metabolism. *Trends in Endocrinology & Metabolism, 29*(7), 492–509.

Baskaran, P., Krishnan, V., Fettel, K., Gao, P., Zhu, Z., Ren, J., & Thyagarajan, B. (2017). TRPV1 activation counters diet-induced obesity through sirtuin-1 activation and PRDM-16 deacetylation in brown adipose tissue. *International Journal of Obesity, 41*(5), 739–749.

Baskaran, P., Krishnan, V., Ren, J., & Thyagarajan, B. (2016). Capsaicin induces browning of white adipose tissue and counters obesity by activating TRPV1 channel-dependent mechanisms. *British journal of pharmacology, 173*(15), 2369–2389.

Baskaran, P., Markert, L., Bennis, J., Zimmerman, L., Fox, J., & Thyagarajan, B. (2019). Assessment of pharmacology, safety, and metabolic activity of capsaicin feeding in mice. *Scientific reports, 9*(1), 8588.

Batista, R. O., Budu, A., Alves-Silva, T., Arakaki, A. M., Gregnani, M. F. S., Húngaro, T. G. R., & Camara, N. O. S. (2020). Paternal exercise protects against liver steatosis in the male offspring of mice submitted to high fat diet. *Life Sciences, 263*, Article 118583.

Bodden, C., Pang, T. Y., Feng, Y., Mridha, F., Kong, G., Li, S., & Hannan, A. J. (2022). Intergenerational effects of a paternal Western diet during adolescence on offspring gut microbiota, stress reactivity, and social behavior. *The FASEB Journal, 36*(1), e21981.

Campbell, J. M., & McPherson, N. O. (2019). Influence of increased paternal BMI on pregnancy and child health outcomes independent of maternal effects: A systematic review and meta-analysis. *Obesity research & clinical practice, 13*(6), 511–521.

Candida, V., Alexandra, J. K., Mark, B., Pei Fang, T., Jason, H., Tessa, P. X. Y., . . . Neerja, K. (2021). Short-term diet intervention alters the small non-coding RNA (sncRNA) landscape of human sperm, *bioRxiv*. 2021.2007.2008.451257. 10.1101/2021.07.08.451257.

Cao, K., Xu, J., Zou, X., Li, Y., Chen, C., Zheng, A., & Shi, Y. (2014). Hydroxytyrosol prevents diet-induced metabolic syndrome and attenuates mitochondrial abnormalities in obese mice. *Free Radical Biology and Medicine, 67*, 396–407.

Carrer, M., Liu, N., Grueter, C. E., Williams, A. H., Frisard, M. I., Hulver, M. W., & Olson, E. N. (2012). Control of mitochondrial metabolism and systemic energy homeostasis by microRNAs 378 and 378. *Proceedings of the National Academy of Sciences, 109*(38), 15330–15335.

Chen, Q., Yan, M., Cao, Z., Li, X., Zhang, Y., Shi, J., & Zhou, Q. (2016). Sperm tsRNAs contribute to intergenerational inheritance of an acquired metabolic disorder. *Science, 351*(6271), 397–400. https://doi.org/10.1126/science.aad7977

Chooi, Y. C., Ding, C., & Magkos, F. (2019). The epidemiology of obesity. *Metabolism, 92*, 6–10.

de Castro Barbosa, T., Ingerslev, L. R., Alm, P. S., Versteyhe, S., Massart, J., Rasmussen, M., & Vetterli, L. (2016). High-fat diet reprograms the epigenome of rat spermatozoa and transgenerationally affects metabolism of the offspring. *Molecular metabolism, 5*(3), 184–197.

Denham, J., O'Brien, B. J., Harvey, J. T., & Charchar, F. J. (2015). Genome-wide sperm DNA methylation changes after 3 months of exercise training in humans. *Epigenomics, 7*(5), 717–731.

Donkin, I., Versteyhe, S., Ingerslev, L. R., Qian, K., Mechta, M., Nordkap, L., & Kristiansen, V. B. (2016). Obesity and bariatric surgery drive epigenetic variation of spermatozoa in humans. *Cell metabolism, 23*(2), 369–378.

Freeman, E., Fletcher, R., Collins, C., Morgan, P., Burrows, T., & Callister, R. (2012). Preventing and treating childhood obesity: Time to target fathers. *International journal of obesity, 36*(1), 12–15.

Fudge, J. R., Bennett, B. L., Simanis, J. P., & Roberts, W. O. (2015). Medical evaluation for exposure extremes: Cold. *Wilderness & Environmental Medicine, 26*(4), 63–68.

Fullston, T., Teague, E. M. C. O., Palmer, N. O., DeBlasio, M. J., Mitchell, M., Corbett, M., & Lane, M. (2013). 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. *The FASEB Journal, 27*(10), 4226–4243.

Grandjean, V., Fourré, S., De Abreu, D. A. F., Derieppe, M.-A., Remy, J.-J., & Rassoulzadegan, M. (2015). RNA-mediated paternal heredity of diet-induced obesity and metabolic disorders. *Scientific reports, 5*(1), 18193.

Gu, Y., Liu, X., Liao, L., Gao, Y., Shi, Y., Ni, J., & He, G. (2021). Relationship between lipid metabolism and hedgehog signaling pathway. *The Journal of Steroid Biochemistry and Molecular Biology, 209*, Article 105825.

Güneş, S., & Kulaç, T. (2013). The role of epigenetics in spermatogenesis. *Turkish journal of urology, 39*(3), 181.

Hosseini, M., Tavalaee, M., Rahmani, M., Eskandari, A., Shaygannia, E., Kiani-Esfahani, A., & Nasr-Esfahani, M. H. (2020). Capsaicin improves sperm quality in rats with experimental varicocele. *Andrologia, 52*(11), e13762.

Hu, Z., Tylavsky, F. A., Han, J. C., Kocak, M., Fowke, J. H., Davis, R. L., & Zhao, Q. (2019). Maternal metabolic factors during pregnancy predict early childhood growth trajectories and obesity risk: The CANDLE study. *International journal of obesity, 43*(10), 1914–1922.

Hwang, L. L., Wang, C. H., Li, T. L., Chang, S. D., Lin, L. C., Chen, C. P., & Yang, W. S. (2010). Sex differences in high-fat diet-induced obesity, metabolic alterations and learning, and synaptic plasticity deficits in mice. *Obesity, 18*(3), 463–469.

Jääskeläinen, A., Pussinen, J., Nuutinen, O., Schwab, U., Pirkola, J., Kolehmainen, M., & Laitinen, J. (2011). Intergenerational transmission of overweight among finnish adolescents and their parents: A 16-year follow-up study. *International journal of obesity, 35*(10), 1289–1294.

Josey, M. J., McCullough, L. E., Hoyo, C., & Williams-DeVane, C. (2019). Overall gestational weight gain mediates the relationship between maternal and child obesity. *BMC Public Health, 19*(1), 1–9.

Kang, S. S., Kurti, A., Fair, D. A., & Fryer, J. D. (2014). Dietary intervention rescues maternal obesity induced behavior deficits and neuroinflammation in offspring. *Journal of neuroinflammation, 11*(1), 1–12.

Kawada, T., Hagihara, K.-I., & Iwai, K. (1986). Effects of capsaicin on lipid metabolism in rats fed a high fat diet. *The Journal of Nutrition, 116*(7), 1272–1278.

Klastrup, L. K., Bak, S. T., & Nielsen, A. L. (2019). The influence of paternal diet on sncRNA-mediated epigenetic inheritance. *Molecular Genetics and Genomics, 294*, 1–11.

Kleeman, E. A., Reisinger, S. N., Adithya, P., Houston, B., Stathatos, G., Garnham, A. L., & Hannan, A. J. (2024). Paternal immune activation by poly I: C modulates sperm noncoding RNA profiles and causes transgenerational changes in offspring behavior. *Brain, Behavior, and Immunity, 115*, 258–279. https://doi.org/10.1016/j.bbi.2023.10.005

Kleiner, D. E., Brunt, E. M., Van Natta, M., Behling, C., Contos, M. J., Cummings, O. W., & Nonalcoholic Steatohepatitis, C. (2005). Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology, 41*(6), 1313–1321. https://doi.org/10.1002/hep.20701

Konstantinidi, M., & Koutelidakis, A. E. (2019). Functional foods and bioactive compounds: A review of its possible role on weight management and obesity's metabolic consequences. *Medicines, 6*(3), 94.

Krist, B., Florczyk, U., Pietraszek-Gremplewicz, K., Józkowicz, A., & Dulak, J. (2015). The role of miR-378a in metabolism, angiogenesis, and muscle biology. *International journal of endocrinology., 2015*.

Krout, D., Roemmich, J. N., Bundy, A., Garcia, R. A., Yan, L., & Claycombe-Larson, K. J. (2018). Paternal exercise protects mouse offspring from high-fat-diet-induced type 2 diabetes risk by increasing skeletal muscle insulin signaling. *The Journal of nutritional biochemistry, 57*, 35–44.

Li, W., Yang, H., & Lu, Y. (2019). Capsaicin alleviates lipid metabolism disorder in high beef fat-fed mice. *Journal of functional foods, 60*, Article 103444.

Li, X. Q., Liu, J., Wang, G. C., Yu, J., Sheng, Y. L., Wang, C., & Ding, G. X. (2015). Determination of UCP1 expression in subcutaneous and perirenal adipose tissues of patients with hypertension. *Endocrine, 50*(2), 413–423. https://doi.org/10.1007/s12020-015-0572-3

Liang, X., Yang, Q., Zhang, L., Maricelli, J. W., Rodgers, B. D., Zhu, M.-J., & Du, M. (2016). Maternal high-fat diet during lactation impairs thermogenic function of brown adipose tissue in offspring mice. *Scientific reports, 6*(1), 34345.

Lin, S. L., Wang, Z. Y., Lin, Y. L., Ge, S. H., Hamzah, S. S., & Hu, J. M. (2019). Bound phenolics from fresh lotus seeds exert anti-obesity effects in 3T3-L1 adipocytes and high-fat diet-fed mice by activation of AMPK. *Journal of Functional Foods, 58*, 74–84. https://doi.org/10.1016/j.jff.2019.04.054

Ma, S., Xi, B., Yang, L., Sun, J., Zhao, M., & Bovet, P. (2021). Trends in the prevalence of overweight, obesity, and abdominal obesity among chinese adults between 1993 and 2015. *International journal of obesity, 45*(2), 427–437.

Macartney-Coxson, D., Danielson, K., Clapham, J., Benton, M. C., Johnstone, A., Jones, A., & Hayes, M. (2020). MicroRNA profiling in adipose before and after

weight loss highlights the role of miR-223-3p and the NLRP3 inflammasome. *Obesity, 28*(3), 570–580.
Machado, I. F., Teodoro, J. S., Palmeira, C. M., & Rolo, A. P. (2020). miR-378a: A new emerging microRNA in metabolism. *Cellular and Molecular Life Sciences, 77*, 1947–1958.
McPherson, N. O., Fullston, T., Aitken, R. J., & Lane, M. (2014). Paternal obesity, interventions, and mechanistic pathways to impaired health in offspring. *Annals of Nutrition and Metabolism, 64*(3–4), 231–238.
McPherson, N. O., Lane, M., Sandeman, L., Owens, J. A., & Fullston, T. (2017). An exercise-only intervention in obese fathers restores glucose and insulin regulation in conjunction with the rescue of pancreatic islet cell morphology and microRNA expression in male offspring. *Nutrients, 9*(2), 122.
McPherson, N. O., Owens, J. A., Fullston, T., & Lane, M. (2015). Preconception diet or exercise intervention in obese fathers normalizes sperm microRNA profile and metabolic syndrome in female offspring. *American Journal of Physiology-Endocrinology and Metabolism, 308*(9), E805–E821.
McPherson, N. O., Shehadeh, H., Fullston, T., Zander-Fox, D. L., & Lane, M. (2019). Dietary micronutrient supplementation for 12 days in obese male mice restores sperm oxidative stress. *Nutrients, 11*(9), 2196.
Nätt, D., Kugelberg, U., Casas, E., Nedstrand, E., Zalavary, S., Henriksson, P., & Öst, A. (2019). Human sperm displays rapid responses to diet. *PLOS Biology, 17*(12), e3000559.
Nätt, D., & Öst, A. (2020). Male reproductive health and intergenerational metabolic responses from a small RNA perspective. *Journal of Internal Medicine, 288*(3), 305–320. https://doi.org/10.1111/joim.13096
Ng, R., Hussain, N. A., Zhang, Q., Chang, C., Li, H., Fu, Y., & Xu, F. (2017). miRNA-32 drives brown fat thermogenesis and trans-activates subcutaneous white fat browning in mice. *Cell reports, 19*(6), 1229–1246.
Nie, Y., Yan, Z., Yan, W., Xia, Q., & Zhang, Y. (2015). Cold exposure stimulates lipid metabolism, induces inflammatory response in the adipose tissue of mice and promotes the osteogenic differentiation of BMMSCs via the p38 MAPK pathway in vitro. *International Journal of Clinical and Experimental Pathology, 8*(9), 10875.
Ravnskjaer, K., Madiraju, A., & Montminy, M. (2016). Role of the cAMP pathway in glucose and lipid metabolism. *Metabolic Control*, 29–49.
Rendina-Ruedy, E., & Rosen, C. J. (2022). Parathyroid hormone (PTH) regulation of metabolic homeostasis: An old dog teaches us new tricks. *Molecular metabolism, 60*, Article 101480.
Rompala, G. R., Mounier, A., Wolfe, C. M., Lin, Q., Lefterov, I., & Homanics, G. E. (2018). Heavy chronic intermittent ethanol exposure alters small noncoding RNAs in mouse sperm and epididymosomes. [original research]. *Frontiers in Genetics, 9*. https://doi.org/10.3389/fgene.2018.00032
Sai, L., Jia, Q., Zhang, Y., Han, R., Geng, X., Yu, G., & Peng, C. (2020). Genome-wide analysis of DNA methylation in testis of male rat exposed to chlorpyrifos. *Toxicology Research, 9*(4), 509–518.
Salinero, A. E., Anderson, B. M., & Zuloaga, K. L. (2018). Sex differences in the metabolic effects of diet-induced obesity vary by age of onset. *International journal of obesity, 42*(5), 1088–1091.
Sánchez-Ceinos, J., Rangel-Zuñiga, O. A., Clemente-Postigo, M., Podadera-Herreros, A., Camargo, A., Alcalá-Diaz, J. F., & Malagón, M. M. (2021). miR-223-3p as a potential biomarker and player for adipose tissue dysfunction preceding type 2 diabetes onset. *Molecular Therapy-Nucleic Acids, 23*, 1035–1052.
Schaefer-Graf, U. M., Graf, K., Kulbacka, I., Kjos, S. L., Dudenhausen, J., Vetter, K., & Herrera, E. (2008). Maternal lipids as strong determinants of fetal environment and growth in pregnancies with gestational diabetes mellitus. *Diabetes Care, 31*(9), 1858–1863.
Sharma, S. K., Vij, A. S., & Sharma, M. (2013). Mechanisms and clinical uses of capsaicin. *European journal of pharmacology, 720*(1–3), 55–62.
Sharma, U., Conine, C. C., Shea, J. M., Boskovic, A., Derr, A. G., Bing, X. Y., & Rando, O. J. (2016). Biogenesis and function of tRNA fragments during sperm maturation and fertilization in mammals. *Science, 351*(6271), 391–396. https://doi.org/10.1126/science.aad6780
Short, A. K., Fennell, K. A., Perreau, V. M., Fox, A., O'Bryan, M. K., Kim, J. H., & Hannan, A. J. (2016). Elevated paternal glucocorticoid exposure alters the small noncoding RNA profile in sperm and modifies anxiety and depressive phenotypes in the offspring. *Translational Psychiatry, 6*(6), e837–e. https://doi.org/10.1038/tp.2016.109
Short, A. K., Yeshurun, S., Powell, R., Perreau, V. M., Fox, A., Kim, J. H., & Hannan, A. J. (2017). Exercise alters mouse sperm small noncoding RNAs and induces a transgenerational modification of male offspring conditioned fear and anxiety. *Translational Psychiatry, 7*(5), e1114–e. https://doi.org/10.1038/tp.2017.82
Speakman, J. R. (2019). Use of high-fat diets to study rodent obesity as a model of human obesity. *International journal of obesity, 43*(8), 1491–1492.
Stanford, K. I., Rasmussen, M., Baer, L. A., Lehnig, A. C., Rowland, L. A., White, J. D., & Patti, M.-E. (2018). Paternal exercise improves glucose metabolism in adult offspring. *Diabetes, 67*(12), 2530–2540.
Stuppia, L., Franzago, M., Ballerini, P., Gatta, V., & Antonucci, I. (2015). Epigenetics and male reproduction: The consequences of paternal lifestyle on fertility, embryo development, and children lifetime health. *Clinical epigenetics, 7*(1), 1–15.
Sugiyama, M., Machida, N., Yasunaga, A., Terai, N., Fukasawa, H., Ono, H. K., & Kurusu, S. (2021). Vaginal mucus in mice: Developmental and gene expression features of epithelial mucous cells during pregnancy. *Biology of reproduction, 105*(5), 1272–1282.
Sun, W., Dong, H., Becker, A. S., Dapito, D. H., Modica, S., Grandl, G., & Sarker, G. (2018). Cold-induced epigenetic programming of the sperm enhances brown adipose tissue activity in the offspring. *Nature medicine, 24*(9), 1372–1383.
Tyebji, S., Hannan, A. J., & Tonkin, C. J. (2020). Pathogenic infection in male mice changes sperm small RNA profiles and transgenerationally alters offspring behavior. *Cell Reports, 31*(4). https://doi.org/10.1016/j.celrep.2020.107573
Weihrauch-Blüher, S., Schwarz, P., & Klusmann, J.-H. (2019). Childhood obesity: Increased risk for cardiometabolic disease and cancer in adulthood. *Metabolism, 92*, 147–152.
Xu, D., Yin, C., Wang, S., & Xiao, Y. (2013). JAK-STAT in lipid metabolism of adipocytes. *Jak-stat, 2*(4), e27203.
Xu, Z., You, W., Zhou, Y., Chen, W., Wang, Y., & Shan, T. (2019). Cold-induced lipid dynamics and transcriptional programs in white adipose tissue. *BMC biology, 17*(1), 1–21.
Yuan, L.-J., Qin, Y., Wang, L., Zeng, Y., Chang, H., Wang, J., & Zhang, Q.-Y. (2016). Capsaicin-containing chili improved postprandial hyperglycemia, hyperinsulinemia, and fasting lipid disorders in women with gestational diabetes mellitus and lowered the incidence of large-for-gestational-age newborns. *Clinical nutrition, 35*(2), 388–393.
Yuan, Z., He, P., Cui, J., & Takeuchi, H. (1998). Hypoglycemic effect of water-soluble polysaccharide from Auricularia auricula-judae Quel. on genetically diabetic KK-ay mice. *Bioscience, biotechnology, and biochemistry, 62*(10), 1898–1903.
Zeng, L., Zhou, J., Zhang, Y., Wang, X., Wang, M., & Su, P. (2022). Differential expression profiles and potential intergenerational functions of tRNA-derived small RNAs in mice after cadmium exposure. *[Original Research]. Frontiers in Cell and Developmental Biology, 9*. https://doi.org/10.3389/fcell.2021.791784
Zhang, C., Liu, J., He, X., Sheng, Y., Yang, C., Li, H., & Huang, K. (2019). Caulis spatholobi ameliorates obesity through activating brown adipose tissue and modulating the composition of gut microbiota. *International journal of molecular sciences, 20*(20), 5150.
Zhang, Y., Ren, L., Sun, X., Zhang, Z., Liu, J., Xin, Y., & He, B. (2021). Angiogenin mediates paternal inflammation-induced metabolic disorders in offspring through sperm tsRNAs. *Nature Communications, 12*(1), 6673. https://doi.org/10.1038/s41467-021-26909-1
Zheng, J., Zheng, S., Feng, Q., Zhang, Q., & Xiao, X. (2017). Dietary capsaicin and its anti-obesity potency: From mechanism to clinical implications. *Bioscience reports, 37*(3).
Zhou, R., Lu, G., Yan, Z., Jiang, R., Sun, Y., & Zhang, P. (2022). Epigenetic mechanisms of DNA methylation in the transgenerational effect of ethylhexyl salicylate on zebrafish. *Chemosphere, 295*, Article 133926.
Zhou, Y., Wu, H., & Huang, H. (2018). Epigenetic effects of male obesity on sperm and offspring. *Journal of Bio-X Research, 1*(03), 105–110.