Paternal hyperglycemia in rats exacerbates the development of obesity in offspring

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Abstract

Parental history with obesity or diabetes will increase the risk for developing metabolic diseases in offspring. However, literatures as to transgenerational inheritance of metabolic dysfunctions through male lineage are relatively scarce. In the current study, we aimed to evaluate influences of paternal hyperglycemia on metabolic phenotypes in offspring. Male SD rats were i.p. injected with streptozotocin (STZ) or citrate buffer (CB, as control). STZ-injected rats with glucose levels higher than 16.7 mM were selected to breed with normal female rats. Offspring from STZ or CB treated fathers (STZ-O and CB-O) were maintained in the identical condition. We monitored body weight and food intake, and tests of glucose and insulin tolerance (GTTs and ITTs), fasting–refeeding and cold exposure were performed. Expression of factors involved in hypothalamic feeding and brown adipose tissue (BAT) thermogenic activity was performed by real-time PCR and Western blot. Adult STZ-O were heavier than CB-O. Impairment of GTTs was observed in STZ-O compared with CB-O at 22 and 32 weeks of age; ITTs results showed decreased insulin sensitivity in STZ-O. Daily food intake and accumulated food intake during 12-h refeeding after fasting were significantly higher in STZ-O. UCP1 levels were downregulated in BAT from STZ-O at room temperature and cold exposure. Finally, STZ-O rats showed suppressed leptin signaling in the hypothalamus as evidenced by upregulated SOCS3, reduced phosphorylation of STAT3, impaired processing POMC and decreased α -MSH production. Our study revealed that paternal hyperglycemia predisposes offspring to developing obesity, which is possibly associated with impaired hypothalamic leptin signaling.

Key Words

- ▶ diabetic father
- ▶ hypothalamus
- ▶ thermogenic capacity
- ▶ obese offspring

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Introduction

In the past decades, the global prevalence of obesity and other metabolic diseases (MS) has dramatically increased in both children and adults (Friend *et al.* 2013, Jaacks *et al.* 2016). The disproportionately early-onset of obesity and signs of MS among children and adolescents can be partially explained by Baker's 'Fetal origins of adult disease' hypothesis (Lane 2014). Animal studies from both rodents (Samuelsson *et al.* 2008, Nivoit *et al.* 2009, Song *et al.* 2012, Muralidarane *et al.* 2013, Elshenawy & Simmons 2016) and nonhuman primates (McCurdy *et al.* 2009) supported the fact that maternal high-fat diet (HFD) intake or hyperglycemia during pregnancy increases the rates of metabolic disorders in offspring. Detrimental impacts of maternal obesity or diabetes during perinatal periods on adiposity and metabolic function in offspring are well established, whereas the contribution of obese or diabetic fathers to these processes is poorly described. In 2010, Ng and coworkers pioneered the role of paternal obesity on the health of offspring and found that when rat fathers were fed a high-fat diet, the resulting female offspring exhibited impaired glucose tolerance and insulin secretion as young adults (Ng *et al.* 2010). Later on, a variety of studies showed that either paternal obesity or mild diabetes can significantly alter metabolic outcomes of offspring, making them more prone to obesity, diabetes and fatty liver diseases (Fullston *et al.* 2013, Wei *et al.* 2014, Cropley *et al.* 2016). Evidence from human studies also suggested that in the context of parent-offspring, the magnitudes of the maternal or paternal influences on offspring phenotype are almost identical (Kivimäki *et al.* 2007, Soubry *et al.* 2013, Wells 2014). Although strong associations between adult disease risk and environmental perturbations experienced during early development have been identified, the impact of paternal metabolic status around conception remains poorly understood. Obese male may associate with reduced pregnancy rates due to the impairment in sperm motility, increased sperm abnormality and decreased blastocyst developmental rates (Ghanayem *et al.* 2010). In addition, in response to paternal HFD (Mitchell *et al.* 2011), low protein diet feeding (Carone *et al.* 2010) or preconceptional fasting (Anderson *et al.* 2006), metabolic profiles of offspring are significantly altered including hepatic lipid metabolism and pancreatic β -cell function, which may possibly be related to changes in DNA methylation status of the imprinting genes.

The majority of studies on intergenerational transmission of metabolic disorders are from rodent

models of diet-induced obesity, which include a variety of metabolic abnormalities such as hyperglycemia, hyperleptinemia, dyslipidemia and metabolic inflammation. Thus, it is difficult to distinguish the role of each metabolic index in mediating transgenerational inheritance of metabolic dysfunction. To address this issue, we produced a relatively pure hyperglycemic rat model by single injection of low-dose streptozocin (STZ) (Deeds *et al.* 2011), and then bred with healthy female rats to generate offspring from STZ fathers (STZ-O), in comparison with those from citrate buffer (CB)-treated euglycemic fathers (CB-O). Our aim was to determine the impact of a paternal hyperglycemia on metabolic homeostasis of adult offspring, with particular focus on analysis of changes in hypothalamus-mediated food intake and energy expenditure.

Materials and methods

Animals

All experimental procedures were approved by the Animal Ethics Committee, the First Affiliated Hospital of Chongqing Medical University (Approval number: 2014–2017). Male and female Sprague–Dawley (SD) rats were purchased from Chongqing Medical University Laboratory Animal Centre (Chongqing, China) and housed under a 12h/12h light/darkness cycle with lights on from 07:00 to 19:00 humidity-controlled rooms at 22°C. The male rats randomly received two different treatments: streptozocin (STZ, S0130, Sigma) and CB. After one-week acclimatization, hyperglycemic model was induced by intraperitoneal injection of STZ (35 mg/kg body weight) dissolved in 0.1 M CB, pH 4.3, and control rats received an equal volume of CB after 16 h of fasting. Glucose levels were measured by using a glucose analyzer (Roche Instruments), and rats with glucose levels were persistently higher than 16.7 mM at the 3rd, 6th and 9th day after STZ injection and immediately before mating (45th day after STZ treatment) were considered as hyperglycemia and used for subsequent experiments. Rats satisfied with these hyperglycemic criteria after STZ injection (STZ, $n=15$) and control CB-treated rats (CB, $n=10$) were mated with age-matched healthy female rats. The males were removed from the cages immediately when the females were confirmed pregnancy. The offspring were labeled according to their fathers, forming two groups: STZ-O (STZ-offspring) and CB-O (CB-offspring).

Subsequently, the litter size was adjusted to eight pups per dam at postpartum day 2. After weaning (postpartum 21 days), all pups were housed by sex. The rats were weighed every week. All animals were maintained on a rodent regular chow diet (Composition: fat 4.5%, protein 20% and carbohydrate 57%; Energy contents: fat 11.6%, protein 23% and carbohydrate 65.4%) and had free access to water throughout the study (Fig. 1A). At the end of the experiment, animals were killed by CO₂ inhalation under the non-fasting condition except for some specific fasting/refeeding test was required. Body weight was monitored based on the sex from all offspring. For the measurement of other metabolic parameters, one male rat from each litter was randomly selected.

Food intake and cold challenge

Measurement of food intake and acute cold challenge test were performed in the individually caged rats. Fasting-refeeding test was implemented in young (6 weeks)

and adult (20 weeks) male offspring. Food intake was measured at a 12-h fasting followed by a 12-h refeeding period. In addition, the daily food consumption of three consecutive weeks (24–26 weeks) *ad libitum* was also monitored every day. Subsequently, cold exposure (4°C) and room temperature (22°C) for 6 h were conducted on male STZ-O and CB-O to examine the changes in the thermogenic capacities.

Glucose tolerance tests (GTTs) and insulin tolerance tests (ITTs)

GTTs were performed at 12, 22 and 32 weeks of age, and ITTs were performed at 30 weeks as our previous reports (Song *et al.* 2012, Wang *et al.* 2015). Briefly, rats were fasted for 16 h, and GTTs were performed as follows: after collecting blood from the tail (0 time), the rats received glucose (2 g/kg body weight) via intraperitoneal injection and blood glucose was measured at 15, 30, 60 and 120 min points. ITTs were performed 4 h after fasting, and

Figure 1

Paternal hyperglycemia induced significant weight gain and obesity in offspring. (A) Study design; (B) body weight of STZ-O and CB-O during lactation (STZ-O: $n=118$ from 15 different litters; CB-O: $n=78$ from 10 different litters); (C) body weight of male STZ-O and CB-O after weaning (STZ-O: $n=68$; CB-O, $n=44$); (D) body size of male offspring at 20 weeks of age; (E) representative H&E staining of WAT from male CB-O and STZ-O; (F) epididymal WAT mass standardized to body weight of male offspring ($n=9$ for each group). The data are expressed as mean $\pm$ s.e.m., and the differences between the two groups were analyzed with Student's *t*-test, $*P<0.05$.

human recombinant insulin (1 unit/kg, Actrapid, Novo Nordisk) was intraperitoneally injected and blood glucose was measured at 0, 30, 60, 90 and 120 min. For GTTs and ITTs, the data were plotted as blood glucose concentration over time.

Adipose tissue histological and immunohistochemistry analysis

Epididymal white adipose tissue (WAT) and interscapular brown adipose tissue (BAT) were carefully isolated, and then fixed in 4% paraformaldehyde at 4°C, embedded in paraffin, serially sectioned, stained with hematoxylin-eosin (H&E) for the assessment of cell morphology study. UCP1 (ab23841) immunohistochemistry was performed in BAT according to the manufacturer's instructions (Chengdu Biological Technology Co., Ltd). The UCP1 positive cells were quantified by Image J software, and expressed as the percentage of UCP1-positive area to the total area.

Plasma leptin and α -MSH assay

The plasma was separated by centrifugation (3000g for 15 min) at 4°C and stored at -80°C until the biochemical tests were performed. The plasma leptin and α -melanocyte stimulating hormone (α -MSH) concentrations were measured in duplicate using immunoenzymatic test kits (Rat leptin ELISA kit, CK-E30492R and rat α -MSH ELISA Kit, CK-E94745R, BIOSAMITE).

RNA isolation and quantitative RT-PCR analysis

Total RNA of the hypothalamus and BAT was extracted with TRIzol (Invitrogen), and the RNA quantity was measured using Nanodrop 2000 system (Thermo Scientific). cDNA was synthesized according to the manufacturer's instructions of the Reverse Transcription Kit (TaKaRa). Quantitative real-time PCR was performed in a 10 μ L final reaction volume with SYBR Green (Roche) on a C1000 Thermal Cycler CFX96 Real-Time System (BioRad) in triplicates. Amplification was carried out at 95°C for 4s, 60°C for 10s and 65°C for 5s. This was repeated for 40 cycles. The mRNA expression levels were calculated using the formula $2^{-\Delta\Delta Ct}$ (where $\Delta\Delta Ct = \Delta Ct$ sample - ΔCt reference). The primers used were listed in Table 1.

Table 1 Sequences of the primer pairs used in the RT-PCR.

Gene	Primers (5'→3')
β -Actin	Forward TCACCAACTGGGACGATA
	Reverse AGGCATACAGGGACAACA
<i>LepR</i>	Forward TCACCCAGCACAATCCAATCA
	Reverse GCCATCTGTCTGTAAGACGCAGT
<i>SOCS3</i>	Forward CCAGTCGGGGACCAAGAACC
	Reverse TGAGTACACAGTCAAAGCGGGG
<i>NPY</i>	Forward CTGCGACACTACATCAATCT
	Reverse ATACAACGACAACAAGGGA
<i>POMC</i>	Forward GATCCGAGATTCTGCTACA
	Reverse TGCTTTCCGTGGTGAGGT
<i>PC1</i>	Forward TCACCCTCTTTGCGTTTGG
	Reverse TCTCCGCCGCCATT
<i>PC2</i>	Forward AAGACGGAGAGGAAGAGG
	Reverse GCATCAGAGTTATAGTTGTAGG
<i>CPE</i>	Forward TGGTAATGAGGCGGTTGGAC
	Reverse TCAAGGAGGCATGATATGGAT
<i>Ucp1</i>	Forward TGGCGTGGCGGTATTCA
	Reverse GGCTTTGTGCTTGCACTCTG

Protein extraction and Western blot analysis

Total protein was extracted from frozen hypothalamus using RIPA buffer (Thermo Scientific) containing protease and phosphatase inhibitors (Roche). The ultrasonic fragmentation was centrifuged (14,000g for 15 min) at 4°C, and the supernatant was collected. Subsequently, the protein concentration was determined with the BCA kit (Beyotime). Fifty microgram protein was loaded onto 10% SDS-PAGE, transferred to 0.45 μ m PVDF membranes and then blocked with 5% bovine serum (BSA) at room temperature for 2 h to prevent nonspecific binding. The membrane was incubated overnight at 4°C with following primary antibodies: p-STAT3 (1:1000, 9145, Tyr705) and STAT3 (1:1000, 9139) (Cell Signaling Technology), and suppressor of cytokine signaling 3 (SOCS3) (1:1000, ab16030), and then the secondary antibodies for 1 h at room temperature. The protein bands were visualized with enhanced chemiluminescence (ECL) detection system. The band intensity was quantified with Fusion software, and all quantitative analyses were normalized to β -actin.

Statistical analysis

Statistical differences were analyzed by two-tailed Student's *t*-test or one-way ANOVA using GraphPad prism analysis software. All experimental data were represented as the mean $\pm$ S.E.M. *P* < 0.05 was considered statistically significant.

Results

Paternal hyperglycemia resulted in significant weight gain and obesity in offspring

Among 25 rats that received single injection of 35 mg/kg of STZ, 15 rats (60%) met our hyperglycemic criteria: glucose levels persistently higher than 16.7 mM at the 3rd, 6th, 9th and 45th day after STZ injection. In these rats, STZ triggered a 3-fold increase in blood glucose levels (data not shown). No obvious changes were observed in the structure of the testis and epididymis in these male hyperglycemic rats (data not shown). Body weight of the offspring born from STZ and CB treated fathers (STZ-O and CB-O) did not differ at birth, during lactation and young adults (Fig. 1B and C). However, beginning with 14 weeks of age, STZ-O gained more weight than their CB-O counterparts (Fig. 1C). In addition, STZ-O also had larger body size (Fig. 1D). White adipose tissue (WAT) H&E staining indicated the presence of extensive adipocyte expansion with increased adipocyte size in STZ-O (Fig. 1E), in correspondence with increased fat mass in STZ-O (Fig. 1F).

Paternal hyperglycemia induced glucose intolerance and reduced insulin sensitivity in male offspring

To test whether paternal hyperglycemia altered glucose homeostasis and insulin sensitivity in the offspring,

GTTs (Fig. 2A, B and C) and ITTs (Fig. 2D) were performed at the different ages of STZ-O and CB-O. At the ages of 12 and 22 weeks (Fig. 2A and B), no significant changes were observed between STZ-O and CB-O in GTTs. However, at the age of 32 weeks (Fig. 2C), significantly higher glucose levels were observed in STZ-O rats at 15 min after glucose loading, suggesting glucose intolerance in STZ-O compared with CB-O. In addition, the hypoglycemic effects after insulin treatment were significantly attenuated in STZ-O rats at 30 weeks of ages (Fig. 2D), suggesting reduced insulin sensitivity.

The offspring of hyperglycemic fathers exhibited hyperphagia

To examine whether more weight gain and adiposity of offspring from paternal hyperglycemia resulted from higher food consumption, we evaluated their ability to adapt energy intake by measuring the amount of refeed food at 1, 2, 6 and 12 h after an overnight fast. As shown in Fig. 3A, at 6 weeks of age, male STZ-O exhibited significantly higher food consumption within 12 h after an overnight fast, and higher food intake within 12 h after an overnight fast was observed in the male STZ-O at 20 weeks of age (Fig. 3B). In addition, we examined daily food intake at *ad libitum* feeding conditions. Remarkably, male STZ-O showed hyperphagia compared to CB-O at 26 weeks of age (Fig. 3C).

Figure 2

Paternal hyperglycemia induced glucose intolerance and reduced insulin sensitivity in male offspring. Glucose tolerance tests (GTTs) were performed at 12 (A), 22 (B), and 32 weeks of age (C). Insulin tolerance tests (ITTs) were performed at the age of 30 weeks (D). The data are expressed as mean $\pm$ S.E.M., $n=6-8$ for each group and test, and the differences between the two groups at the same time point were analyzed with Student's *t*-test, * $P<0.05$.

Figure 3

The offspring of hyperglycemic fathers exhibited hyperphagia. Food intake at 1-, 2-, 6- and 12-h after an overnight fast in male offspring at 6 week of age (A); food intake at 2- and 12-h after an overnight fast of male offspring at 20 weeks of age (B); average daily food intake of male offspring at 26 weeks of age (C). Values are expressed as mean $\pm$ S.E.M., $n=8$ for each group, and the differences between the two groups were analyzed with Student's *t*-test, * $P<0.05$.

Paternal hyperglycemia impaired BAT thermogenic capacity in male offspring

To test whether energy expenditure is involved in paternal hyperglycemia-induced obesity in offspring, we examined BAT histological changes and expression of thermogenic gene UCP1 in STZ-O and CB-O. Figure 4A and B shows histological changes in STZ-O and CB-O rats maintained at 21–22°C (RT). Brown adipocytes from CB-O rats exhibited typically multilocular, with the bulk of the cell being occupied by numerous circular lipid droplets with various sizes, whereas in STZ-O BAT, larger

and more circular lipid droplets were obvious, similar to the histological characteristics of WATs. Impaired thermogenic capacities in STZ-O rats were further confirmed by UCP1 expression. There were plentiful UCP1 positive cells in BAT from CB-O rats at room temperature (Fig. 4C and E). Compared with CB-O, only fewer UCP1 positive cells were observed in BAT from STZ-O rats (Fig. 4D and E). Acute exposure of normal animals to 4°C (cold exposure) is a well-established method investigating functional activity of BAT-mediated nonshivering thermogenesis. Both basal and cold-induced *Ucp1* mRNA

Figure 4

Paternal hyperglycemia impaired BAT thermogenic capacity in male offspring. Representative H&E staining of BAT from CB-O (A) and STZ-O (B); representative UCP1 immunohistochemistry of BAT from CB-O (C) and STZ-O (D); quantified UCP1 positive cells in CB-O and STZ-O (E); relative expression of *Ucp1* mRNA in BAT from CB-O and STZ-O at room temperature (22°C) and cold conditions (4°C) (F). The data are expressed as mean $\pm$ S.E.M., $n=8$ for each group and treatment, and the differences between the two groups were analyzed with Student's *t*-test, * $P<0.05$.

expression showed a remarkable reduction in BAT from STZ-O in comparison with those from CB-O rats (Fig. 4F), indicating a reduced BAT nonshivering thermogenesis in STZ-O animals.

Paternal hyperglycemia affected hypothalamic α -MSH processing and circulating α -MSH levels in male offspring

Proopiomelanocortin (POMC)-containing neurons represent the master subset of hypothalamic anorexigenic neurons, being the source of the α -melanocyte-stimulating hormone (α -MSH), which suppresses feeding and/or stimulates energy expenditure. As shown in Fig. 5A, under conditions of fasting, serum α -MSH levels of STZ-O rats were lower than CB-O, and lower α -MSH levels of STZ-O rats were maintained at 2 and 12 h after refeeding. α -MSH was produced from POMC through a series of enzymatic cleavage including type

1 and type 2 prohormone convertase (PC1 and PC2) and carboxypeptidase E (CPE) (Nillni 2016) (Fig. 5B). To test if changes in POMC expression and processing contribute to reduction of α -MSH in STZ-O rats, we detected hypothalamic mRNA levels of *Pomc* and α -MSH-producing enzymes. As shown in Fig. 5C, D, E and F, hypothalamic mRNA levels of *Pomc* and *Pc1*, *Pc2* and *Cpe* were consistently downregulated in STZ-O rats, suggesting downregulated POMC expressing and impairment in α -MSH-processing contribute to the lower levels of α -MSH in STZ-O rats.

Paternal hyperglycemia affected hypothalamic leptin signaling in male offspring

Leptin signaling plays crucial role in the control of food intake and energy expenditure in response to an altered energy state (Kwon *et al.* 2016). To investigate whether paternal hyperglycemia had effect on central leptin

Figure 5

Paternal hyperglycemia affected hypothalamic POMC processing and circulating α -MSH levels in male offspring. (A) Serum α -MSH levels; (B) schematic representation of POMC cleavage and α -MSH production; transcriptional levels of prohormone convertase (PC1, C) and PC2 (D), and carboxypeptidase E (CPE, E). The data were expressed as mean $\pm$ S.E.M., $n=8$ for each group, and the differences between the two groups were analyzed with Student's *t*-test, * $P<0.05$.

Figure 6

Paternal hyperglycemia affected hypothalamic leptin signaling in male offspring (A) Serum leptin levels; hypothalamic mRNA levels of neuropeptide Y (*Npy*, B), suppressor of cytokine signaling 3 (*Socs3*, C) and leptin receptor (*LepR*, D); (E) Western blot analysis of phosphorylated signal transducer and activator of transcription 3 (p-STAT3) and SOCS3 protein levels in hypothalamic extracts. (F) Quantified p-STAT3 and SOCS3 protein levels. The data were expressed as mean $\pm$ S.E.M., $n=6-8$ for each group, and the differences between the two groups were analyzed with Student's *t*-test, * $P<0.05$.

signaling, the key components of the JAK/STAT signaling pathways were examined in the hypothalamus. STZ-O rats exhibited hyperleptinemia (Fig. 6A), but without inhibitory effect on food intake (Fig. 4), suggesting a typical leptin resistance. Neuropeptide Y (NPY) is the most important orexigenic peptide, which was significantly upregulated in the hypothalamus of STZ-O rats (Fig. 6B). Suppressor of cytokine signaling 3 (SOCS3) is an inhibitory protein of the leptin signaling pathway. In this study, hypothalamic *Socs3* mRNA (Fig. 6C) and protein levels (Fig. 6E and F) were higher in STZ-O rats, while *LepR* mRNA levels (Fig. 6D) did not differ. Phosphorylated signaling transducers and activators of transcription 3 (p-STAT3) is the key hallmark of leptin signaling activation. Compared with CB-O, p-STAT3 was decreased in STZ-O rats (Fig. 6E and F), suggesting the impaired leptin signaling in STZ-O rats. These findings indicate that paternal hyperglycemia leads to leptin resistance in the offspring.

Discussion

Our present study revealed that compared to the euglycemic fathers, paternal hyperglycemia could induce more weight gain in the offspring, and offspring from hyperglycemic fathers displayed hyperphagia and impaired BAT thermogenesis, which may possibly contribute to their increased adiposity. Excess energy intake and obesity altered multiple metabolic organs and resulted in reduced insulin sensitivity and impaired glucose tolerance. Furthermore, these offspring displayed significant defects in hypothalamic leptin signaling, evidenced by reduction of anorexigenic peptide α -MSH levels and hypothalamic mRNA levels of its precursors (POMC) and processing enzymes (PC1, PC2 and CPE), increased expression of orexigenic NPY and decreased phosphorylation of STAT3. Hence, these findings strongly indicated that paternal hyperglycemia has a long-term impact on the regulation of energy homeostasis and

increases the risk of developing metabolic diseases in their next generation.

The transgenerational inheritance of acquired metabolic disorders may contribute to recent obesity and diabetes pandemic. Typically, the majority of studies as to whether and how early-life events influence later susceptibility to metabolic diseases, including ours, are from mothers (Song *et al.* 2012, Wang *et al.* 2015, Damm *et al.* 2016, Godfrey *et al.* 2016). Although maternal nutrition and metabolic status during gestation and lactation are undeniably linked to health of offspring, recent studies have shown that paternal environmental, nutritional or behavioral factors affect the phenotype of the offspring as well (Ng *et al.* 2010, Fullston *et al.* 2013, Braun & Champagne 2014, Wei *et al.* 2014, Cropley *et al.* 2016, Wu *et al.* 2016). In this study, offspring from paternal hyperglycemia were born normal and maintained normal body weight as young adult under standard diets. These data were generally consistent with the results of paternal obese (Cropley *et al.* 2016, Masuyama *et al.* 2016) or prediabetes (Wei *et al.* 2014) models. In comparison with Wei's findings, which utilized paternal obesity plus minor hyperglycemia (fasting blood glucose levels were at 7–11 mM, prediabetes) model and body weight of offspring was observed at relatively young age (16 week), our study uniquely revealed that severe paternal hyperglycemia *per se* significantly altered metabolic characters and induced a late onset weight gain in offspring. At birth and early developmental stages, the normal body weight of offspring born from diabetic fathers was possibly attributable to the protective mechanisms against an inadequate metabolic environment such as excess of metabolites and hormones. After adolescents, offspring from diabetic fathers gained more weight and adiposity overtaking their control counterparts, in agreeing with the results observed from paternal obesity (Masuyama *et al.* 2016). In addition to adiposity, paternal hyperglycemia was found to be linked to other metabolic abnormalities in offspring, including glucose intolerance, reduced insulin sensitivity, dyslipidemia and hyperleptinemia, which were observed in the offspring of paternal obesity (Ng *et al.* 2010, Fullston *et al.* 2013, 2015, Ornellas *et al.* 2015). The obesity is a very complicated condition, confounded with a broad spectrum of metabolic abnormalities, including adiposity, metabolic inflammation, glucose intolerance, β -cell dysfunction and altered secretion of hormones and cytokines (Gregor & Hotamisligil 2011, Dali-Youcef *et al.* 2013). Therefore, it is hard to distinguish which individual factor contributes to metabolic programming in the model

paternal obesity. In our study, a single low dose of STZ was used, which has been widely used to induce experimental diabetes and diabetic complications in rodents (Tesch & Allen 2007, Lenzen 2008, Song *et al.* 2012). The most important mechanism of streptozotocin-induced diabetes is pancreatic β -cell death due to DNA alkylation. In addition, generation of reactive oxygen/nitrogen species (ROS/RNS) also contributes to streptozotocin toxic effect on β cells and diabetogenic effect (Elsner *et al.* 2000, Lenzen 2008). STZ has a short life due to the quick metabolizing and elimination and its half-life time is about 15 min in the serum after intravenous injection (Eleazu *et al.* 2013). Based on its short life time, the acute toxicity of STZ could be neglected after persistent hyperglycemia is obtained, and any further functional changes in metabolism may be attributed to the effects of diabetic hyperglycemia after STZ is eliminated out of the body. Thus, our data provide important evidence to confirm that in addition to obesity and obesity-related metabolic abnormalities, paternal hyperglycemia *per se* can significantly affect offspring phenotype.

STZ-O were born normal and maintained healthy body weight during youth and adolescents. Surprisingly, at 6 weeks of age, STZ-O were evident in higher food intake but maintained normal body weight as their CB-O counterparts, which may possibly be attributable to diet-induced thermogenesis and an activation of brown fat, where UCP1 could serve to burn off excess calories to maintain energy balance, thus opposing weight gain (Westerterp 2013). However, STZ-O gained more weight and adiposity after young adults, and this has been observed in other rodent (Berends *et al.* 2013) and nonhuman primate (Sullivan *et al.* 2017) metabolic programming models. The body weight change between STZ-O and CB-O may be possibly through metabolic regulation of the hypothalamic feeding circuits, which could further alter food intake and energy expenditure (Gao & Sun 2016, Lage *et al.* 2016). Leptin activates anorexigenic neurons and inhibits orexigenic neurons in the hypothalamus via Janus tyrosine kinase 2 (JAK2) and signal transducer and activator of transcription 3 (STAT3)-mediated pathways, resulting in reduced food intake and increased energy expenditure (Williams & Schwartz 2011, Roh *et al.* 2016). A hallmark of obesity is leptin resistance, in which high-circulating leptin levels are unable to promote its central anorexigenic effects. As abundant food availability and feeding behavior play an important role in the obesity epidemic (Ellacott *et al.* 2010), we thus performed fast-refeed test and measured daily food intake in adult STZ-O

and CB-O as young and adults under standard chow diets. Food deprivation generally results in a reduction in plasma concentration of leptin, and low plasma leptin levels trigger a series of adaptive neuroendocrine responses to increase food intake, and amount of the consumed food after refeeding is an ideal indicator as appetite (Ahima 2000). At 6 weeks of age, male STZ-O exhibited higher appetite as reflected by higher food consumed within 12 h after an overnight fast, while female STZ-O did not differ, suggesting there is a potential sex-specific feeding pattern in rats (Zandian *et al.* 2011, Fukushima *et al.* 2015). Sexual differences of offspring in response to changes in paternal glycemia may be attributable to the activational role of sex hormones at puberty in male and females, as rise in estrogen acts as a reducing factor of feeding (Xu *et al.* 2011, Fukushima *et al.* 2015). Of note, no obvious changes in body weight were observed between STZ-O and CB-O at this age. We speculate that in response to higher intake of caloric energy, a metabolic adaptation was triggered in young STZ-O animals as an attempt to control weight gain, possibly by consuming the excess energy through increased thermogenesis. Higher food intake within 12 h after an overnight fast and daily food intake was observed after 20 weeks of age in STZ-O, in consistence with increased weight gain and adiposity. BAT is a specialized fat depot that can increase energy expenditure through non-shivering thermogenesis (Sidossis & Kajimura 2015, Kalinovich *et al.* 2017). Changes in histological features and *Ucp1* mRNA and protein levels suggested that brown fat from adult STZ-O was less active compared with CB-O at neutral temperature. In addition, cold-induced *Ucp1* mRNA expression was significantly reduced in adult STZ-O rats. All these data implied that both increased food intake and impaired energy expenditure contribute to the phenotype of adult STZ-O rats.

Excess weight gain and obesity result from an imbalance between energy intake and energy expenditure. Hypothalamic leptin signaling plays a crucial role in the control of food intake and energy expenditure (Caron & Richard 2016, Kwon *et al.* 2016, Roh *et al.* 2016). In this study, STAT3 phosphorylation (pSTAT3), a key component of leptin signaling, was detected by immunoblotting tests in the hypothalamus from both STZ-O and CB-O. Compared with CB-O, pSTAT3 proteins were significantly reduced in STZ-O, suggesting significant hypothalamic leptin resistance. Physiologically, the activation of leptin signaling may further modulate the action of a series of neuropeptides, mainly inhibiting orexigenic neuropeptide

Y (NPY) and promoting anorectic proopiomelanocortin (POMC) in the hypothalamus (Kageyama *et al.* 2012). NPY stimulates food intake, and the POMC product α -MSH inhibits food intake (Shi *et al.* 2013, Loh *et al.* 2015). In our study, STZ-O showed a significantly high hypothalamic NPY expression, reduced mRNA levels of *Pomc* and α -MSH-processing enzymes, and more importantly, low-circulating α -MSH concentrations.

Due to the technical difficulty, we are unable to detect the levels of α -MSH in cerebrospinal fluid (CSF) or hypothalamic tissues. However, peripheral serum levels of α -MSH may well correlate with any parameter of adiposity, and a substantial amount of α -MSH in the blood was produced by the pituitary (Gavrila *et al.* 2005, Enriori *et al.* 2016). These data could help explain leptin resistance in STZ-O rats.

In summary, our work suggests that paternal hyperglycemia programs hypothalamic feeding circuits and exacerbates the development of obesity in offspring. Our studies provide additional evidence that abnormal metabolic state of fathers can be passed to the subsequent generation; this process may be associated with hypothalamic leptin resistance. Although a clearly inherited phenotype of overweight/obesity in the second generation was observed, current study failed to address the mechanisms involved in the transgenerational inheritance through male lineage. In the future study, we will examine whether epigenetic modification is involved in transgenerational inheritance of metabolic disturbance in this specific model of paternal hyperglycemia.

Declaration of interest

The authors who have taken part in this study declared that there is no conflict of interest to disclose regarding this manuscript.

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Author contribution statement

XX, XS and XL conceptualized data collection instruments. XX, XS, XL, QL and WC designed data collection instruments. XS, XL, YH, XC, YZ, HW, HW, CP and JB executed the data. XX, WC, QL, XS, XL and JL interpreted the data. XX, XS and XL helped in manuscript preparation.

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