

Maternal and post-weaning high-fat, high-sucrose diet modulates glucose homeostasis and hypothalamic *POMC* promoter methylation in mouse offspring

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Received: 6 February 2015 / Accepted: 23 April 2015 / Published online: 5 May 2015
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Abstract Substantial evidence demonstrated that maternal dietary nutrients can significantly determine the susceptibility to developing metabolic disorders in the offspring. Therefore, we aimed to investigate the later-life effects of maternal and postweaning diets interaction on epigenetic modification of the central nervous system in the offspring. We examined the effects of dams fed a high-fat, high-sucrose (FS) diet during pregnancy and lactation and weaned to FS diet continuously until 32 weeks of age. Then, DNA methylation and gene expressions of hypothalamic proopiomelanocortin (*POMC*) and melanocortin receptor 4 (*MC4R*) were determined in the offspring. Offspring of FS diet had heavier body weight, impaired glucose tolerance, decreased insulin sensitivity and higher serum leptin level at 32-week age ($p < 0.05$). The expression of *POMC* and *MC4R* genes were significantly increased in offspring exposed to FS diet during gestation, lactation and into 32-week age ($p < 0.05$). Consistently, hypomethylation of *POMC* promoter in the hypothalamus occurred in the FS diet offspring ($p < 0.05$), compared with the C group. However, no methylation was detected of *MC4R* promoter in both the two groups. Furthermore, *POMC*-specific methylation (%) was negatively associated with glucose response to a glucose load ($r = -0.273$, $p = 0.039$). Maternal and post-weaning high-fat diet predisposes the offspring for obesity, glucose intolerance and insulin resistance in later life. Our

findings can advance our thinking around the DNA methylation status of the promoter of the *POMC* and *MC4R* genes between long-term high-fat, high-sucrose diet and glucose homeostasis in mouse.

Keywords High-fat · High-sucrose diet · Glucose homeostasis · Hypothalamus · DNA methylation · Offspring

Introduction

The developmental origins of health and disease (DOHaD) hypothesis proposes that exposures during early life may play a critical role in determining the risk of developing metabolic diseases in adulthood (Skogen and Overland 2012). Substantial epidemiological studies and animal experiments demonstrated that maternal malnutrition including both overnutrition and undernutrition can significantly increase the susceptibility to some metabolic disorders such as obesity, insulin resistance and diabetes mellitus in the adult offspring, which can be further exacerbated by the postweaning malnutrition (Painter et al. 2005; Elahi et al. 2009; Curhan et al. 1996; Carone et al. 2010). Previous studies have indicated that a maternal high fat diet and/or a postnatal weaning diet rich in fat can increase susceptibility to obesity, glucose intolerance and insulin resistance in the adult offspring (Zhang et al. 2009; Yokomizo et al. 2014; McKay et al. 2014).

However, the molecular mechanisms underlying the interaction between nutrition and metabolic disorders in offspring has not been clearly understood. The hypothesis that epigenetic mechanisms may link such nutritional disturbances with the risks of aforementioned diseases has been widely accepted in recent years (Jang and Serra 2014). Epigenetics can be defined as the study of heritable changes in gene expression without any alterations in the DNA sequence and it could be

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inherited between generations steadily (Holliday 2006). Epigenetic modifications play a significant role in regulating tissue specific gene expressions and hence alterations in these processes may induce long-term changes in gene function and metabolism which persist throughout the lifecourse (Gluckman et al. 2009). DNA methylation was the first recognized and the most well-characterized epigenetic modification (Holliday and Pugh 1975).

Previous studies have indicated that a maternal high fat (HF) diet and/or a postnatal weaning diet rich in fat can increase susceptibility to obesity, glucose intolerance and insulin resistance in the adult offspring (Yokomizo et al. 2014). Most peripheral organs including liver, adipose tissue, pancreas, skeletal muscle and brain appear to be imprinted by these early disturbances (Warner and Ozanne 2010). However, investigations into the later-life effects of maternal and postweaning diets interaction on epigenetic modification of the brain like hypothalamus in the offspring are limited. In the recent years, it is increasingly apparent that the brain plays a central role in glucose homeostasis (Vogt and Bruning 2013). Especially, it is important given the central role of neuropeptides expressed in neurons within nuclei located in the mediobasal hypothalamus which can keep balance between food intake and energy expenditure. On the one hand, orexigenic neuropeptides, such as neuropeptide Y (*NPY*) and agouti-related protein (*AgRP*), increase food intake and body weight. For counter regulation, anorexigenic neuropeptides, such as proopiomelanocortin (*POMC*), α -melanocyte-stimulating hormone (α -*MSH*) and melanocortin-4 receptor (*MC4R*), mediate satiety and increase energy expenditure, thus lose weight (Marco et al. 2013; Berglund et al. 2014). Some studies have focused on the effect of high fat diet and/or maternal obesity on hypothalamic neuropeptides that affect food intake. It indicates increased (to varying extents) expressions of *NPY*, *AgRP*, *POMC* and *MC4R* in the offspring (Chen and Morris 2009; Muhlhausler et al. 2006; Vucetic et al. 2010). Furthermore, epigenetic mechanisms have been associated with long-term programming of gene expression after various in utero insults. It indicated global and gene-specific like dopamine reuptake transporter (*DAT*), μ -opioid receptor (*MOR*) and preproenkephalin (*PENK*) promoter DNA hypomethylation in the brains of offspring from dams that consumed the high fat diet (Vucetic et al. 2010). Another study demonstrated a long-term effect of maternal high fat diet on CpG methylation of the *Pomc* promoter in the offspring, which was not reprogrammed by standard chow from weaning (Marco et al. 2014).

However, little information is known about the long-term effects and epigenetic modification of the central nervous system of long-term overnutrition including maternal and postweaning high-fat, high-sucrose (FS) diet. This long-term FS diet mice model is particularly important in the light of the increasing consumption of refined foods with a high glycaemic index and fat content among men and women

(Agency 2006). Given the interdependent relationship among epigenetic modifications, diets and glucose homeostasis, our objective was to explore the effect of maternal and postweaning diet interaction on food intake, body weight, glucose metabolism, hypothalamic gene expression of feeding-related neuropeptides and DNA methylation status in the offspring.

Materials and methods

Animals

Seven-week-old C57BL/6 J mice were obtained from the Institute of Laboratory Animal Science, Chinese Academy of Medical Sciences and Peking Union Medical College (Beijing, China, SCXK-2013-0107). The animals were maintained under controlled conditions (room temperature at 22 ± 2 °C; 12-h light/dark cycle). Food and water were provided ad libitum throughout the study period. All procedures were approved by the animal care and use committee of the Peking Union Medical College Hospital (Beijing, China, MC-07-6004) and were conducted in compliance with the Guide for the Care and Use of Laboratory Animals, 8th ed, 2011.

Experimental design and diets

Before mating, all the mice were fed with the control (C) diet for 1 week for adaptation. Mating was performed by housing females with adult males fed with C diet for 3 days. Females were checked daily for postcopulatory plugs, and the presence of a plug in the morning after mating was taken as 0.5 day of pregnancy. Then, the pregnant mice were randomly fed on FS diet or C diet during pregnancy and lactation. The purified FS diet was composed of 58 % of energy as fat, 25.6 % carbohydrates and 16.4 % protein. Both the C diet and FS diet were produced by Research Diets (New Brunswick, NJ, USA) and the ingredient compositions were shown in Table 1. At day 1 after birth, the litter sizes of both the FS and C groups were standardized to six pups, to ensure no litter was nutritionally biased. All offspring were weaned at 3 weeks of age. At weaning, the male offspring from dams fed on C diet were weaned C diet and the offspring from dams fed on FS diet weaned to FS diet for the following 29 weeks. The specific study design is shown in Fig. 1a.

Exsanguination and sampling

At the end of the experimental period (32 weeks of age), all mice ($n=10$ per group) from five different litters ($n=2$ per litter) were sacrificed. Blood samples were collected from the intraorbital retrobulbar plexus after 10-h fasted anesthetized mice. Whole brains of offspring were quickly removed, snap frozen on top of dry ice, and then stored at -80 °C for further analysis. The hypothalamus were dissected separately

Table 1 Ingredient compositions of the high-fat and control diets

Ingredient	C diet		HF diet	
	g	kcal	g	kcal
Casein	200	800	228	912
Amino acid	3	12	2	0
Corn Starch	397	1590	0	0
Maltodextrin	132	528	170	680
Sucrose	100	400	175	700
Soybean Oil	70	630	25	225
Choline Bitartrate	2.5	0	2	0
Mineral	35	0	40	0
Vitamin	10	40	10	40
Coconut Oil	0	0	333.5	3001.5
kcal/g	3.9		5.56	

Diet abbreviations: C Control, HF, High-fat

on ice according to the method previously (Vucetic et al. 2010; Cho et al. 2013).

Food intake and body weight

The offspring had free access to food. 72-h food intake was measured biweekly from 3 to 32 weeks old and their average food intake (g) was calculated by carefully collecting and weighing the remaining food including the spillage in the cage, and then subtracting the total remaining food from the known amount given before. The total energy (kJ) consumed by the

offspring was calculated by multiplying the food intake (g) by the energy information (kcal/g) according to the nutritional composition of the FS and C diets fed to mice. Body weight was measured at birth, at weaning and per week post-weaning for each mouse (Fig. 1b) (Cho et al. 2013; Rajia et al. 2010).

Intraperitoneal glucose tolerance test (IPGTT)

IPGTT of the offspring was performed at an interval of about 8 weeks (Fig. 1b). Specifically, after the mice had fasted for 10 h, glucose (2.0 g/kg body weight) was intraperitoneally administered. BG (Blood Glucose) levels were measured before injection (time 0) and at 30, 60, and 120 min after injection from a tail bleed using a Contour TS glucometer (Bayer, Japan). The area under the curve (AUC) of intraperitoneal glucose tolerance test (IPGTT) was calculated by the trapezoid formula: $AUC = 0.5 \times (BG \text{ 0 min} + BG \text{ 30 min}) / 2 + 0.5 \times (BG \text{ 30 min} + BG \text{ 60 min}) / 2 + 1 \times (BG \text{ 60 min} + BG \text{ 120 min}) / 2$ (Purves 1992).

Blood biochemistry and hormone analysis

At 32 weeks of age of the mice offspring, blood samples were collected after euthanasia. The blood samples were centrifuged at 4000 g for 10 min and serum was stored in aliquots at -80°C . Serum insulin concentration was measured using the Mouse Ultrasensitive Insulin ELISA kit from ALPCO Diagnostics (80-INSMSU-E01, Salem, NH). The intra-assay coefficient of variation for insulin measurements was 4.2 %. Insulin sensitivity was assessed using the homeostasis model assessment of insulin resistance (HOMA-IR). The HOMA-IR was calculated as fasting insulin concentration ($\mu\text{U/mL}$) \times fasting glucose concentration (mmol/L) / 22.5. Fasting serum leptin concentrations in mice were determined using mouse leptin ELISA kit by following the manufacturer's instructions (EZML-82 K, Linco, St. Charles, MO). The inter- and intra-assay coefficients of variation were 3.0–4.6 and 1.1–1.8 %, respectively.

Hypothalamic gene expression

Total RNA from the hypothalamus was extracted using TRIzol reagent (Life Technologies Inc., Carlsbad, CA, USA) according to the manufacturer's instructions. The concentrations of total RNA were measured using a Nanodrop (ND-1000, NanoDrop products, Bancroft Building, Wilmington, DE, USA). All samples had high quality and showed no signs of DNA contamination or RNA degradation. The RNA samples were immediately frozen and stored at -80°C until further analysis. Prior to PCR, each total RNA was processed with Rnase-free Dnase (Qiagen, New York, USA). Then, the RNA was reverse transcribed by 1 μg of total RNA from each sample using the Power cDNA Synthesis kit (A3500, Promega BioSciences LLC, Sunnyvale, CA, USA). The cDNA (2 μL) was amplified with a SYBR[®] Green PCR

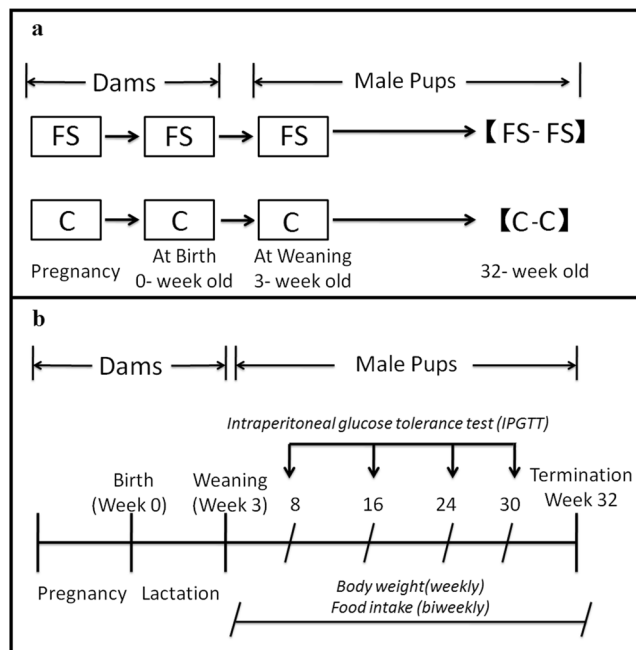


Fig. 1 Schematic sketch of the study design (a) and protocol (b) for male offspring. Diet abbreviations: C, Control; FS, High-fat, high-sucrose. Dam and pup diets denoted before and after the dashed line, respectively

Master Mix (RR420A, Takara Bio Inc., Otsu, Shiga, Japan) in a final volume of 20 μ L. Changes in gene expression were calculated using relative quantitation of a target gene against endogenous, unchanged β -actin standard. Primers were constructed using the NCBI online database (www.ncbi.nlm.nih.gov) and Primer 3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). The sequences of the primers are as following: *POMC*, forward 5'-CGACAGGCAGGAGACTGAAC-3' reverse 5'-CGCAGAGAAACGAGGGTTTG-3'; *MC4R*, forward 5'-TGAACTTCTGAGAGGCTGCG-3' reverse 5'-TTCTCGTTGACCAGTCTGC-3'; β -actin, forward 5'-TGTTACCAACTGGGACGACA-3' reverse 5'-GGGGTGTGTAAGGTCTCAA-3'. The reaction production was accurately measured in the exponential phase of amplification by the ABI prism Vii7 Sequence Detection System (ABI Prism[®] Vii7, Applied Biosystems, Life Technologies). The reaction conditions consisted of an initial activation step (30 s at 95 °C) and a cycling step (denaturation for 5 s at 95 °C and annealing for 34 s at 60 °C for 40 cycles). All reactions were carried out with three biological replicates, and each analysis consisted of three technical replicates and a melting curve analysis that was performed after each run. The relative expression levels were quantified by the $2^{-\Delta\Delta C_t}$ method, with the relative fold changes normalized to the control values (Livak and Schmittgen 2001).

Bisulfite sequencing PCR

The methylation status of CpG sites within the *POMC* and *MC4R* promoters were examined by bisulfite sequencing using genomic DNA extracted from hypothalamus tissues using a E.Z.N.A.[®] Tissue DNA Kit (Omega Bio-tek, Norcross, GA, U.S.). DNA samples were treated with sodium bisulfite, using EZ DNA Methylation Kit[™] (Zymo Research, HiSS Diagnostics, Germany) according to the manufacturer's instructions. The quality of the DNA after bisulfite reaction was evaluated using an agarose gel to verify that the DNA was intact and not nicked. The DNA methylation status of the *POMC* and *MC4R* promoters were evaluated as described previously (Marco et al. 2013). Briefly, *POMC* and *MC4R* promoter areas were amplified with PCR using the following primers: *POMC*, forward 5'-GATTGGTTTTTGGGAGATTT-3' reverse 5'-ATTTCAAACCTTAAACAATTCCCT-3'; *MC4R*, forward 5'-TTTAAAATTTGGAAAGGAAAATTT-3' reverse 5'-TACTAAAAACAAAATCAAAAACAAC-3'. PCR amplification of genomic fragment of *POMC* and *MC4R* promoters were performed using BIOTAQ DNA Polymerase (Bioline USA Inc, Taunton, MA, USA). The PCR cycles were as follows: an initial incubation at 95 °C for 5 min, 40 cycles including denaturation at 95 °C for 30 s, annealing at 58 °C for 30 s, and elongation at 72 °C for 45 s. The PCR products were separated on 1.5 % agarose gel followed by gel extraction with QIAquick Gel

Extraction Kit (QIAGEN, Hilden, Germany) and cloning into PGEMT-easy vectors (Promega, Madison, WI) according to the manufacturer's instructions. PGEMT-easy vectors were multiplied using JM109 competent *Escherichia coli* cells using standard procedures and then purified from the bacteria with QIAprep Spin Miniprep Kit (QIAGEN). There are a minimum of 20 positive bacterial clones in each sample. Clones with complete sequencing data and a minimum of 95 % bisulfite conversion were included in subsequent analyses. Figure generation, sequence analysis and quality control were performed using BiQ Analyzer software.

Statistical analysis

The data were normally distributed and were expressed as mean \pm standard deviation (SD). Body weight and IPGTT were analyzed by two-way analysis of variance (ANOVA) followed by Bonferroni's post hoc test. Group differences in fasting blood glucose, insulin, HOMA-IR, leptin levels and mRNA expression levels were analyzed by Student's t-tests. Differences in methylation patterns were analyzed by chi-square analyses. Correlation analyses between DNA methylation status and glucose response to a glucose load were performed by using Spearman's correlation analyses. *P* value < 0.05 were considered to be statistically significant. All statistical analysis was calculated with SPSS 21.0 (SPSS, Inc., Chicago, IL, USA).

Results

Effects of diets on body weight and food intake in offspring

There was no significant difference of birth weight and body weight at weaning between the C-C and FS-FS groups (*p*>0.05). FS-FS offspring had higher body weight than C-C group from 16 to 32 weeks of age (*p*<0.001) (Fig. 2). No

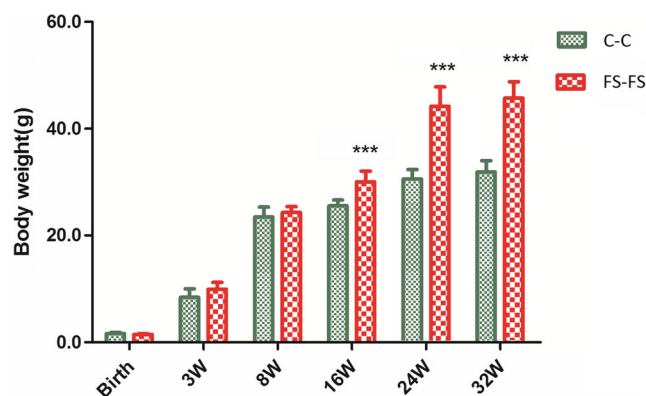


Fig. 2 Body weight for male offspring from birth to 32 weeks. Data represents as mean \pm S.D. (*n*=10, in each group). ****p*<0.001 FS-FS vs the C-C group. Diet abbreviations: C, Control; FS, High-fat, high-sucrose. Dam and pup diets denoted before and after the dashed line, respectively. W, Week

difference in food consumption (g/day) was observed in the two groups throughout the experiment (data not shown). Because FS diet contains more calorie than C diet (5.56 vs 3.9 kcal/g), as a result, FS offspring consumed significantly more kcal per day compared with C offspring.

Effects of diets on glucose response in offspring from 8- to 30-week old

Offspring of FS-FS group had impaired glucose tolerance from 8-week old. More specifically, the blood glucose levels of the male offspring at 8-week old from the FS-FS group were significantly higher at 30 min ($p<0.001$) and 60 min ($p<0.01$) after intraperitoneal glucose administration compared with those of the C-C group. AUC of IPGTT in FS-FS group was larger than C-C group ($p<0.05$). Then the impaired glucose tolerance was exacerbated in the offspring of FS-FS group

during the period from 8 to 30 weeks. At 30-week old, the FS-FS offspring were significantly higher at 30 min ($p<0.01$), 60 min ($p<0.001$) and 120 min ($p<0.001$) compared with those of the C-C group. Consistently, AUC of IPGTT in FS-FS group was significantly larger than C-C group ($p<0.001$) (Fig. 3).

Effects of diets on insulin sensitivity and serum hormones in offspring

To determine insulin sensitivity of the offspring, the levels of fasting blood glucose and insulin were determined. No significant difference of insulin level was observed between the FS-FS and C-C groups ($p>0.05$). However, both fasting blood glucose and HOMA-IR of the offspring were significantly higher in FS-FS group at 32 weeks of age ($p<0.01$ and $p<0.05$, respectively). Because leptin is one of the most important peripheral hormones which can regulate the activity of

Fig. 3 Glucose tolerance test for male offspring from 8 to 30 weeks. Data represents as mean \pm S.D.($n=10$, in each group). * $p<0.05$, ** $p<0.01$, *** $p<0.001$ FS-FS vs. the C-C group. Diet abbreviations: C, Control; FS, High-fat, high-sucrose. Dam and pup diets denoted before and after the dashed line, respectively

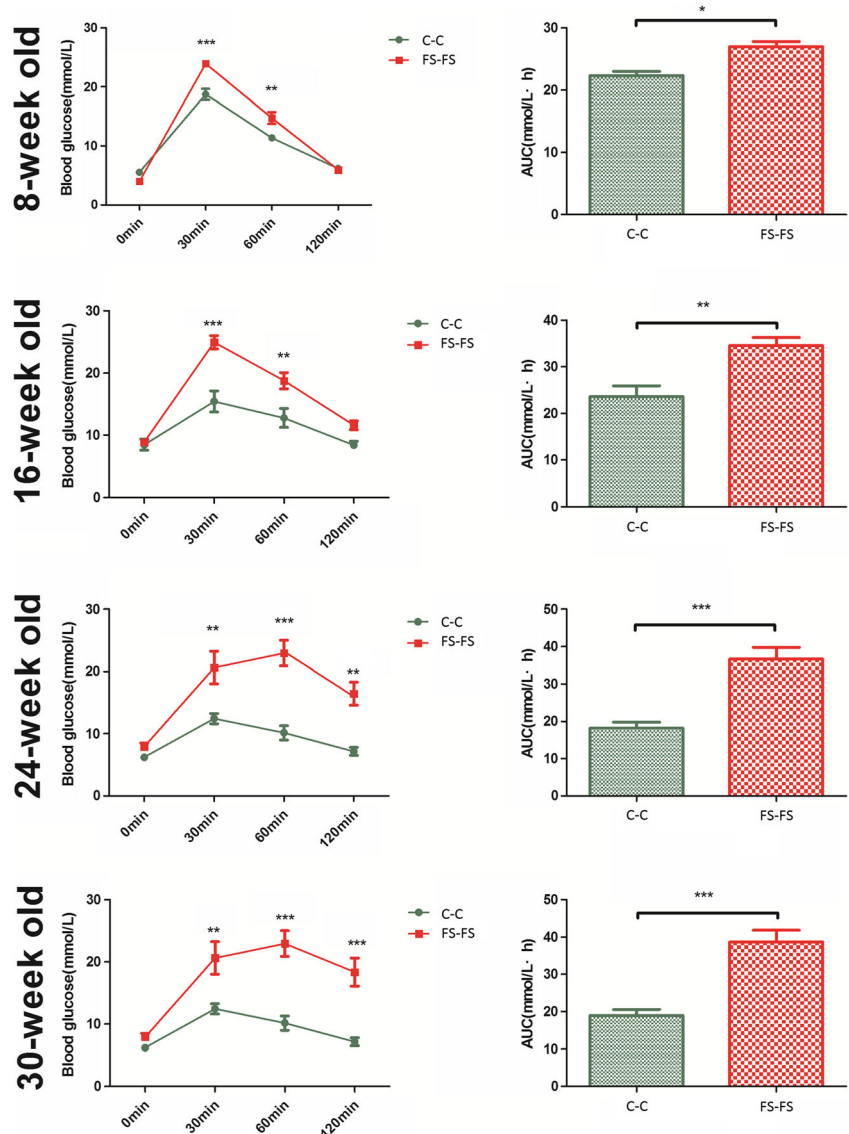
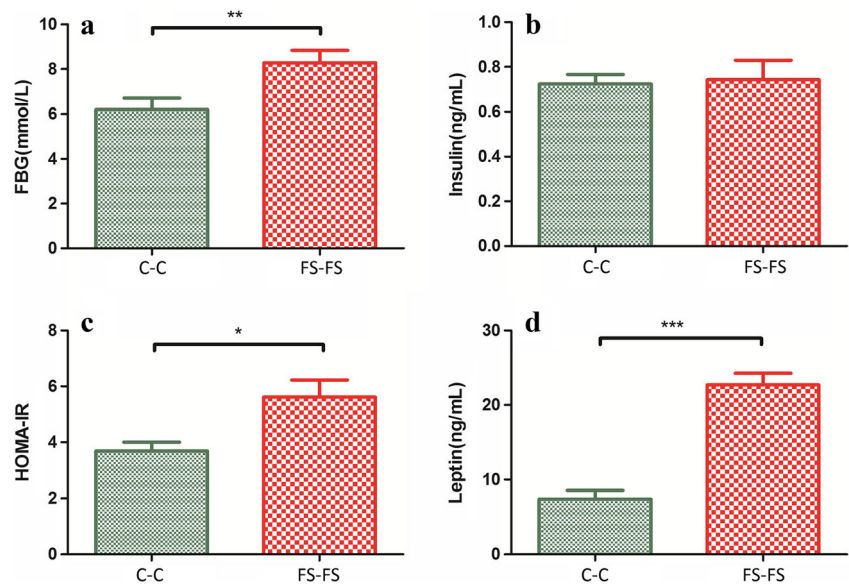


Fig. 4 The comparison of biochemical parameters of mice offspring at 32 weeks. Data represents as mean \pm S.D. ($n=10$, in each group). FBG (a), serum insulin (b), HOMA-IR (c) and serum leptin (d). * $p<0.05$, ** $p<0.01$, *** $p<0.001$ FS-FS vs. the C-C group. Diet abbreviations: C, Control; FS, High-fat, high-sucrose. Dam and pup diets denoted before and after the dashed line, respectively. FBG: Fasting blood glucose



neurons in hypothalamus, we further examined the serum leptin in the male offspring. Leptin also can reflect the fat storages in the body. Since the FS group offspring were heavier, as expected, the leptin level was significantly higher in FS-FS offspring than C-C offspring ($p<0.001$) (Fig. 4).

Effects of diets on hypothalamic gene expressions in offspring

To further assess potential perturbations in the neuroendocrine control of body weight and energy homeostasis, we examined *POMC* and *MC4R* gene expressions of hypothalamus in the offspring. As expected, the expression of *POMC* and *MC4R* in the hypothalamus both significantly increased in the FS-FS offspring, compared with the C-C group ($p<0.01$) (Fig. 5).

Effects of diets on hypothalamic gene methylation in offspring

Since expression levels of *POMC* and *MC4R* were significantly affected by the FS diet, then, we examined the epigenetic mechanisms which regulate the altered expressions of *POMC* and

MC4R genes, concentrating on the CpG methylation of their promoters, respectively. A 329 base-pair fragment of the *POMC* promoter that contains 8 CpG sites (Fig. 6b) and a 223 base-pair fragment of the *MC4R* promoter that contains 10 CpG sites were investigated. The average methylation of 8 CpG sites in the promoter region of the *POMC* was found to be significantly decreased in the FS-FS offspring, compared with the C-C group ($37.5 \pm 1.7\%$ vs. $46.3 \pm 3.5\%$, $p=0.03$) (Fig. 6a and b). Unexpectedly, no methylation was detected of *MC4R* promoter in all the samples in both the FS-FS and C-C groups (data not shown).

Correlation analyses

To evaluate the hypothesis that metabolic alterations due to overfeeding could be responsible for modified promoter methylation in FS diet offspring mice, correlation analyses between *POMC*-specific methylation (%) and glucose response to a glucose load were performed by using Spearman's correlation analyses. The glucose response to a glucose load was assessed by AUC of IPGTT. Remarkably, it indicated that *POMC*-specific methylation (%) was negatively associated with AUC of IPGTT ($r=-0.273$, $p=0.039$) (Fig. 7).

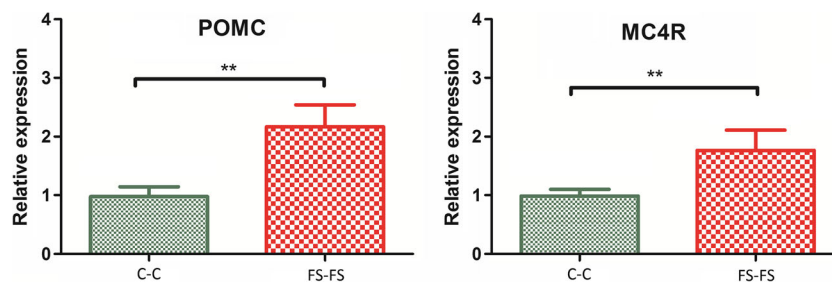


Fig. 5 Relative expression of genes in the hypothalamus in the male offspring at 32 weeks. Data represents as mean \pm S.D. ($n=10$, in each group). ** $p<0.01$ FS-FS vs. the C-C group. *POMC*:

proopiomelanocortin, *MC4R*: melanocortin-4 receptor, Diet abbreviations: C, Control; FS, High-fat, high-sucrose. Dam and pup diets denoted before and after the dashed line, respectively

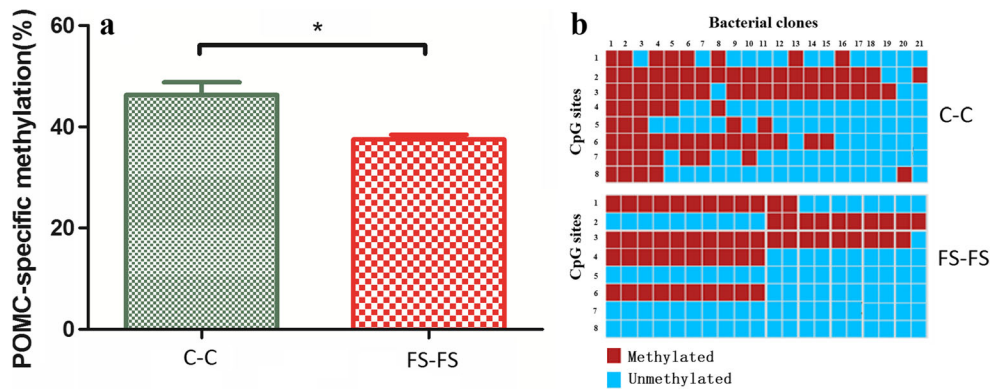


Fig. 6 *POMC*-specific methylation in the offspring. Data represents as mean ± S.D.(*n*=10, in each group). *POMC*-specific methylation(%) (a), Bacterial clones of *POMC*-specific methylation (b). **p*<0.05 FS-FS vs.

the C-C group. *POMC*: proopiomelanocortin, Diet abbreviations: C, Control; FS, High-fat, high-sucrose. Dam and pup diets denoted before and after the dashed line, respectively

Discussion

Our study was designed to examine the effect of maternal and post-weaning FS diet interaction on hypothalamic gene expression of feeding-related neuropeptides, DNA methylation status and glucose homeostasis in the offspring. Similar to other studies (Page et al. 2009; Bruce et al. 2009), we have shown that offspring from FS dams fed on FS diet from weaning to later life had heavier body weight, impaired glucose tolerance, insulin resistance at 32 weeks of age. In our present study, it also indicated hyperleptinemia in the offspring of FS diet. It has been reported that plasma leptin levels were higher in obese subjects, compared with lean individuals (Schwartz et al. 2000). Thus, our study indicated that maternal and post-weaning high-fat diet interaction could contribute to obesity, abnormal glucose metabolism and impaired response of the brain to the leptin signal in later-life of the offspring.

The anorexigenic melanocortinergic system has been suggested to be the best-characterized hypothalamic circuit involved in the regulation of energy balance (Watez et al. 2013). In the hypothalamus, *POMC* is one of the major

anorexigenic neurohormone which regulates food intake and body weight via *MC4R* in the paraventricular nucleus (Plagemann et al. 2009). Our present study indicated that the expression of *POMC* and *MC4R* in the hypothalamus both significantly increased in the FS-FS offspring. A previous studies in Sprague–Dawley rats also indicated that *POMC* expression and serum leptin were also significantly increased in offspring exposed to excess saturated fat during gestation and into adulthood (Page et al. 2009). Also, the offspring of Wistar rats exposed to prenatal undernutrition and postnatal high-fat diet exhibited increased food intake and fat mass, which correlated with an increase in hypothalamic *POMC* and elevations in circulating levels of both plasma leptin and insulin levels (Ikenasio-Thorpe et al. 2007). Leptin is one of the most important peripheral circulating hormones which can regulate the hypothalamic expression of anorexigenic neuropeptides. Consistent to our study, the expression, synthesis and release of *POMC* are physiologically increased in the presence of elevated leptin levels (Cone 2005).

Furthermore, we investigate the epigenetic mechanisms of *POMC* and *MC4R* expressions affected by the maternal and postweaning FS diet. It indicated that the methylation of *POMC* promoter in the hypothalamus was significantly decreased in the FS diet offspring. However, no methylation was detected of *MC4R* promoter in both the FS-FS and C-C groups. It is widely acknowledged that hypermethylation of genes may reduce gene expression and repress transcription, while gene hypomethylation is related to transcriptional activation, thus increase gene expressions (Reik and Dean 2001). In our present study, decreased methylation of *POMC* gene promoter was consistently related with increased gene expression. However, such correlation was not been observed in *MC4R* gene which indicated that the increased expression of *MC4R* gene may be modulated by other regulating factors. As previously reported, there was marked hypomethylation (62 % decrease) of the *POMC* gene promoter in the offspring of maternal undernutrition (Stevens et al. 2010). Although

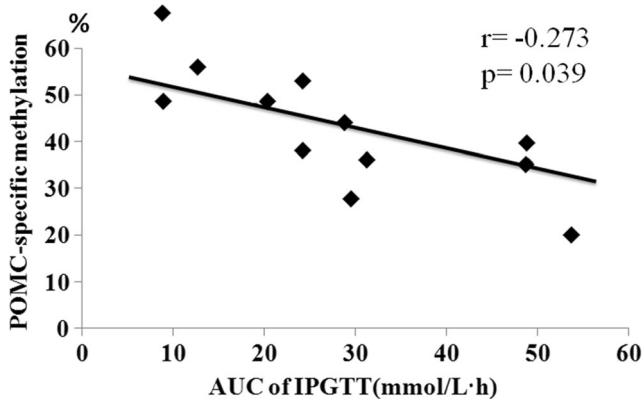


Fig. 7 Correlation analyses between *POMC*-specific methylation (%) and glucose response to a glucose load. *POMC*: proopiomelanocortin, AUC: area under the curve, IPGTT: intraperitoneal glucose tolerance test

studies have shown that epigenetic modifications occur mainly during early development, recent evidence from animal experiments showed that epigenetic modifications may continue throughout the lifespan (Roth 2012). Similarly, our study also indicates that maternal malnutrition can regulate epigenetic modifications of the mice offspring at 32 weeks of age. This provides evidence for epigenetic modifications that could act as a programming mechanism for the hypothalamic *POMC* gene and predispose hypothalamic feeding centres to abnormal glucose metabolism later in life. Of interest, our present study showed *POMC*-specific methylation (%) was negatively associated with glucose response to a glucose load. It indicated *POMC*-specific methylation might be importantly epigenetic modification in regulating glucose metabolism in the offspring. In view of the reversibility of epigenetic mechanisms, *POMC*-specific methylation may be utilized as a new theoretical foundation of diabetes prevention and intervention.

In conclusion, our study demonstrates that maternal and post-weaning overfeeding predisposes the offspring for obesity, glucose intolerance and insulin resistance in later life. Furthermore, these data identify that an epigenetic modification (promoter region hypomethylation) may be as a potential mechanism for increased long-term expression of hypothalamic *POMC* gene in the offspring.

Acknowledgments This project was supported by National Natural Science Foundation of China (No. 81170736), National Natural Science Foundation for Young Scholars of China (No. 81300649), National Key Program of Clinical Science, and Peking Union Medical College Hospital and Postgraduate Student Innovation Fund in Peking Union Medical College (No. 2013-1002-33).

Conflict of interest The authors declare that there is no conflict of interest.

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