Original article

MOLECULAR METABOLISM

High-fat diet reprograms the epigenome of rat spermatozoa and transgenerationally affects metabolism of the offspring

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ABSTRACT

Objectives: Chronic and high consumption of fat constitutes an environmental stress that leads to metabolic diseases. We hypothesized that high-fat diet (HFD) transgenerationally remodels the epigenome of spermatozoa and metabolism of the offspring.

Methods: F0-male rats fed either HFD or chow diet for 12 weeks were mated with chow-fed dams to generate F1 and F2 offspring. Motile spermatozoa were isolated from F0 and F1 breeders to determine DNA methylation and small non-coding RNA (sncRNA) expression pattern by deep sequencing.

Results: Newborn offspring of HFD-fed fathers had reduced body weight and pancreatic beta-cell mass. Adult female, but not male, offspring of HFD-fed fathers were glucose intolerant and resistant to HFD-induced weight gain. This phenotype was perpetuated in the F2 progeny, indicating transgenerational epigenetic inheritance. The epigenome of spermatozoa from HFD-fed F0 and their F1 male offspring showed common DNA methylation and small non-coding RNA expression signatures. Altered expression of sperm miRNA let-7c was passed down to metabolic tissues of the offspring, inducing a transcriptomic shift of the let-7c predicted targets.

Conclusion: Our results provide insight into mechanisms by which HFD transgenerationally reprograms the epigenome of sperm cells, thereby affecting metabolic tissues of offspring throughout two generations.

Keywords Epigenetics; Obesity; Spermatozoa; DNA methylation; microRNA

1. INTRODUCTION

Obesity is a metabolic disorder caused by a chronic imbalance between energy intake and expenditure that increases the risk of developing type 2 diabetes, cardiovascular disease and malignancies. Epidemiological data showing that children of obese parents are at higher risk of developing metabolic disease later in life support the notion that a heritable component contributes to obesity and obesity-related traits [1]. Several lines of evidence reveal that the nutritional status of the parents can affect the metabolic phenotype of the offspring [2–4]. Although cultural factors could be involved in this phenomenon in humans, animal studies support the notion that gametes of obese parents contain an environmentally-induced message that influences glucose and energy metabolism.

Epigenetic inheritance describes transmission of a phenotype from one generation to the next that is not carried by the DNA code itself. By modulating the early stages of embryonic development, epigenetic information contained in the gamete has the potential to alter the phenotype of an offspring after birth [5,6]. Epigenetic modifications such as DNA methylation and hydroxymethylation, histone modifications and non-coding RNA expression modulate the access of the transcription machinery to the chromatin, the repression of transposable elements or the regulation of mRNA targets, thereby regulating gene expression in time and space [7]. Most of the epigenetic marks are erased upon gametogenesis and fertilization to allow for *de novo* programming of the embryo, but specific epigenetic marks escape reprogramming and are potential carriers of environmentally-induced information to program phenotypes from one generation to the next.

Animal models of paternal epigenetic inheritance have been used to investigate the possible transfer of epigenetic information from one generation to next in order to exclude any confounding influence of gestational effects on somatic tissues during embryological development. Using these types of models, the nutritional status of the father has been reported to impair metabolism in the offspring, which strongly implicates that the spermatozoa carry information that is influenced by dietary factors [2,8,9]. However the nature and influence

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of the gametic epigenetic signature on metabolic features such as glucose metabolism and the predisposition towards developing obesity is unknown.

Here, we determined how paternal diet affected the epigenetic signature of spermatozoa and the metabolic function of the offspring over two generations. We provide evidence that a paternal high-fat diet induces a robust, sex specific disturbance in glucose metabolism and energy homeostasis within two following generations. We identified common altered DNA methylation signatures and small non-coding RNA expression profiles in the spermatozoa from F0 and F1 males, providing a mechanism for the propagation of metabolic dysfunction to the next generation. The predicted pathways affected by these epigenetic marks were perturbed in metabolic tissues of the offspring. Our results support the existence of transgenerational reprogramming of the gametic epigenome and inheritance of diet-induced metabolic dysfunction throughout two generations.

2. MATERIAL AND METHODS

2.1. Animal care

Male and female Sprague–Dawley founder rats were obtained from Charles River Laboratories (Germany). At 4 weeks of age, F0 male breeders were fed either with a high-fat diet (HFD; TD.88137/TD.08811, 42/45% energy from fat, Harlan Laboratories, USA) or a control chow diet (R36-Lab For Lactamin, Sweden) for 12 weeks (Figure 1A). Water and food were provided *ad libitum*.

To obtain the F1 offspring, one F0 male breeder was housed together with a 12 week-old female rat, with free access to chow diet from 7:00 to 18:00, for 8 consecutive days. Male F0 breeders returned to their respective cages with the original diet, while F0 female rats consumed only chow diet throughout mating, gestation and lactation. To assure that there were no differences between female breeders, body and tissue weight, as well as blood glucose levels were evaluated (Table T2). To control for postnatal nutrition, litter sizes were standardized to 12 pups at day-1 after birth. At day-3, all pups were weighed and at day-5 litter sizes were reduced to 8 pups (4 males and 4 females). The pups were weaned from mothers at 21 days of age, and male or female siblings were housed together and fed a chow diet. At week 10 of age, one of the F1 siblings was subjected to a HFD for 12 weeks, and another sibling was kept on chow diet (control group) (Figure 1A).

To generate F2 offspring, only chow-fed F1 male rats were mated with 12 week-old females from an independent line. During mating, one F1 male was housed with one female for 10 consecutive days. Exactly as for F1, the F2 offspring litter size was measured and pups were weaned to a chow diet at 21 days of age. At week 10, a subgroup of the F2 offspring was also subjected to a HFD treatment for 12 weeks (Figure 1A). Body weight and food intake of F0 male breeders and F1 and F2 offspring were monitored weekly by collecting and weighing offered and remaining food.

All the rats were maintained under a temperature-controlled environment and 12:12-h light:dark cycle at the animal facility at the Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden. All the experimental procedures were approved by the animal ethical committee, Stockholm North, and conducted in accordance with regulations for protection of laboratory animals. All the experiments were repeated using two independent cohorts of F0 breeders and F1 and F2 offspring. Statistical analysis related to transgenerational effects was made considering one sibling per litter. If more than one sibling per litter was used, the number is stated in the figure legend.

2.2. Intraperitoneal glucose tolerance test (ipGTT)

The ipGTT was performed in the F0 male breeders and F1 and F2 offspring after 12 weeks under the assigned diet (control or HFD). Rats underwent an overnight fast (15 h) prior to the ipGTT. On the day of the experiment, the animals received an intraperitoneal injection of 2 g glucose/kg body weight. The blood collected from the tail was used to measure glucose levels at basal (0), 15, 30, 60, 90 and 120 min after glucose injection. Blood glucose levels were measured using Accu Check Advantage Glucometer (Roche) and insulin levels were measured using an ELISA kit (see details below).

2.3. Intraperitoneal insulin tolerance test (ipITT)

The ipITT was performed in the F0 male breeders after 12 weeks on the assigned diet. On the day of the experiment, food was removed from the cages 3 h prior to the ipITT. Insulin (Actrapid, Novo Nordisk; 1 U/kg) was administrated intraperitoneally. The blood collected from the tail was used to measure the blood glucose levels at basal (0), 10, 30, 45, 60 and 90 min after insulin injection. A different subset of animals underwent the glucose tolerance test or the insulin tolerance test to avoid stress response associated to injections and blood sampling.

2.4. Endpoint

Rats were subjected to food deprivation from 4 h prior to the termination. Animals were anesthetized with sodium pentobarbital (100 mg/kg, ip). Blood was collected and the plasma was stored at −20 °C until use. Tissues were harvested, snap-frozen in liquid nitrogen and stored at −80 °C until use.

2.5. Blood analysis

Blood insulin and leptin levels were measured using the ultrasensitive rat ELISA kits (#90060 and #90040, respectively, Crystal Chem Inc., USA). Blood triglycerides and free fatty acid levels were determined by colorimetric assays (#ab65336 and #ab65341, Abcam, UK). The assays were performed according to manufacturer's instructions.

2.6. Pancreas optical projection tomography (OPT)

The entire pancreas from 5 day-old F1 female offspring was fixed in 4% paraformaldehyde (PFA) for 2 h at 4 °C, followed by a subsequent dehydration in methanol 33%, 66% and 100% for 15 min at each step. To quench the autofluorescence, tissues were incubated for 24 h in a methanol:DMSO:H2O2 (2:1:3) solution, followed by two washes in methanol for 30 min. Samples were brought to −80 °C for 1 h and brought back to room temperature 3 times to ensure that antigens in the deeper parts of the tissue were rendered accessible. Samples were rehydrated in Tris-Buffered Saline and Tween 20 buffer (TBST) 33%, 66% and 100% for 15 min at each step, and blocked in 10% rat serum in TBST for 24 h. Samples were incubated for 48 h with primary insulin antibody, followed by 48 h incubation with secondary antibody. Finally, tissues were mounted in 1% low melting agarose and dehydrated in 100% methanol for 24 h. Samples were washed in BABB solution (1:2 Benzyle alcohol, Benzyle benzoate) for 24 h, scanned, visualized and quantified in a stereomicroscope equipped for epifluorescence fluorochrome (Alexa 488, Alexa 594, Cy 3).

2.7. Isolation of motile spermatozoa

The *cauda epididymis* was dissected from the anesthetized animal and punctured in a Petri dish containing sperm isolation buffer (Earle's Balanced Salt Solution, 25 mM Hepes, 48.5 mM bovine serum albumin) pre-warmed to 37 °C. Samples were transferred to a 14 ml round bottom tube, overlaid with isolation buffer and subjected to a swim-up