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Research paper

Caloric restriction can affect one-carbon metabolism during pregnancy in the rat: A transgenerational model

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ABSTRACT

One-carbon metabolism is critical to pregnancy outcomes, because it determines the availability of nutrients involved in cell divisions and DNA methylation. The aim of this study was to analyze how 50% prenatal calorie restriction affected one-carbon metabolism in pregnant Wistar rats of the F0 to F2 generations. Mean choline ($p < 0.001$), betaine ($p < 0.001$), and S-adenosylmethionine (SAM) ($p < 0.05$) concentrations were respectively about 40%, 45%, and 20% lower in the F0_R (R – restricted diet) than in the F0_C (C – control diet). Homocysteine, S-adenosylhomocysteine (SAH), and trimethylamine oxide concentrations were unaffected. In the F1_R, the SAM-to-SAH ratio was 25% higher ($p < 0.05$) than in the F1_C. No differences between the C and R groups were observed in the F2 generation. The SAM concentrations in the F1_R were higher than in the F0_R and the F2_R ($p < 0.01$). The relative transcript levels of *Mat1a*, *Bhmt*, *Cbs*, *Pemt*, and *Mthfr* were only slightly affected by the diet, with changes of less than a factor of 2.0. *Cbs* activity in the F2_R was significantly higher than in the F2_C ($p < 0.001$). Food deprivation may affect one-carbon metabolism in pregnant rats, but it does not stimulate persistent metabolic changes that can be observed during the pregnancy of their progeny of the F1 or F2 generations.

1. Introduction

Pregnancy outcomes partly depend on the intrauterine environment, but the effects of prenatal stimuli can also be observed later in life [1]. These effects include altered metabolism and physiology, which affect the risk of chronic diseases [2–5]. This phenomenon has been called fetal programming [6]. Different animal models of fetal programming have been tested to date [7,8], but total calorie restriction (food deprivation) and protein restriction models are the most commonly used models in investigating dietary deficiencies [3]. Pregnancy leads to several metabolic changes and increased nutrient requirements to meet the needs of the developing fetus [9]. The overall availability of nutrients to the fetus depends on a combination of factors, including current dietary intake, maternal metabolic status, and the functioning of the placenta [10,11]. Moreover, in the event of a nutrient deficiency, the mother can mobilize nutrients from her own tissues to make up the deficit [12].

One-carbon metabolism occurs in the liver and is central to fetal programming, as it involves reactions that are crucial to the availability of folate and choline—two nutrients that play key roles in cell division and in the development of the fetus [13,14]. Secondly, the methyl groups for methylation reactions come from this cycle, with different methyltransferases using S-adenosylmethionine (SAM) as a methyl donor to produce S-adenosylhomocysteine (SAH). SAH is then converted to homocysteine (Hcy; see Fig. 1). A cell's ratio of SAM to SAH is referred to as its methylation potential. SAM formation is driven by methionine adenosyltransferase (MAT). Hcy can be metabolized in three ways: it may be methylated to methionine by betaine-homocysteine S-methyltransferase (BHMT), or alternatively by the simultaneous action of methionine synthase (MS) and MS reductase (MSR). The latter reaction requires 5-methyltetrahydrofolate (methyl-THF) to be provided by

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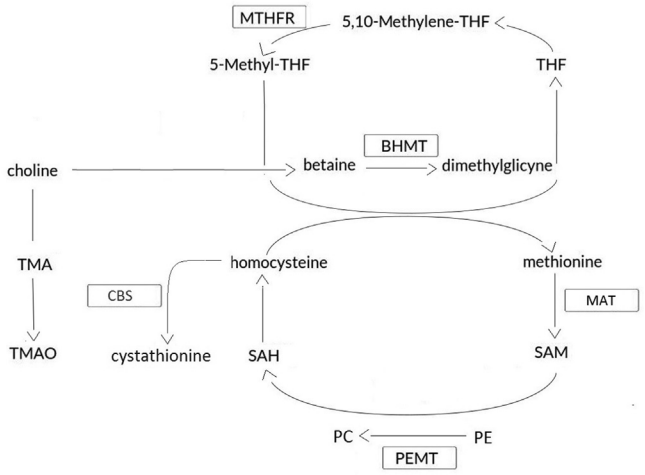

Fig. 1. Overview of one-carbon metabolism. Only the enzymes which genes were analyzed are shown.

methylenetetrahydrofolate reductase (MTHFR). Hcy can also be removed from the cycle through a transsulfuration pathway, the first step of which is catalyzed by cystathionine β-synthase (CBS) [15]. Choline metabolism is closely related to the metabolism of methionine and folate. For example, the betaine used in the methylation of Hcy can be derived from the oxidation of choline [16]. The flow of one-carbon metabolism depends on the availability of nutrients and the functioning of the enzymes involved in this cycle [15,17]. We have previously shown that the protein or folic acid content of the maternal diet may affect the transcription *of Cbs*, *Pemt*, and *Bhmt*, but does not determine Hcy concentrations in the rat [18].

The molecular mechanisms responsible for fetal programming are extremely complex, but epigenetics is most likely the bottom line [7,19]. Epigenetic mechanisms, such as chromatin remodeling, histone modifications, and DNA methylation, may affect gene expression, and thus phenotype, without affecting the sequence of nucleotides [20]. As mentioned above, methyl donors for DNA methylation are derived from one-carbon metabolism. Additionally, it has been proposed that the effects of developmental programming are transmitted transgenerationally, which means that exposure in early life can affect not only the F1 generation, but also future generations [21]. However, the data that might support these hypotheses are very limited [22,23].

We have hypothesized that prenatal total calorie restriction can alter one-carbon metabolism of pregnant rats and program this pathway in later life. We have also assumed that the differences between the progeny of normal-diet and calorie-restricted dams may be more pronounced during pregnancy. Since pregnancy is an additional challenge for the body, metabolic inefficiencies programmed by prenatal caloric restriction may be revealed during that period. The aim of this study was thus to analyze how a 50% prenatal calorie restriction affected one carbon metabolism in pregnant rats of the F0 to F2 generations. To this end, the expression of genes involved in one-carbon metabolism in the liver was analyzed. Blood and liver metabolite concentrations were also measured.

2. Material and methods

2.1. Animals and diets

This experiment was a part of a larger experiment [24]; for the purpose of this article, only relevant parts are mentioned. All experimental procedures were carried out in compliance with the international principles for laboratory animals, and the study protocol was approved by the local ethics committee (approval no. 37/2014). Healthy Wistar rats aged ten weeks (average weight 242.7 ± 15.8 g) were purchased from Charles River Laboratories (Germany). The rats were housed in individual cages on a 12-h light-dark cycle at a temperature of 20 ± 1 °C. After one week of acclimatization, 34 virgin female rats were mated with 15 males. Successful mating was confirmed by the presence of a vaginal plug, and the female rats were then assigned to either the control diet (C group) or the caloric-restriction diet (R group). The control diet was the AIN-93G diet *ad libitum* [25]. The R group was fed 50% of the typical food intake of the C group, with a correction for body mass. After delivery, the litters were culled to a maximum of eight pups. Following parturition, all rats were introduced to the AIN-93G diet *ad libitum*. The rats were weaned at the age of 4 weeks; some of the females were then anesthetized and sacrificed for tissue collection. At least two randomly selected female rats from each litter were kept alive and used for mating, which occurred when the rats were aged 8–10 weeks. In each generation F0–F2, 6–10 pregnant dams were randomly selected on day of pregnancy 19 (DOP 19) and sacrificed. The same procedure was continued until the F2 generation. The design of the experiment is presented in Fig. 2.

Body weight was measured weekly using electronic scales. Food intake was measured from DOP 0 to DOP 15. Food intake was normalized to body weight (as g of food intake per day per 100 g body weight), and was calculated as the average daily calorie intake in kcal per day per 100 g body weight.

The animals were anesthetized by CO_2 inhalation and euthanized by cardiac puncture. The fetuses were sacrificed by decapitation. The body weight of the fetuses was measured using electronic scales. Liver samples were taken, immediately frozen in liquid nitrogen, and stored at −80 °C for further analyses. Blood samples for Hcy analysis were placed in tubes containing EDTA. Plasma was separated from blood cells by centrifugation for 10 min at 4 °C and stored at −80 °C until analysis.

2.2. Measurement of metabolites

2.2.1. Blood choline, betaine, and trimethylamine-N-oxide

Total serum choline, betaine, and trimethylamine-N-oxide (TMAO) concentrations were measured using hydrophilic interaction liquid chromatography/mass spectrometry with electrospray ionization (HILIC-ESI-MS). Concisely, serum samples were prepared by protein precipitation with methanol (MeOH), which leads to a better peak shape, according to [26]. 225 μL of MeOH with two internal standards (ISTD) was added to 25 μL of plasma. The TMAO-d9 and choline-d9 deuterated ISTDs were introduced to the samples at a final concentration of 0.2 and 0.1 μg/mL of injected sample, respectively. The prepared sample was vortexed (5 s) and centrifuged (15,000 g, 10 min); 10 μL of supernatant was injected into the LC-ESI-MS system. The HILIC-ESI-MS analysis was performed using a Dionex UltiMate 3000 UHPLC (Thermo Fisher Scientific, Sunnyvale, CA, USA) coupled to a Bruker maXis impact ultrahigh resolution orthogonal quadrupole-time-of-flight accelerator (qTOF) equipped with an ESI source and operated in positive-ion mode (Bruker Daltonik, Bremen, Germany). Chromatographic separation was achieved using a Kinetex® 1.7 μm HILIC 100 Å column, 100 × 2.1 mm coupled with SecurityGuard ULTRA Cartridges, UPLC/HILIC (Phenomenex, Torrance, CA, USA). The ESI-MS settings were as follows: capillary voltage 4500 V, nebulizing gas 1.5 bar, and dry gas 5 L/min at 220 °C. The mobile phase was composed of acetonitrile/water (90/10) containing 5 mM ammonium formate (A) and 5 mM solution of ammonium formate in water (B). The flow rate was 0.16 mL/min with an elution gradient of 0%–90% B over