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Journal of Nutritional Biochemistry 23 (2012) 1121–1127

Journal of Nutritional Biochemistry

Intrauterine growth restriction leads to changes in sulfur amino acid metabolism, but not global DNA methylation, in Yucatan miniature piglets☆

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Received 31 August 2010; received in revised form 31 May 2011; accepted 23 June 2011

Abstract

Intrauterine growth restriction (IUGR), in both animals and humans, has been linked to metabolic syndrome later in life. There has been recent evidence that perturbations in sulfur amino acid metabolism may be involved in this early programming phenomenon. Methionine is the precursor for cellular methylation reactions and for the synthesis of cysteine. It has been suggested that the mechanism behind the "fetal origins" of adult diseases may be epigenetic, involving DNA methylation. Because we have recently demonstrated the fetal origins phenomenon in Yucatan miniature swine, we hypothesized that sulfur amino acid metabolism is altered in IUGR piglets. In this study, metabolites and the activities of sulfur amino acid cycle enzymes were analyzed in liver samples of 3- to 5-day-old runt (IUGR: 0.85±0.13 kg) and large (1.36±0.21 kg) Yucatan miniature pig littermates (n=6 pairs). The IUGR piglets had significantly lower specific and total activities of betaine-homocysteine methyltransferase (BHMT) and cystathionine γ-lyase (CGL) than larger littermates (P<.05). Expression of CGL (but not BHMT) mRNA was also lower in IUGR piglets (P<.05). This low CGL reduced cysteine and taurine concentrations in IUGR pigs and led to an accumulation of hepatic cystathionine, with lower homocysteine concentrations. Methylation index and liver global DNA methylation were unaltered. Reduced prenatal growth in Yucatan miniature piglets impairs their remethylation capacity as well as their ability to remove cystathionine and synthesize cysteine and taurine, which could have important implications on long-term health outcomes of IUGR neonates.

Keywords: Methionine; Enzyme activity; Homocysteine; Epigenetics; Early origins of adult diseases

1. Introduction

The "fetal origins" of adult disease hypothesizes that factors *in utero* can affect the development of disease later in life [1]. Many studies in both animals and humans have demonstrated links between prenatal growth rate and adult diseases, including obesity, cardiovascular diseases and type 2 diabetes [2]. Because the initial hypotheses generated from epidemiological data implicated small birth weight, the most successful animal model in fetal programming has been the protein-deficient rat dam [3]. The intrauterine growth-restricted (IUGR) rat pups became adult rats that exhibited symptoms of various chronic diseases such as obesity, coronary heart disease, hypertension, glucose intolerance, appetite dysregulation and osteoporosis [3]. In addition, postnatal catch-up growth in growth-retarded pups also led to reduced longevity and increased hypertension, similar to epidemiological data [4]. Recently, the protein-deficient rat dams were found to be hyperhomocysteinemic because the low-protein diet was disproportionately high in methionine and low in cysteine (Fig. 1) [5–7]. Hyperhomocysteinemia in pregnancy has been linked to intrauterine growth retardation [8], and hyperhomocysteinemia in children has been linked to development of obesity and hypertension [9]. Because the mediating mechanism of fetal programming does not seem to be protein deficiency per se, focus has been more recently directed toward imbalance of sulfur amino acid metabolism as a potential mechanism for this phenomenon. Indeed, Rees [7] concluded that changes in cell function only occur when the metabolism of sulfur amino acids is disturbed.

Methionine is required for protein synthesis and for oxidation to synthesize cysteine; the oxidation to cysteine involves both transmethylation and transsulfuration pathways (Fig. 1). However, the oxidative enzymes in the transsulfuration pathway are low *in utero* and may be limiting for cysteine synthesis [10,11]. With intrauterine growth retardation, where nutrient flow to the fetus is reduced, oxidative pathways are further reduced due to less substrate

Abbreviations: BHMT, betaine-homocysteine methyltransferase; CBS, cystathionine β-synthase; CGL, cystathionine γ-lyase; MAT, methionine adenosyltransferase; MS, methionine synthase; MTHFR, methylenetetrahydrofolate reductase; SAH, *S*-adenosyl-L-homocysteine; SAM, *S*-adenosyl-L-methionine; IUGR, intrauterine growth-restricted.

☆ Grants, sponsors and funding sources: This work was supported in part by grants to R.F.B from the Canadian Institutes of Health Research and from the International Life Sciences Institute North America (ILSI N.A.) [for further information about ILSI N.A., call (202) 659-0074 or email ilsina@ilsi.org]. The opinions expressed herein are those of the authors and do not necessarily represent the views of ILSI N.A.

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doi:10.1016/j.jnutbio.2011.06.005

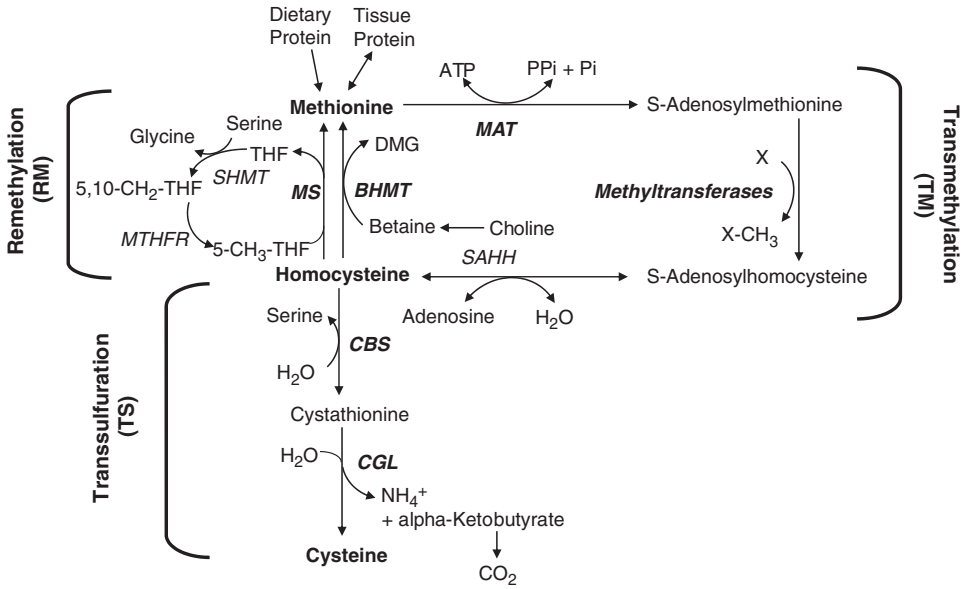

Fig. 1. Schematic of pathways in sulfur amino acid metabolism. Abbreviations: SAHH, *S*-adenosyl homocysteine hydrolase; SHMT, serine hydroxymethyltransferase; THF, tetrahydrofolate; DMG, dimethylglycine.

availability. In the postnatal situation, compensatory growth following IUGR involves more efficient use of nutrients that, for amino acids, usually also involves a down-regulation of oxidative pathways [12]. So IUGR neonates have an imbalanced methionine metabolism such that oxidation, or transsulfuration, is reduced, leading to a potential accumulation of homocysteine.

One of the key transmethylation reactions is methylation of DNA, which is an inheritable mechanism regulating gene expression [13]. Indeed, such epigenetic changes have been proposed as the underlying mechanisms in the plasticity associated with early development that can plausibly program risk for disease in later life [13,14]. Methylation of DNA can be affected by dietary levels of methyl-donor components, such as methionine, choline and folate [13,15]. Moreover, in fetuses of rat dams fed methionine-imbalanced low-protein diets, global methylation of DNA was increased in several tissues, including the fetal liver [6]. Thus, changes in sulfur amino acid metabolism have been suggested as a possible mechanism by which environmental influences during fetal development could permanently affect fetal nutrition [7].

More recently, we [16,17] and others [18,19] have validated the pig as a model for early programming by demonstrating that the naturally occurring runt IUGR pig develops biomarkers for hypertension, diabetes, obesity and dyslipidemia early in adulthood compared to larger siblings. The pig has distinct advantages over rodent models of this phenomenon in that postnatal metabolism can be studied and the nutritional requirements of the pig are more similar to those of the human, especially with respect to amino acids [20]. Therefore, using the Yucatan miniature piglet as a model, we hypothesized that the lowered fetal nutrition that caused the subsequent low birth weight of the runt would also result in a decrease in the activity of the enzymes that facilitate the removal of excess sulfur amino acids, such as methionine and homocysteine. The imbalanced metabolic pathways and accumulation of homocysteine would also modify the methylation of DNA, thereby affecting the long-term regulation of gene expression and perhaps explaining the higher risk for chronic diseases later in life observed in IUGR piglets [16–19]. The main objectives of this study are (a) to determine if IUGR limits the capacity of certain sulfur amino acid enzymes, (b) to determine if IUGR affects expression of altered sulfur amino acid enzymes and (c) to determine if IUGR affects global methylation of DNA as a result of sulfur amino acid enzyme alterations.

2. Methods and materials

2.1. Reagents

L-[3-^{14}C]serine was obtained from American Radiolabeled Chemicals, Inc. (St. Louis, MO, USA), and 5-[^{14}C]methyl-tetrahydrofolic acid barium salt was from Amersham Biosciences UK Limited (Buckinghamshire, UK). L-[1-^{14}C]methionine and *N,N,N*-trimethyl[methyl-^{14}C]glycine (^{14}C-betaine) were acquired from Moravek Biochemicals (Brea, CA, USA). The poly-prep prefilled chromatography columns (AG 1-X8, AG1-X4 and AG50W-X4 resins, 200–400 mesh, 0.8×4 cm) were obtained from Bio-Rad Laboratories, Inc. (Hercules, CA, USA). All other chemicals were of analytical grade and were from Sigma (St. Louis, MO, USA) or Fisher Scientific (Fair Lawn, NJ, USA).

2.2. Animals

Yucatan miniature piglets (n=12) were obtained from the Memorial University of Newfoundland breeding colony. Animal care and handling procedures were approved by the Institutional Animal Care Committee and in accordance with the guidelines of the Canadian Council on Animal Care. Six pairs of littermates, each consisting of a spontaneously occurring runt (three males, three females) and its largest littermate (two males, four females), were removed from sows at 3–5 days of age. For the purposes of this study, a runt (i.e., IUGR) was defined as the smallest piglet in a litter that was at least 25% smaller than its largest littermate at birth. In this herd of Yucatan pigs, piglets fitting these parameters occur naturally in ~90% of litters. Within 1 h of removal from the sow, pigs were anesthetized with halothane, and blood was sampled via cardiac puncture and centrifuged immediately at 1500*g* for 10 min at 4°C to isolate plasma; given that pigs were within 1.5–2 h of last suckling, piglets were in fed state. Organs (liver, kidneys, colon, small intestine, lung and heart) were removed quickly under anesthesia and weighed, and samples were immediately frozen in liquid nitrogen and stored at −80°C until further analyses.

2.3. Liver homogenate preparation

Approximately 0.5–1.5 g of frozen liver was weighed and kept on ice. A homogenate of liver and 50 mM potassium phosphate dibasic (pH 7.0) buffer (1:5) was freshly prepared on ice using a Polytron homogenizer (Brinkmann Instruments, Mississauga, ON, Canada) for 30 s at 50% output. The homogenate was centrifuged at 20 000*g* for 30 min at 4°C (Beckman L8-M Ultracentrifuge), and the supernatant was removed and used immediately to measure the activities of sulfur amino acid metabolism enzymes. Homogenates were centrifuged at 13 000*g* for 5 min at 4°C. Protein concentration of homogenates was determined using the Biuret assay using porcine serum albumin as a standard.