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Developmental changes in hepatic glucose metabolism in a newborn piglet model: A comparative analysis for suckling period and early weaning period

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ABSTRACT

The liver glucose metabolism, supplying sufficient energy for glucose-dependent tissues, is important in suckling or weaned animals, although there are few studies with piglet model. To better understand the development of glucose metabolism in the piglets during suckling period and early weaning period, we determined the hepatic glycogen content, and investigated the relative protein expression of key enzymes of glucogenesis (GNG) and mRNA levels of some glucose metabolism-related genes. During suckling period, the protein level of G6Pase in the liver of suckling piglets progressively declined with day of age compared with that of newborn piglets (at 1 day of age), whereas the PEPCK level stabilized until day 21 of age, indicating that hepatic GNG capacity gradually weakened in suckling piglets. The synthesis of hepatic glycogen, which was consistent with the fluctuation of glycolytic key genes PFKL and PKLR that gradually decreased after birth and was more or less steady during latter suckling period, although both the mRNA levels of GCK and key glucose transporter GLUT2 presented uptrend in suckling piglets. However, early weaning significantly suppressed the hepatic GNG in the weaned piglets, especially at d 3–5 of weaning period, then gradually recovered at d 7 of weaning period. Meanwhile, PFKL, PKLR and GLUT2 showed the similar trend during weaning period. On the contrast, the hepatic glycogen reached the maximum value when the G6Pase and PEPCK protein expression were at the lowest level, although the GCK level maintained increasing through 7 days of weaning period. Altogether, our study provides evidence that hepatic GNG and glycolysis in newborn piglets were more active than other days during suckling period, and early weaning could significantly suppressed glucose metabolism in liver, but this inhibition would progressively recover at day 7 after weaning.

1. Introduction

Maintaining blood glucose concentration within a relatively narrow range through periods of fasting or excess nutrient availability is essential to the survival of the organism. This is achieved through an intricate balance between glucose uptake and endogenous glucose production to maintain constant glucose concentrations [1]. Liver plays a crucial role in the maintenance of systemic glucose homeostasis, and this glucostat function is based on the reversible shift between glycogen synthesis and degradation as

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well as between glycolysis and GNG [2]. In the absorptive state, the liver increases glucose uptake via the absorption of glucose by hepatocytes and subsequent transformation into glycogen and lipids. In the fasting state, hepatocytes provide glucose via glycogenolysis and GNG to maintain glucose homeostasis [3]. GNG is conventionally considered as the formation of new glucose from non-glucose precursors. The available data show that in the human newborn, GNG appears soon after birth and contributes 30%–70% to glucose produced [4]. Newborn piglet requires regularity of nursing and substantial gluconeogenic activity to maintain normoglycemia. Estimates suggest that approximately 50% of the glucose may be derived through synthesis, even if the piglet is able to nurse ad libitum [5]. However, a strict definition of GNG should include not only the formation of glucose but also that of glycogen [4]. When nutrients become scarce, insulin levels are decreased and glucagon is secreted to promote hepatic glucose production to meet brain and red blood cells energetic demands. Hepatic glucose production is achieved by glycogen breakdown (glycogenolysis) as well as by de novo glucose synthesis from available precursors [6].

Piglets are born with limited energy stores [7], and limited capacity for oxidizing fatty acid and AA [8]. Therefore, mortality of piglets is increased during the first few days of life, and most deaths are caused by inadequate energy supply [9]. The liver is the major source of net endogenous glucose production, and maintaining hepatic GNG contributed to reducing the rate of piglet hypoglycemia, and improved the growth rate of piglets [10]. Although there were many studies about the development of GNG in neonate [4,11], few detailed studies on the GNG in the liver of piglets, have been reported. In the other hand, most studies used rats as models to gain insight into human metabolic regulation, but pigs are considered a better model for metabolic studies, for they are more similar to humans in morphology, physiology, metabolism, and omnivorous habits [12]. The aim of this study is to gain further insight into the development of hepatic glucose metabolism during suckling period, and investigate effects of early weaning on hepatic glucose metabolism in weaned piglets.

2. Materials and methods

This study was conducted according to the guidelines for the treatment of animal subjects as approved by the Animal Care Committee of the Institute of Subtropical Agriculture, Chinese Academy of Science.

2.1. Animals and experimental design

In this experiment, 64 neonatal piglets (Duroc × Landrace × Large Yorkshire) from 8 litters (8 piglets/litter) were assigned into 8 groups on the basis of different litter origins and the similar body weights. All piglets were housed in an environmentally controlled farrowing cage with hard plastic slatted flooring and had free access to drinking water. Some piglets were nursed by sows until 21 days old, other piglets were weaned at 14 days old and housed in the same farrowing cage without sow and fed with creep feed (Artificial milk 101, Anyou Feed, China).

After electrical stunning, one piglet was slaughtered from each litter at day 1 (d 1), 7 (d 7), 14 (d 14) and 21 (d 21) of age during suckling period (8 piglets/day of age). Moreover, other piglets weaned at day 14 of age were also slaughtered at day 15 (w 1d), 17 (w 3d), 19 (w 5d), and 21 (w 7d) (8 piglets/day of age). Then, part of the liver tissue was immediately collected and snap-frozen in liquid nitrogen and stored at −80 °C for later analysis.

2.2. Determination of hepatic glycogen in the liver

The liver tissue (~100 mg) was homogenized in a nine-fold volume of PBS (0.1 M, pH 7.2) and then centrifuged at 3000× g for 10 min at 4 °C to obtain the supernatant. The content of hepatic glycogen was measured using a commercial kit (Nanjing Jiancheng, Nanjing, China) according to the manufacturer's instructions.

2.3. RNA extraction and cDNA synthesis

Total RNA in the liver was isolated using the TRIZOL reagent (Invitrogen, Carlsbad, CA, USA) and treated with DNase. The RNA quality was checked by 1% agarose gel electrophoresis, stained with 10 μg/ml ethidium bromide, and the OD_{260}:OD_{280} ratio of extracted RNA was between 1.9 and 2.05. Reverse transcription was performed using PrimeScript RT reagent Kit with gDNA eraser (Takara, Dalian, China).

2.4. Real-time PCR

Primers used in Real-time PCR were designed with Primer 5.0 according to the gene sequence of pig (http://www.ncbi.nlm.nih.gov/pubmed) to produce an amplification product. The following primer sequences for the selected genes were used: forward: 5′-CTGATCCCACTGCGTATGGT-3′, reverse 5′-AATAATCCTCTGGCGTCGTG-3′ for GSase; forward: 5′-CCGACTTCCTGGACAAGCAT-3′, reverse 5′-ATCGTGGCCA-CAGTGTCATT-3′ for GCK; forward: 5′-ACTCCCTTCGACCGGAACTA-3′, reverse 5′-TGCTCAAAGTCGGTGTCCTC-3′ for PFKL; forward: 5′-CCCACTGAAGTCACCGCTAT-3′, reverse 5′-GAGGAAGCCACG-GAGTTTT-3′ for PKLR; forward: 5′-GGTTCATGGTGGCCGAGTT-3′, reverse 5′-ATTGCGGGTCCAGTTGC-3′ for GLUT2; forward: 5′-CGTTGGCTGGTTGAGAATC-3′, reverse 5′-CGGCAAGACAGAAATGA-CAA-3′ for β-actin. The amplification reactions were carried out in an ABI Prism 7900 HT sequence detection system (Applied Biosystems, Foster, CA). The relative level of mRNA expression was calculated using the $2^{-(\triangle\triangle Ct)}$ method, where $\Delta\Delta Ct = (Ct_{target} - Ct_{\beta\text{-}actin})_{treatment} - (Ct_{target} - Ct_{\beta\text{-}actin})_{control}$. The piglet at d 1 of age was taken as control group, and other groups were expressed as a ratio to the expression in control group. Therefore, relative gene expressions were reported as a fold change from the control value, and relative expression of target genes in control group (d 1 of age) was 1.0.

2.5. Total protein extraction

Frozen liver samples were powdered under liquid nitrogen, and lysed in RIPA buffer (150 mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris–HCl at pH 7.4) (Beyotime Biotechnology, China), plus a protease inhibitor cocktail (Roche, Shanghai, China). After the sample was centrifuged at 12,000× g, 4 °C, for 10 min, the protein concentration in the supernatant was determined using a Bicinchoninic Acid assay (Beyotime Biotechnology, China).

2.6. Western blotting

The total proteins from the liver were separated using SDS-PAGE (10% polyacrylamide gel), and transferred to PVDF membranes (Millipore, Billerica, MA) for 2 h using a Bio-Rad transblot apparatus (Hercules, CA). The membranes were blocked with 5% fat-free milk in Tris–Tween buffered saline (TBST: 20 mM Tris/150 mM NaCl, pH 7.5, and 0.1% Tween-20) for 3 h and then incubated with primary antibodies specific to key enzymes of GNG, i.e., phosphoenolpyruvate carboxykinase-mitochondria (PEPCK) (Santa Cruz Inc., USA),