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Effect of caloric restriction and subsequent re-alimentation on oxidative stress in the liver of Hu sheep ram lambs

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ABSTRACT

Growing animals under moderate caloric restriction (CR) exhibit compensatory growth upon *ad libitum* feeding, while the influence of oxidative stress under this phonotype in ruminants is poorly understood. The objective of this study was to investigate the antioxidant defenses and mitochondrial function in the liver of Hu sheep ram lamb in response to two months of CR (55% feeding) and subsequent three months of *ad libitum*. In this study, the expressions of antioxidase related genes (*SOD2*, *CAT* and *GPX1*) and mitochondrial biogenesis related genes (*AMPK*, *PPARGC1A* and *TFAM*) were examined in the liver, as well as the activities of antioxidase enzymes and the hepatic mitochondrial function. Results indicated that lambs tended to compensate for growth loss, and liver oxidative stress was limited by CR and subsequent re-alimentation. CR also activated PPARGC1A and TFAM, and elevated the relative mtDNA copy number, which could be recovered by subsequent re-alimentation. Likewise, re-alimentation restored the reduced complex I activity and H_2O_2 production associated with low mitochondrial potential with CR treatment. In addition, AMPK phosphorylation at Thr172 was activated and SIRT1 expression was enhanced in CR lambs, while showed a decreased tendency after re-alimentation. Altogether, our findings demonstrated that moderate CR and subsequent re-alimentation induced "efficient" mitochondria biogenesis as an adaptation mechanism, which maintained the dynamic balance of oxidant-antioxidant system by activating AMPK-PPARGC1A-TFAM pathway in the liver of Hu sheep ram lambs.

1. Introduction

Compensatory growth, an accelerated growth phenomenon caused by calorie restriction (CR) and subsequent re-alimentation,

Abbreviations: AA, Antimycin A; ADFI, average daily feed intake; ADG, average daily gain; AMPK, AMP-activated protein kinase; ATP, adenosine triphosphate; CAT, catalase; CR, caloric restriction; CS, citrate synthase; cyt *b*, cytochrome *b*; ESRRA, estrogen-related receptor alpha; ETC, electron transport chain; GAPDH, glyceraldehyde -3- phosphate dehydrogenase; GPx, glutathione peroxidase; GPX1, glutathione peroxidase 1; GSH, glutathione; GSSG, oxidized glutathione; H_2O_2, hydrogen peroxide; mtDNA, mitochondrial DNA; NAD, nicotinamide adenine dinucleotide; NADH, nicotinamide adenine dinucleotide hydride; NRF1, nuclear respiratory factor 1; NRF-2, nuclear respiratory factor-2; PPARGC1A, peroxisome proliferator-activated receptor gamma, coactivator 1 alpha; PRKAA1, protein kinase, AMP-activated, alpha 1 catalytic subunit; qPCR, real-time quantative polymerase chain reaction; ROS, reactive oxygen species; ROT, rotenone; SIRT1, sirtuin 1; SIRT3, sirtuin 3; SOD, superoxide dismutase; SOD2, superoxide dismutase 2; TFAM, transcription factor A, mitochondrial

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was incorporated into many animal production systems worldwide to reduce costs and produce animals with high feed efficiency (Keogh et al., 2016). Extensive studies have shown that compensatory growth acted as a metabolic adaptation involved in the regulation of the major pathways of intermediary metabolism, particularly in the liver, which was the central organ maintaining metabolic homeostasis during the shifts of fasting-refeeding periods (Geisler et al., 2016). Therefore, understanding the elasticity of hepatic metabolite flux and the central roles of liver in providing nutrients to peripheral tissues is essential to study the metabolic perturbations. Although many investigations focused on the liver metabolic pathway shifts or the mechanisms of cell adaptive during the fasting-refeeding transition (Ramalingam et al., 2017), the literature lacks studies to evaluate hepatic oxidative stress, which is believed to involve metabolic dysfunction and the pathogenesis of numerous diseases.

Mitochondrion, as the major organelle for adenosine triphosphate (ATP) and reactive oxygen species (ROS) production, played critical roles in oxidative damage regualtion (Scheffler, 2001). Evidence from the literature has demonstrated that mitochondrial function tightly linked to nutrient availability (Huang et al., 2010). It has been reported that reduced mitochondrial ROS production and oxidative damage with CR treatment in rodents (Lopez-Torres et al., 2002), while others have discrepancy results that CR had no effects on oxidative damage (Ramsey et al., 2004; Sorensen et al., 2006). In addition, till now there are only few reports regarding hepatic mitochondrial function in relation to CR and re-alimentation in ruminant animals.

AMP-activated protein kinase (AMPK), sirtuin 1 (SIRT1), and peroxisome proliferator-activated receptor gamma, coactivator 1 alpha (PPARGC1A) constitute an energy sensing cellular network that controls mitochondrial biogenesis (Canto and Auwerx, 2009). The phosphorylation of AMPK could be activated by CR, then phosphorylated AMPK activates its downstream PPARGC1A to regulate mitochondrial function (Canto and Auwerx, 2011). To et al. (2007) reported that the down-regulation of AMPK caused by CR could be beneficial for metabolic adaptation to the frequent fluctuations of food availability. In addition, CR could induce a molecular adaptation by increasing nicotinamide adenine dinucleotide (NAD)/nicotinamide adenine dinucleotide hydride (NADH) ratio toward activiting SIRT1, then increased the transcriptional activity of PPARGC1A (Rodgers et al., 2005). Additionlly, PPARGC1A was known as a upstream of nuclear respiratory factors (NRF) 1 and 2, and transcription factor A, mitochondrial (TFAM), and served as a nutrient sensing system by increasing mitochondrial biogenesis (Rodgers et al., 2005). Although CR has been reported to affect on the activities of AMPK, SIRT1 and PPARGC1A, the regulation of mitochondrial metabolism under fasting-refeeding transition remains to be elucidated.

The objective of this study was to investigate the effects of CR and subsequent re-alimentation on liver antioxidant defenses and mitochondrial function, and to detect whether those responses were associated with the activation of key metabolic sensors AMPK, SIRT1 and PPARGC1A in the liver of Hu sheep ram lambs.

2. Materials and methods

2.1. Animals, experimental design and sample collection

All protocols involving in the use of animals were in accordance with the Guidelines for Animal Experiments of Nanjing Agricultural University, and approved by Animal Care and Use Committee of Nanjing Agricultural University. Forty Hu sheep ram lambs (3 ± 0.1 months of age weighing 22.3 ± 0.624 kg) with body condition scores of 2.51 ± 0.08 units were supplied by a local breeding colony (Taizhou Hailun Sheep Farm, Jiangsu, China). The lambs were housed in individual cages with free access to water.

All lambs were fed with normal diet to acclimatize for two weeks. Then CR stage started for two months, the lambs were randomly divided into two groups as following: control group was fed with normal diet (100% of energy requirements, 12.9 MJ/d; n = 20), and CR group was fed with CR diet (55% of energy requirements, 7.11 MJ/d; n = 20). After CR, half of the lambs (10 lambs per group) were euthanized, while the other lambs were used in re-alimentation stage for three months with *ad libitum* feeding, at the end of re-alimentation, all animals were euthanized. For each phase, the experimental diets were isonitrogenous, and the requirements of dietary energy were calculated based on the feeding standards for meat-producing sheep and goats (NY/T 816-2004, China). Details of diet composition and nutrient composition content were listed in Table 1, and the *in vitro* digestibility of the experimental diets was showed in Table 2.

After euthanized, the liver was rapidly removed, and representative liver samples from the right hepatic lobe were immediately frozen in liquid nitrogen for further analysis. Meanwhile, suit aliquots of liver tissues were collected and used for mitochondrial isolation and function analysis.

2.2. Assessment of ATP levels, NAD and NADH content, and antioxidant enzymes activities in the liver homogenates

Liver tissues were homogenized in lysis buffer and centrifuged at 15,000*g* for 15 min at 4 °C. The supernatant was separated to measure ATP, NAD and NADH contents, and the activity of antioxidant enzymes. The protein concentration was determined by using bicinchoninic acid (BCA) Protein Assay Kit (No. P0012S, Beyotime, Nantong, China). ATP levels (ATP Determination Kit, No.A22066, Invitrogen, CA, USA), NADH and NAD content (NAD/NADH Quantification Kit, No. MAK037, Sigma, MO, USA), and enzyme activity of superoxide dismutase (SOD, SOD Assay Kit, No.S311-10, Dojindo Molecular Technologies, Tokyo, Japan) were determined by using the commercial kits. Catalase (CAT), glutathione peroxidase (GPx), glutathione (GSH) and oxidized glutathione (GSSG) were determined by using commercially available assay kits from Beyotime Biotechnology (Catalase Assay Kit, No. S0051; Cellular Glutathione Peroxidase Assay Kit, No. S0056; GSH and GSSG Assay Kit, No. S0053; respectively; Nantong, China) and strictly following the manufacturer's instruction.