Available online at www.sciencedirect.com

Metabolism

www.metabolismjournal.com

Arginine reverses growth hormone resistance through the inhibition of toll-like receptor 4-mediated inflammatory pathway

Jingren Xu[a,1], Canjun Zhu[a,1], Mengyuan Zhang[a], Qingchun Tong[c], Xiaojuan Wan[a], Zhengrui Liao[a], Xingcai Cai[a], Yaqiong Xu[a], Yexian Yuan[a], Lina Wang[a], Xiaotong Zhu[a], Songbo Wang[a], Ping Gao[a], Qianyun Xi[a,b], Yong Xu[d], Qingyan Jiang[a,b,*], Gang Shu[a,b,*]

[a] Guangdong Province Key Laboratory of Animal Nutritional Regulation, 483 Wushan Road, Tianhe District, Guangzhou, Guangdong 510642, China
[b] National Engineering Research Center for Breeding Swine Industry, College of Animal Science, South China Agricultural University, 483 Wushan Road, Tianhe District, Guangzhou, Guangdong 510642, China
[c] Brown Foundation Institute of Molecular Medicine, The University of Texas Health Science Center at Houston, 7000 Fannin, Suite 1800, Houston, TX 77030, USA
[d] Department of Molecular and Cellular Biology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA

ARTICLE INFO

Article history:
Received 24 June 2017
Accepted 4 October 2017

Keywords:
Arginine
IGF-1
Liver
Malnutrition
TLR4

ABSTRACT

Objective. Growth hormone stimulates growth by increasing insulin-like growth factor 1 expression and secretion. In the presence of insufficient nutrients, GH increases, whereas IGF-1 expression becomes severely suppressed, leading to GH resistance. This study aimed to explore the effect of arginine (Arg) on GH resistance during malnutrition and to describe its underlying mechanism.

Methods. C57BL/6 J mice were injected intraperitoneally with Arg for 1 h or subjected to caloric restriction with Arg supplement in drinking water for 18 days. HepG2 cells were exposed to different Arg concentrations for 24 h. Signaling pathway agonists/inhibitors, siRNA, and overexpression plasmids were used to investigate the underlying molecular mechanism. Liver-specific toll-like receptor (TLR4) knockout mice were utilized to clarify the role of TLR4 in Arg-induced IGF-I expression and secretion.

Results. Arg inhibited the TLR4 downstream pathway by binding to TLR4 and consequently activated Janus kinase 2/signal transducer and activator of transcription 5 signaling pathway. As a result, IGF-1 transcription and secretion increased. Arg activity was absent in liver-specific TLR4 knockout mice and was greatly suppressed in liver with overexpressed TLR4, suggesting that hepatic TLR4 was required and sufficient to induce GH resistance. By contrast, the mammalian target of rapamycin pathway was unnecessary for Arg activity. Arg not only significantly increased IGF-1 expression and secretion under acute fasting and chronic CR conditions but also attenuated body weight loss.

Abbreviations: Ala, alanine; Arg, arginine; CR, caloric restriction; GH, growth hormone; GHR, growth hormone receptor; GHRH, growth hormone-releasing hormone; IGF-1, insulin-like growth factor 1; IL, interleukin; IKKα/β, inhibitor of nuclear factor kappa-B kinase α/β; JAK2, Janus kinase 2; LPS, lipopolysaccharide; mTOR, mammalian target of rapamycin; PTP1B, protein tyrosine phosphatase 1B; SOCS3, suppressor of cytokine signaling 3; STAT5, signal transducers and activators of transcription 5; SST, somatostatin; TNFα, tumor necrosis factor α; TLR, toll-like receptor.

* *Corresponding authors:* Guangdong Province Key Laboratory of Animal Nutritional Regulation, National Engineering Research Center for Breeding Swine Industry, College of Animal Science, South China Agricultural University, 483 Wushan Road, Tianhe District, Guangzhou, Guangdong 510642, China.
E-mail addresses: qyjiang@scau.edu.cn (Q. Jiang), shugang@scau.edu.cn (G. Shu).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.metabol.2017.10.006

Conclusions. Our results demonstrate a previously unappreciated pathway involving Arg that reverses GH resistance and alleviates malnutrition-induced growth restriction through the inhibition of TLR4-mediated inflammatory pathway.

1. Introduction

Malnutrition, marked by lack of variant nutrients, is a leading cause of mal-development and growth deficits worldwide, especially in economically poor regions [1]. Body growth is synergistically controlled by nutritional status and endocrine signaling. Under normal conditions, growth hormone (GH) potently stimulates insulin-like growth factor 1 (IGF-1) expression and secretion in liver [2]. Under low-nutrition states, including acute fasting and chronic caloric restriction (CR), IGF-1 becomes greatly suppressed, whereas GH increases [3–5]. An increase in GH is part of counter-regulatory responses for glucose homeostasis, whereas a decrease in IGF-1 diverts nutrient requirement for development to maintain normal cell function and enhance survival [6,7]. However, the underlying mechanism for this apparent GH resistance, that is, increased GH but decreased IGF-1, remains unclear.

IGF-1, produced predominantly by the liver, plays a vital role in various physiological and pathological processes [8–10], including muscle development [11], bone growth [12,13], glucose metabolism [14,15], and aging [16]. GH is a major endocrine hormone that stimulates hepatic IGF-1 expression via Janus kinase 2/signal transducer and activator of transcription 5 (JAK2/STAT5) signaling pathway [17,18]. JAK2 is a non-receptor tyrosine kinase, which phosphorylates and activates STAT5, leading to its dimerization and translocation to the nucleus, where it binds to the regulatory elements of IGF-1 and promotes the expression and production of IGF-1 [19–21].

In addition to GH, nutrients, such as dietary protein and amino acids, are necessary to maintain circulating IGF-1 [18,22,23]. The deficiency of some essential amino acids in rodents or a low protein intake in humans reduces serum IGF-1 levels [5,24]. Although the concentration of most of serum amino acids decreases, changes in hepatic amino acid levels under malnourished conditions are inconsistent. A recent study showed that hepatic lysine and glycine concentrations increase after overnight starvation, whereas arginine (Arg) and alanine (Ala) concentrations decrease by 58.3% and 50.8%, respectively [25]. This finding suggests that some crucial amino acids, especially Arg, are probably associated with malnutrition-induced hepatic IGF-1 suppression [25].

Arg is an important conditional essential amino acid [26], which possesses various metabolic and regulatory roles, such as promoting the growth and development of animals, stimulating muscle protein synthesis [27], enhancing cell division [28] and wound healing [28], and regulating inflammatory processes [29–31]. These effects are at least partly due to the ability of Arg to activate mammalian target of rapamycin (mTOR). mTOR is an important signaling hub that regulates cellular growth, proliferation, and protein synthesis and acts as a master nutrient controller that senses the intake and deficiency of various amino acids, especially Arg, a known key activator of mTORC1 [32–34].

This study aimed to examine whether Arg contributes to GH resistance during fasting. We found that Arg promoted hepatic IGF-1 expression and secretion and alleviated malnutrition-induced growth restriction in C57BL/6 J mice. These phenomena were mediated by inhibiting the interaction between TLR4-mediated inflammation and JAK2/STAT5 signaling pathway. These findings indicated the crucial role of Arg in malnutrition-induced hepatic IGF-I suppression and growth arrest. Providing a therapeutic potential of malnutrition with Arg or TLR4 inhibitor.

2. Materials and Methods

2.1. Animal Studies

The experimental procedures and methods described in this study were approved by the College of Animal Science, South China Agricultural University. All experiments were conducted in compliance with "The Instructive Notions with Respect to Caring for Laboratory Animals" issued by the Ministry of Science and Technology of the People's Republic of China. C57BL6/J mice were purchased from the Animal Experiment Center of Guangdong Province [permission number: SYXK(Canton)2013-0002]. Liver-specific TLR4-KO mice (*TLR4*$^{-/-}$*-Alb-Cre*) were generated by crossing B6(Cg)-*Tlr4*$^{tm1.1Karp}$/J and B6·FVB(129)-Tg(Alb1-cre)1Dlr/J mice (The Jackson Laboratory). All mice were housed in individual cages and were given access to standard pellets (crude protein 18%, crude fat 4%, and crude ash 8%) before the experimental period and maintained under constant light for 12 h and a 12 h dark cycle at a temperature of 23 °C ± 3 °C and relative humidity of 70% ± 10% throughout the experimental period.

In the fasting experiment, six-week-old male mice were fasted overnight, then injected (i.p.) with 1.6 g/kg Arg or equimolar Ala for 1 h before sacrificed. Blood samples and liver tissues were collected for further analysis.

In the caloric restriction experiment, twenty twelve-week-old male littermates were received 60% of the average value of food intake for 18 days. Arg was supplemented by drinking water (1.5%, pH 7.0). The water was replaced three times a week and body weight was checked every three days. At the end of the experiment, mice were sacrificed to collect blood samples and liver tissue for further testing.

In the acute experiment, six-week-old male littermates were divided into three groups and injected (i.p.) with saline, 0.8 g/kg and 1.6 g/kg of Arg (Sigma) for 1 h, respectively. To explore the underlying mechanism of Arg action in vivo, mice were injected (i.p.) with vehicle control, Arg (1.6 g/kg, 1 h), drugs (mTOR inhibitor rapamycin, Sigma, Shanghai, China, 5 mg/kg, 5 h; JAK2 inhibitor AZD1480, Selleck, Shanghai, China, 30 mg/kg, 3 h; LPS, Sigma, 100 μg/kg, 19 h and 4 h, twice) or Arg + drugs. Then mice were sacrificed (about