

## TRANSLATIONAL MEDICINE: BENCH TO BEDSIDE

# Muscle at Risk: The Multiple Impacts of Ammonia on Sarcopenia and Frailty in Cirrhosis

Hui-Wei Chen, MD<sup>1</sup> and Michael A. Dunn, MD<sup>1</sup>

*Clinical and Translational Gastroenterology* (2016) 7, e170; doi:10.1038/ctg.2016.33; published online 26 May 2016

Muscle wasting is a cardinal manifestation of advanced cirrhosis.<sup>1</sup> Its key consequence is frailty, a state of vulnerability to stress, that is now recognized in transplant hepatology as an important risk factor for functional decline, waitlist removals, hospital and transplant deaths, and major transplant complications.<sup>2,3</sup> Frailty assessments in cirrhosis are gaining attention, both as measurements of anatomic muscle loss (sarcopenia), and measurements of functional decline in physical performance. Different measurement methods may be associated with discrepant clinical outcomes in the same patient cohort, as would be expected from the complexity of the underlying metabolic processes driving the problem.<sup>4</sup> Excess ammonia as a pivotal cause of muscle loss and functional impairment in cirrhosis has long been understood in terms of a shift in its predominant metabolism from liver to muscle. Three new mechanisms now help to account for ammonia's adverse impacts on muscle in cirrhosis.

## Cirrhosis Drives Inter-Organ Shifting of Ammonia Metabolism

In health, ammonia is predominantly metabolized in the liver to urea. Skeletal muscle and brain also process ammonia to a much lesser extent for utilization and detoxification via glutamine synthesis from glutamate. Cirrhotic patients with progressive hepatocyte damage have deficient urea synthesis, and shunting of ammonia past hepatocytes also contributes to its escape from the liver with increased muscle uptake. The shift to skeletal muscle as the prime site for ammonia metabolism requires diversion of branched-chain amino acids to generate the glutamate needed for ammonia detoxification, leading to the well-described depletion of these substrates needed for protein synthesis and maintenance of muscle mass.<sup>5,6</sup> Mathematical modeling suggests that shunting in cirrhosis is sufficient to explain excess delivery of ammonia to muscle with its consequent adverse impact on protein synthesis.<sup>7</sup>

## Ammonia Mediates Myostatin Transcription and Expression

Myostatin is a potent autocrine growth inhibitor produced by myocytes that inhibits skeletal muscle growth and reduces muscle mass in cirrhosis.<sup>8,9</sup> Qiu *et al.*<sup>10</sup> recently showed that exposure of mouse skeletal muscle myotubes in culture to ammonium acetate caused a time- and concentration-dependent increase in myostatin mRNA and protein expression. They found that hyperammonemia-activated transcription factor p65 NF- $\kappa$ B bound to the myostatin promoter with transcriptional upregulation. Pharmacologic and genetic silencing of NF- $\kappa$ B during hyperammonemia decreased myostatin expression. They also found that myotube diameter was significantly greater in the NF- $\kappa$ B knockdown cells compared with the control cells, further supporting their proposal that NF- $\kappa$ B regulates myostatin expression during hyperammonemia. Their observations show that hyperammonemia induces myostatin expression in myotubes via an NF- $\kappa$ B-dependent pathway.

## Ammonia Mediates Muscle Autophagy

Autophagy is a normal process through which damaged proteins are degraded or recycled to maintain essential cellular function. Qiu *et al.*<sup>10,11</sup> studied autophagy in skeletal muscle from 13 cirrhotic patients undergoing liver transplantation and 13 control

<sup>1</sup>Division of Gastroenterology, Hepatology and Nutrition, Center for Liver Diseases, and Thomas E. Starzl Transplantation Institute, University of Pittsburgh, Pittsburgh, Pennsylvania, USA

Correspondence: Michael A. Dunn, MD, Division of Gastroenterology, Hepatology and Nutrition, Center for Liver Diseases, and Thomas E. Starzl Transplantation Institute, University of Pittsburgh, Kaufmann 916, 3459 Fifth Avenue, Pittsburgh, Pennsylvania 15213, USA. E-mail: dunnma@upmc.edu

patients having elective abdominal surgery. They found that expression of three autophagy pathway components, beclin-1, LC3-I cytosolic protein, and p62/SQSTM1, was enhanced in cirrhotic human muscle, in the same pattern that they observed with hyperammonemia-induced change of the same autophagy marker components in portacaval-shunted rats. They also found that hyperammonemia induced the formation of autophagosome vesicles observed by electron microscopy of ammonia-treated murine myotubes in culture.

## Ammonia Impairs Skeletal Muscle Contractility

McDaniel *et al.*<sup>12</sup> recently explored the effect of hyperammonemia on skeletal muscle contractile function, independent of muscle mass. They found that hyperammonemic portacaval-shunted rats showed impaired initial maximum grip strength compared with controls. They also found that rat soleus muscles treated with ammonium acetate generated significantly less contractile force than did control muscles, and that the rates of force development and relaxation were depressed in the ammonia-treated muscles, replicating observations in cirrhotic patients. Although the mechanism by which hyperammonemia impairs muscle contractile function remains unclear, this report shows that hyperammonemia contributes to muscle dysfunction.

## Two Unanswered Questions

As noted above, a modeling study suggested that shunting may be largely responsible for liver to muscle shifting of ammonia with consequent impaired protein synthesis.<sup>7</sup> That proposal seems challenging to reconcile with the observation that muscle mass frequently improves after placement of a transjugular intrahepatic shunt in cirrhotic patients.<sup>13</sup> The finding suggests that the effects of shunting on muscle mass are more complex than can be explained by ammonia alone.

Myostatin is also well-described as an important regulatory molecule in cardiac muscle, where it may prevent hypertrophy and protect against heart failure.<sup>14</sup> Experimental or clinical studies of cardiac myostatin in hyperammonemia or chronic liver disease have not yet been reported.

## Ammonia as a Myotoxin—an Old Culprit With New Injury Mechanisms

To conclude, ammonia is now implicated in three recently reported muscle injury pathways involving myostatin, autophagy, and functional muscle impairment, all described by Dasarathy and colleagues from the Cleveland Clinic.<sup>9–12</sup> These new injury mechanisms appear to amplify the well-known muscle wasting impact of inter-organ shifting of ammonia metabolism to deplete essential amino acid protein substrates. The relative importance of each of these new mechanisms for functional impairment or decline of patients is not yet clear. Taken together, however, the findings underline the need to identify and minimize the impact of ammonia in cirrhosis not only on cognition but on the disabling and lethal potential of sarcopenia and frailty.

### CONFLICT OF INTEREST

**Guarantor of the article:** Michael A. Dunn, MD.

**Specific author contributions:** Both authors drafted and approved the final manuscript as written.

**Financial support:** None.

**Potential competing interest:** None.

1. Dasarathy S. Consilience in sarcopenia of cirrhosis. *J Cachexia Sarcopenia Muscle* 2012; **3**: 225–237.
2. Montano-Loza AJ, Meza-Junco J, Prado CM *et al.* Muscle wasting is associated with mortality in patients with cirrhosis. *Clin Gastroenterol Hepatol* 2012; **10**: 166–173.
3. Lai JC, Dodge JL, Sen S *et al.* Functional decline in patients with cirrhosis awaiting liver transplantation: results from the Functional Assessment in Liver Transplantation (FRAILT) study. *Hepatology* 2015; **63**: 574–580.
4. Yadav A, Chang YH, Carpenter S *et al.* Relationship between sarcopenia, six-minute walk distance and health-related quality of life in liver transplant candidates. *Clin Transplant* 2015; **29**: 134–141.
5. Granda OP, Ruderman NB. Muscle nitrogen metabolism in chronic hepatic insufficiency. *Metabolism* 1976; **25**: 427–435.
6. Lockwood AH, McDonald JM, Reiman RE *et al.* The dynamic of ammonia metabolism in man. Effects of liver disease and hyperammonemia. *J Clin Invest* 1979; **63**: 449–460.
7. Noiret L, Baigent S, Jalan R. Arterial ammonia levels in cirrhosis are determined by systemic and hepatic hemodynamics, and by organ function: a quantitative modelling study. *Liver Int* 2014; **34**: e45–e55.
8. Garcia PS, Cabbabe A, Kambadur R *et al.* Brief-reports: elevated myostatin levels in patients with liver disease: a potential contributor to skeletal muscle wasting. *Anesth Analg* 2010; **111**: 707–709.
9. Dasarathy S, Dodig M, Muc SM *et al.* Skeletal muscle atrophy is associated with an increased expression of myostatin and impaired satellite cell function in the portacaval anastomosis rat. *Am J Physiol Gastrointest Liver Physiol* 2004; **287**: G1124–G1130.

10. Qiu J, Thapaliya S, Runkana A *et al*. Hyperammonemia in cirrhosis induces transcriptional regulation of myostatin by an NF- $\kappa$ B-mediated mechanism. *PNAS* 2013; **110**: 18162–18167.
11. Qiu J, Tsien C, Samjhana T *et al*. Hyperammonemia-mediated autophagy in skeletal muscle contributes to sarcopenia of cirrhosis. *Am J Physiol Endocrinol Metab* 2012; **303**: E983–E993.
12. McDaniel J, Davuluri G, Hill E *et al*. Hyperammonemia results in reduced muscle function independent of muscle mass. *Am J Physiol Gastrointest Liver Physiol* 2016; **310**: G163–G170.
13. Tsien C, Shah SN, McCullough AJ *et al*. Reversal of sarcopenia predicts survival after a transjugular intrahepatic portosystemic stent. *Eur J Gastroenterol Hepatol* 2013; **25**: 85–93.
14. Biesemann N, Mender L, Wietelmann A *et al*. Myostatin regulates energy homeostasis in the heart and prevents heart failure. *Circ Res* 2014; **115**: 296–310.



**Clinical and Translational Gastroenterology** is an open-access journal published by *Nature Publishing Group*. This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International License. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>