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**Title: Targeting the muscle for the treatment and prevention of hepatic encephalopathy**

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4 Muscle mass loss or sarcopenia is a principle component of malnutrition which prevails in 65-90%  
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6 of patients with end-stage liver disease [1]. Intuitively, the roots of malnutrition play a precipitating  
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8 role in muscle catabolism. Undernutrition frequently occurs in cirrhosis since an inadequate diet is  
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10 compounded by a hypermetabolic energy demand. However, multiple other factors contribute to the  
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12 pathogenesis of malnutrition including malabsorption of nutrients, metabolic alterations, increased  
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14 intestinal protein losses, reduced protein synthesis, increased protein catabolism and disturbance of  
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16 substrate utilization [2,3]. Sarcopenia adversely affects quality of life, leads to longer hospitalizations,  
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18 increases susceptibility to infections, negatively impacts clinical outcomes pre- and post-liver  
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20 transplantation and is an independent prognostic factor for survival in patients with cirrhosis [4]. The  
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22 pathophysiological pathways triggering a reduction in muscle protein synthesis and/or an increase in  
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24 proteolysis, resulting in loss of muscle mass, remain elusive. Furthermore, in addition to loss of  
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26 quantity, quality of muscle is also affected during muscle wasting. Altered muscle metabolism and  
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28 contractile function, concomitant with a reduction in muscle mass, contributes to the onset of frailty as  
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30 well as functional decline in physical performance, leading to increased morbidity in patients with  
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32 cirrhosis [5].  
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41 The liver is a powerful metabolic organ which in addition to being the center for the metabolism of  
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43 nutrients (glucose and lipids), also plays a major role in ammonia disposal. Exclusively expressing all  
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45 the enzymes of the urea cycle, the liver regulates the circulating blood ammonia levels arising from the  
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47 gut (primary source of ammonia generation). Thus, severe liver impairment, leads to the occurrence of  
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49 hyperammonemia. At physiological pH, the majority (98%) of ammonia is found in ionic form ( $\text{NH}_4^+$ )  
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51 with ~ 2% arising in gas form ( $\text{NH}_3$ ). Both forms are capable of crossing cellular membranes.  $\text{NH}_3$  via  
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53 diffusion and  $\text{NH}_4^+$  via  $\text{K}^+$ -channels and cotransporters since  $\text{NH}_4^+$  has very similar ionic properties  
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55 (ionic radius and diffusion coefficient) to  $\text{K}^+$ . In addition, specific ammonia transporters have also been  
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57 identified [6]. Subsequently, following its concentration gradient, ammonia is dispersed throughout the  
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3 body, entering all cells, organs and tissues. Elevated concentrations of ammonia (including increased  
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5 flux (uptake) of ammonia across cell membranes) lead to changes in pH, adjusted membrane potential  
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7 and altered cell metabolism, which independently and/or collectively lead to a cascade of  
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9 pathophysiological events [7]. Since ammonia easily crosses the blood-brain barrier, blood-derived  
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11 ammonia leads to neurotoxic levels of ammonia in the brain which is a fundamental component  
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13 implicated in the pathogenesis of hepatic encephalopathy (HE).  
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18       Aside from primarily affecting the brain, the toxicity of ammonia has also demonstrated to affect  
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20 other organs and tissues, including muscle. Muscle plays a significant compensatory role in detoxifying  
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22 ammonia during liver disease since it houses the enzyme glutamine synthetase (GS), an important  
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24 ammonia removing pathway during the amidation of glutamate to glutamine. Therefore, in the setting  
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26 of liver disease, reduced capacity to remove ammonia in the liver, aggregated with muscle mass  
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28 depletion, further reduces the body's capacity to clear ammonia which in turn leads to a higher risk of  
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30 developing hyperammonemia and HE [8]. However, paradoxically, within the last 5 years, Dasarathy  
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32 and colleagues have provided solid evidence that elevated levels of ammonia cause detrimental effects  
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34 to the muscle. It has been shown that elevated ammonia i) upregulates myostatin (an autocrine growth  
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36 inhibitor) in myotubes through a NF- $\kappa$ B-dependent pathway [9], ii) stimulates muscle autophagy [10]  
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38 and iii) impairs skeletal muscle contractility and strength [11]. In the recent issue (JHEPAT-D-15-  
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40 02306R2), Davuluri and colleagues, using human tissue as well as *in vitro* and *in vivo* models of  
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42 hyperammonemia (including knockout and knockdown of a number of molecular targets), elegantly  
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44 identified the molecular pathways implicated in the inhibition of muscle protein synthesis in patients  
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46 with cirrhosis and hyperammonemia [12]. The authors found that ammonia activated the general  
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48 control nonderepressible 2 (GCN2) kinase (amino acid deficiency sensor) which inactivated the  
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50 eukaryotic initiation factor 2 (eIF2 $\alpha$ ) (via phosphorylation of the  $\alpha$  subunit) and additionally inactivated  
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52 mTORC1, resulting in global repression of mRNA translation and hence protein synthesis in the  
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3 skeletal muscle. Under physiological conditions, phosphorylation of eIF2 $\alpha$  is followed by an adaptive  
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5 integrated stress response (ISR) that is mediated via upregulation of activating transcription factor 4  
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7 (ATF4) which through downstream signalling pathways leads to the reversible dephosphorylation of  
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9 phospho-eIF2 $\alpha$ . Here, authors demonstrated that loss of feedback negative loop of ISR during  
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11 hyperammonemia, as evidenced by failure in ATF4 induction, results in chronic and persistent low  
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13 protein synthesis. Since the effects of increased ammonia (pH, membrane potential and metabolism)  
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15 touch all cells within the body and therefore, are not specific to the brain, the importance of  
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17 hyperammonemia and its impact on clinical outcomes merits to be thoroughly investigated.  
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23 Davuluri and colleagues also demonstrated in this issue (JHEPAT-D-15-02306R2) the  
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25 therapeutic benefit of L-leucine on muscle protein synthesis that was due to the metabolic adaptation of  
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27 muscle to hyperammonemia. Leucine, a branched-chain amino acid (BCAA), is capable of activating  
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29 mTORC1 and activating protein synthesis [13]. Furthermore, the authors demonstrated that L-leucine  
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31 reversed GCN2-eIF2 $\alpha$  phosphorylation, removing protein synthesis inhibition. The authors determined  
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33 that leucine “starvation”, provoked by ammonia, resulted in a compensatory upregulation of the leucine  
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35 transporter (leucine/glutamine exchanger (SLC7A5)) and therefore leucine supplementation rescued  
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37 the inhibition of protein synthesis.  
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43 Davaluri and colleagues administered 15g of leucine-enriched BCAA (7.5g L-leucine, 3.75g L-  
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45 isoleucine and 3.75g L-valine) to 6 hyperammonemic patients with cirrhosis and demonstrated a  
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47 reversal in muscle protein synthesis inhibition. However, muscle mass (quantity or quality) was not  
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49 evaluated, most likely due to short treatment time of 7 hours. A supporting study by Les et al., 2011  
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51 demonstrated leucine-enriched BCAAs (13.5g L-leucine, 9g L-isoleucine and 7.5g L-valine) given as a  
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53 nutritional supplementation for 56 weeks lead to an improvement in muscle mass (mid-arm muscle  
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55 circumference) in 46 patients with cirrhosis [14]. To date, only one study has tested the independent  
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57 effect of L-leucine and, following 10g/day supplementation for 12 weeks to 9 patients with cirrhosis,  
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3 thigh circumference was not enhanced [15]. This study puts in question the beneficial effect of L-  
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5 leucine on optimizing muscle mass. However, following the positive results of Davuluri and  
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7 colleagues, a time-dependent, dose-response study evaluating the effect of L-leucine on muscle mass  
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9 merits to be thoroughly conducted.

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12 The beneficial effect of L-leucine on muscle protein synthesis and subsequently muscle wasting  
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14 may also be a result of lowering ammonia. BCAAs are highly metabolized in the muscle and are  
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16 capable of replenishing  $\alpha$ -ketoglutarate, believed to be depleted during hyperammonemia as  $\alpha$ -  
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18 ketoglutarate is aminated to glutamate which subsequently is amidated to glutamine. These ammonia-  
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20 removing pathways are stimulated during hyperammonemia in attempt to reduce ammonia. BCAAs  
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22 have been reported to lower blood ammonia following 3 months of daily supplementation (0.24g/kg;  
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24 50% L-leucine, 25% isoleucine and 25% valine) in patients with cirrhosis which was associated with an  
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26 improvement in HE [16]. The treatment time and dose appear to be vital as 3-hour administration of  
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28 BCAAs (0.45g/kg; 45.5% L-leucine, 30% isoleucine and 24.5% valine) did not lower blood ammonia  
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30 in patients with cirrhosis but did increase BCAA-derived ammonia clearance in muscle [17]. It remains  
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32 to be defined whether the beneficial effect of L-leucine (BCAA) on muscle protein synthesis is due to a  
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34 lowering of blood ammonia.

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37 Patients with cirrhosis and sarcopenia have a higher risk of developing HE [8] as loss of muscle  
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39 mass, in addition to liver impairment, further reduces the capacity of ammonia removal, leading to  
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41 higher risk of hyperammonemia [18]. There is an invested interest in improving extra-hepatic ammonia  
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43 clearance (targeting GS in the muscle) for the treatment of HE [19]. Both L-ornithine L-aspartate and  
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45 L-ornithine phenylacetate have demonstrated to lower blood ammonia by generating glutamate and  
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47 stimulating GS activity in the muscle [20,21]. However, L-ornithine phenylacetate may be more  
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49 efficient by chelating glutamine released during muscle catabolism and preventing ammonia generation  
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51 through glutaminase activity. Overall, increasing the muscle's capacity to clear ammonia by either  
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53 stimulating GS activity and/or optimizing muscle mass are attractive strategies for the treatment and  
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3 prevention of HE. A recent meta-analysis revealed that BCAAs have a beneficial effect on HE [22];  
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5 however the precise mechanisms remain unclear. Interestingly, exercise has been shown to be  
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7 advantageous in optimizing muscle mass in patients with liver disease [23] and furthermore, in  
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9 combination with L-leucine supplementation, exercise demonstrated to have a greater impact on muscle  
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11 mass which was associated with an improvement in minimal HE compared to L-leucine alone [15]. The  
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13 independent effect of exercise on HE has not been evaluated.  
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17 In conclusion, the integrated relationship between muscle (mass and activity of GS), ammonia  
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19 and cognitive function remains to be comprehensively investigated. Studies evaluating the beneficial  
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21 effect of improving muscle mass on ammonia clearance and the treatment and prevention of HE are  
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23 lacking. The data presented by Davuluri and colleagues highlights the important contribution of  
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25 hyperammonemia on the inhibition of muscle protein synthesis and muscle mass wasting in cirrhosis.  
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27 This depicts the toxicity of ammonia lies beyond the brain and provides compelling evidence that long-  
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29 term treatment of hyperammonemia can be beneficial for the muscle and the brain in cirrhosis,  
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31 improving clinical outcomes pre- and post-liver transplantation.  
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