Oral administration of L-arginine improves gastrointestinal function in dystrophic mdx mice

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Duchenne muscular dystrophy (DMD) is a devastating muscle wasting disorder which results from mutations in the dystrophin (*dmd*) gene. It is characterized by progressive weakness and wasting of both skeletal and cardiac muscle, leading to loss of ambulation, respiratory complications and death. However, since the dmd gene encodes multiple dystrophin protein isoforms of various length and tissue distribution, the loss of dystrophin also affects other organ systems with serious impact on patient quality of life such as clinical manifestations of abnormal gastric and colonic motor activities. Constipation, bloating, and feelings of fullness are reported as matters of fact in DMD patients and those with other muscular dystrophies. Studies in *mdx* mice (the most widely used animal model of DMD) show reduced gastric and small intestinal motility, which is counteracted by addition of relaxin, a modulator of nitric oxide (NO) production. Since dystrophin deficiency affects NO production in skeletal muscle fibres, it is likely that dysregulated NO production causes gastrointestinal (GI) dysfunction in DMD. Indeed, studies have revealed both myogenic NOS and endogenous NO production are defective in colons of *mdx* mice (Mule *et al*., 2001). The addition of L-arginine [which is converted to NO by NO synthase (NOS)] restores normal motor activity in isolated colons from *mdx* mice (Azzena *et al*., 1999). Therefore, compounds that increase activity of NOS and/or NO production are likely to improve GI dysfunction in muscular dystrophy. We investigated the effect of systemic L-arginine and its metabolic precursor L-citrulline administration on GI function in the mdx mouse.

All experiments were approved by the Animal Ethics Committee of The University of Melbourne and conducted in accordance with the Australian code of practice for the care and use of animals for scientific purposes (NH&MRC). To assess the ability of a dietary intervention to improve GI function, 4-week-old male C57BL/10 and *mdx* mice received either vehicle, L-arginine (1 g/kg/day), the amino acid control L-alanine (1 g/kg/day) or L-citrulline (1 g/kg/day) in the feed for 8 weeks. At the end of the treatment, mice were killed by cervical dislocation. Colons were excised, faecal pellets analysed, and colon motility examined by spatiotemporal mapping *ex vivo* (Swaminathan *et al*, 2016). The *quadriceps*, *gastrocnemius*, *soleus*, *extensor digitorum longus*, and *tibialis anterior* muscles as well as the heart were excised and weighed to assess any protective effect of each diet on the dystrophic pathology.

No significant differences in faecal pellet length, width or mass were observed between groups. Resting colon diameter was unchanged between C57BL/10 and mdx mice in the proximal and mid colon, but the diameter of the distal colon was significantly smaller in mdx mice relative to C57BL/10 ($P < 0.05$; genotype main effect). This was attenuated in *mdx* mice fed L-arginine. Using spatiotemporal mapping of video recordings of colon contractions *ex vivo*, we observed an overall genotype effect for increased colon contractions in the proximal, mid, and distal colons of *mdx* mice relative to C57BL/10 mice when fed the control diet which was most pronounced in the distal colon ($P < 0.0001$). Interestingly, diet did not alter contraction number between genotypes in either the proximal or mid colon, but administration of either L-citrulline or L-arginine attenuated contraction number in the distal colons of *mdx* mice relative to C57BL/10 mice. The mass of the *quadriceps*, *gastrocnemius*, *soleus*, *extensor digitorum longus*, and *tibialis anterior* muscles (P P < 0.05 genotype main effect) were increased in mdx mice compared to C57BL/10 mice and not altered by diet.

Together these data suggest an altered phenotype within the distal colon of mdx mice that may be attenuated by L-citrulline or L-arginine supplementation to improve GI function. The ability to modulate GI dysfunction in muscular dystrophy using compounds already clinically approved has the potential to immediately improve quality of life for DMD patients.

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