Choline as a nutritional intervention to alleviate the dystrophic pathology in mdx mice

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Duchenne muscular dystrophy (DMD) is a devastating muscle wasting disorder caused by a variety of mutations in the dystrophin gene. It is characterized by progressive muscle wasting and weakness leading to loss of ambulation and premature death from cardiorespiratory complications. A lack of dystrophin protein renders muscle fibres fragile and prone to membrane tears leading to impaired Ca^{2+} homeostasis, excessive inflammation, increased muscle breakdown and altered metabolism in other tissues (Stapleton *et al*., 2014). A cure for DMD may eventually come from corrective gene or cell therapies, but in the interim, other treatments are needed urgently to counteract the progressive muscle loss and weakness. Choline, an essential water-soluble nutrient, is involved in multiple biological processes, including modulation of inflammation and oxidative stress, and it forms a substrate for membrane phospholipids. Based on these properties, we tested the hypothesis that choline supplementation would ameliorate the dystrophic pathology in mdx mice.

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 (NHMRC). To assess whether a dietary intervention could slow the progression of the dystrophic pathology, three-week old male *mdx* mice (n=40) were fed choline-enriched feed containing 5 g/kg choline (MCHL; n=20), or a control purified laboratory feed (MCON; n=20) for four weeks. Rotarod performance, grip strength and running (wheel) distance were assessed during treatment. At the end of treatment, mice were anaesthetized deeply with sodium pentobarbitone (60 mg/kg, *i.p.*), and selected muscles and the liver were excised. Mice were killed by cardiac excision, while anaesthetized deeply. Maximal sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) activity was measured as a proxy of Ca^{2+} handling capacity (Gehrig *et al*., 2012). Muscle structure was assessed using (immuno)histochemical analyses, protein expression was assessed from western immunoblotting, while muscle and liver gene expression were analysed by qPCR.

Choline treatment did not improve functional performance in *mdx* mice but in the severely affected diaphragm, it blunted inflammation [macrophage infiltration (CD68 -33.0%, $P<0.05$)] and reduced collagen infiltration (-34.0%, $P<0.05$). In *quadriceps* muscles, choline treatment increased maximal SERCA activity (37.8%, $P<0.05$) and reduced markers of inflammation (*Tnfα*, *F4/80* and *Cd206* mRNA, $P<0.05$). Choline treatment reduced *Acta2* mRNA expression (-34.0%, $P<0.05$) but did not alter triglyceride accumulation or other markers of inflammation and fibrosis in livers of *mdx* mice.

Together these data suggest that choline supplementation slowed progression of the dystrophic pathology, evident from reductions in diaphragm fibrosis and inflammation, and it enhanced maximal SERCA activity in *quadriceps* muscles. The reduction in fibrosis is clinically relevant for increasing the efficacy of future gene, cell and drug therapies for DMD.

Stapleton DI, Lau X, Flores M, Trieu J, Gehrig SM, Chee A, Naim T, Lynch GS & Koopman R. (2014). *PLoS ONE* **9,** e91514.

Gehrig SM, van der Poel C, Sayer TA, Schertzer JD, Henstridge DC, Church JE, Lamon S, Russell AP, Davies KE, Febbraio MA & Lynch GS. (2012). *Nature* **484,** 394-398.

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