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Viewpoint:

Title: The antioxidants Neopterin/7,8-dihydroneopterin - novel biomarker and muscle protectant in Duchenne muscular dystrophy.

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In the current issue of *Experimental Physiology,* Lindsay and colleagues (2018) have shown accumulation of inflammatory cells in patients with Duchenne muscular dystrophy (DMD) results in an increase in the immune cell synthesised antioxidants Neopterin and 7,8-dihydroneopterin. Furthermore, elevated levels of this antioxidant appear to be protective; improving strength and reducing the effects of contraction induced muscle damage in muscles isolated from the *mdx* mouse model of DMD. While this research highlights 7,8-dihydroneopterin as a novel biomarker for the assessment of disease progression in DMD, further work is needed to determine the potential therapeutic effects of these antioxidants *in vivo*.

Duchenne muscular dystrophy (DMD) is an X-linked inherited muscle disease caused by the absence of dystrophin, a key protein that connects the cytoskeleton of a muscle fiber to the surrounding extracellular matrix. This progressive disorder is characterised by muscle weakness, resulting in loss of ambulation, and ultimately early death due to cardiac and respiratory failure. The hallmark of this disease is an increase in susceptibility to skeletal muscle damage due to disruption of the muscles structure, which causes chronic inflammation and the accumulation of immune cells. The *mdx* mouse model of DMD has been used to study this condition for over 30 years (Bulfield et al., 1984) and while the mice represent a useful tool– the search continues for effective therapies and ultimately a cure for DMD.

Inflammation is known to cause an increase in reactive oxygen species (ROS), with subsequent immune cell (macrophage) activation increasing antioxidant production to counter this increase in ROS and oxidative stress. Neopterin, a key antioxidant released by immune cells, is increased in disorders associated with oxidative stress, including autoimmune disorders (i.e. HIV), viral infections and cardiovascular disease (Dale and Brilot, 2010, Mangge et al., 2014). Lindsay et al., highlight recent research linking the importance of increased oxidative stress and immune cell inflammation in DMD. In this study they provide clear evidence for increased neopterin and 7,8-dihydroneopterin in the urine of DMD patients, compared to age matched controls. This novel finding provides a useful new biomarker for the analysis of inflammation and disease progression in DMD as the accumulation of 7,8-dihydroneopterin is linked with loss of ambulation. The prognostic benefits of 7,8-dihydroneopterins makes it a valuable biomarker, due to the non-invasive nature of its collection and its presence in readily available source (urine), meaning that

multiple time points could be assessed with little impact to the patient. However, while examining the levels of neopterin Lindsay et al highlight that the correction of metabolites in urine can be difficult due to a lack of clear controls. They provide strong evidence for the use of both creatinine and specific gravity as effective normalisers for this purpose in DMD patients and acknowledge the current confusion in the field while providing clear evidence for both normalisation methods in this study.

Furthermore, due to a potent antioxidant effect of 7,8-dihydroneopterin (Gieseg et al., 2008) Lyndsey et al., hypothesised that the presence of this antioxidant may protect skeletal muscle from contraction induced force loss seen in *mdx* mice. By introducing 7,8-dihydroneopterin into a bath containing an isolated extensor digitorum longus (EDL) muscle they were able to show a modest reduction in the damaging effects of contraction induced muscle damage. In wild type muscles exposed to three different ROS, 7,8-dihydroneopterin provided some protection from hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl), but not SIN-1. Similarly, *mdx* muscles exposed to 6 mM of 7,8-dihydroneopterin show approximately 14% higher force and 11% protection from eccentric muscle contraction force loss compared to untreated *mdx* muscles. It is thought that this is the result of 7,8-dihydroneopterin scavenging key ROS and reducing the effect of oxidative damage in both wild type and *mdx* muscles.

While these findings present a tantalising association between the antioxidant 7,8-dihydroneopterin as a biomarker in DMD and a potential protective effect for this antioxidant in isolated muscles from the *mdx* mouse, there still remains the important question of whether the use of this antioxidant can provide protection from dystrophic muscle damage *in vivo* and over a longer period of treatment. Preclinical trials that use the antioxidant N-acetylcysteine (NAC) have recently been called into question by (Pinniger et al., 2017), who report a significant drop in body weight, liver mass and skeletal muscle mass in *mdx* mice treated with 2% NAC for 6 weeks in the drinking water. While Pinniger et al., show an improvement in grip strength and isolated muscle force in the *mdx* mouse treated with NAC, the highlighted effects on body weight and tissue mass raise into question the utility of this antioxidant therapy due to the potential effects of systemic use in growing mice. While Lindsay et al report an exciting new antioxidant biomarker in DMD patients and potential therapeutic target for the reduction in ROS and oxidative stress induced muscle damage, additional preclinical trials need to be carried out to explore the systemic effects of this antioxidant *in vivo* and determine its efficacy as a future novel therapeutic agent for the treatment of DMD.

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