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Letter to the Editor

Rebuttal to: Simvastatin Treatment Does Not Ameliorate Muscle Pathophysiology in a Mouse Model for Duchenne Muscular Dystrophy, Verhaart et al. 2020

N.P. Whitehead, M.J. Kim, K.L. Bible, M.E. Adams and S.C. Froehner
University of Washington, Seattle, Washington, USA

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In 2015, we provided evidence that simvastatin, a widely used cholesterol lowering drug, has potential to be a novel treatment of Duchenne muscular dystrophy (DMD) [1]. Oral delivery of simvastatin to *mdx* mice in the food resulted in drug plasma levels of 403 nM (169 ng/ml) using a dose within the normal range prescribed for humans, based on mouse-to-human equivalence calculations. Long-term simvastatin treatment resulted in a dramatic improvement in overall muscle health. Serum creatine kinase, inflammation and fibrosis were all markedly reduced. Functionally, simvastatin treatment enhanced hindlimb specific force, protected against muscle fatigue and improved force recovery after fatigue. Furthermore, simvastatin improved diaphragm function in old *mdx* mice and reversed fibrosis. We found no evidence that simvastatin impairs muscle regeneration or myogenesis in *mdx* mice. Mechanistically, simvastatin enhances autophagy and reduces oxidative stress, due to reduction in the high levels of NADPH oxidase 2 observed in *mdx* muscle [2]. More recently, we have shown that cardiac function in the *mdx* mouse is also improved by simvastatin, due in part to the prevention of fibrosis [3]. In strong support of our findings, two recent studies have independently validated our results by showing positive effects of simvastatin treatment in *mdx* mice [4, 5]. In one of these studies, significant improvements in muscle function (force and fatigue resistance), as well as reduced inflammation, fibrosis and plasma CK were comparable to those in our original paper [5]. In the other paper, widespread perturbations in muscle cholesterol metabolism were discovered in DMD and *mdx* muscles, and these changes were normalized by simvastatin treatment in dystrophic mice along with reduced plasma CK [4]. Together, our findings and those from two independent research groups provide robust evidence that cholesterol metabolism is abnormal in dystrophic muscles and that simvastatin could provide a novel, inexpensive, and widely used treatment for DMD.

Surprisingly, in a recent paper published by the laboratories of Annemieke Aartsma-Rus and Dominic Wells, it is claimed that they are unable to replicate our results [6]. A major flaw in this conclusion is that neither laboratory was able to achieve therapeutic levels of plasma simvastatin. In fact, the levels they reported (1–3 ng/ml) are *∼50–150 times lower* than we achieved in our study [1]. We concur that these very low levels of simvastatin would not improve *mdx* muscle health and function, as is the case with all drug studies where therapeutic levels are not achieved. In view of this, our contention is that the title of their paper, "Simvastatin Treatment Does Not Ameliorate Muscle Pathophysiology in a Mouse

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Model for Duchenne Muscular Dystrophy", is highly misleading and scientifically unjustified because of the failure to achieve therapeutic levels of simvastatin.

During the last 5 years, we have conducted many studies, some unpublished, using several batches of simvastatin in the chow, prepared by Research Diets, on at least three refreshed colonies of *mdx* mice. All of the batches have shown an improvement in *mdx* skeletal and cardiac muscle health and function. Many other laboratories have achieved therapeutic effect of simvastatin by delivery in the chow (for example, refs [7–9]).

Failure to achieve therapeutic levels of simvastatin in the Verhaart et al. paper could result from any number of methodological differences from our studies. However, regardless of the reason(s) why, the critical point is that neither laboratory achieved therapeutic plasma levels of simvastatin and therefore their data cannot be directly compared to our findings where we achieved much higher drug exposure.

Replication of results by an independent laboratory is the foundation of scientific inquiry. We welcome a careful effort to replicate our results. In fact, we consulted with the Aartsma-Rus laboratory and advised them on various aspects of their study. Unfortunately, Verhaart et al. failed to meet the most critical aspect of animal drug studies – levels of drug exposure in the therapeutic range – which they admit to in the abstract of their paper. Ultimately, this issue is not merely a scientific disagreement between research laboratories but instead has real-world impacts on DMD patients and their families in terms of providing accurate information for potential therapeutic approaches.

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