Does an Exercise Mimetic Improve Brain Function?

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Abstract:

Alzheimer's (AD) and vascular dementia (VD) have become increasingly prevalent in elderly populations in the U.S. in recent years. In addition to cognitive decline, another developing health risk is loss of skeletal muscle mass and function in the aged. A lack of activity is a significant risk factor for the progression of AD/VD in later life, and exercise has been shown to prevent or blunt the progression of these diseases. It has been shown that brain tissue in those afflicted with AD/VD is exposed to higher levels of oxidative stress, an imbalance in the production of reactive oxygen species (ROS) and antioxidant defenses. Exercise is known to reduce oxidant stress, but the aged are not always able to exercise at a level that conveys benefit. The deletion of myostatin, a negative muscle regulator protein, has been shown to increase muscle mass and improve glucose handling, which improves oxidant stress.

In our lab, we use mouse models of myostatin (myo) deletion in young and aged mice, as well as examining sex differences. Our groups include lean control and lean muscular (MYO KO) in both sexes at 12- 20 months old (20-30 years old in humans) and 24-28 months old mice (69-81 years old in humans). We sacrifice mice and use fine dissection to carefully isolate and remove carotid arteries. These tissues have been analyzed for oxidant stress enzymes (ex: NOX1, NOX2, and NOX4). We also used pressure myography to examine vascular function. We observed animal movement using San Diego Instruments Photobeam Activity System (SID PAS) to assess fine motor

skills, balance and rearing. This is an in vivo non-terminal experiment that resides in our lab. We found that myostatin deletion in young mice reduces the oxidant stress enzyme NOX1 and upregulates the antioxidant NOX4. In aged control mice, we see endothelial function is impaired but returned to the level of control with myostatin deletion. Importantly, we observed that, with aging, both fine motor, balance, and rearing is impaired, but that myostatin deletion improved all aspects of motor function. Interestingly, this improvement in motor function was especially significant in the aged male mice.

Introduction:

An important variable in brain health is oxidative stress, an imbalance in the production of reactive oxygen species (ROS) and antioxidant defenses (Gella & Durany, 2009). Oxidative damage to the brain can cause neurodegenerative diseases such as Huntington's, and Parkinson's, and can also cause numerous neuropsychiatric disorders (Salim, 2017). The accumulation of oxidative stress has been shown as a possible contributor to Alzheimer's disease and cognitive aging (Ionescu-Tucker & Cotman, 2021). Reactive oxygen species (ROS) can cause oxidative damage to biomolecules in the body, and this accumulation of oxidative damage is what is known as aging (Mariani et al., 2005). A known source of oxidative stress and ROS generation is NADPH oxidases (NOX), which have been shown to cause endothelial dysfunction and oxidative stress in renal vasculature (Muñoz et al., 2020).

NADPH oxidases catalyze oxygen in order to produce reactive oxygen species (Guo & Chen, 2015). Superoxide, a type of ROS, affects the amount of NADPH oxidase

1 (NOX1), a key regulator of vascular tone and endothelial health (Qiu et al., 2014). The deletion of myostatin, a protein that is a negative regulator of muscle mass, has been shown to mitigate age-related muscle loss (Morissette et al., 2009). The deletion of myostatin also improves insulin resistance, which then improves oxidant stress notably by expression of NOX1 (Qiu, Athanassios, et al., 2014). NOX1, which has previously been shown to be upregulated in the systemic arterioles, becomes downregulated after myostatin is deleted (Sharma et al., 1999). Oxidant stress also causes an imbalance in NOX4, which produces hydrogen peroxide as its main product, and has been shown to mediate endothelial dysfunction such as hypertension and cardiac hypertrophy (Guo et al. 2015). mRNA levels of NOX4 in cerebral vasculature of rodents have been shown to be higher than in peripheral blood vessels (Wingler et al., 2011).

The brain is particularly vulnerable to oxidative stress. As neurons are non-dividing cells that cannot be replaced when damaged, there is an increase in oxidative biomarkers, such as DNA damage, as the brain ages (Ionescu-Tucker et al., 2021). In Alzheimer's disease (AD) there are increased levels of DNA strand breaks, believed to be caused by oxidative damage to DNA bases (Gella et al., 2009). In a brain affected by AD there is excess ROS present, and one of the first indicators of developing Alzheimer's disease is the presence of increased oxidative damage to mitochondrial DNA (Ionescu-Tucker et al., 2021). DNA damage caused by oxidative stress presents as DNA breaks and oxidized purines and pyrimidines (Ionescu-Tucker et al., 2021). There are high levels of DNA damage in both healthy aged brains and AD-affected aged brains, and DNA damage is believed to be a root cause of the aging process (Ionescu-Tucker et al., 2021).

In Alzheimer's disease specifically, there is an increase in dysfunctional repair of DNA as compared to deficits in DNA repair in normal aging (Ionescu-Tucker et al., 2021).

Exercise has been studied as a way to decrease the role oxidative stress plays in aging and development of neurodegenerative diseases. During strenuous exercise there are high levels of ROS production in muscles, thus increasing antioxidant enzyme activity to combat it (Ji et al., 1998). Heightened antioxidant enzyme activity has been shown to be an adaptive response in muscle tissue to age-related oxidative stress (Ji et al., 1998). Well-exercised muscles have increased levels of antioxidant enzyme activity and are more successful at ROS removal (Ji et al., 1998). However, the aged are not always able to exercise at a level that conveys this benefit. Myostatin, as previously stated, is a protein that negatively regulates muscle mass, and inhibition of this protein has been shown to mitigate age-related muscle loss. Removal of myostatin in mice increases skeletal muscle mass and decreases adiposity (Morissette et al., 2009). As exercised muscle mass is beneficial for decreasing oxidative stress, the removal of myostatin is potentially a way to decrease the harmful effects of oxidative stress on brain health and aging.

The goal of our study was to determine whether the removal of myostatin could act as an exercise mimetic to decrease the harmful effects of oxidative stress on brain aging. To do this we studied how myostatin deletion effects NADPH oxidase production in brain aging in myostatin knockout and control mice models. We also used pressure myography to examine vascular function. Lastly, we observed mice movement to assess

fine motor skills, balance and rearing as proxy measurements for brain activity and function.

Methods

The study groups for our project were two *Mus musculus* mouse models: $H_{db}H_{myo}$ (lean control), $H_{db}K_{myo}$ (lean muscular). K_{myo} was a myostatin knockout mouse and H_{myo} was a non-myostatin knockout mouse (Butcher et al., 2018). The *Mus musculus* mouse model, the most often used mammalian model for research, has a high reproduction rate and are ease to maintain and handle (Bacchini et al, 1992).

We used a San Diego Instrument Photobeam Activity System (SID PAS) to assess brain function and activity by recording fine twitch movement, horizontal movement across the cage, and how often the mice reared up on their hind legs. Mice rearing indicates that the mouse is active and able to explore its environment. The SID PAS measured these data points with a photobeam grid that recorded when it was broken by fine twitch movements (recorded as XF), horizontal movements (recorded as XA) and when the mouse reared up (recorded as Rear). The purpose of these tests is to determine how the activity of the mice differs between the lean muscular (MYO KO) and the lean control mice. These tests also provide an analysis of how receptive these mice's bodies would be to insulin and other medications.

After getting data for these external study focuses, we sacrificed a sample of the mice to remove brain tissue and carotid arteries. We analyzed these tissues for oxidant stress enzymes, in specific, NOX1, NOX2, and NOX4. In order to quantify specific

sequences of the mice's RNA, we used RT-PCR (Reverse Transcription Polymerase Chain Reaction) to convert RNA to DNA (Rio, 2014). We also assessed mesenteric blood vessel function using pressure myography. Dilation of blood vessels indicates endothelial health and function.

Results

We assessed the mRNA expression of oxidative stress enzymes of NOX1, NOX2, and NOX4 in the carotid artery of young $H_{db}H_{myo}$ and $H_{db}K_{myo}$ male mice. In Figure 1, our results show the expression of NOX1 was decreased in the myostatin knockout mice, however, the expression of NOX4 was upregulated. This shows that myostatin deletion decreased harmful ROS production while increasing the protective effects of antioxidant defenses in young male $H_{db}K_{myo}$ mice. The levels of NOX2 recorded in the carotid artery of myostatin knockout and control mice were not significantly affected. As NOX4 promotes cell proliferation and growth in endothelial cells, the upregulation of NOX4 in the myostatin knockout mice would have beneficial effects on vascular health (Guo et al., 2015). NOX1, however, has been shown to be a major source of ROS production in vascular diseases (Guo et al., 2015). The reduction of NOX1 in the myostatin knockout mice would therefore also be beneficial for vascular health and the reduction of oxidative stress. As NOX4 produces hydrogen peroxide, unlike NOX1 which produces oxygen, there is no reaction with nitric oxide to produce peroxynitrite, which is damaging to DNA and proteins (Guo et al., 2015). Therefore, the upregulation of NOX4 and the downregulation of NOX1 in mRNA expression of carotid arteries of young male myostatin knockout mice is overall beneficial in reducing oxidative stress and increasing vascular health.

The dilation of mesenteric blood vessels is a good indicator of endothelial function and vascular health. Using pressure myography, we assessed the dilation of mesenteric blood vessels to acetylcholine in aged $H_{db}K_{myo}$, aged $H_{db}H_{myo}$, and young $H_{db}H_{myo}$ mice. Figure 2 shows that the aged control mice had blunted dilator function as compared to the young control mice. However, in the aged myostatin knockout mice, dilator function was shown to improve. Individuals who suffer from dysfunction of the cerebrovascular and Alzheimer's disease are shown to have greater cognitive impairment than those who suffer from only one of the diseases (Bomboi et al., 2010). Studies show that vascular dysregulation is a precursor to AD and improving endothelial function could be a beneficial treatment (Bomboi et al., 2010). As our aged myostatin knockout mice showed improved endothelial function, the deletion of myostatin could be considered a possible treatment to vascular dysregulation and AD.

We ran SID PAS trials on four groups of male and female mice: young $H_{db}H_{myo}$, young $H_{db}K_{myo}$, aged $H_{db}H_{myo}$, and aged $H_{db}K_{myo}$. The trials recorded fine twitch movement (XF), horizontal movement (XA) and rearing (Rear). In Figure 3, our female mice showed a significant decrease in XA and Rear movement between the young control and the aged control. However, there was an increase in XF and Rear between the young control and the young myostatin knockout. There was also an increase in XF, XA, and Rear movement comparing the aged control and the aged myostatin knockout. Therefore, aging was shown to significantly blunt motor movements in control mice, but myostatin deletion in aged mice brings the activity levels back to control. In Figure 4, showing the male mice, there was a significant decrease in XF movement between the young control and the aged control that was completely restored with myostatin deletion. There was also a significant increase in XF, XA, and Rear movements between the aged control and the aged myostatin knockout. Myostatin deletion was therefore

shown to be effective in restoring motor function to aged male mice. As exercise is beneficial for decreasing oxidative stress, the deletion of myostatin allows the aged mice to have increased motor function and therefore could be beneficial for reducing oxidative stress levels.

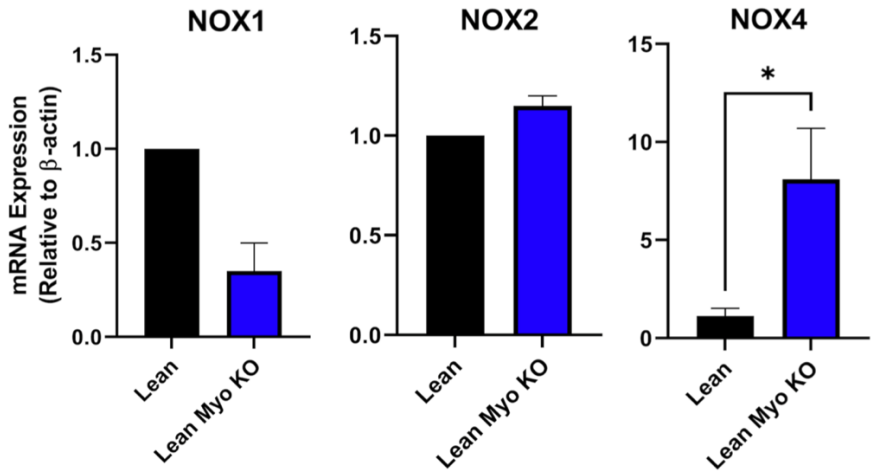

*Figure 1: The above figure shows the mRNA expression of NOX1, NOX2, and NOX4 in the carotid artery of young male control (Lean) and young male myostatin knockout (Lean Myo KO) mice. N = 3 for all groups, * denotes p<0.05*

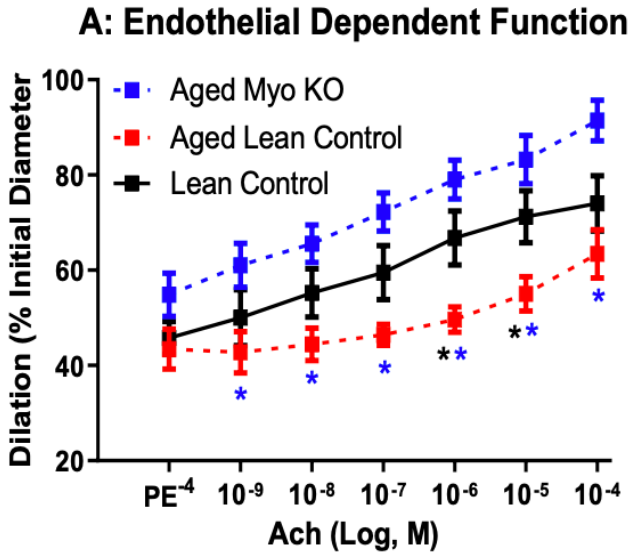

*Figure 2: The above figure shows the vasodilation of mesenteric blood vessels to acetylcholine in aged myostatin knockout (Aged Myo KO), aged control (Aged Lean Control), and young control (Lean Control) mice. N = 5 for all groups, * denotes p<0.05 versus similar acetylcholine concentration.*

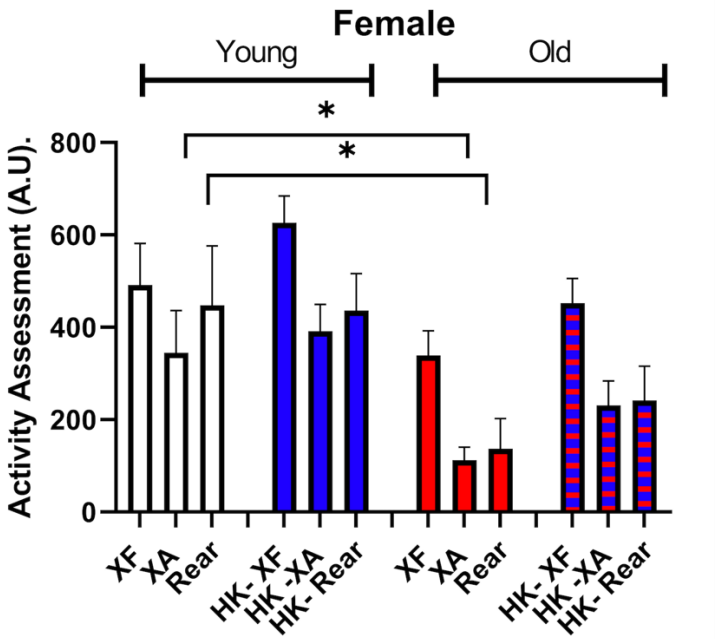

*Figure 3: The above figure shows the SID PAS recorded movements of fine twitch (XF), horizontal (XA), and rearing (Rear) in female young control (white), young myostatin knockout (blue), aged control (red), and aged myostatin knockout (striped blue and red) mice. N = 4 to 8 per group, * denotes p<0.05*

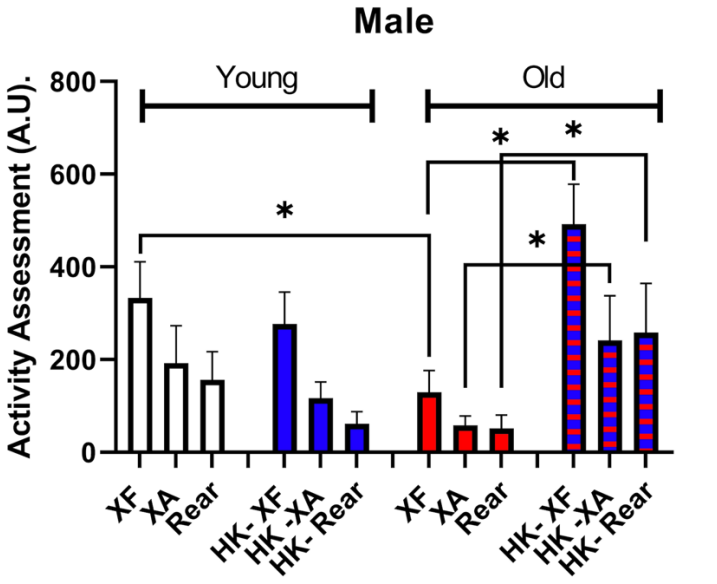

*Figure 4: The above figure shows the SID PAS recorded movements of fine twitch (XF), horizontal (XA), and rearing (Rear) in male young control (white), young myostatin knockout (blue), aged control (red), and aged myostatin knockout (striped blue and red) mice. N = 4 to 8 per group, * denotes p<0.05*

Discussion

The results found support our hypothesis that myostatin could be used as an exercise mimetic to reduce the harmful effects of oxidative stress on brain aging. The diminished levels of NOX1 has beneficial effects on decreasing harmful ROS and superoxide production. As there is excess ROS present in a brain with Alzheimer's disease, the reduced NOX1 production could be effective in decreasing the risk of AD or other neurodegenerative diseases. Our results also show increased levels of NOX4 in the mRNA expression of carotid arteries. NOX4 has a protective effect in vascular diseases and is the most abundant NADPH oxidase in endothelium. NOX4 has been shown to have a beneficial effect on vasodilator function, and our results support this as they showed both increased NOX4 production and improved dilation of mesenteric blood vessels in myostatin knockout mice.

Our results also show an increase in motor movement in the myostatin knockout mice. Well-exercised muscles are shown to have higher levels of antioxidant enzymes, thus decreasing oxidative stress. Motor movement is a proxy measurement for brain function, and as the myostatin knockout mice has increased movement this could be indicative of healthier brain activity. As physical activity improves cognition in age-related neurodegenerative diseases and recovery from brain injury, the improved mobility of the aged myostatin knockout mice would indicate better health in relation to AD and other cognitive decline (Alkadhi, 2018). Overall, exercise can be used as a preventative measure for cognitive decline, and our result show that myostatin deletion has beneficial effects on increasing mobility and activity of aged mice.

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