**Author’s response to reviews**

**Title:** Exercise and Resveratrol Increase Fracture Resistance in the 3xTg-AD Mouse Model of Alzheimer’s Disease

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**RESPONSE TO THE REVIEWERS**

Authors: We thank the Reviewers for the suggested improvements to the manuscript. We appreciate the effort that went into the careful and thoughtful reviews of our study. In the following, we have attempted to address the concerns of the Reviewers. The changed parts in the revised manuscript are shown in red font. We sincerely believe that all the revisions suggested by the Reviewers helped us improve the manuscript and we are tremendously grateful.

Reviewer reports:
Fangxia Guan (Reviewer 1): Review comments In this manuscript, Alkhouli et al. investigated whether treatment with resveratrol or (and) exercise could improve fracture resistance in an AD mouse model. The authors report that treatment with resveratrol or (and) exercise improves fracture resistance and cross-sectional geometric indicators of bone strength.

I have the flowing concerns.

1. There are some mistakes in the manuscript, especially in the title.

AUTHORS: The error in the title was corrected: “Exercise and Resveratrol Increase Fracture Resistance in the 3xTg-AD Mouse Model of Alzheimer’s Disease”. The manuscript was checked for additional mistakes, which were corrected.

2. As reported, whether exercise had beneficial effects on AD was controversial, because treadmill running is a forced exercise. In the manuscript, there was no control group for exercise group. So, I want to understand the detailed exercise procedure of mice in the exercise group.

AUTHORS: Grant funding did not cover the cost of additional mice for a wild type exercise group. Thus, our experimental design was set up as follows: wild type and 3xTg-AD mice were compared to identify deficits in the AD group, then exercise and resveratrol 3xTg-AD groups were compared to 3xTg-AD controls to identify improvements from the AD baseline.

As requested, we added more information on the exercise procedure to the Methods: “Exercise training consisted of forced running on a motor-driven treadmill designed for mice (Exer 3/6 treadmill; Columbus Instruments, Columbus, OH, USA). Mice were initially placed in separate lanes of the treadmill while it was off (0 m/min) and gradually acclimated to daily 10-minute running sessions at 10 m/min for a period of one week. The duration and intensity were increased to 20 min at 10 m/min on week 2, followed by 30 min at 12 m/min on week 3. From week 4 to the end of the training regimen, running activity consisted of 45 min at 15 m/min, 5 day/week, corresponding to an estimated submaximal VO2 of ~50 mL/kg/min [21]. At this intensity, mice were running without reluctance and were able to continue for the entire 45-minute session.”
3. It's not clear how did the authors choose the dosage of resveratrol.

AUTHORS: We added the following explanation to the Methods: “Mice in the resveratrol groups were administered resveratrol (Lalilab Inc., Durham, NC, USA) in diet (4g/kg, AIN-93G, Dyets Inc., Bethlehem, PA), while the control group received regular diet without resveratrol. The resveratrol dosage was selected based on previous studies showing this amount provides sufficient bioavailability to have an active effect with dietary administration in mice (equivalent to ~146 mg kg\(^{-1}\) day\(^{-1}\)) [17, 18]. In addition, this dosage exerts an insulin-mimetic effect, reduces oxidative stress, and activates the sirtuin 1 anti-apoptotic signaling pathway [18-20].”

Rong Sheng (Reviewer 2): The manuscript report the efficacy of resveratrol and exercise in ameliorating fracture risk in the 3xTg-AD mouse model for AD. The manuscript is well prepared and can be published in BMC complementary and alternative medicine after finishing following minor revision.

1) The relationship of resveratrol with AD and osteoporosis need to be described in detail, and please explain why choose a very high dose (4g/kg) of resveratrol in the experiments.

AUTHORS: We added the following description of resveratrol and osteoporosis to the Introduction: “Resveratrol (3,5,4-trihydroxy-trans-stilbene) is a naturally occurring polyphenol found in relatively high concentrations in grapes, seeds, and nuts is known to exert significant bone-protective effects [16]. Specifically, dietary resveratrol activates antioxidative and osteoblastic proliferative pathways (SIRT1 and Fox01), increasing biochemical indicators of bone quality and stimulating osteogenesis to improve bone mineral density in osteoporotic animals [17, 18, 19]. Activation of the SIRT1 pathway by resveratrol also improves cognitive and memory deficits and is being investigated as a treatment for AD [20].”

We added the following explanation of the dose to the Methods: “The resveratrol dosage was selected based on previous studies showing this amount provides sufficient bioavailability to have an active effect with dietary administration in mice (equivalent to ~146 mg kg\(^{-1}\) day\(^{-1}\)) [21, 22]. In addition, this dosage exerts an insulin-mimetic effect, reduces oxidative stress, and activates the SIRT1 anti-apoptotic signaling pathway [22, 23, 24].”
2) I suggest the manuscript should provide some biochemical indicators related to bone quality of 3xTg-AD Mice group with group of resveratrol, exercise and resveratrol + exercise

AUTHORS: We assessed bone quality indirectly by measuring fluorescence of bone ECM. However, due to insufficient funding, we were unable to collect biochemical data indicative of bone quality. However, apart from adding the statement mentioned above to the Introduction on biochemical indicators of bone quality from the literature, we added also added the following explanation to the Discussion: “While we did not directly assess biochemical markers of bone quality, we measured CTBF, which reflects post-translational modification of structural collagen type I through nonenzymatic glycation. 3xTg-AD mice treated with resveratrol and exercise have CTBF values similar to wild type mice, suggesting treatment yields improvements in the quality of bone matrix. This finding is likely an effect of resveratrol treatment, which has been shown to inhibit the formation of AGEs, as well as stimulate expression of collagen type I by osteoblasts [17, 48, 49, 50]. Of the two treatments in our study, resveratrol yields the greatest improvements in elastic modulus and stiffness, both of which are greatly affected by AGEs and are significant contributors to bone toughness [51]. Exercise treatment has also been shown to reduce AGE formation, although the mechanism is not as well understood [52]. Our results suggest actions of resveratrol and exercise may improve bone quality in 3xTg-AD mice, thereby improving fracture resistance.”

We also added references showing the effects of resveratrol on bone formation and how this relates to our findings: “3xTg-AD mice treated with resveratrol and exercise also had increased bone diameter and total and cortical cross-sectional areas in comparison with untreated 3xTg-AD mice. Resveratrol has been shown to increase alkaline phosphatase activity, calcium deposition, and expression of anti-apoptotic and osteogenic regulatory proteins and transcription factors (osteocalcin, Osterix, Runx2/Cbfa1, Wnt, Sirt1) [41, 42, 43]. These stimulatory effects likely explicate the observed increases in bone diameter, area, and fluorescence, which contribute to the improved fracture resistance in these mice.”