Geranylgeraniol Supplementation Mitigates Soleus Muscle Atrophy via Changes in Mitochondrial Quality Control in Diabetic Rats

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ABSTRACT

With diabetes, skeletal muscle mitochondrial quality control (mitochondrial fusion, fission & macro-autophagy) is impaired. Geranylgeraniol (GG) is shown to have a protective effect on preventing mitochondrial damage and muscle health; however, the effect of GG on a diabetic model is not known. PURPOSE: To determine the effect of GG on mitochondrial quality control and muscle cross-sectional area (CSA) in diabetic rats. **METHODS:** Thirty-five Sprague-Dawley rats were divided into three diet groups: control diet (CON), high-fat diet with 35 mg/kg body weight of streptozotocin (HFD), and HFD with 800 mg/kg body weight of GG (GG). Due to the limited sample, a total of 21 (CON: n = 7; HFD: n = 7; GG: n = 7) rats' muscle samples were used for this report. The soleus muscles were harvested after 7-weeks of feeding and were analyzed for OPA1, MFN2, DRP1, pDRP, PINK1, Parkin, LC3A, and LC3B protein content using western blot analysis. Muscle CSAs were assessed using Image J. **RESULTS:** A significant ($p < 0.05$) condition effect was observed for MFN2, DRP1, LC3A, and LC3B protein contents and muscle CSA. For mitochondrial fusion, GG (0.21 ± 0.08) had lower MFN2 than CON (0.43 ± 0.04; p = 0.007) and HFD (0.65 ± 0.08; p = 0.010). For mitochondrial fission, GG (0.26 ± 0.07) had lower DRP1 than HFD (0.59 ± 0.07; p = 0.019). For macro-autophagy, GG (1.08 ± 0.28) had lower LC3A than CON (2.81 ± 0.55; p = 0.028) and HFD (3.99 ± 0.57; p = 0.010); whereas GG (0.63 ± 0.21) had lower LC3B than HFD (1.93 ± 0.24; p = 0.012). No significant differences were observed for OPA1, pDRP, PINK1, Parkin, and LC3B/A. For muscle size, CON (10,092.88 ± 104.67µm2) had larger CSA than GG (7284.69 ± 70.91µm2, p = 0.001) and HFD (5615.59 ± 59.97µm2; p = 0.001), whereas GG (7284.69 ± 70.91µm2) had larger CSA than HFD (5615.59 ± 59.97µm2; p = 0.001). **CONCLUSION:** GG supplementation could prevent mitochondrial fragmentation (reduction in DRP1), thus, potentially resulting in a decreased demand for mitochondrial fusion (reduction in MFN2). In addition, a greater rate of autophagosome degradation than formation (reduction in LC3A and LC3B) was observed (indicative of an increase in macro-autophagy). Improvement in mitochondrial quality could potentially contribute to attenuating the reduction of muscle size in diabetic rats with GG supplementation.

INTRODUCTION

- Increased inflammation and oxidative stress can result in mitochondrial dysfunction, a potential pathogenic contributor to insulin resistance.
- Mitochondrial quality control (mitochondrial fission, fusion, & macro-autophagy) is a mechanism to maintain healthy mitochondria and prevent mitochondrial dysfunction.
- Individuals with Type 2 diabetes had increased fission (increased in DRP1), decreased fusion (decreased in MFN2), and reduced capacity to remove damaged mitochondria (decrease in PINK1, Parkin, and LC3B).
- Geranylgeraniol (GGOH) supplementation has been shown to reduce inflammatory markers and prevent mitochondrial damage in neuronal cells and preserve muscle cross-sectional area in the skeletal muscle.
- Improving mitochondrial quality is essential to improve metabolic regulation in diabetic populations; however, to date, the effect of GGOH on mitochondrial quality control and muscle cross-sectional area in a diabetic model is not known.

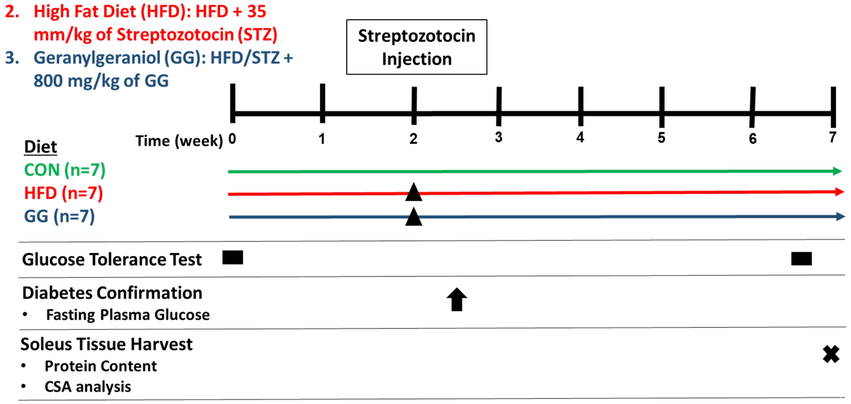
PURPOSE

To determine the effect of GGOH on mitochondrial quality control and muscle cross-sectional area in diabetic rats.

METHODS

Brief Overview

Sprague-Dawley Rats (n=21)
1. CON: Normal Diet
2. High Fat Diet (HFD): HFD + 35 mm/kg of Streptozotocin (STZ)
3. Geranylgeraniol (GG): HFD/STZ + 800 mg/kg of GG



Muscle Preparation and Protein Analyses

- Muscle samples were homogenized, agitated, and centrifuged.
- Protein content for mitochondrial fission (DRP1 & pDRP), fusion (MFN2 & OPA1), and macro-autophagy (mitophagy: PINK1 & Parkin; autophagy: LC3A & LC3B*)* were analyzed using western blot analysis. GAPDH was used as the loading control.
- Chemiluminescent substrate and the C-Digit imaging system were used to visualize the stained protein bands.
- Image Studio Digits Ver 4.0 was used for band densitometry.

Muscle Cross-Sectional Area (CSA) Analysis

- Muscle samples were sectioned at 10 µm followed by Hematoxylin and Eosin staining.
- Slides were visualized with a microscope and muscle CSA was analyzed using Image J.
- 100 muscle fibers from each rat were analyzed.

Data Analyses

- Data was analyzed using a one-way ANOVA.
- Bonferroni post hoc tests were used for pairwise comparisons.
- Statistical significance was set at $p \leq 0.05$.
- Data are reported as mean ± SE.

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RESULTS

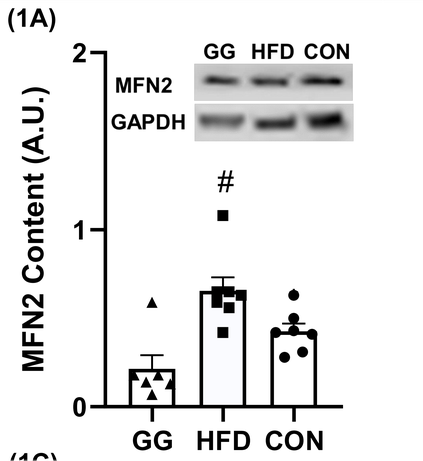

Figure 1. Protein content for **(A)** MFN2, **(B)** DRP1, **(C)** LC3A & B. A significant condition effect was observed for MFN2, DRP1, LC3A and LC3B. # P< 0.05 vs. GG

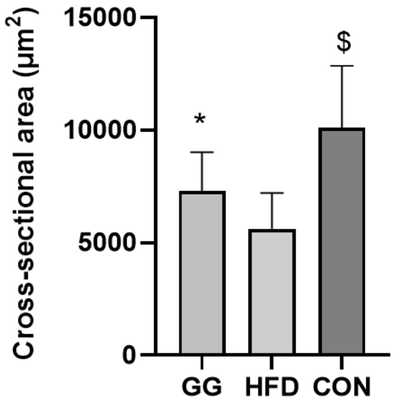

Figure 2. Soleus muscle CSA. 700 muscle fibers were counted for each group. A significant condition effect was observed for muscle CSA of the soleus. $ P< 0.05 vs. GG and HFD. * P< 0.05 vs. HFD.

CONCLUSIONS

- Supplementing GGOH to diabetic rats was able to mitigate the CSA reduction observed in diabetic rats without GGOH.
- Additionally, a lower mitochondrial fragmentation (decrease in DRP1) and a greater autophagosome degradation (decreased in LC3A and LC3B) was observed in diabetic rats with GGOH when compared to diabetic rats without GGOH.

ACKNOWLEDGEMENTS

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