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

Nigel C. Jiwan1, Casey Apple2, Rui Wang4, Chwan-U Shen3,4, & Hui-Ying Luk1

1Department of Kinesiology and Sport Management, Texas Tech University, Lubbock, TX
2Department of Kinesiology and Sport Management, Texas Tech University, Lubbock, TX
3Department of Pathology, Center of Excellence for Integrative Health; 4Center of Excellence for Translational Neuroscience and Therapeutics, Texas Tech University Health Sciences Center, Lubbock, TX

ABSTRACT

With diabetes, skeletal muscle mitochondrial quality control (mitochondrial fusion, fission & macro-autophagy) is impaired. Geranylgeraniol (GGOH) is shown to have a protective effect on preventing mitochondrial damage and muscle health; however, the effect of GGOH on a diabetic model is not known. PURPOSE: To determine the effect of GGOH 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 GGOH (GG). Due to the limited sample, a total of 21 (CON: n = 7, HFD: n = 7, GGO: n = 7) rats’ muscle samples were used for this report. The soleus muscles were harvested after 7 weeks of feeding and were analyzed for OPAL, MFN1, DRP1, pDRP1, 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, GGOH (0.21 ± 0.08) had lower MFN2 than CON (0.43 ± 0.04; p = 0.007) and HFD (0.65 ± 0.48; p = 0.010). For mitochondrial fission, GGOH (0.26 ± 0.07) had lower DRP1 than HFD (0.59 ± 0.07; p = 0.033). For autophagy, GGOH (0.38 ± 0.28) had lower LC3A than CON (0.21 ± 0.05; p = 0.028) and HFD (0.39 ± 0.57; p = 0.101); whereas GC (0.63 ± 0.21) had higher LC3B than HFD (1.91 ± 0.24; p = 0.002). No significant differences were observed for OPAL, pDRP1, Parkin, and LC3B/A. For muscle size, CON (10.92 ± 2.67 mm²) had lower CSA than HFD (2584.69 ± 70.11 mm²; p = 0.001) and GGOH (1651.59 ± 59.97 mm²; p = 0.001). CONCLUSION: GGOH 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 control could potentially contribute to attenuating the reduction of muscle size in diabetic rats with GGOH supplementation.

INTRODUCTION

• Increased inflammation and oxidative stress can result in mitochondrial dysfunction, a potential pathogenic contributor to insulin resistance.
• Mitochondrial quality control (mitochondrial fusion, fission, & macro-autophagy) is a mechanism to maintain healthy mitochondria and prevent mitochondrial dysfunction.
• Individuals with Type 2 diabetes have increased fission (in DRP1), decreased fusion (in MFN2), and reduced capacity to remove damaged mitochondria (decrease in PARK1, PARK2, 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 +75 ± 800 mg/kg of GG

RESULTS

1. CON (n=7) HFD (n=7) GG (n=7)

2. Diet

3. Streptozotocin Injection

Time (weeks)

0 1 2 3 4 5 6 7

Glucose Tolerance Test

Diabetes Confirmation

Fasting Plasma Glucose

Soleus Tissue Harvest

Protein Content

CSA Analysis

Muscle Preparation and Protein Analyses

• Muscle samples were homogenized, agitated, and centrifuged.
• Protein content for mitochondrial fission (DRP1 & pDRP1), fusion (MFN2 & QP1A), and macro-autophagy (mitophagy: PARK1 & 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 ≤ 0.05.
• Data are reported as mean ± SE.

ACKNOWLEDGEMENTS

This project was supported by the by the Texas Tech University internal funding.