Ginger Root Extract Supplementation Attenuated Mitochondrial Fusion and Improved Skeletal Muscle Size in Type 2 Diabetic Rats

Casey Appell [1], Nigel C. Jiwan [1], Chwan-Li Shen[2,3,4], Hui-Ying Luk [1]
[1] Applied Physiology Laboratory, Department of Kinesiology and Sport Management
Texas Tech University, Lubbock, TX
[2] Department of Pathology, [3]Center of Excellence of Integrative Health,
[4]Center of Excellence for Translational Neuroscience and Therapeutics; Texas Tech University Health Scienc

TEXAS TECH UNIVERSITY
Department *of* Kinesiology & Sport Management™

Abstract

Backgrounds: Type 2 diabetes mellitus has been associated with dysfunctional mitochondrial dynamics (biogenesis, fission, and fusion) and decreased muscle cross sectional areas (CSA). We previously reported that ginger root extract supplementation enhanced the mRNA expression of mitochondrial biogenesis-associated genes in diabetic rats. However, the effect of ginger root extract on the protein expression of basal mitochondrial dynamics (fission and fusion) and CSA in diabetic rats has not been elucidated.

Objectives: We investigated the effects of gingerol-enriched ginger (GEG) supplementation on mitochondrial dynamics and CSA in diabetic rats.

Methods: Thirty-three male rats were assigned to three groups: low-fat diet (CON group), high-fat-diet+streptozotocin (single dose 35mg/kg BW) (T2DM group), and high-fat-diet+streptozotocin+0.75%GEG in diet (GEG group) for 42 days. Soleus tissues were collected and analyzed for cross sectional area (CSA) using H&E stain and the protein expression of fission (DRP1, PDPR1), fusion (OPA1, and MFN2), and mitophagy (Pink1, Parkin, LC3A/B) using western blot. The data of CSA and protein expression of groups was presented as the fold-change relative to CON group.

Results: Compared to the CON group, T2DM had greater MFN2 protein expression in soleus (p=0.002). Supplementation of GEG into the diet suppressed MFN2 protein expression in soleus than those in T2DM group (p=0.05). There was no difference in soleus MFN2 protein expression between GEG group and CON group. Unlike MFN2 protein expression, these was no difference in soleus OPA1 protein expression between the CON group and the T2DM group. Intriguingly, GEG supplementation resulted in decreased protein expression of OPA1 in diabetic rats (p=0.006).
No differences in protein expression levels of DRP1, PDRP1, Pink1, Parkin, and LC3A/B were observed among the groups. In terms of CSA, the T2DM rats had decreased CSA than those in the CON rats (p<0.001). Supplementation of GEG into diet significantly increased CSA in diabetic rats (p<0.001). No significant difference in CSA was observed between the CON group and the GEG group.

Conclusion: In T2DM, GEG supplementation attenuated both mitochondrial outer membrane fusion (MFN2) and mitochondrial inner membrane fusion (OPA1) in diabetic rats, without modulation the mitochondrial fission (DRP1, PDRP1) and mitophagy (Pink1, Parkin, LC3A/B).

Backgrounds

- In 2017, type 2 Diabetes Mellitus resulted in over 1 million death worldwide and an estimated economic burden of $327 billion in the US alone.
- Diabetes (T2DM) is accompanied an increased ROS production which damages mitochondria.
- Damaged mitochondrial produce more ROS and contribute to insulin resistance, together resulting in a vicious cycle of ROS production and mitochondrial damage.
- Insulin resistance of skeletal muscle is the primary contributor to hyperglycemia and further metabolic disorder associated with T2DM.
- Ginger has been used in traditional Chinese medicine historically and contains the active ingredients; 6-gingerol and 6-shogaol.
- Ginger's bioactive role centers primarily around its ability to scavenge ROS which has been suggested to contribute to its anti-diabetic potential to improve insulin resistance.
- Additionally, in skeletal muscle ginger has been shown to improve mitochondrial dynamics (mitochondrial biogenesis) in obese rats, acting as a protective mechanism against mitochondrial damage.
- However, the effect of gingerol-enriched ginger on the basal mitochondria fission/fusion and mitophagy state has not been elucidated.

Objectives

- We investigated the effects of gingerol-enriched ginger (GEG) supplementation on mitochondrial dynamics and CSA in diabetic rats.

Methods

Methods

Groups and diet preparation

Low-fat-diet (CON group):

- Animals were given a standard diet (AIN-93G) throughout the study period.
- After two weeks of feeding, animals were given vehicle citrate buffer in a dose volume of 1mg/kg

High-fat-diet+streptozotocin (T2DM group):

- Animals were given a high-fat diet (45% calories from fat) throughout the study period
- After 2 weeks of feeding, animals were given a single streptozotocin (STZ) injection (35mg/kg BW, at 0.1mmol/L citrate buffer; pH4.4)

High-fat-diet+streptozotocin+0.75%GEG in diet (GEG group):

- Animals were given a high-fat diet (45% calories from fat) throughout the study period
- Additionally, Animals were given a GRE diet throughout the study period (0.75% GRE, w/w diet)
- After 2 weeks of feeding, animals were given a single streptozotocin injection (35mg/kg BW, at 0.1mmol/L citrate buffer; pH4.4)

	Low-fat-diet (CON)	High-fat-diet+streptozotocin (T2DM)	High-fat-diet+streptozotocin +0.75%GEG (GEG)
N	11	11	11

Table 1. Descriptive statistics.

Sample collection

- Due to limited samples 7 rats from each group were used for analyses (21 rats total)
- Fresh soleus skeletal muscle samples were excised from the left hind limb
- Muscle samples were cataloged and snap frozen in liquid nitrogen
- Muscle samples were stored at -80 ºC

Western blot analyses

- Frozen soleus muscle samples were homogenized. Supernatant was separated and analyzed for total protein using bicinchoninic acid assay.
- SDS-PAGE gel was used to separate total protein. Completed gels were transferred to PVDF membranes for blotting. Samples from each group were loaded in the same gel in duplicate.
- DRP1, PDPR1, OPA1, MFN2, Pink1, Parkin, and LC3A/B protein expression were analyzed and normalized to GAPDH.
- Stained protein bands were visualized using a chemiluminescent substrate.
- C-Digit imaging system was used, and band densitometry was performed by using Image Studio Digits Ver 4.0.

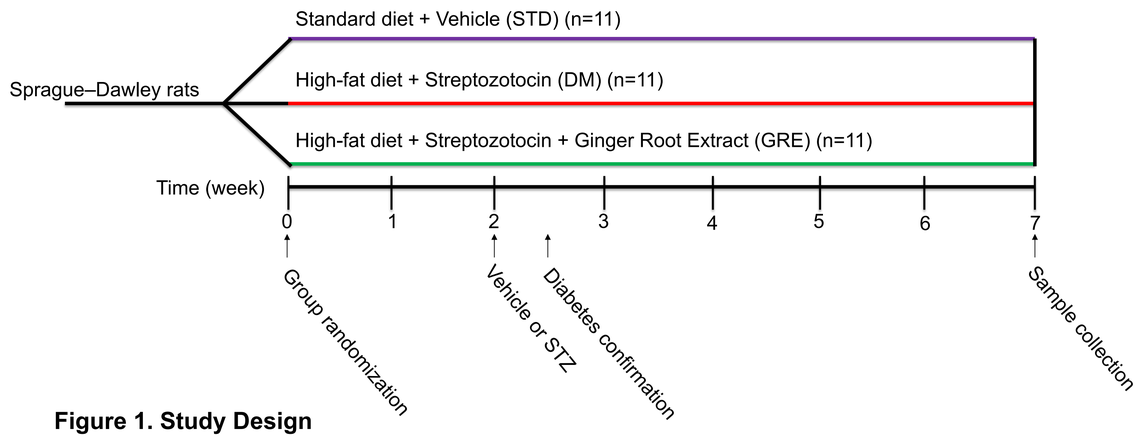

Figure 1. Study Design

Hematoxylin & Eosin histochemical stain

- Soleus muscle was frozen in O.C.T. compound and sectioned (10 mm thick) at -25°C in a cryostat
- Sections were placed on positive charged microscope slides
- *Slides were incubated with hematoxylin solution for 5 min followed by incubation with 0.5% Eosin solution with 1:100 acetic acid for 10 minutes.*
- *Samples were imaged at 5X magnification using a Ziess Axiovert 200m Inverted Fluorescent Motorized Microscope.*
- *100 muscle fibers in each sample were measured for cross sectional area using ImageJ software*

Data Analyses

- Data was analyzed using a one-way ANOVA, and the alpha level was set at $p < 0.05$.

Results

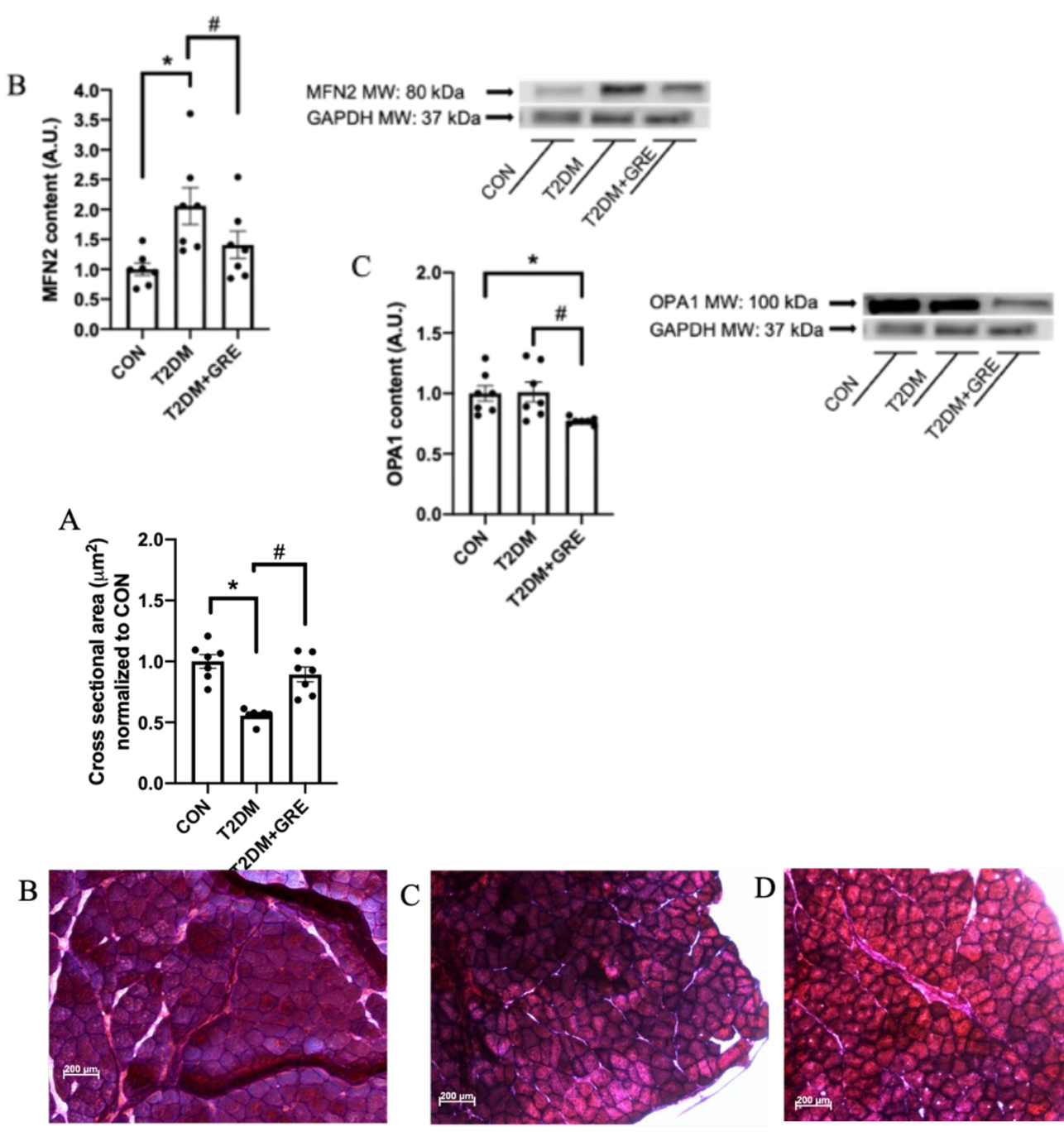

Figure 1: Protein content of MFN2 (A) and OPA1 (B) with representative western blot images and corresponding GAPDH. Data was normalized to CON. Values are mean ± SEM (CON: n=7; T2DM: n=7; T2DM+GRE: n=7). MW, molecular weight (kDa). *p<0.05 vs. CON. #p<0.05 vs. T2DM.
Figure 2: Skeletal muscle cross sectional area. (A) Skeletal muscle cross sectional area (mm2). Averaged of muscle fibers (n=100) counted per rat. Data was normalized to CON. Values are mean ± SEM (CON: n=7; T2DM: n=7; T2DM+GRE: n=7). *p<0.05 vs. CON, #p<0.05 vs. T2DM. Representative hematoxylin and eosin stain from CON group (B), T2DM group (C), and GEG group (D).

Conclusion

- In T2DM rats, GEG supplementation increased basal mRNA expression of mitochondria cleavage protein (i.e., *DRP*), mitophagy degradation tag (i.e., *PINK1*), and autophagolysosome formation (i.e., *LC3A, LC3B, p62*, autophagic flux) markers.
- Increase in the mRNA expression of these proteins suggests a potential increased in mitochondrial fission and mitophagy capacity
- Increase mitochondrial fission and mitophagy has been shown to improve mitochondrial health and improve type 2 diabetes related characteristics of the skeletal muscle

Acknowledgement

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