# Direct and Indirect effects of Guggulsterone on the Induction of Beiging in Mature 3T3-L1 Adipocytes

[1]Colette N. Miller, [2]Yusra Azhar, [2]Ashish Parmar, [2]Janaiya Samuels, [2]Rangaiah Shashidharamurthy, [2]Srujana Rayalam

[1]Department of Foods and Nutrition, University of Georgia, Athens, GA [2]Department of Pharmaceutical Sciences, School of Pharmacy, Philadelphia College of Osteopathic Medicine- GA Campus, Suwanee, GA

## Abstract

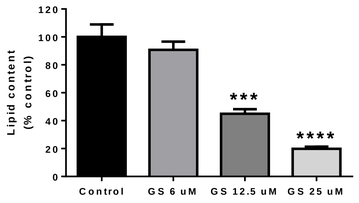
Phytochemicals have long demonstrated anti-obesity properties in adipocytes. Their ability, however, to induce browning in white adipose tissue is only beginning to emerge. We have recently established that the white adipocyte cell line, 3T3-L1, is capable of beiging under beta-adrenergic conditions. Using this information, we sought to investigate if the plant steroid guggulsterone (GS) can induce beiging in 3T3-L1s. 3T3-L1 preadipocytes were differentiated using established protocols supplemented with rosiglitazone and thyroid hormone. Direct effects of GS were measured by treating mature 3T3-L1s for 24 hours. Indirect effects were measured by treating mature 3T3-L1s with conditioned media from GS-treated RAW 264.7 macrophages. Direct treatment of 3T3-L1s with GS resulted in increased lipolysis, increased mitochondrial activity (11%), and increased peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α) levels by 250% more than control. 3T3-L1s also demonstrated an increase in uncoupling protein 1 (UCP1) expression by 200% and beige marker, T-box protein 1 (TBX1) expression by 80% more than control. Furthermore, this was accompanied by increased levels of G protein-coupled bile acid receptor (TGR5) and its downstream target iodothyronine deiodinase 2 (DIO2). Treatment of RAW 264.7 macrophages with GS induced a 60% increase in catecholamine release into the media compared to control. Using this conditioned media from macrophages, 3T3-L1 adipocytes increased the expression of DIO2 and UCP1 following 24 hours of incubation. Results from this study demonstrate that GS can potentially induce beiging in white adipose tissue through two distinct mechanisms: (1) direct signaling through the TGR5-cAMP-DIO2 pathway and (2) indirectly through stimulating catecholamine release in macrophages. Thus, it is reasonable to conclude that GS may improve the metabolic capacity of adipose tissue thereby counteracting the effects of obesity.

## Research Goals

- Determine if the anti-obesity phytochemical, guggulsterone, can promote mitochondrial biogenesis and beiging in 3T3-L1 adipocytes.
- Investigate if guggulsterone mediates mitochondrial biogenesis through direct or indirect signaling.

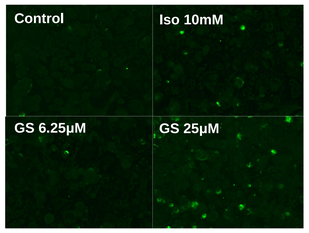
## Direct Effects of GS on 3T3-L1 Adipocytes

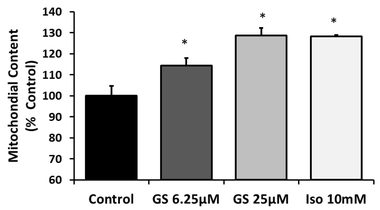
### GS decreases adipogenesis

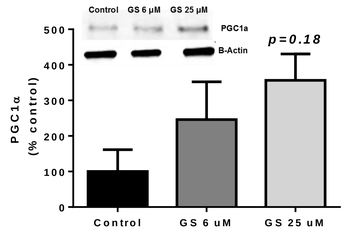



## Direct Effects of GS on 3T3-L1 Adipocytes

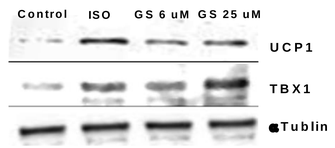
### GS induces mitochondrial biogenesis

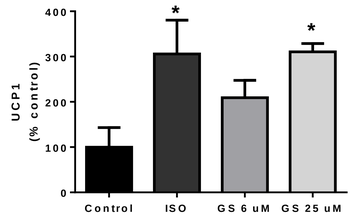


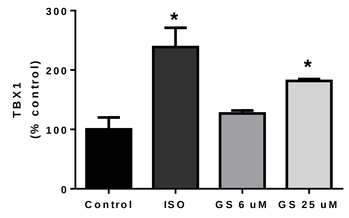




### GS induces adipocyte beiging

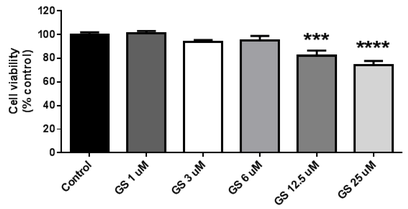


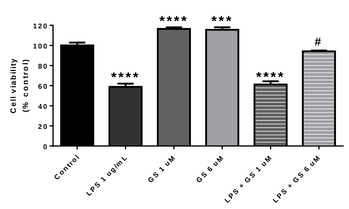



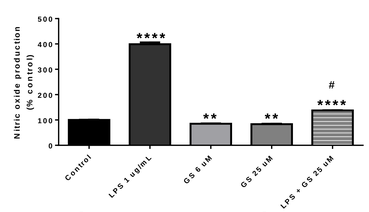




## Indirect Effects of GS on 3T3-L1 Adipocytes

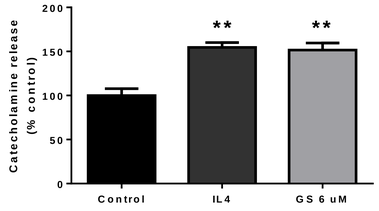
### GS protects against LPS-injury in RAW 264.7 macrophages

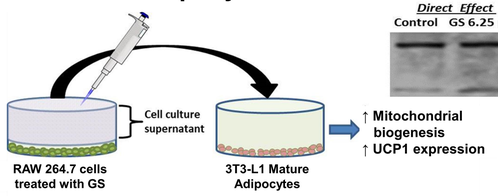


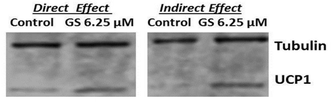




### GS induces catecholamine secretion in RAW 264.7 macrophages that can upregulate UCP1 in adipocytes



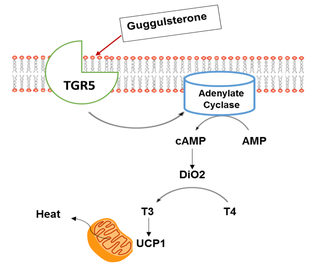



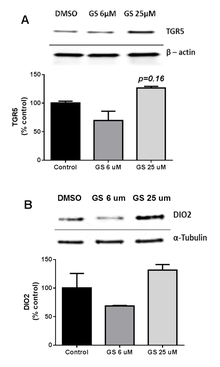

*p<0.05
**p<0.01
***p<0.001
****p<0.0001
#p<0.05 vs. + control

## GS signaling

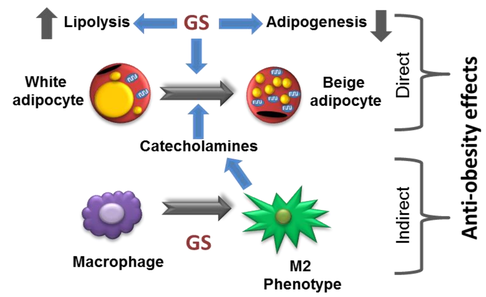
***Phytochemicals may induce beiging through nuclear receptor activation.***




***GS treatment appears to upregulate TGR5-DIO2 pathway in 3T3-L1s.***




## Working Model




## Conclusions

GS appears to have 2 distinct effects on adipocyte beiging:

1) Direct signaling in adipocytes leading to upregulation of thermogenic/ beiging makers and enhanced mitochondrial biogenesis. This effect appears to be partly mediated through TGR5 signaling.
2) Indirect signaling in adipocytes through macrophage M2 polarization and catecholamine secretion.

## Financial Support

Funding was provided by the Philadelphia College of Osteopathic Medicine Biomedical Research Program.