Sex hormone-binding globulin restores mitochondrial integrity in PPARγ-depleted mesenchymal stromal cells

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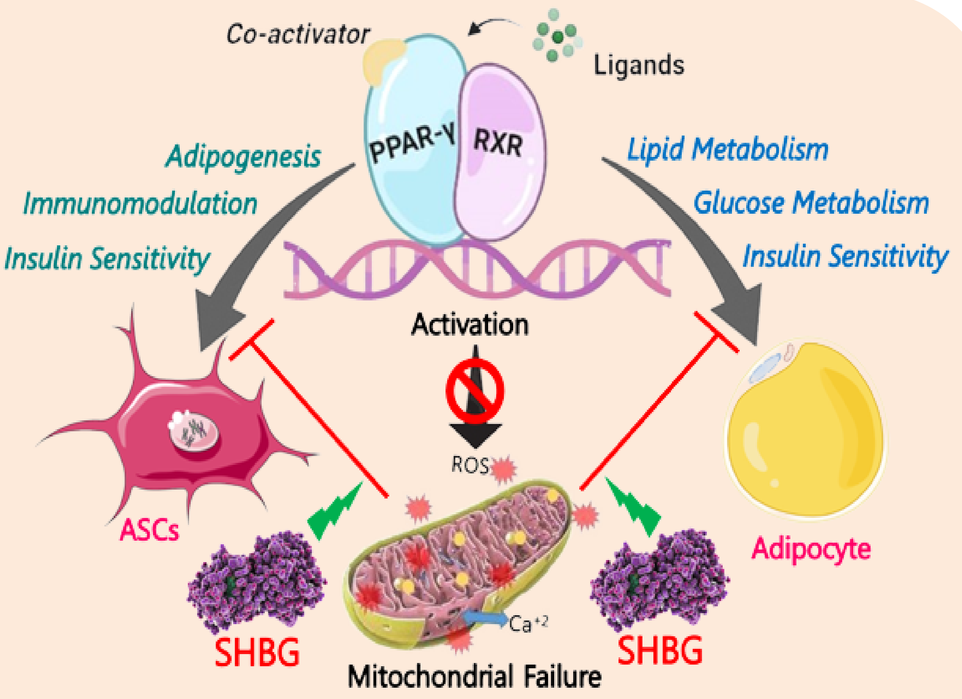
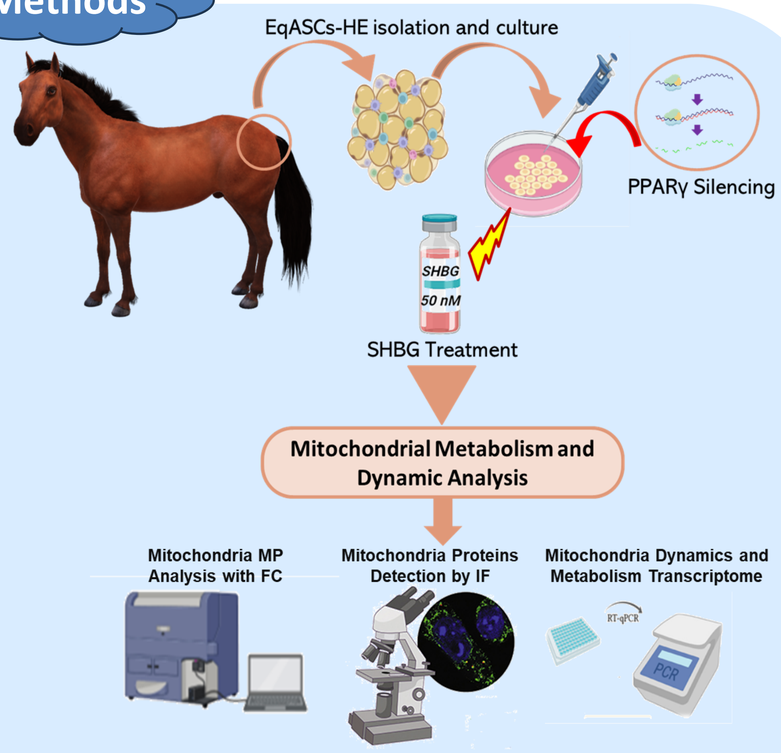
Background

PPARγ is a nuclear receptor protein that plays a crucial role in regulating gene expression associated with adipocyte differentiation and function. often referred to as the "master regulator" of adipogenesis, it is responsible for initiating and controlling the development of fat cells. It promotes the storage of lipids and regulates various aspects of fat cell metabolism including insulin sensitivity. PPARγ has a multifaceted relationship with mitochondrial function. It can influence mitochondrial biogenesis, quality control, energy metabolism, and oxidative stress. Dysregulation of PPARγ is associated with metabolic disorders that often involve mitochondrial dysfunction.

Sex Hormone-Binding Globulin (SHBG), primarily responsible for binding to and transporting certain sex hormones, has been recently proposed as a pleiotropic metabolic regulator, correlating with insulin sensitivity. Besides changes in both SHBG and PPARγ availability seem to be implicated in the pathogenesis of adipose tissue metabolic dysfunctions, whether both mediators' crosstalk in the modulation of cellular homeostasis, remains still unknown. **Therefore, this study aimed at verifying the impact of SHBG on PPARγ activity in adipose derived stromal cells' mitochondrial integrity.**

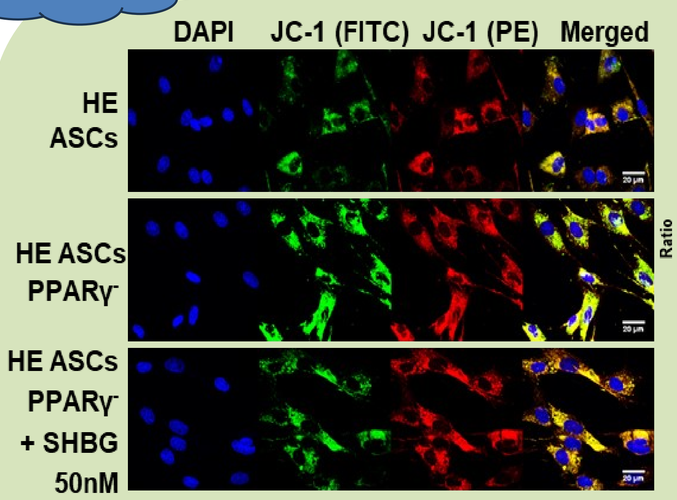


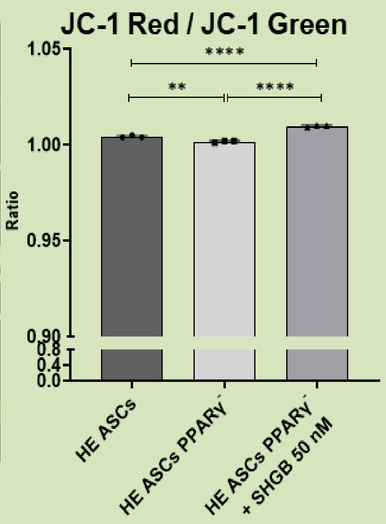
Methods

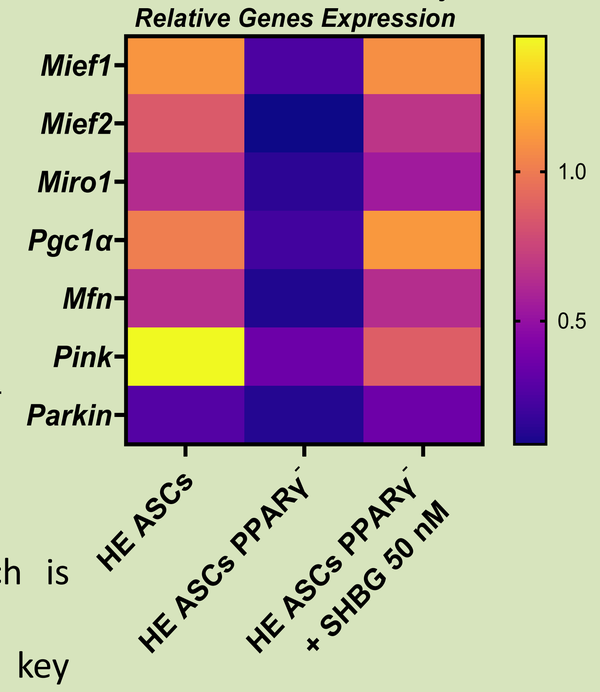


In the study the equine ASCs were used. The cultures were maintained under standard conditions (constant and aseptic conditions in a CO2 incubator at 37°C, and 95% humidity). The PPARγ gene expression was knockdown with the use of small interfering RNA and Lipofectamine RNAiMAX Reagent. Prior to the experiment, the optimal - 20 nM siRNA concentration was chosen based on a screening test and a concentration and the PPARγ gene ex-pression was suppressed for a duration of 72 hours. Next, cells with suppressed PPARγ expression supplemented with SHBG protein at a concentration of 50 nM for 24 hours and then the cells were made ready for analysis. In order to monitor mitochondrial health, the membrane-permeant MitoProbeTM JC-1 Assay Kit for Flow Cytometry. Additionally, active mitochondria have been examined through confocal microscopy. Next the expression of selected markers involved in metabolism and dynamics of mitochondrial was assessed using RT-qPCR analysis.

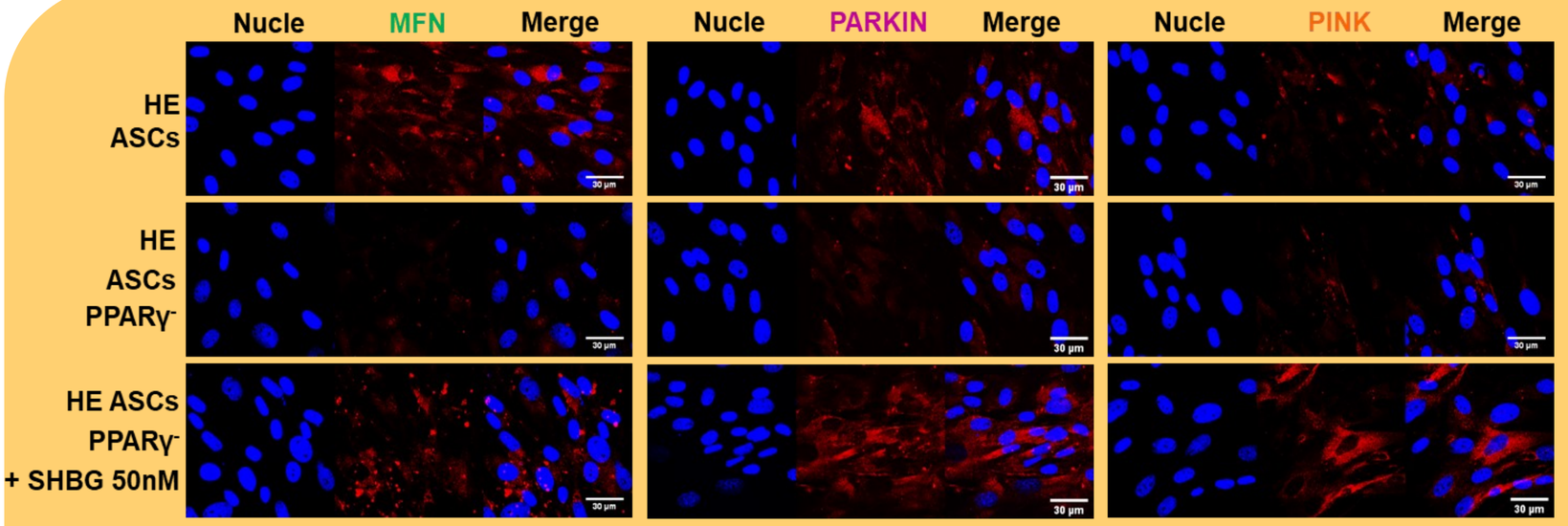
Results







- ✓ PPARγ silencing triggers profound collapse in MMP which is restored following SHBG treatment.
- ✓ Loss in PPARγ expression resulted in lower expression of key markers involved in mitochondrial dynamics, mitophagy and metabolism which was upregulated in the ASCs exposed to SHBG.



- ✓ PPARγ silenced cells displayed imbalanced MFN/PARKIN/PINK axis which was visibly normalized after SHBG supplementation.

Conclusion

Present study evidenced PPARγ loss in equine ASCs leads to profound mitochondrial dysfunction with increased transmembrane depolarization and gradual suppression of metabolism, dynamics and mitophagy master regulators expression at both mRNA and protein level. SHBG protein showed remarkable PPARγ mimicking effects in the modulation of mitochondrial activity in treated ASCs. Noteworthy, SHBG treatment substantially improved the expression of PCG1a, which is naturally activated by PPARγ. Therefore, the presented findings suggest that SHBG may serve as a potential PPARγ agonist that mimics its biological effects, and thus could be considered as a valuable therapeutic agent for the modulation of mitochondrial activity. However, further in-depth studies are necessary to elucidate the exact mechanisms underlying the observed SHBG effects and whether they could be attributed to a PPARγ agonising pathway.

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