The Role Of Mitochondria In Diabetic Bone

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Introduction

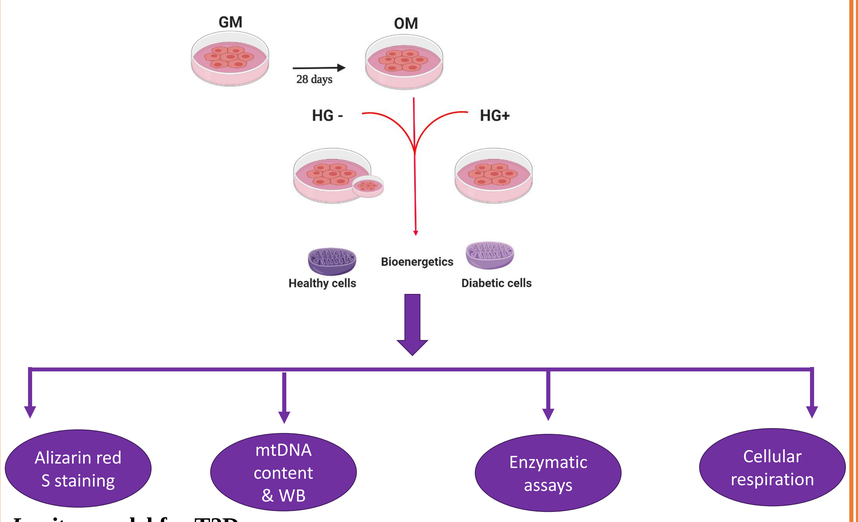
- Diabetes mellitus (DM) is global public health crisis
- DM is comprised of type 1 diabetes (T1D) and type 2 diabetes (T2D)
- T2D has a higher incidence in the population
- The β cells of diabetic patients fail to secrete sufficient levels of insulin
- Bone quality and function are compromised in T2D
- Diabetic patients have increased risk of fracture
- Bone matrix mineralization is impaired in type 2 diabetes (T2D)
- Mitochondrion is the organelle that generates energy for the cell
- Mitochondria preferably use glucose via oxidative phosphorylation in pre-osteoblasts
- Mitochondria use glucose via both oxidative phosphorylation and glycolysis in early stages of osteoblast differentiation
- Differentiated bone cells exhibit a preference for glycolysis
- Diabetes promotes mitochondrial dysfunction

Objectives

In the present study, we aim to bridge the gap between bone disorders and T2D, focusing on Bioenergetic needs during differentiation by:

- Assessing calcium deposition throughout differentiation in the presence of low and high glucose
- Measuring altered protein expression of bone markers involved in differentiation
- Determining changes in expression and function of key mitochondrial proteins involved in the Tricarboxylic acid (TCA) cycle
- Measuring cellular respiration
- Investigating alternative fuel oxidation needs in disease state

Materials and Methods



***In vitro* model for T2D.**

- Pre- osteoblastic (MC3T3-E1) cell differentiation was carried out for 28 days
- Low glucose (5.5mM) and high glucose (25 mM) conditions mimic normal and T2D, respectively, *in vitro*
- Alizarin red S staining was used to assess calcium deposition
- Quantitative PCR (qPCR) and western blot analysis were used to assess mtDNA content and protein expression, respectively
- Enzymatic assays and cellular respiration were performed at week 3 of treatment
- GraphPad was used for data statistical analysis

Mineralization is inhibited in diabetic osteoblasts

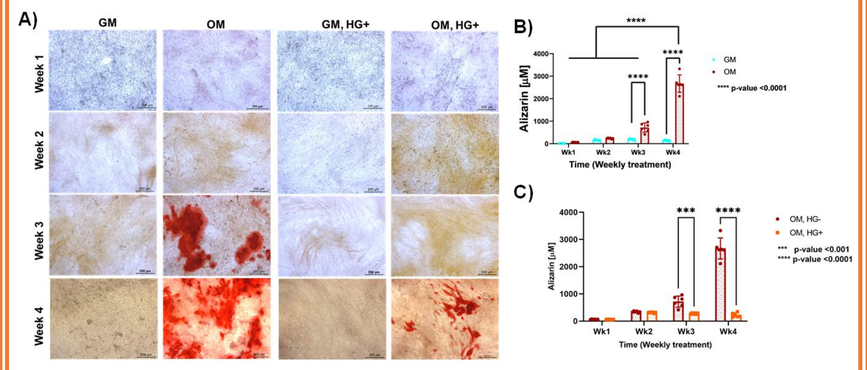
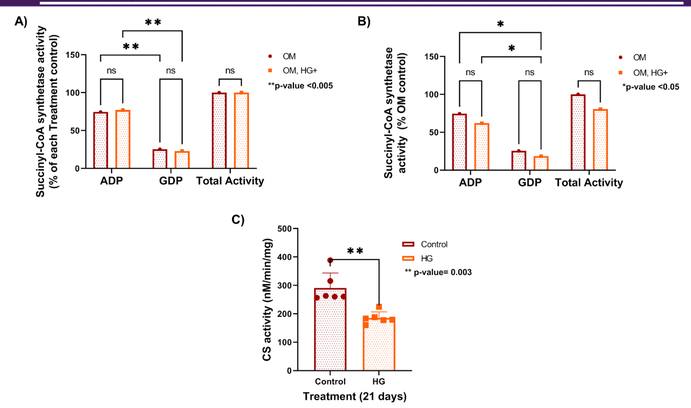


Figure 2. Calcium deposition was detected by Alizarin red S staining.

- Calcium deposition was increased in differentiated cells at week 3
- Calcium deposition was delayed in HG treated cells until week 4
- Alizarin concentration was increased in differentiated cells (OM)
- Alizarin concentration was decreased in diabetic bone cells (OM, HG+)

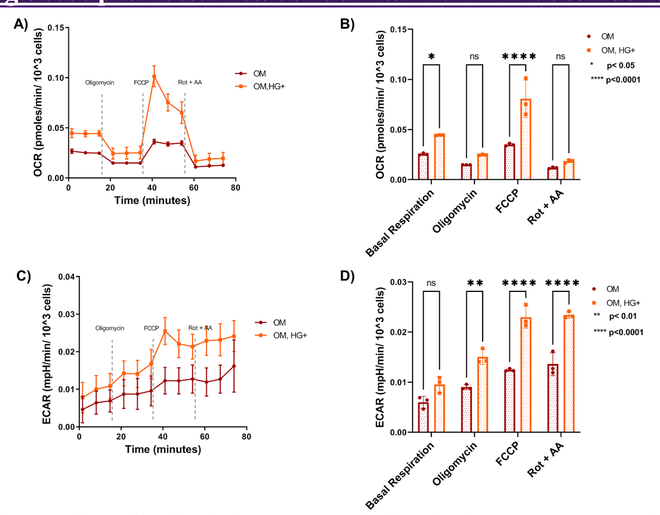
Mitochondria content is reduced under HG conditions



Activity of TCA cycle enzymes.

- Both normal and diabetic cells exhibited substrate preference for ADP
- SCS function was decreased in diabetic bone cells
- Citrate synthase (CS) function was significant reduced in diabetic bone cells

High glucose promotes metabolic shifts in differentiated bone cells



Diabetic bone cells utilize both OXPHOS and Glycolysis to meet energy demands.

- Mitochondrial respiration was increased in diabetic bone cells
- OCR levels were increased in two channels in diabetic bone cells
- Glycolysis was increased in diabetic cells.
- ECAR levels were increased in three channels in diabetic bone cells

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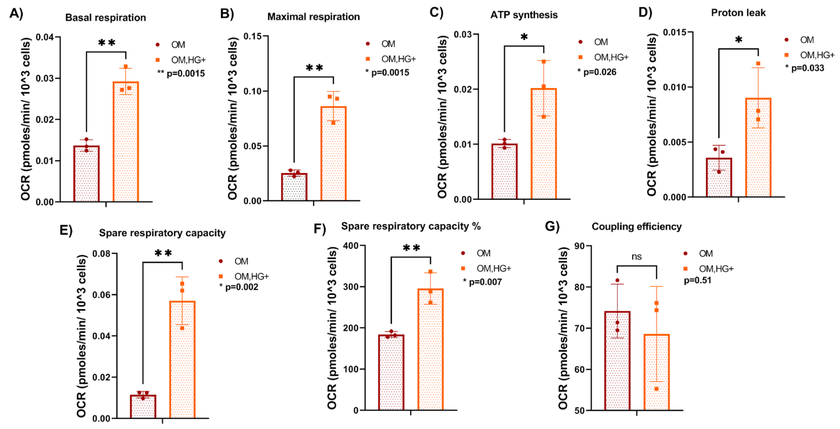
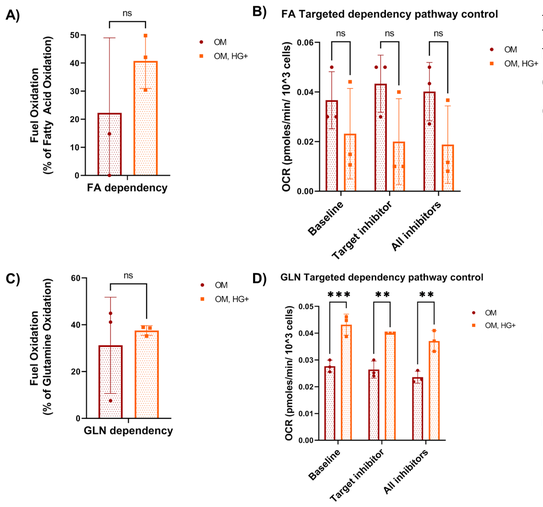
Diabetic bone cells exhibit increase in mitochondrial respiration



Most fundamental parameters of mitochondrial respiration are significantly elevated in diabetic bone cells.

- Minimal rate of oxygen consumption to maintain the cell's function was increased
- Maximal oxygen consumption at a rapid oxidation rate was elevated
- ATP synthesis was increased to meet energy demands in diabetic bone cells
- Incomplete coupling of oxygen substrate and ATP synthesis was increased
- Reserve capacity was increased suggesting that ATP synthesis via OXPHOS in diabetic cells
- Coupling mechanism of oxidation and phosphorylation was increased, but not significantly
- Mitochondrial respiration parameters were increased suggesting that HG-treated bone cells met energy demands via OXPHOS similarly to what has been reported during early stages of osteoblast differentiation

Diabetic bone cells partially rely on GLN to meet enegy demands



Diabetic bone cells partially utilize GLN as a fuel oxidation to maintain energy demands.

- FA oxidation dependency was increased in diabetic bone cells
- FA targeted pathway dependency control was reduced in diabetic bone cells
- GLN oxidation dependency was increased in diabetic bone cells
- GLN targeted pathway dependency control was elevated in diabetic cells.

Conclusions and Future Directions

- Calcium accumulation was decreased: mineralization was delayed in diabetic osteoblasts
- Both normal and diabetic bone cells prefer ADP-β subunit as substrate for energy production
- CS activity is decreased indicating that intact mitochondrial content is reduced
- Oxygen consumption via mitochondrial respiration was elevated suggesting a metabolic shift in T2D
- Glutamine was partially involved in the maintenance of minimal energy requirements in T2D cells

Overall, these findings demonstrate that hyperglycemia promotes a delay in mineral deposition and altered cellular metabolism

- Future work will focus on AKT phosphorylated states and AKT modulators, Citrate levels, TCA cycle intermediate, and other potential fuels