Obesity and the Sustainability of Calcium Oscillations in Hepatocytes: Explicitly Modeling Mitochondria-associated ER Membranes

Jungmin Han and Vipul Periwal

jungmin.han@nih.gov

Laboratory of Biological Modeling, National Institute of Diabetes Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD

Motivation

Multiple cellular organelles tightly orchestrate intracellular calcium (Ca^{2+}) dynamics to regulate cellular activities and maintain homeostasis. Throughout the cell domain, the endoplasmic reticulum (ER) and mitochondria form Ca^{2+} "hot spots" called mitochondria-associated ER membranes (MAMs). It has been proposed that the properties of these microdomains are closely associated with mitochondrial dysfunction in obesity.

Arruda et al. [1] showed that in mouse hepatocytes, obesity is linked with a higher degree of MAM formation, and higher expression levels of IP_3 receptors (IPRs) and mitochondrial calcium uniporters (MCUs). In obese mouse hepatocytes, ATP-induced mitochondrial Ca^{2+} signals showed higher peaks, compared to wild type, while the peaks of cytosolic Ca^{2+} signals were similar across the cells.

We constructed a mathematical model to reproduce the experimental observations in Arruda et al., and make predictions about the effects of obesity on Ca^{2+} dynamics in hepatocytes.

Our model simulations predict that in obese mouse hepatocytes:

(1) Ca^{2+} oscillations are faster
(2) mitochondrial Ca^{2+} oscillations are on a much higher base level, and
(3) Ca^{2+} oscillations are more likely to be abolished under higher levels of agonist

IPR: Ca^{2+} releasing channels on the ER membrane; MCU: Ca^{2+} sequestering channels on the mitochondrial membrane

Ca^{2+} Model

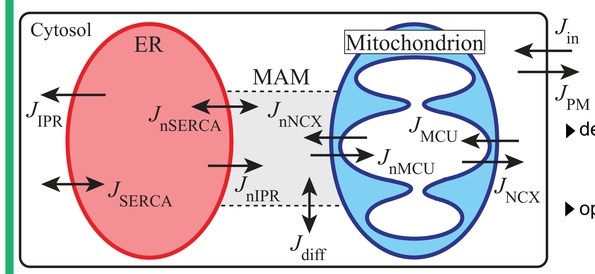
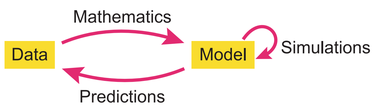


Figure 1.
Schematic diagram of Ca^{2+} fluxes in the model

- deterministic: synchronized activities of the IPRs
- open-cell: Ca^{2+} fluxes across the plasma membrane

$$R_{S1} = \frac{\text{SA of the ER that adjoins the MAM}}{\text{the total ER SA}}$$

$$R_{S2} = \frac{\text{SA of mitochondrion that adjoins the MAM}}{\text{the total mitochondrion SA}}$$



Cellular changes	Model modifications
Increased MAMs	↑R_{S1} & R_{S2}
Associated with obesity	↑R_{S1} & R_{S2} ↑J_{IPR} & J_{nIPR} ↑J_{MCU} & J_{nMCU}

Table 1.
Model modifications for different conditions

Conclusions

- Obesity is associated with the upregulation of MAMs, IPRs and MCUs in mouse hepatocytes.
- Arruda et al. showed that upon ATP stimulation, obese mouse hepatocytes exhibit higher peaks of mitochondrial Ca^{2+} signals, compared to wild type hepatocytes.
- Our mathematical model explicitly includes MAM Ca^{2+} dynamics.
- The model simulations are consistent with the experimental observations.

Our model predicts that:

- Obesity induces an increase in the oscillation frequency and the base level of mitochondrial Ca^{2+} oscillations.
- Obesity induces instability on hepatocytes Ca^{2+} oscillations under high concentrations of agonist.

Model Validation

- We performed model simulations with some parameter modifications shown in Table 1 to reproduce the experimental observations reported by Arruda et al. [1].

Control vs. Increased MAMs

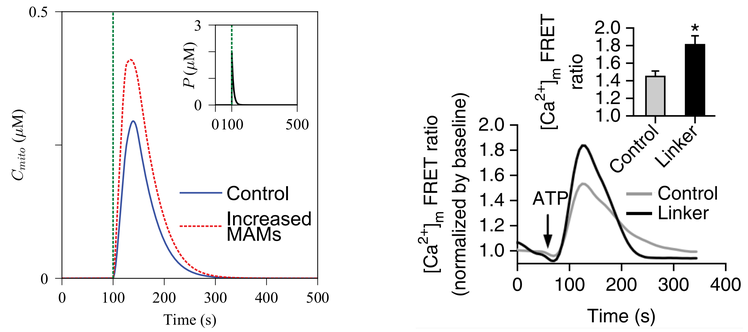


Figure 2.
Model simulations (left) and experimental data (right, Arruda et al., Fig. 5C) showing effects of increased MAMs on the amplitude of mitochondrial Ca^{2+} activity.

- The model was a given a pulse of stimulus P shown by the inset graph. The green dashed lines indicate the onset of the pulse.
- An increase in the proportion of MAMs induced about 30% increase in the peak of mitochondrial Ca^{2+} trajectory.

Control vs. Obesity

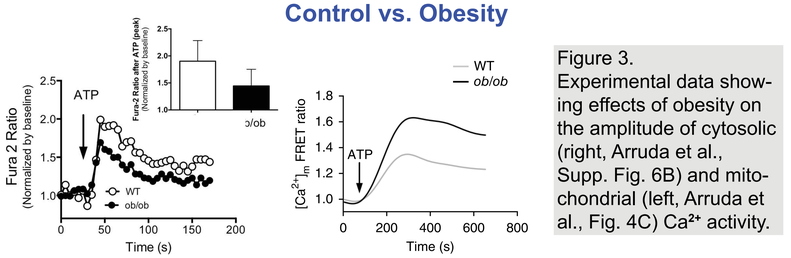


Figure 3.
Experimental data showing effects of obesity on the amplitude of cytosolic (right, Arruda et al., Supp. Fig. 6B) and mitochondrial (left, Arruda et al., Fig. 4C) Ca^{2+} activity.

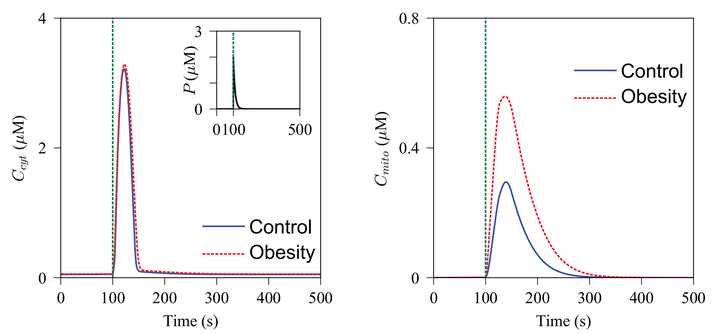


Figure 4.
Model simulations showing effects of the cellular changes associated with obesity on the amplitudes of cytosolic (left) and mitochondrial (right) Ca^{2+} transients

- The same pulse was applied to the model under two different environments, one that represents the control condition and the other that mimics the cellular conditions associated with obesity.
- The peak of mitochondrial Ca^{2+} trajectory was increased under the obesity condition, while that of cytosolic Ca^{2+} trajectory showed a negligible change.

Model Predictions

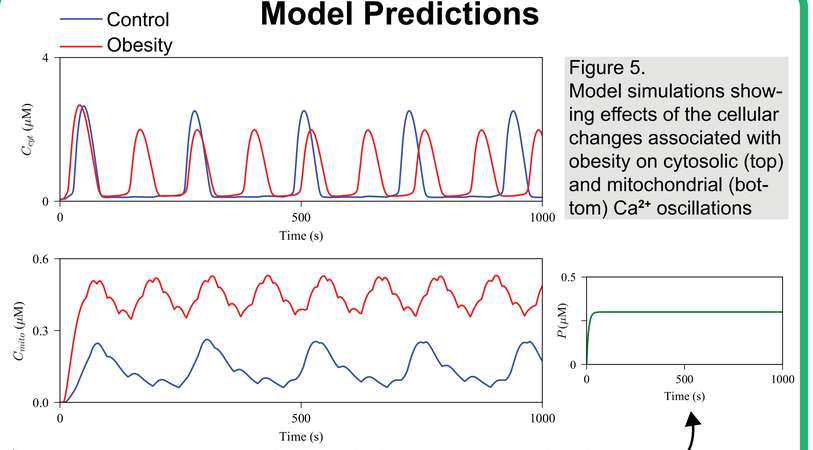


Figure 5.
Model simulations showing effects of the cellular changes associated with obesity on cytosolic (top) and mitochondrial (bottom) Ca^{2+} oscillations

- The model was simulated with a constant level of stimulus P continuously.
- The same stimulation was given to the model under the control condition and the obesity condition.
- The Ca^{2+} oscillations generated under the obesity condition:
  - have a higher frequency,
  - have a higher base level in mitochondria

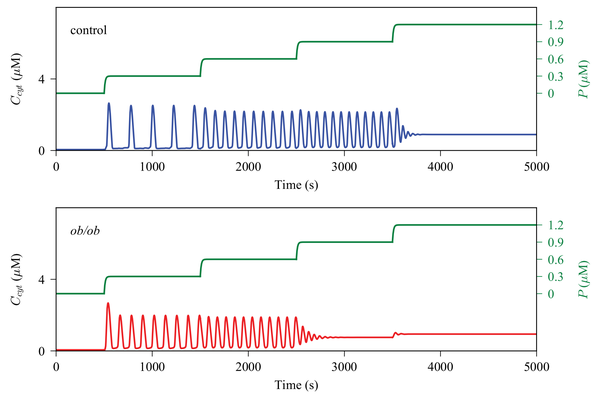


Figure 6.
Model simulations showing the Ca^{2+} oscillations generated under the control condition (top) and the obesity condition (bottom), at different levels of stimus P

- The level of stimulus was incrementally increased from 0μM to 1.2μM.
- Ca^{2+} oscillations generated under the control condition tolerated a wider window of stimulus level, compared to those from the obesity condition.
- These suggest that, at higher levels of stimulus, obesity compromises the robustness of Ca^{2+} oscillations.

References:

[1] A. P. Arruda et al., Chronic enrichment of hepatic endoplasmic reticulum-mitochondria contact leads to mitochondrial dysfunction in obesity. *Nature Medicine.* **20**(12):1427-1435, 2014.

[2] B. Wacquier, L. Combettes, G. Tran Van Nhieu, and G. Dupont. Interplay between intracellular Ca^{2+} oscillations and Ca^{2+}-stimulated mitochondrial metabolism. *Scientific Reports.* **6**:19316

Acknowledgements:

This work was supported by Intramural Research Program of the National Institutes of Health, NIDDK.