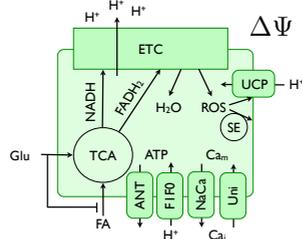


Abstract: Reactive oxygen species (ROS) are hypothesized to underlie many well-defined diseases and clinically-relevant complications, including those associated with diabetes; however, details about ROS production and its specific roles in cellular signaling and tissue damage in living cells in response to nutrients over time are not well understood. Based on the current published data and mathematical models derived from first principles, we present a simple model that captures the responses of mitochondrial respiration, ATP synthesis, and ROS production to nutrients in pancreatic β -cells. Although the model was developed with β -cells in mind, it can be useful on a broader scale through model transference. Our model is consistent with experimental observations of the non-obhm rise in the passive proton-leak rate at high membrane potential and its dependence on increased ROS production. We show that while increasing uncoupling protein activity can effectively reduce ROS levels, it also has the adverse effect of decreasing glucose-stimulated insulin secretion. An effective strategy to decrease oxidative stress while increasing insulin secretion may be to increase mitochondrial density while decreasing uncoupling protein activity, for which we present supporting results. By using glucose and fatty acid profiles from individuals in two independent studies, we calculate corresponding ROS and ATP profiles and find strong correlations between total ROS-per-ATP levels and various physical parameters: most notably of which is a negative correlation with insulin sensitivity. We also find that an increase in plasma-fatty acid levels causes an increase in the amount of ROS produced per ATP. Such relationships could have important implications for physiology as well as tissue-specific cellular function as homeostatic mechanisms are gradually overwhelmed in the long term.

ODEs:

$$\begin{aligned} \frac{dNADH_m}{dt} &= \gamma (J_{Glu,N} + J_{FA,N} - J_{O,N} - J_{ROS_p,N}) \\ \frac{dFADH_2_m}{dt} &= \gamma (J_{Glu,F} + J_{FA,F} - J_{O,F} - J_{ROS_p,F}) \\ \frac{dADP_m}{dt} &= \gamma (J_{ANT} - J_{F1FO} - J_{TCA,Glu} - J_{TCA,FA}) \\ \frac{dC_m}{dt} &= f_m (J_{uni} - J_{NaCa}) \\ \frac{d\Delta\psi}{dt} &= (J_{H,res,N} + J_{H,res,F} + J_{H,ros,N} + J_{H,ros,F} - J_{H,atp} \\ &\quad - J_{H,leak} - J_{ANT} - J_{NaCa} - 2J_{uni}) / C_m \\ \frac{dROS}{dt} &= 2 (J_{ROS_p,N} + J_{ROS_p,F} - 2J_{SE,i}) - J_{UCP,a} + J_{UCP,i} \\ \frac{dSE_i}{dt} &= J_{SE,a} - J_{SE,i} \\ \frac{dUCP_i}{dt} &= J_{UCP,a} - J_{UCP,i} \end{aligned}$$



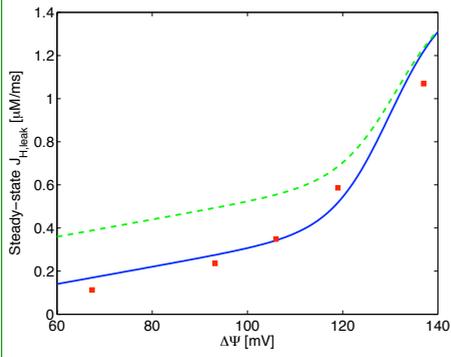
Fluxes:

$$\begin{aligned} J_{glu} &= p_1 \sqrt{Glu/1mM} \\ J_{FA} &= \frac{p_2}{Glu + p_3} \sqrt{FA/1mM} \\ J_{uni} &= p_4 (\Delta\psi - p_5) \left(\frac{C_m^2}{1mM^2} \right) \\ J_{NaCa} &= \frac{p_6 C_m}{p_7 + C_m} e^{p_8 \Delta\psi} \\ J_{Glu,N} &= p_9 J_{glu} \left(\frac{C_m}{p_{10} + C_m} \right) \\ J_{Glu,F} &= p_{11} J_{glu} \left(\frac{C_m}{p_{10} + C_m} \right) \\ J_{FA,N} &= p_{12} J_{FA} \left(\frac{C_m}{p_{10} + C_m} \right) \\ J_{FA,F} &= p_{13} J_{FA} \left(\frac{C_m}{p_{10} + C_m} \right) \end{aligned}$$

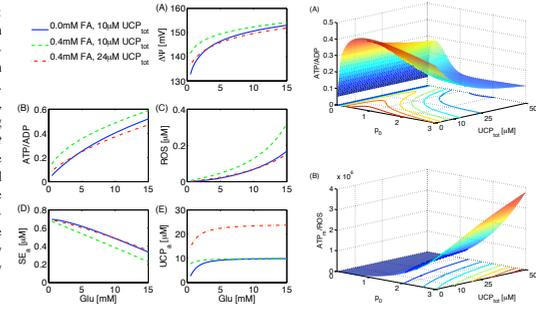
Parameters:

$\gamma = 0.001mM^{-1}ms^{-1}$	$p_{14} = 3.8\mu Mms^{-1}$	$p_{20} = 1772\mu Mms^{-1}$
$f_m = 0.0003$	$p_{15} = 0.5mM$	$p_{21} = 20.3\mu Mms^{-1}$
$p_6 = 1.8\mu Mms^{-1}$	$p_{16} = 162mV$	$p_{22} = 6.6$
$p_1 = 0.035\mu Mms^{-1}$	$p_{17} = 6mV$	$p_{23} = 8.3mV$
$p_2 = 0.18\mu Mms^{-1}$	$p_{18} = 45.6\mu Mms^{-1}$	$p_{24} = 0.75mM^{-1}ms^{-1}$
$p_3 = 14.98mM$	$p_{19} = 0.144\mu Mms^{-1}$	$p_{25} = 0.17mM^{-1}$
$p_4 = 0.0279mMms^{-1}mV^{-1}$	$p_{20} = 208mV$	$p_{26} = 1\mu M^{-1}ms^{-1}$
$p_5 = 85mV$	$p_{21} = 6.8mV$	$p_{27} = 0.0017mM^{-1}$
$p_6 = 0.043mMms^{-1}$	$p_{22} = 1.1313\mu Mms^{-1}$	$p_{28} = 0.004\mu Mms^{-1}mV^{-1}$
$p_7 = 3.75mM$	$p_{23} = 0.5$	$p_{29} = -25mV$
$p_8 = 0.0185mV^{-1}$	$p_{24} = 1.06$	$p_{30} = 0.1ms^{-1}$
$p_9 = 4.25$	$p_{25} = 4.25$	$NAD_{tot} = NAD_m + NADH_m = 10mM$
$p_{10} = 0.1\mu M$	$p_{26} = 590.7\mu Mms^{-1}$	$FAD_{tot} = FAD_m + FADH_2_m = 2.75mM$
$p_{11} = 2.125$	$p_{27} = 87.4mM$	$ADP_a = ADP_m + ATP_m = 15mM$
$p_{12} = 16.47$	$p_{28} = 164mV$	$SE_{tot} = SE_a + SE_i = 0.7\mu M$
$p_{13} = 7.97$	$p_{29} = 8mV$	$UCP_{tot} = UCP_a + UCP_i = 10\mu M$

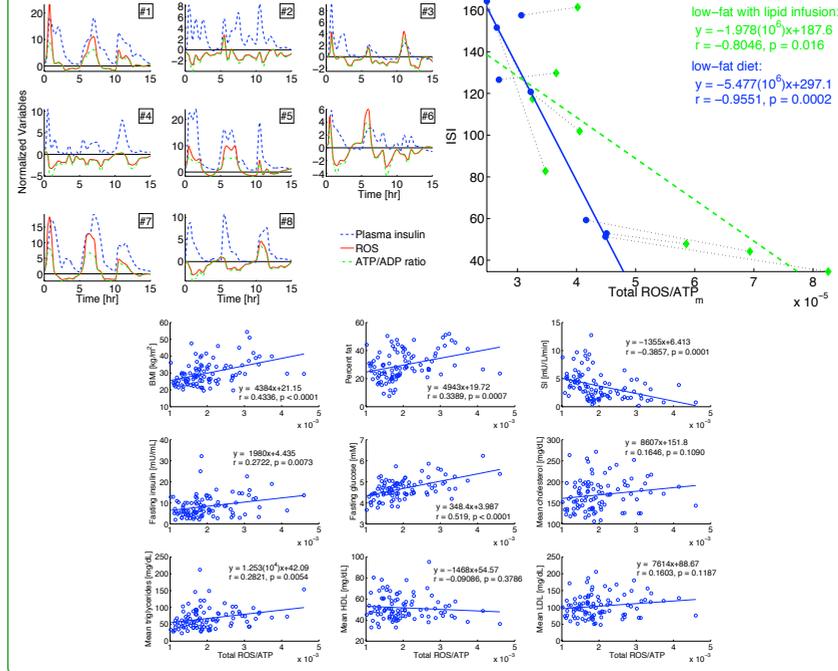
Proton leak rate: A non-obhm relationship exists between the passive proton leak rate and the membrane potential (blue curve compared to scaled data from Echstay *et al.* (2002)). Exogenous ROS production, such as from xanthine/xanthine oxidase, increases this proton conductance at each membrane potential (dashed-green curve calculated by adding $J_{ROS,ex} = 0.001\mu M/ms$ to the ROS production rate).



Fatty acids, UCPs, and mitochondrial density: Under low plasma-glucose conditions, β -cells in the presence of fatty acids have a higher ATP-to-ADP ratio, and therefore increased basal insulin secretion, than cells in the absence of fatty acids. Increasing glucose levels increases ROS levels, especially for fatty acid treated cells assuming UCP_{tot} concentration remains fixed. Lameloise *et al.* (2001) showed fatty acid treated β -cells have two and a half times as much UCP as untreated β -cells. Under such conditions, cells have increased ROS protection, but inhibited glucose-stimulated insulin secretion. In order to increase insulin secretion while keeping ROS low, it may be reasonable to increase mitochondrial density while decreasing UCP activity.



Inferences related to insulin sensitivity: Using plasma-glucose and fatty acid profiles from individuals in two independent diet studies, one of mixed ethnicity (Knuth *et al.*, 2008) and one of African-American subjects (Periwal *et al.*, 2008), we calculate corresponding ROS and ATP profiles postulating that a standard β -cell is placed in each subject and exposed to the individual profiles. We find strong correlations between total ROS-per-ATP and various physical parameters: most notably a negative correlation with insulin sensitivity.



Conclusions: The model we developed goes beyond the models upon which it was based by combining both glucose and fatty acid inputs and incorporating ROS production and the activity of scavenging enzymes and uncoupling proteins. Its simplicity is also an advantage in that it allows easy manipulation and transference, making it useful in pursuing research investigating the integrative physiology of mitochondria. Having been developed for pancreatic β -cell mitochondria, the model allowed us to make inferences and propose hypotheses related to insulin secretion and the effects of diet and exercise. Our results suggest a pathway by which increases in plasma-fatty acid levels cause ROS overproduction in relation to ATP production and potentially contribute to mitochondrial dysfunction through age-associated accumulation of damage from ROS and decreases in mitochondrial density through autophagy. Furthermore, we found a negative correlation between insulin sensitivity and the total ROS-per-ATP predicted by the model in data from two independent studies, one of mixed ethnicity and one of African-American subjects. These two data sets had different measures of insulin sensitivity and different protocols of nutrient intake. These facts suggest that this correlation may hold in general.

Acknowledgments: This work was supported by the Intramural Research Program, NIDDK, NIH.