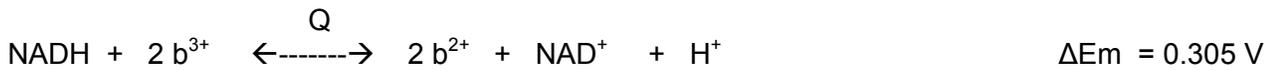


A model for glucose sensing by the β -cells of the pancreas

The steady state rate expression. The function of the first two sites of mitochondrial oxidative phosphorylation, from NAD to cytochrome c, can be represented as two steps:



and



These two reactions have been shown to be freely reversible and near equilibrium under physiological conditions. The difference between the half reduction potentials of the two parts of the redox reaction (ΔE_m) is given on the right. The difference between the oxidation-reduction potentials is the energy coupled to ATP synthesis (Q) this is the same for both reactions. When glucose is present, G-6-P is produced by glucokinase at a rate:

$$v_G = V_m * [G]^{1.7} / (K_g + [G]^{1.7})$$

and this G-6-P goes through glycolysis, producing 2 moles each of ATP, NADH, and pyruvate for each mole of G-6-P. The NADH is reoxidized and the reducing equivalents transferred to the respiratory chain at the level of cytochrome b where the isopotential group includes 2 b cytochromes, ubiquinone, and iron sulfur proteins. There are 2 moles of NADH or 4 moles of reducing equivalents (e^-) for each mole of G-6-P so rate of reduction of cytochrome b (V_s), ignoring the less than 20% loss due to formation of lactate, is:

$$V_s = 4 * v_G e^- s^{-1}$$

The increase in the rate of reduction of NAD^+ has been calculated assuming 20% of the pyruvate formed enters the mitochondria and is oxidized through PDH. The combination of PDH and the citric acid cycle produces the equivalent of 5 NADH for each pyruvate, and 2 pyruvate are formed per G-6-P oxidized. As a result, the input of reducing equivalents from pyruvate oxidation is approximately equal to that into the b cytochrome pool from the glycerol phosphate shuttle. The input into the b cytochrome region of the respiratory chain also decreases the rate of oxidation of NADH decreases the rate of NADH oxidation and the input into the NAD pool can be described as:

$$V_b = 4 * 5 * f * v_G e^- s^{-1} = 2 * V_s M s^{-1}$$

For reactions the ratio of the forward (V_f) and reverse (V_r) rates is directly related to the change in Gibbs free energy:

$$\Delta G = -2.303 RT \log(V_f/V_r) \quad (7)$$

Where 2.303 converts the natural logarithm to the logarithm to the base 10, R is the gas constant, and T the absolute temperature. At equilibrium, the ratio is 1.0, but in metabolism there is almost always a net flux in either the forward or reverse direction. As a result, the ratio is not 1.0 and ΔG is not zero. For reactions near equilibrium ($V_f/V_r < 5$ and > 0.2) and for which there are no changes in reaction mechanism or in environmental factors that can activate or inhibit the reaction, the change in ΔG can be obtained from the ratio after taking into account the ancillary reactions that add to or subtract from V_f and V_r . When the net flux is in the forward direction the ratio > 1 and ΔG is negative whereas when it is in the reverse direction the ratio is > 1 and the ΔG is positive. The first two coupling sites of oxidative phosphorylation are freely reversible and near equilibrium. In the absence of glucose the reverse rate is less than the forward rate by the rate of oxygen consumption (V_o). As the concentration of glucose increases the increasing input of reducing equivalents from pyruvate is added to V_r and the ratio becomes:

$$V_f / (V_r - V_o + V_b)$$

The resulting ratio is used to calculate a change in ΔG due to the input of reducing equivalents from glucose into the NAD pool. This makes the electrochemical potential of the NAD couple more negative and increases the value of Q, the energy couple to ATP synthesis. These calculations show the change relative to the initial state. The initial value for Q, that prior to adding glucose, is calculated from the cytochrome c oxidase portion of the model and in this case has a value of 0.295 V. The value of Q increases due to increasing glucose concentration. Q is then used to calculate the energy state as well as the levels of reduction of cytochrome b ($E_{mb} = -0.030V$), the NAD couple ($E_{mn} = -0.335 V$, pH 7.4) and of cytochrome c ($E_{mc} = 0.235 V$). Cytochrome c is the electron donor for cytochrome c oxidase.

The program for the portion of oxidative phosphorylation from cytochrome c to oxygen is used as published. **Note:** the cytochrome c concentration in beta cells is assumed to be 8 μM while oxidative phosphorylation is calculated for 2 μM and then the rates multiplied by 4.

MatLab program:

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k1 = 3.5*10^9; % a3^3+-Cu^2+ + c^2+ ---> a3^3+ - Cu^1+ + c^3+
k1r = 3.5*10^7; % a3^3+-Cu^1+ + c^3+ <--- a3^3+-Cu^2+ + c^2+
k2 = 3.5*10^8; % a3^3+-Cu^1+ + O2 ---> a3^3+-Cu^1+-O2
k2r = 1*10^1; % a3^3+-Cu^1+-O2 <--- a3^3+-Cu^1+ + O2
K3 = 2*10^6; % a3^3+-Cu^1+-O2 + H+ <---> O2-a3^2+-Cu^2+
k4a = 1.2*10^8; % a3^3+-Cu^1+-O2 + c^2+ ----> a3^3+-O2^2--Cu^2+
k4b = 4*10^7; % O2-a3^2+-Cu^2+ + c^2+ ----> a3^3+-O2^2--Cu^2+
K5 = 1*10^25; % a3^3+-O2^2--Cu^2+ + 2H+ + 2c^2+ <--> a3^3+-Cu^2+ + 2c^3++H2O
a3t = 1*10^-6; % cytochrome oxidase concentration (a, 2 Cu, a3)
ct = 2*10^-6; % cytochrome c concentration (2 uM)
ATP = 5*10^-3; % ATP concentration
Pi = 6*10^-3; % inorganic phosphate concentration
Vm = 3.2 *10^-4; % GK max rate (G-6-P x 8 = Me-/sec; 8 uM cyt c)
Vf = 1.3*10^-5; % NAD reduction in Me-/sec, no G, 8uM cyt c
Kg = 3.98 * 10^-4; % 50% saturation of GK with glucose @ 10 mM
Emc = 0.235; % cyt c half reduction potential (Em in Volts)
W = 7.1; % pH
H = 10^-W; % hydrogen ion concentration
Emn = -0.335; % Em NAD couple @ pH 7.4 in mito matrix;
O = 5*10^-5; % O is oxygen concentration
for i=1:60; % Used to set glucose concentrations
    Gc(i) = i.*4.*10^-4 - 2.8.*10^-4; % Gc = glucose concentration
    Vb(i) = Vm.*(Gc(i)^1.7./(Kg+Gc(i)^1.7))./4; % GK input @ 2 uM cyt c
    Vo(i) = 1.0.*10^-5 + Vb(i)./4; % net rate of respiration
    Gn(i) = -1.42.*log10(Vf./(Vf-Vo(i)+Vb(i))); % free Energy diff
    dE(i) = Gn(i)./46.18; % effect of G-6-P input (NAD couple) in Volts
    Q(i) = 0.295 + dE(i); % sets energy state with GP input
    G(i) = Q(i) .* 46.183; % Gibb's energy in kcal for 2 e- transfer
    S(i) = Q(i) ./0.059; % coupling to energy conservation in volts
    z(i) = 10.^S(i); % effect of coupling on rate or equilibrium constants
    kf1(i) = k1 ./z(i).^0.5; % couples k1 to energy state
    kr1(i) = k1r .*z(i).^0.5; % couples k1r to energy state
for q = 1:50; % generates levels of cyt c reduction
    cr(q) = 3 .* 10^-7+ q.*3 .* 10^-8; % cr is reduced cyt c (M)
    co(q) = ct - cr(q); % co is oxidized cytochrome c
    Ehc(q)= Emc + 0.059.*log(co(q)./cr(q)); % Volts rel to H elec
    A(q) = (k2r + k4a.*cr(q)+k4b.*cr(q).*K3.*H)./(k2.*O); % var A
    B(q,i)=(k2.*O.*A(q)+kr1(i).*co(q).*A(q)-k2r)./(kf1(i).*cr(q)); %
    C(q,i)= K5.^-1.*(1/H).^2.*(co(q)./cr(q)).^2.*z(i).^2.*B(q,i); %
    Ill(q,i) = a3t./(1 + K3.*H + A(q) + B(q,i) + C(q,i)); % intermediate Ill conc.

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I(q,i) = B(q,i) .* III(q,i);
II(q,i) = A(q) .* III(q,i);
IV(q,i) = K3 .* H .* III(q,i);
V(q,i) = C(q,i) .* III(q,i);
y(q,i)=(k4a.*cr(q)+k4b.*cr(q).*K3.*H).*III(q,i).*4./ct;
Ehb(q,i) = Ehc(q) - Q(i);
Ehn(q,i) = Ehc(q) - 2.*Q(i);
ADP(i) = (ATP/Pi)./(10.^((G(i)-7.6)./1.42));
AMP(i) = ADP(i).^2./ATP;
end
end
plot(cr,y);
axis([0.4*10^-6 1.8*10^-6 0 20])

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% conc. of intermediate I
% conc. of intermediate II
% conc. of intermediate IV
% conc. of intermediate V
% cyt TN s^-1
% cyt b redox potential (Volts)
% potential for NAD couple (Volts)
% [ADP]
% [AMP] assuming Keq = 1

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% plot cyt c reduced vs cyt c TN at diff. energy states
% sets graph x and y axis limits

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