Using phase relations to identify potential mechanisms for metabolic oscillations in isolated β-cell mitochondria

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There is a great deal of evidence for the existence of metabolic oscillations in pancreatic β-cells. Mechanisms that have been proposed for these oscillations include glycolytic oscillations; oscillations due to the feedback of Ca^{2+} onto the mitochondrial inner membrane and on dehydrogenases; and oscillations intrinsic to the tricarboxylic (TCA) cycle or the downstream reactions of oxidative phosphorylation. MacDonald and co-workers (J Biol Chem 2003; 278:51894–900) showed examples of oscillations in TCA intermediates in isolated mitochondria from liver cells and pancreatic β-cells. These oscillations were clearly not due to oscillations in glycolysis or Ca^{2+} feedback. In this article we consider several potential mechanisms for these TCA oscillations, using mathematical modeling to determine the phase relations that would result between the citrate and NAD^+ concentrations in each case. We demonstrate that negative feedback at only one feedback point, isocitrate dehydrogenase, produces the correct phase relation if oscillations are intrinsic to the TCA cycle. Alternatively, the correct phase relation results if oscillations are due to oscillations in oxidative phosphorylation feeding back onto the TCA cycle. This analysis shows that the observed phase relation between citrate and NAD(P) places strict limits on the potential mechanism for the metabolic oscillations in isolated mitochondria that were observed by MacDonald and co-workers.

Introduction

Metabolic oscillations in pancreatic islets have been described by several labs.[1-6] Considerable recent research efforts have explored whether these oscillations are inherent in metabolism within β-cells or are due to the feedback of cytosolic Ca^{2+} onto mitochondrial metabolism (reviewed in ref. 7). However, in one experimental setting it is clear that the observed metabolic oscillations are intrinsic to metabolism.[8] Here, isolated mitochondria from liver, pancreatic islets and INS-1 insulinoma cells were used and citric acid cycle intermediates as well as NAD(P) and ATP were measured in the presence of pyruvate. It was shown

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that several of the intermediates, as well as NAD(P) and ATP, exhibited clear oscillations, with a period of approximately two minutes.[8] Further, it was shown that the citrate oscillations and the NAD(P) oscillations were largely in phase with one another. Since these oscillations were produced in isolated mitochondria, the mechanism did not involve glycolytic oscillations[9] or Ca^{2+} feedback.[10-12]

In this paper we use mathematical modeling to analyze several possible mechanisms for the oscillations described by MacDonald et al.[8] We consider two classes of oscillation mechanism: those arising from within the tricarboxylic cycle (TCA cycle) and those arising from downstream oxidative phosphorylation. We focus on the phase relation between the citrate and NAD^+ variables, since the MacDonald measurements showed that these variables oscillated in phase with one another. Hence, a mechanism in which the model citrate and NAD^+ variables oscillate in phase is more likely to be valid than one in which in-phase oscillations are not produced. In addition, we provide an explanation for why each oscillation mechanism produces the citrate/NAD^+ relation that it does. While the mechanism for oscillations does depend on the details of the model, the phase relations (in-phase or antiphase) that exist during oscillations depend only on the sequences of chemical reactions that make up the TCA cycle and the target of negative feedback onto the cycle. This analysis helps to restrict the range of possibilities for the biochemical mechanism of the metabolic oscillations reported in.[8]

Results

Intrinsic oscillations in the TCA cycle. The data from MacDonald et al.[8] demonstrate the existence of oscillations in TCA cycle intermediates and in ADP. The mechanism for these oscillations could either come directly from the TCA cycle (**Fig. 1**) or from oscillations in oxidative phosphorylation feeding back onto the TCA cycle. We begin with the first mechanism. Oscillations intrinsic to the TCA cycle are facilitated by negative feedback of several intermediates and by NADH. In our model, we consider three feedback points: citrate synthase (CS) where there is negative feedback from its product citrate (CIT) and from NADH, isocitrate dehydrogenase (IDH) where there is negative feedback from NADH, and α-ketogluterate dehydrogenase (KGDH)
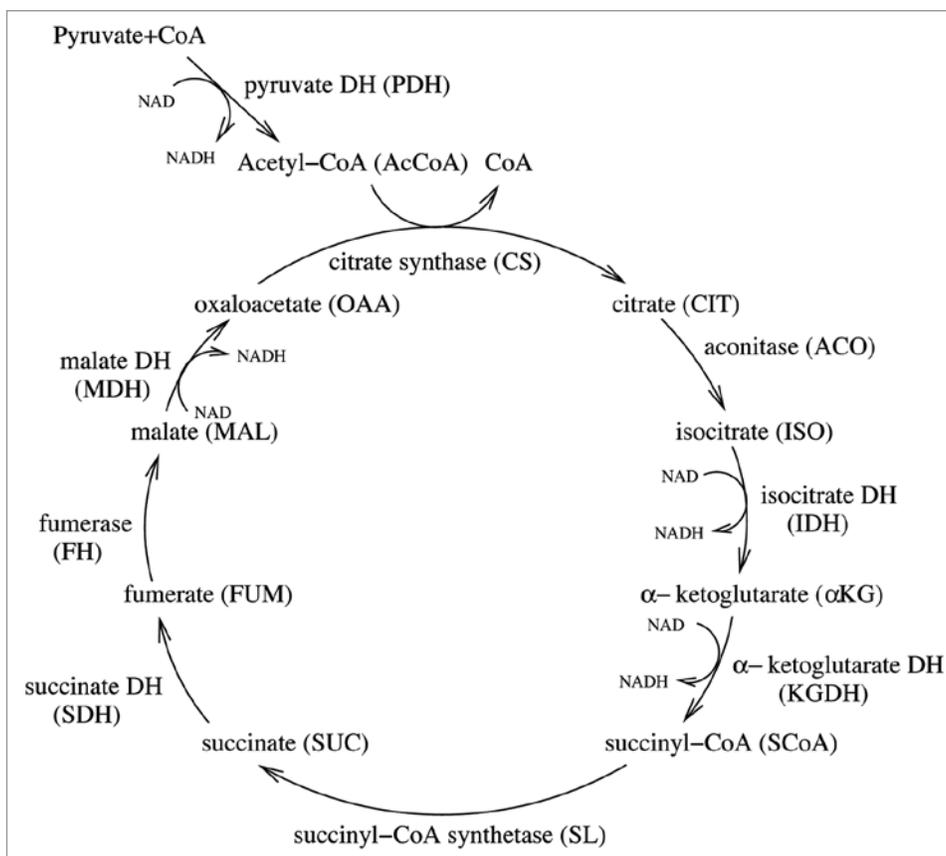
Figure 1. Illustration of the TCA cycle, highlighting the reactions that lead to NADH production.

where there is negative feedback from NADH (see Methods). An additional element that is necessary for oscillations is that the negative feedback should be slow or delayed. We therefore introduce an explicit time delay (τ = 45 sec) at each feedback target, one by one, as we investigate the phase relation between CIT and NAD⁺ that is produced from the resulting oscillation. While we do not speculate on the source of the time delay, we point out that such a slow or delayed feedback must occur for oscillations to be generated. In our model, time delays as short as 3 sec produce oscillations. However, the period of the oscillations is shorter for smaller time delays, and 45 sec was chosen based on the period of oscillations exhibited in the experimental data. Our focus is not on the source of the delay, but on phase relations that occur during the resulting oscillation.

We first consider delayed negative feedback of CIT onto CS (**Fig. 2A**). This is accomplished by replacing *CIT* with *CIT$_\tau$* in Eq. (9), where *CIT$_\tau$* means the citrate concentration delayed by τ seconds. (Note the CIT represents citrate, while *CIT* represents the citrate concentration. A similar convention, using italics for concentrations, is used for other variables. In particular, *NAD* represents the NAD⁺ concentration). All other negative feedback (i.e., the NADH feedback) is retained without delay. This produces oscillations in the TCA cycle as shown in **Figure 3A**. (The *CIT* and *NAD* time courses are scaled to facilitate display on the same figure). The important point to note is that the *CIT*

and *NAD* oscillations are out of phase. That is, when the citrate concentration (solid curve) increases the NAD⁺ concentration (dashed) decreases. The explanation for this will be given in the next section. This phase relation is the opposite of what was demonstrated in,[8] where the CIT and NAD(P) concentrations oscillated in phase.

Next, we consider the oscillations obtained through delayed negative feedback of NADH on CS (**Fig. 2B**). Here we replace *NADH* with *NADH$_\tau$* in Eq. 9 (and use the non-delayed *CIT* in the same equation). In the resulting oscillations, *CIT* and *NAD* are again antiphasic (**Fig. 3B**), in contrast with the experimental data.

We next demonstrate the oscillations produced by delayed negative feedback of NADH onto isocitrate dehydrogenase (**Fig. 2C**). We replace *NADH* with *NADH$_\tau$* in the expression for V_{IDH}, while using non-delayed values for *CIT* and *NADH* in Eq. 9. For the first time, this produces oscillations in which *CIT* and *NADH* oscillate in phase (**Fig. 3C**), consistent with the experimental data. This suggests that of the three feedback pathways for intrinsic TCA oscillations examined, only feedback of NADH onto IDH produces oscillations with the correct phase relation between the CIT and NAD⁺ concentrations.

Explanation of the phase relations. We now examine why the *CIT-NAD* phases in **Figure 3** are in-phase or antiphase. To do this, we mimic the inhibitory feedback onto the various

target points by reducing the maximum catalytic rates of the enzymes by a factor of 10 and investigating the effects. Thus, we apply the inhibition directly. While CIT and NADH still provide negative feedback, there is no delay, so no oscillations are produced.

Figure 4A shows what happens when the maximum catalytic rate for citrate synthase (k_{cat}^{CS}) is transiently reduced from 1.6×10^{-4} ms^{-1} to 1.6×10^{-5} ms^{-1} (indicated by an overbar). This reduction causes the product CIT to decline and the substrate OAA to increase. The reduction in CIT causes a reduction in the flux through the TCA cycle, so less NADH is produced from NAD$^+$. Therefore, the concentration of NAD$^+$ increases. This illustrates why *CIT* and *NAD* oscillate out of phase when the target of delayed feedback inhibition is CS: the inhibition causes *CIT* to go down and *NAD* to go up.

Figure 4B demonstrates the effect of transiently reducing the maximum catalytic rate of isocitrate dehydrogenase (k_{cat}^{IDH}) from 0.0163 ms^{-1} to 0.00163 ms^{-1}. As expected, the substrate concentration (*ISOC*) increases due to the reduced enzymatic flux. This causes the intermediate that is earlier in the cycle, CIT, to increase as well. The reduction of flux through IDH results in lower flux through the TCA cycle, so less NADH is made, and therefore the NAD$^+$ concentration increases. In this way, *CIT* and *NAD* change in the same direction when the point of feedback inhibition is IDH, resulting in an in-phase oscillation when inhibition is through delayed negative feedback onto IDH.

In **Figure 4C** we look at α-ketogluterate dehydrogenase (KGDH), a dehydrogenase that is downstream of IDH and which is known to be negatively modulated by succinyl-CaA and NADH.[8,13] We mimic this by transiently reducing the maximum catalytic rate k_{cat}^{KGDH} from 1.5×10^{-3} ms^{-1} to 1.5×10^{-4} ms^{-1}. This results in an increase in the substrate concentration (α*KG*) and a decrease in flux through the TCA cycle, so *NAD* increases and the NADH concentration decreases. Since NAD$^+$ stimulates IDH and NADH inhibits it, both concentration changes work together to stimulate IDH. This results in a reduction in the concentration of the IDH substrate ISOC, as well as other intermediates that are earlier in the cycle, in particular, *CIT*. Thus, inhibition of KGDH results in an increase in *NAD* and a decrease in *CIT*, so that oscillations produced by delayed negative feedback of NADH onto KGDH produce antiphasic changes in *NAD* and *CIT* (**Fig. 3D**).

For the same reason that negative feedback onto KGDH produces antiphasic changes in *NAD* and *CIT*, inhibitory feedback onto enzymes further upstream such as succinate dehydrogenase and malate dehydrogenase also produce antiphasic changes in *NAD* and *CIT*. That is, NAD$^+$ levels increase and NADH levels decrease due to reduced TCA flux, and the combined stimulatory effect of the two changes on IDH lower the substrate *ISOC* and the upstream intermediate *CIT*.
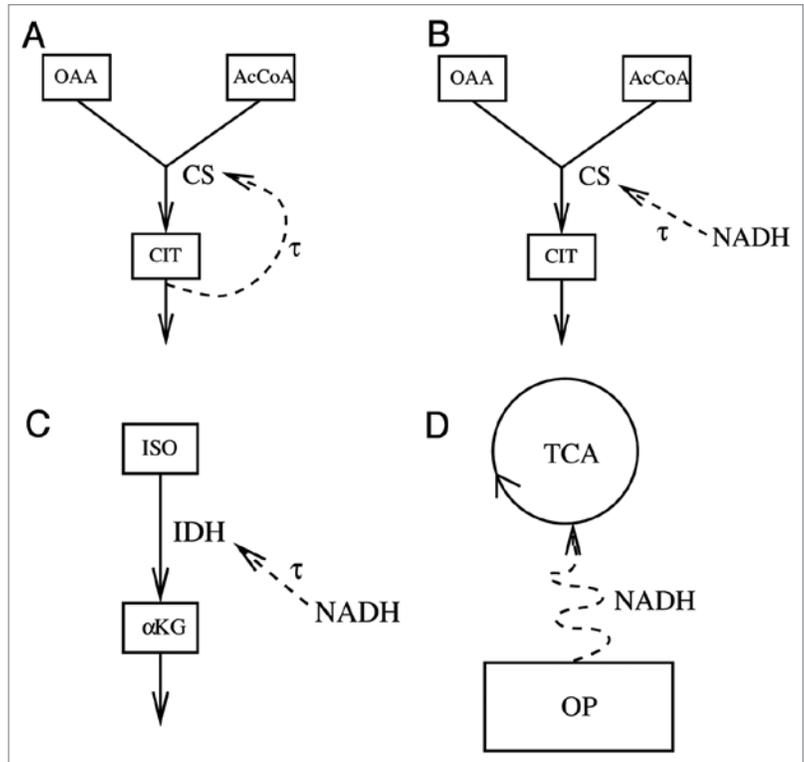

Figure 2. Illustrations of the different mechanisms examined for the production of oscillations in the TCA cycle and in NAD$^+$. Solid lines represent enzymatic reactions and dashed lines represent negative feedback, either delayed by τ sec or oscillatory. In the first three cases considered the oscillations are intrinsic to the TCA cycle. In the fourth case the oscillations are produced during oxidative phosphorylation, and are then transmitted to the TCA cycle through the negative feedback of NAD$^+$ and NADH onto various dehydrogenases in the cycle.

Oscillations intrinsic to oxidative phosphorylation. We focused above on oscillations that are intrinsic to the TCA cycle. Another alternative explanation for the findings of MacDonald et al.[8] is that the oscillations are produced downstream of the TCA cycle, during oxidative phosphorylation (OP), and that the feedback of NAD$^+$ and NADH onto TCA enzymes causes oscillations in TCA intermediates (**Fig. 2D**). We examine this possibility now, again focusing on the resulting phase relation between the NAD$^+$ and CIT concentrations.

We begin with a very simple test: we impose a sinusoidal oscillation onto the NADH concentration, representing an oscillation in OP produced by some unspecified mechanism. The NADH concentration is then described by Eq. 17, which includes a term for sinusoidal forcing (Eq. 18). The forcing function causes the NADH concentration to oscillate, as well as the NAD$^+$ concentration since the sum of the two is assumed to be conserved (Eq. 10). One or both of the *NADH* and the *NAD* variables then feed back onto the TCA cycle at several feedback points (CS, IDH, KGDH and MDH).

Figure 5A shows the oscillations in *NADH* that result from the imposed oscillation. The subsequent oscillations in *CIT* are shown in **Figure 5B**, superimposed on the *NAD* oscillations (both scaled). In this scenario *CIT* and *NAD* oscillate in phase,
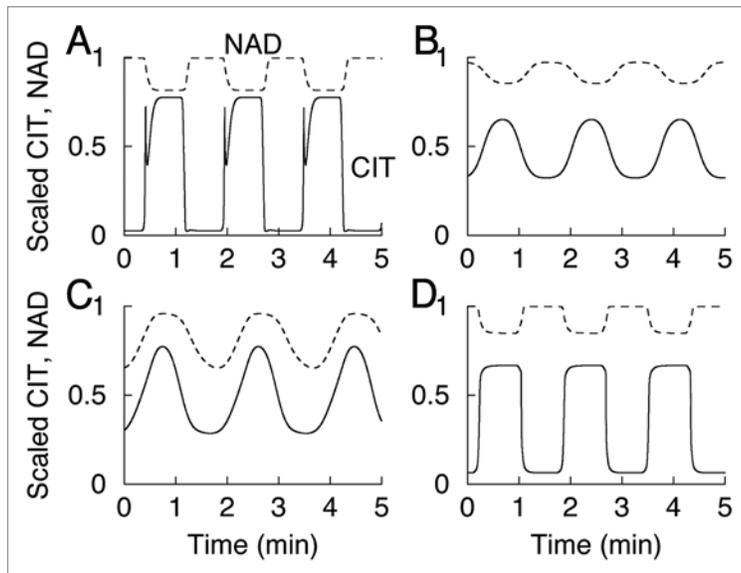
Figure 3. Oscillations in citrate (*CIT*, solid) and *NAD* (dashed) produced through four different mechanisms intrinsic to the TCA cycle. (A) Antiphase oscillations produced by the delayed negative feedback of CIT onto citrate synthase (CS). (B) Antiphase oscillations produced by the delayed negative feedback of NADH onto CS. (C) In-phase oscillations produced by the delayed negative feedback of NADH onto isocitrate dehydrogenase (IDH). (D) Antiphase oscillations produced by delayed negative feedback of NADH onto α-ketogluterate dehydrogenase (KGDH).
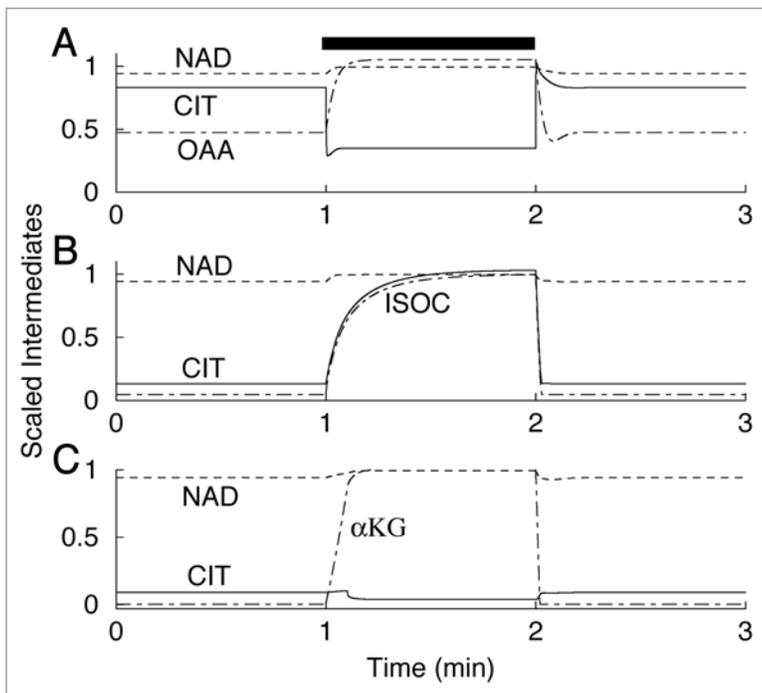
Figure 4. The effects of reducing maximum catalytic rates by a factor of 10 (indicated by the black bar). Feedback of products and nucleotides onto enzymes is present, without delay. (A) Ten-fold reduction of the catalytic rate of citrate synthase causes *NAD* (dashed) to increase and *CIT* (solid) to decrease. (B) Reduction of the catalytic rate of isocitrate dehydrogenase causes both *NAD* and *CIT* to increase. (C) Reduction of the catalytic rate of α-ketogluterate dehydrogenase causes *NAD* to increase and *CIT* to decrease. All curves have been scaled to facilitate superposition.

in accordance with the experimental observations. The in-phase oscillation occurs because NADH inhibits CS, so that a peak in NADH results in a decline in CS activity, and thus a decline in the CIT concentration. Thus, *NADH* and *CIT* are antiphasic, and *NAD* and *CIT* are in phase. The correct phase relation depends critically on the NADH feedback onto CS. If this is not present or is extremely weak, then the first feedback point in the TCA cycle is at IDH, and inhibition of IDH by a peak of NADH causes isocitrate and thus CIT to build up, so that *NADH* and *CIT* are in phase and there is an antiphasic phase relationship between *NAD* and *CIT*.

Finally, we use a biophysical model for oscillations in OP (see Methods), rather than the ad hoc sinusoidal NADH forcing function above, to once again examine the resulting *NAD-CIT* phase relation. In this model,[14] oscillations are produced by the interaction of the mitochondrial inner membrane anion channels (IMACs)[15] and the mitochondrial centum-picosiemen (mCS) channels.[16] These two channel types interact to create oscillations in ΔΨ, which result in oscillations in other mitochondrial variables, including NADH. The model was developed to describe metabolic oscillations in mitochondria under ischemic conditions, as have been seen in isolated cardiomyocytes.[15] Other mechanisms for OP oscillations under ischemic conditions have been suggested, such as oscillations due to reactive oxygen species,[17] but for our purposes the mechanism for the OP oscillations is not critical. To our knowledge, there is no direct evidence for oscillations intrinsic to OP under normoxic conditions.

Figure 6A shows the oscillations in mitochondrial inner membrane potential (ΔΨ) that reflect the oscillations intrinsic to OP. The resulting oscillations in *NADH* are shown in **Figure 6B**. The NADH concentration rises rapidly during the hyperpolarized phase of the inner membrane and declines rapidly during the depolarized phase. On top of the elevated plateau are small oscillations that reflect the interactions of the OP variables. **Figure 6C** shows *NAD* and *CIT* plotted together (scaled to facilitate comparison). The CIT concentration, and the concentrations of other TCA intermediates, are affected by the oscillations in *NADH* produced by OP. When *NADH* rises (*NAD* falls) at the beginning of the ΔΨ hyperpolarization there is an initial decline in *CIT*, due to the inhibition of CS by NADH. This is followed by a rise in *CIT* due to the small decline in *NADH* on the *NADH* plateau, again reflecting the inhibitory action of NADH on CS. Comparison of the scaled *NAD* and *CIT* levels reveals that the two variables oscillate in phase with one another, in accordance with the experimental data.[8]

Oscillations in Acetyl-CoA. Acetyle-CoA (AcCoA) is a product of pyruvate dehydrogenase (PDH), an

enzyme that is positively regulated by Ca^{2+} and negatively regulated by ATP, NADH and AcCoA.[8] In addition, oscillations in glycolysis would produce oscillations in the substrate of PDH, pyruvate, resulting in oscillations in AcCoA. So in the intact β-cell, where glycolytic oscillations are likely to occur,[7,9] AcCoA would be oscillatory. Thus, the AcCoA concentration could be oscillatory due to either delayed negative feedback of a modulator such as NADH acting on PDH, or it could be oscillatory due to upstream glycolytic oscillations (in the case of an intact cell). In either case, what phase relation would this produce between the NAD+ and CIT concentrations?

We examine this by imposing an oscillation onto the AcCoA concentration, with the understanding that the origin of this oscillation could be either of the mechanisms discussed above. We use $AcCoA = 0.002 + AS(t)$, where $A = 0.0015$ mM and $S(t)$ is the sinusoid function defined in Eq. 18. The sinusoidal AcCoA input is shown in **Figure 7A**, while the effect on *NAD* and *CIT* is shown in **Figure 7B**. The two concentration time courses oscillate in antiphase. The reason for this is that when AcCoA is at its peak there will be an increase of CIT production by CS. This increases the flux through the TCA cycle, resulting in an increased production of NADH at the expense of NAD+. Thus, *CIT* increases while *NAD* decreases. This demonstrates that delayed negative feedback onto PDH is an unlikely mechanism for the oscillations in *CIT* and *NAD* reported[8] since the phase relation is wrong. Also, it predicts that CIT and NAD would oscillate in antiphase in an intact cell that exhibits glycolytic oscillations (assuming that no other oscillatory mechanism is simultaneously active).

Discussion

We have considered several potential mechanisms for oscillations in TCA intermediates in isolated mitochondria, as have been observed by MacDonald et al.[8] Using a mathematical model of the TCA cycle[18] coupled to a model of oxidative phosphorylation,[19] we demonstrated that oscillations intrinsic to the TCA cycle are possible if a delay is introduced into one of several negative feedback targets (**Fig. 3**). We also demonstrated that the only delayed feedback target that produces oscillations in the NAD+ and citrate concentrations which agree with the experimental data is the negative feedback of NADH onto isocitrate dehydrogenase (IDH). The explanation for this is based on the location of the feedback target in the TCA cycle. In most cases, partial inhibition of the target enzyme leads to a decline in the citrate concentration, but inhibition of IDH leads to an increase in the citrate concentration (**Fig. 4**). Since the NAD+ concentration rises (and
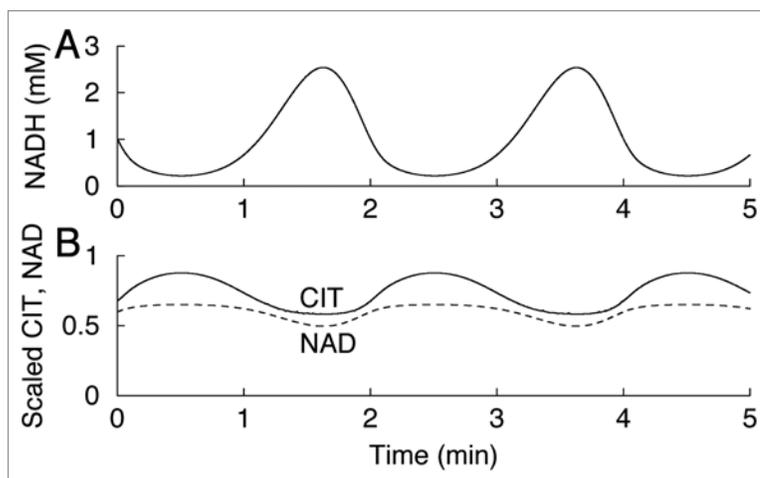

Figure 5. (A) Oscillations in *NADH* imposed through sinusoidal forcing, reflecting *NADH* oscillations produced downstream of the TCA cycle in oxidative phosphorylation. (B) Scaled *NAD* (dashed) and *CIT* (solid) levels show that oscillations in these variables are in phase.
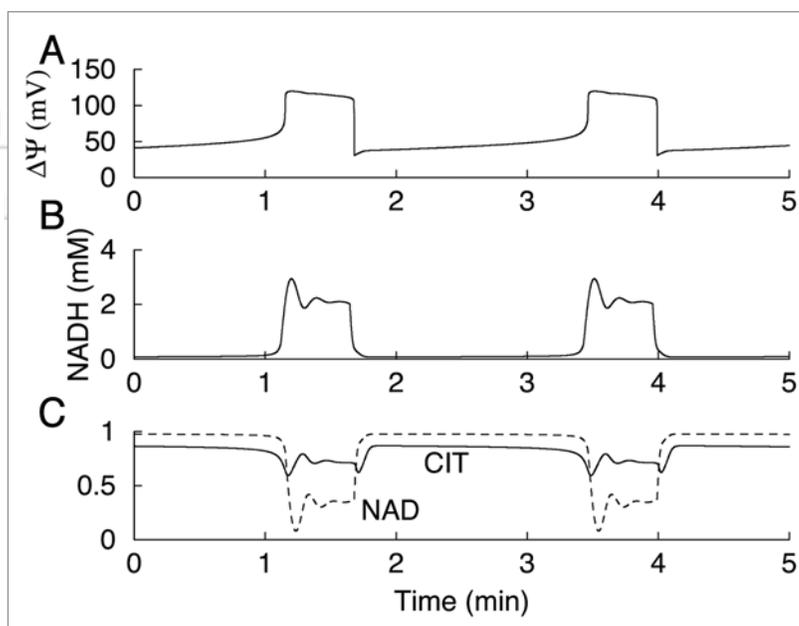
Figure 6. Oxidative phosphorylation oscillations produced with a mechanistic model.[14] (A) Oscillations in the mitochondrial inner membrane potential (ΔΨ) reflecting oscillations intrinsic to OP. The membrane is depolarized when ΔΨ is low and hyperpolarized when ΔΨ is high. (B) Oscillations in NADH. (C) Scaled *NAD* (dashed) and *CIT* (solid) levels exhibit in-phase oscillations.

the NADH concentration falls) in all cases, only negative feedback onto IDH results in the in-phase oscillations in citrate and NAD+ that were observed experimentally.

If the negative feedback is delayed at more than one point, then results can be complicated. For example, in the model with default parameter values, if the NADH feedback onto both IDH and citrate synthase (CS) is delayed, then oscillations are
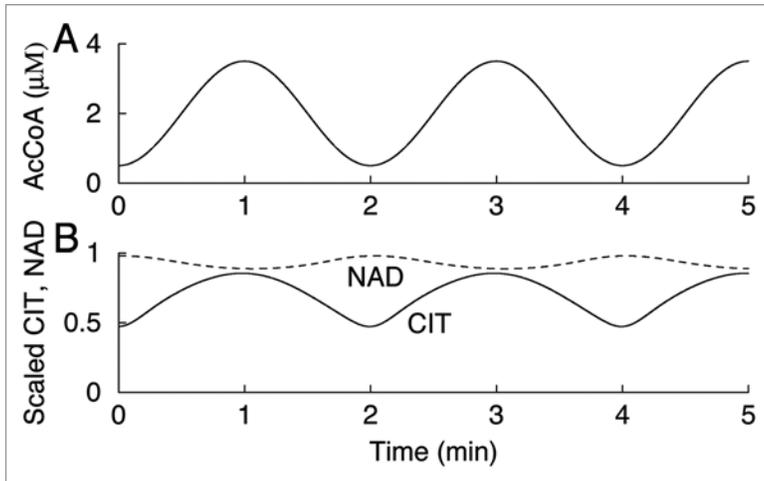
Figure 7. (A) Imposed oscillations in AcCoA and (B) their effect on *NAD* and *CIT*. The result of AcCoA oscillations, which could be due to delayed negative feedback of a modulator of PDH or to glycolytic oscillations in an intact cell, is antiphasic oscillations in *NAD* and *CIT*.

produced in which the NAD+ and citrate concentrations are antiphasic. Likewise, if negative feedback of both NADH and citrate onto CS is delayed, then oscillations with antiphasic phase relations occur. This indicates that the delayed NADH feedback onto IDH is necessary, but not sufficient, for in-phase oscillations.

Another explanation for oscillations in TCA intermediates from isolated mitochondria is that the oscillations are produced downstream of the TCA cycle, in the reactions comprising oxidative phosphorylation. We demonstrated that oscillations produced in this way result in oscillations in citrate and NAD+ that are in phase (**Figs. 5 and 6**), consistent with the experimental data. Thus, it is possible to reproduce the experimentally-determined phase relation reported by MacDonald et al. through oscillation mechanisms intrinsic to the TCA cycle or intrinsic to oxidative phosphorylation. The large time delay (45 seconds in the model) required for TCA-produced oscillations with an appropriate period suggests that the oxidative phosphorylation-driven mechanism is more likely, although we are unaware of direct data showing oxidative phosphorylation-driven metabolic oscillations in normoxic conditions. To check this, one could manipulate oxidative phosphorylation in such a way that oscillations intrinsic to oxidative phosphorylation are prevented. For example, agents that block the mitochondrial inner membrane anion channels (IMACS) have been shown to block metabolic oscillations.[15] These blockers would presumably leave TCA-driven oscillations intact. In this way, one could discriminate between oscillations driven by the TCA cycle and those driven by oxidative phosphorylation.

Oscillations in metabolism have been reported in isolated cardiac mitochondria[19,20] and brain mitochondria,[21] complementing the observation of oscillations in TCA cycle intermediates in liver and β-cell mitochondria.[8] It is therefore evident that at least one oscillatory mechanism exists within mitochondria. However, this does not imply that the mechanism for oscillations in isolated mitochondria is the same mechanism responsible for the oscillations in metabolism or metabolic variables observed in single β-cells or β-cells within intact pancreatic islets.[2,3,22-28] In these physiological settings there are indirect data suggesting that the metabolic oscillations are mediated by oscillations in glycolysis, acting on mitochondria to produce oscillations in the ATP/ADP ratio that drives bursting oscillations in electrical activity and results in pulsatile insulin secretion.[7,9] The glycolytic oscillations could be mediated by the allosteric enzyme phosphofructokinase-1 (PFK-1). The product of the PFK-1 enzyme (fructose 1,6-bisphosphate) feeds back onto and stimulates PFK-1, leading to substrate (fructose 6-phosphate) depletion. It has been demonstrated that this is a viable mechanism for glycolytic oscillations,[29] and glycolytic oscillations produced in this way have been observed in muscle extracts[9,30] that have the same isoform of PFK-1 that is dominant in β-cells.[31] An additional factor that can contribute to metabolic oscillations in intact cells is intracellular Ca^{2+}. This enters mitochondria through Ca^{2+} uniporters and in so doing depolarizes the mitochondrial inner membrane potential.[10,11,26,28] Calcium within the mitochondria stimulates pyruvate dehydrogenase, isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase.[8,12] Since the cytosolic Ca^{2+} concentration oscillates in glucose-stimulated islets, the actions of Ca^{2+} on mitochondria provide an additional source for metabolic oscillations. Indeed, in reference 8 no Ca^{2+} buffer was used to maintain the extramitochondrial Ca^{2+} concentration at a physiological level, and the resulting elevated Ca^{2+} concentration could have triggered metabolic oscillations.

Methods

Model of the tricarboxylic acid cycle. We use the model of the TCA cycle (**Fig. 1**) developed by Cortassa et al.[18] This consists of a set of ordinary differential equations for the TCA cycle intermediates isocitrate (*ISO*), α-ketoglutarate (α*KG*), succinyl-CoA (*SCoA*), succinate (*SUC*), fumerate (*FUM*), malate (*MAL*) and oxaloacetate (*OAA*). We assume for simplicity that there is no anaplerosis or cataplerosis; neither process would affect the phase relations that are the focus of our attention. Since the total concentration of citric acid intermediates is therefore conserved, the citrate (*CIT*) concentration can be obtained through a conservation equation:

$$CIT = TOT - ISO - \alpha KG - SCoA - SUC - FUM - MAL - OAA$$
(1)

where *TOT* = 1 mM is the total concentration of TCA cycle intermediates that we assume (reviewed in ref. 18). For simplicity, we use CIT to denote citrate and *CIT*, in italics, to denote its concentration (in units of mM), and similarly for other variables. The differential equations are:

$$\frac{dISO}{dt} = V_{ACO} - V_{IDH} \qquad (2)$$

$$\frac{d\alpha KG}{dt} = V_{IDH} - V_{KGDH} \qquad (3)$$

$$\frac{dSCoA}{dt} = V_{KGDH} - V_{SL} \qquad (4)$$

$$\frac{dSUC}{dt} = V_{SL} - V_{SDH} \qquad (5)$$

$$\frac{dFUM}{dt} = V_{SDH} - V_{FH} \qquad (6)$$

$$\frac{dMAL}{dt} = V_{FH} - V_{MDH} \qquad (7)$$

$$\frac{dOAA}{dt} = V_{MDH} - V_{CS} \qquad (8)$$

where subscripted V is the reaction rate for aconitase (ACO), isocitrate dehydrogenase (IDH), α-ketogluterate dehydrogenase (KGDH), succinyl-CoA synthetase (SL), succinate dehydrogenase (SDH), fumerase (FH), malate dehydrogenase (MDH) and citrate synthase (CS). Mathematical expressions for these reaction rates are given in Cortassa et al.[18] We describe in detail only those that are the target of negative feedback in the various oscillation mechanisms that we examine. This negative feedback is required to generate oscillations, and we examine several feedback points that are known to exist.

There are several known targets of negative feedback in mammalian mitochondria (reviewed in ref. 13). One target is citrate synthase (CS). This enzyme is inhibited by citrate and NADH, among other modulators. We modify the expression used in[18] to include these negative feedback pathways:

$$V_{CS} = \frac{k_{cat}^{CS} E_T^{CS}}{1 + \frac{K_M^{AcCoA}}{AcCoA} + \frac{K_M^{OAA}}{OAA} + \frac{K_M^{AcCoA}}{AcCoA} \frac{K_M^{OAA}}{OAA}} \frac{1}{CIT(1 + \frac{NADH}{K_I^{NADH}})} \qquad (9)$$

The parameters and parameter values that we use are: $k_{cat}^{CS} = 0.00016$ ms^{-1} is the CS catalytic rate, $E_T^{CS} = 0.04$ mM is the CS concentration, $K_M^{AcCoA} = 0.0126$ mM is the AcCoA Michaelis constant, $K_M^{OAA} = 6.4 \times 10^{-4}$ mM is the OAA Michaelis constant, and $K_I^{NADH} = 0.5$ mM is the NADH inhibition constant.

A second target for negative feedback is isocitrate dehydrogenase. This enzyme is inhibited by NADH, and the feedback is contained within the expression for V_{IDH} used in.[18] The feedback enters into the reaction rate as it does in Eq. 9. All parameter values for V_{IDH} are the same as in,[18] except $K_M^{NAL} = 5$ mM.

Model of oxidative phosphorylation. We use the model of oxidative phosphorylation (OP) that was developed in Bertram et al.[19] This is a simplification of the model developed earlier by Magnus and Keizer.[10,11,32] The full model consists of the TCA compartment described above coupled to the OP compartment described below. The input to the OP compartment is the NADH from the TCA cycle, and the primary output is mitochondrial ATP. The variables are NADH concentration, mitochondrial ADP concentration (*ADP*), mitochondrial inner membrane potential (ΔΨ), and the mitochondrial Ca^{2+} concentration (*Ca*). The adenine and pyridine nucleotides are assumed to be conserved, so that

$$NAD + NADH = NAD_{tot} \qquad (10)$$

$$ADP + ATP = A_{tot} \qquad (11)$$

where we use $NAD_{tot} = 10$ mM and $A_{tot} = 15$ mM. The variables change in time according to the following differential equations:

$$\frac{dNADH}{dt} = V_{DH} - V_o \qquad (12)$$

$$\frac{dADP}{dt} = V_{ANT} - V_{F1F0} \qquad (13)$$

$$\frac{d\Delta\Psi}{dt} = (V_{H,res} - V_{H,atp} - V_{ANT} - V_{H,leak} - V_{NaCa} - 2V_{uni})/C_m \qquad (14)$$

$$\frac{dCa}{dt} = f_m(V_{uni} - V_{NaCa}) \qquad (15)$$

where units of time are ms, units of *NADH* and *ADP* are mM, units of ΔΨ are mV, and units of *Ca* are μM. The reaction rates V are for NADH production through dehydrogenases (DH), NADH utilization reflected by oxygen consumption (o) (we omit the contribution from FADH$_2$), exchange of ATP for ADP through the mitochondrial antiporter (ANT), ATP synthesis through the ATP synthase (F1F0 ATPase), membrane hyperpolarization through the respiration-driven proton flux (H,res), depolarization through the ATP synthase proton flux (H,atp), depolarization through proton leakage (H,leak), Ca^{2+} influx through the uniporter (uni) and efflux through the Na$^+$/Ca^{2+} exchanger (NaCa). The extra-mitochondrial Ca^{2+} concentration is held fixed at 0.1 μM. The TCA cycle component influences the OP component through the dehydrogenase rate:

$$V_{DH} = V_{IDH} + V_{KGDH} + V_{MDH} \qquad (16)$$

The OP component influences the TCA cycle component through NADH, which inhibits CS and IDH.

Model of oscillations in oxidative phosphorylation. To simulate oscillations that are intrinsic to oxidative phosphorylation we first add a sinusoidal forcing function to the differential equation for NADH concentration:

$$\frac{dNADH}{dt} = V_{DH} - V_o + S(t) \qquad (17)$$

where V_{DH} is the summed reaction rates of the dehydrogenases, V_o is the oxygen consumption rate, and $S(t)$ is the sinusoidal forcing function,

$$S(t) = A\sin\left(\frac{2\pi t}{T}\right) \qquad (18)$$

where A is the amplitude of the imposed oscillations and $T = 120,000$ ms (2 min) is the period. This model is used in **Figure 5**.

We use a separate model for oscillations in oxidative phosphorylation. This model, developed by Jafri and Kotulska,[14] describes oscillations in cardiac mitochondria under conditions of metabolic stress, such as ischemia. It is coupled to a model of the TCA cycle developed by Jafri.[14] The key players in the production of the oscillations are the mitochondrial inner membrane anion channels (IMACs)[15] and the mitochondrial centum-picosiemen (mCS) channels.[16] In this model, these two channel types interact to create oscillations in $\Delta\Psi$, which result in oscillations in other mitochondrial variables, including NADH. The NADH oscillations then feed back onto the TCA cycle through inhibition of CS and IDH, resulting in TCA oscillations. Thus, in this scenario the oscillations are produced by OP and cause oscillations in TCA cycle intermediates, including CIT.

Computer programs for both models are available for free download from www.math.fsu.edu/~bertram/software/islet. Differential equations in model 1 were solved numerically using the CVODE solver in the XPPAUT software package.[33] Differential equations in model 2 were solved numerically using the forward Euler method implemented in FORTRAN.

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