Molecular BioSystems

Cite this: *Mol. BioSyst.*, 2011, **7**, 3335–3342

www.rsc.org/molecularbiosystems

**PAPER**

# *In silico* studies on the sensitivity of myocardial PCr/ATP to changes in mitochondrial enzyme activity and oxygen concentration†

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***Received 28th July 2011, Accepted 21st September 2011***
**DOI: 10.1039/c1mb05310h**

The ratio of myocardial phosphocreatine (PCr)/ATP reflects the balance of energy consumption and energy supply in the heart. It is reduced in a range of important physiological conditions including during and after acute hypoxia, after a prolonged visit to high-altitude, and in those suffering from both type 2 diabetes mellitus and various forms of heart failure. Yet despite its significance, the factors underlying the reduced PCr/ATP ratio seen in heart failure remain poorly understood. Given that oxidative phosphorylation is the only viable steady-state provider of ATP in the heart, the argument has been put forward that the observed reduction in myocardial PCr/ATP in all these conditions can be accounted for by some form of mitochondrial insufficiency. Thus we used a computer model of oxidative phosphorylation, coupled with creatine kinase, to study the effects of hypoxia and mitochondrial dysfunction on myocardial PCr/ATP. In physiological normoxia, all oxidative phosphorylation complexes, NADH supply and proton leak exerted comparable (of the same order of magnitude) control over PCr/ATP, as defined within Metabolic Control Analysis (MCA). Under hypoxia, the control increased considerably for all components of the system, especially for cytochrome oxidase and mitochondrial proton leak. Hypoxia alone, without any changes in other factors, exerted a pronounced effect on PCr/ATP. Our simulations support three important ideas: First, that mitochondrial abnormalities can contribute considerably to a blunted PCr/ATP; second, that hypoxia and mitochondrial dysfunction can interact in important ways to determine the energy status of the failing heart; and third, that hypoxia alone can account for significant decreases in cardiac PCr/ATP.



## Introduction

The ratio of phosphocreatine (PCr) to adenosine 5′-triphosphate (ATP) in the working heart provides an index of the balance between the rates of cellular energy consumption and energy supply. It is reduced in a range of important physiological, environmental and clinical conditions, including during and after acute hypoxia,[1,2] after a prolonged visit to high-altitude,[3] and in those suffering from both type 2 diabetes mellitus[4] and various forms of heart failure.[5,6] Indeed, in those with heart failure a reduced myocardial PCr/ATP may predict mortality better than conventional measures.[7]

Given that oxidative phosphorylation is the only viable steady-state provider of ATP for contractile and other work in the heart, the argument has been put forward that the observed reduction in myocardial PCr/ATP in all these conditions can be accounted for by some form of mitochondrial insufficiency; either due to defects in key mitochondrial enzymes, increased mitochondrial proton leak, impaired supply of reducing equivalents or insufficient mitochondrial $PO_2$. Furthermore, a reduction in PCr/ATP and an increase in Pi/PCr means a reduction in the free energy available from hydrolysis of ATP ($\Delta G'_{ATP}$) and an increase in free ADP, *via* the Lohmann 'reaction'.[8] This, in the heart, leads to diastolic dysfunction, possibly *via* slowed cross bridge cycling.[9,10] Therefore myocardial PCr/ATP is important not only as an estimate of the degree of energetic mismatch in the working heart, but also as a driver of dysfunction and/or pathogenesis in its own right.

Yet despite its potential scientific and clinical significance, the factors underlying environmentally and pathologically-induced reductions in PCr/ATP ratio in working heart remain poorly understood. Thus we sought to investigate and semi-quantitatively analyze the effect of (and interactions between) changes in mitochondrial enzyme and pathway activity, reduced NADH supply and lowered oxygen concentration on the PCr/ATP ratio in working heart. We chose as our experimental platform a well characterized and validated *in silico* model of cardiac energy metabolism combined with the formalism of metabolic control analysis (MCA).

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† Electronic supplementary information (ESI) available. See DOI: 10.1039/c1mb05310h

## Methods

An existing, well-characterised model of oxidative phosphorylation in intact heart[11,12] was used for this study. This model (Fig. 1) centres on a system of ordinary differential equations (ODEs) describing the rates of change of the following mitochondrial metabolites: NADH, reduced ubiquinone, reduced cytochrome c, oxygen, protons, ATP and inorganic phosphate; and the rates of change of the following metabolites in the cytosol: ATP, ADP, inorganic phosphate and phosphocreatine (PCr). The concentrations of other metabolites are calculated from these, assuming equilibrium and moiety conservation.

The model has been tested for numerous variable values/system properties (in isolated mitochondria, isolated hepatocytes, intact skeletal muscle and intact heart) (see ref. 15 for review). They comprise, among others: (1) the values of fluxes (oxygen consumption and ATP turnover) and concentrations of metabolites (ADP, ATP, $P_i$, PCr, Cr, NADH/$NAD^+$, reduction level of cytochrome c, reduction level of cytochrome $a_3$, $\Delta p$—protonmotive force and $O_2$) in different steady-states imposed by various energy demands and oxygen concentrations; (2) changes over time of fluxes and metabolite concentrations during transitions between different steady-states (rest-to-work transition or low-to-high work transition, aerobiosis-to-anaerobiosis transition); (3) the values of the flux control coefficients (defined within Metabolic Control Analysis[16]) quantifying the control of particular enzymes, processes, and metabolic blocks over the oxygen consumption flux in different steady-states; (4) the dependence of the respiration rate on the activities of different enzymes, obtained by the titration of particular oxidative phosphorylation complexes with specific inhibitors.

The model was adapted from the original FORTAN and reprogrammed in Mathematica 6 (Wolfram Research, Champaign, Illinois, USA). The model's system of differential equations was solved numerically using a backwards differentiation algorithm, *via* Mathematica's NDSolve command.

Multiplying the appropriate rate equation by a scalar simulated changes in mitochondrial enzyme or pathway activity. In order to quantify the sensitivity of the PCr/ATP ratio to changes in enzyme or pathway flux rate, we used the formalism of Metabolic Control Analysis (MCA) suggested by Fell.[16] We calculated the concentration control coefficient, defined as the fractional change in PCr/ATP for an infinitesimal change in enzyme concentration/activity that caused this change, thus:

$$C_E^{\mathrm{PCr/ATP}} = \frac{d(\mathrm{PCr/ATP})/(\mathrm{PCr/ATP})}{(dE/E)} \qquad (1)$$

where $E$ is the concentration/activity of the enzyme or pathway of interest and $C_E^{\mathrm{PCr/ATP}}$ is the concentration control coefficient of the enzyme of interest over myocardial PCr/ATP. In order to calculate $C_E^{\mathrm{PCr/ATP}}$, we increased the activity of the enzyme or pathway of interest by 1% and recorded the fractional change in PCr/ATP after reaching a new steady-state. We then calculated $C_E^{\mathrm{PCr/ATP}}$ using (1).

In the present paper we calculate PCr/ATP using cytosolic ATP. In the heart, the cytosol occupies 75–80% of the cell volume and contains the contractile proteins; it is the energy status of the heart at the site of contraction that is of most interest here. The mitochondrial ATP/ADP is lower than, but changes in the same direction as the cytosolic ATP/ADP. The only exception is when ATP/ADP carrier activity is lowered: in this case the mitochondrial ATP/ADP increases, while the cytosolic ATP/ADP decreases.

In order to study the effect of changes in cell oxygen concentration, we repeated our simulations under three conditions—saturating $[O_2]$ (defined as 240 μM[17]), physiological normoxia (defined as 10% saturating $[O_2]$[18]) and hypoxia

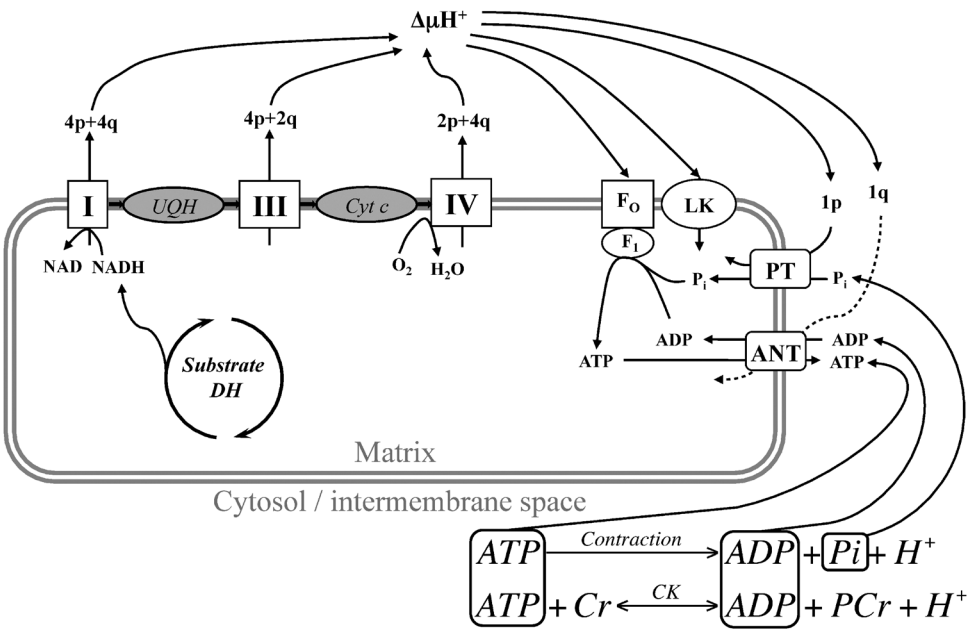


**Fig. 1** The elements of the bioenergetic system in heart (oxidative phosphorylation coupled with substrate dehydrogenation, phosphate and adenine nucleotide transport and creatine kinase) taken into account explicitly within the computer model used in the present study. Abbreviations: p = proton, q = charge.

 

(defined as 1% saturating $[O_2]$[19]). By repeating all our simulations under these three conditions were able to study not only the 'direct' effect of $[O_2]$ on myocardial PCr/ATP but also interactions between changes in enzyme or pathway activity and cell oxygen content. It should be stressed that our model was intended to involve *extra*-, and not *intra*-cellular oxygen concentrations, and that this was the reference point for experimental data.

All concentrations are reported in μM and all fluxes in μM $min^{-1}$. The complete, basic Mathematica program is available from the corresponding author on request while a complete mathematical description of the model can be found in the Supplementary Materials.

## Results

We first simulated the effect on myocardial PCr/ATP of changes in the activity of enzymes or pathways that contribute to (Fig. 2) or consume (Fig. 3) the proton gradient. In all cases there was little effect until enzyme or pathway activity was reduced to around one fifth of normal in the presence of saturating $[O_2]$ (Fig. 2A and 3A). When enzyme activities were reduced beyond this point, PCr/ATP dropped and the corresponding control coefficients climbed sharply. These effects could be seen particularly clearly when the control coefficients of each enzyme (or pathway) over PCr/ATP were plotted as a function of enzyme activity, as in Fig. 2B and 3B. Under these conditions (of saturating $[O_2]$) the adenine nucleotide translocase (ANT) had, at 0.14, the highest $C_E^{PCr/ATP}$ of any single enzyme in our analysis, while Complex I and ATP synthase had the lowest $C_E^{PCr/ATP}$ (0.023 and 0.022, respectively). Generally, all concentration control coefficient were of the same order of magnitude (differed less than sevenfold), indicating that all components of the system have comparable impact on PCr/ATP. A summary of all the control coefficients, ordered by absolute magnitude, is given in Table 1. The concentration control coefficients presented in Table 1 are calculated for normal (100%) activity of enzymes (from the initial slopes of the curves presented in Fig. 2A, C and E and Fig. 3A, C and E).

We then repeated our simulations under conditions of (a) physiological normoxia (Fig. 2C, D, 3C and D) and (b) critical hypoxia (Fig. 2E, F, 3E and F). In normoxia, the changes in PCr/ATP and corresponding concentration control coefficients were broadly similar to those under saturating $[O_2]$. Nevertheless, it is worth noticing that PCr/ATP was noticeably lower even at normal complex activities. The critical threshold occurred at around one fifth normal enzyme activity, although the threshold was noticeably higher for certain enzymes (*cf.* Complex III (Fig. 2C) and ANT (Fig. 3C)). However, the slope of the relationships between enzyme activity and PCr/ATP was steeper at close to normal enzyme activity, signifying an increased sensitivity of myocardial PCr/ATP to changes in

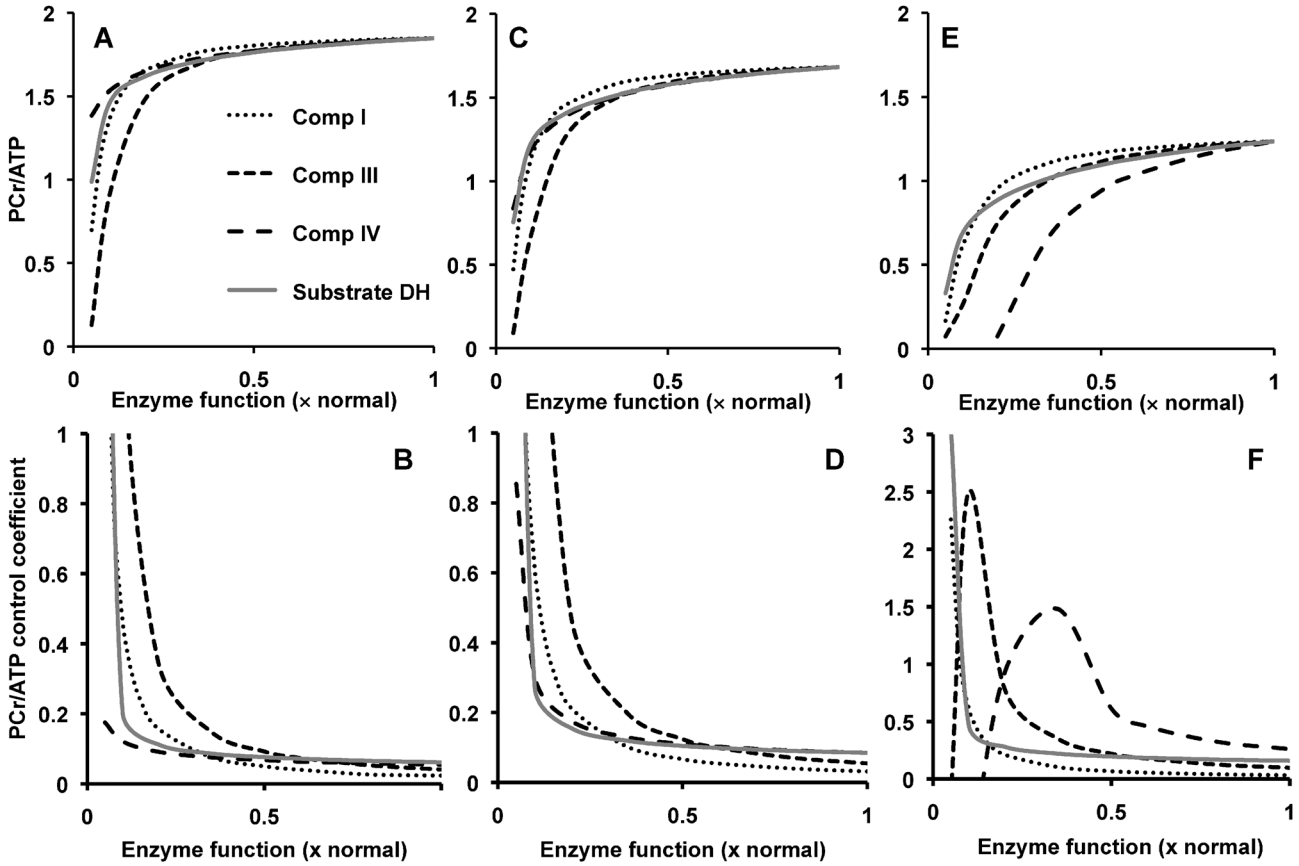


**Fig. 2** The effect on myocardial PCr/ATP of changes in the activity of enzymes/pathways that contribute to the proton gradient under a) saturating $O_2$ (**2A**, **2B**), (b) physiological normoxia (**2C**, **2D**) and (c) hypoxia (**2E**, **2F**). Abbreviations: Comp = complex; Substrate DH = substrate dehydrogenation.

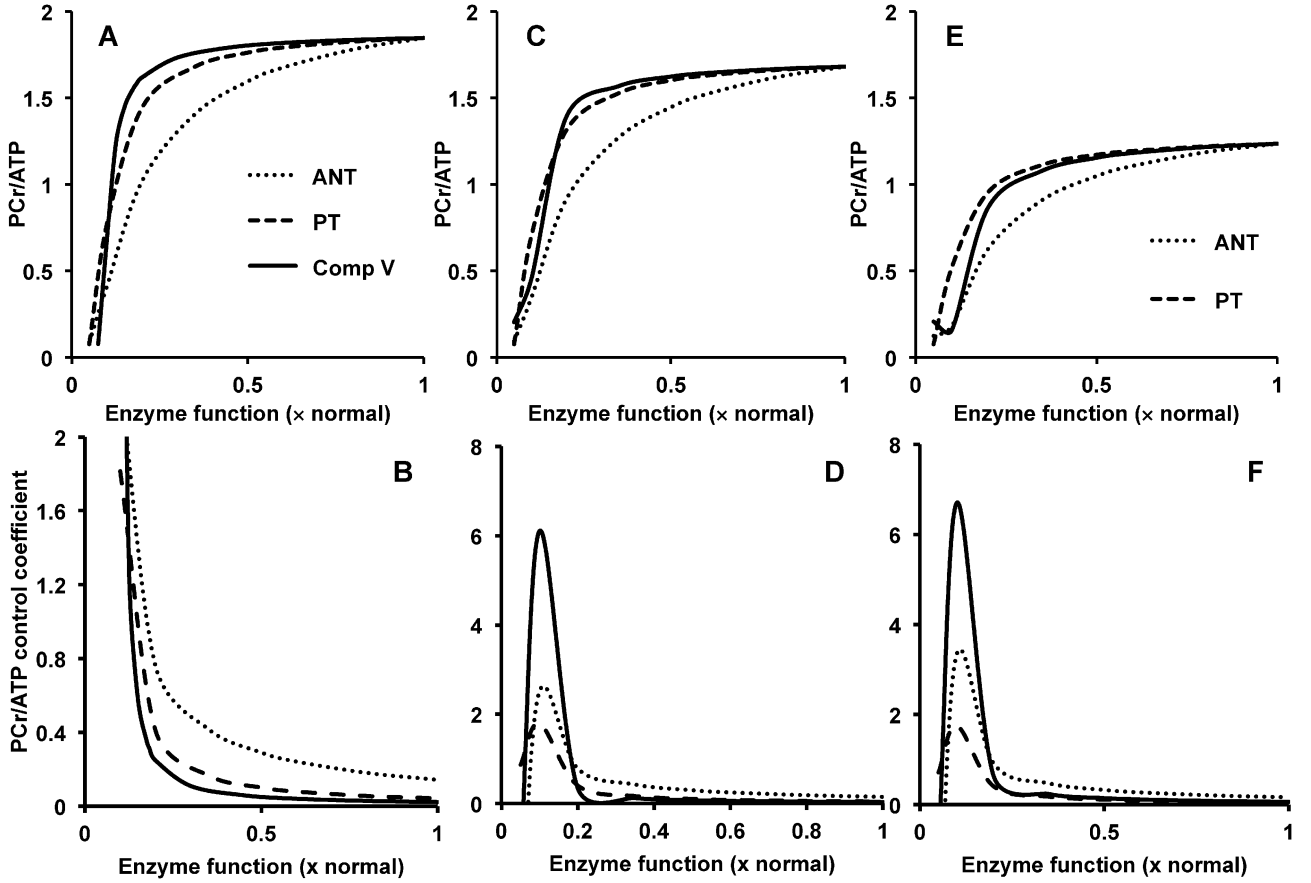


**Fig. 3** The effect on myocardial PCr/ATP of changes in the activity of enzymes/pathways that consume the proton gradient under a) saturating $O_2$ (**3A**, **3B**), (b) physiological normoxia (**3C**, **3D**) and (c) hypoxia (**3E**, **3F**). Abbreviations: Comp = complex; ANT = adenine nucleotide translocase; PT = phosphate transporter.

**Table 1** Concentration control coefficients for mitochondrial enzymes and pathways over myocardial PCr/ATP under conditions of (a) saturating $O_2$, (b) physiological normoxia and c) hypoxia

| Enzyme | Saturating $O_2$ (240 μM) | Normoxia (24 μM) | Hypoxia (2.4 μM) |
|---|---|---|---|
| Adenine nucleotide translocase | 0.143 | 0.149 | 0.164 |
| Substrate dehydrogenation | 0.061 | 0.084 | 0.157 |
| Cytochrome oxidase (Complex IV) | 0.054 | 0.084 | 0.259 |
| Pi transporter | 0.042 | 0.044 | 0.050 |
| Complex III | 0.040 | 0.054 | 0.096 |
| Proton leak (normal leak) | −0.031 | −0.038 | −0.062 |
| Proton leak (3 × normal leak) | −0.081 | −0.100 | −0.172 |
| Complex I | 0.023 | 0.031 | 0.055 |
| ATP-synthase (Complex V) | 0.022 | 0.032 | 0.064 |

enzyme activity under physiological normoxia when compared with saturating $O_2$.

In hypoxia the effect of low [$O_2$] was very significant. First, PCr/ATP was much lower than in normoxia and at saturated [$O_2$] even at normal enzyme activity. Second, the threshold for the PCr/ATP-[$O_2$] dependence was shifted to higher values of enzyme activities. This is especially evident for cytochrome oxidase (complex IV) and cytochrome III—the enzymes that are 'closest' to oxygen in the respiratory chain (the strongest effect is for cytochrome oxidase for which $O_2$ is a substrate). The initial slope of the PCr/ATP-[$O_2$] dependence was also significantly increased for most complexes.

The effect of lowered oxygen is further evidenced by the increased concentration control coefficients $C_E^{PCr/ATP}$ shown in Table 1. First of all, the values of all coefficients gradually increased during passage from saturated [$O_2$] through normoxia to critical hypoxia. This takes place because at lower [$O_2$] the activity of the system is less 'excessive' in order to sustain constant PCr/ATP. The greatest increase, both in absolute and relative terms (from $C_E^{PCr/ATP}$ = 0.05 to 0.26), occurred for cytochrome oxidase. This is completely understandable, because oxygen is a substrate for this enzyme.

During these simulations of modified oxygen content combined with altered enzyme activity we began to observe maxima in $C_E^{PCr/ATP}$ at low activities of Δp-consuming enzymes (Fig. 3D for normoxia). Although ATP homeostasis was well maintained in our simulations, [ATP] did fall at critical levels of enzyme flux leading to instability in the relationship between enzyme flux and PCr/ATP. Thus these maxima could be taken as the first evidence of a loss of ATP homeostasis. Under hypoxia, these patterns were further accentuated and concerned a greater number of complexes (both Δp-consuming and producing) (Fig. 2F and 3F). For example, we observed a local maximum in $C_E^{PCr/ATP}$ when cytochrome oxidase activity was reduced to just 40% of normal under hypoxic conditions. This behavior pattern did not concern substrate dehydrogenation and complex I that did not cause ATP depletion in the range of their activities

 

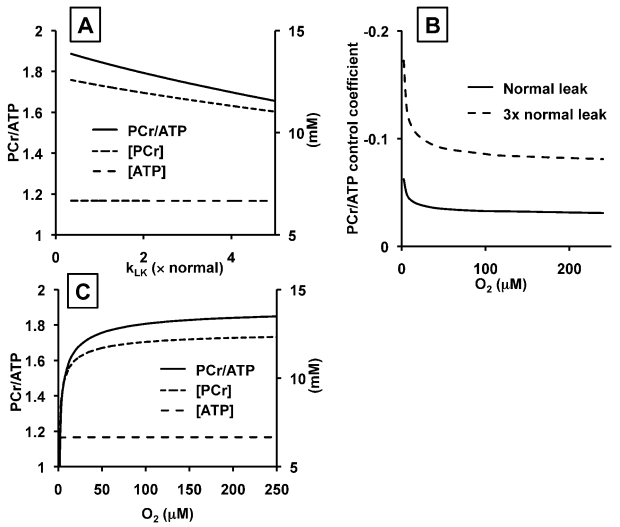


**Fig. 4** The effect of changes in proton leak activity (rate constant) on myocardial [PCr], [ATP] and PCr/ATP at saturating [$O_2$]. Fig. 4B: The effect of intracellular [$O_2$] on the concentration control coefficient of proton leak over myocardial PCr/ATP at normal (solid line) and 3x normal (dashed line) proton leak activities. Fig. 4C: The effect of variations in intracellular [$O_2$] on [PCr], [ATP] and PCr/ATP. Abbreviations: $k_{LK}$ = rate constant of the proton leak rate equation; PCr = phosphocreatine; ATP = adenosine triphosphate.

(down to 5% of normal) applied in the simulations (limitation of NADH supply was not crucial under critical hypoxia conditions).

We simulated the effect of changes in proton leak activity (by varying the proton leak rate constant) on myocardial PCr/ATP. Our findings are shown in Fig. 4A and B. We found that in normoxia PCr/ATP was relatively insensitive to increases in proton leak, reflected in the low concentration control coefficient of −0.03 (the control coefficient is negative because increased proton leak reduces PCr/ATP). To study any potential interaction between proton leak and ischaemia we calculated $C_E^{PCr/ATP}$ of proton leak over PCr/ATP at varying oxygen tensions. These simulations revealed a sharp rise in $C_E^{PCr/ATP}$ at low, but still physiological oxygen tensions (Fig. 4B). At normal leak activities, however, $C_E^{PCr/ATP}$ was only −0.06 even at 1% saturating [$O_2$]. Finally, we studied the effect of increased uncoupling and hypoxia together by increasing uncoupling threefold and, as before, calculating $C_E^{PCr/ATP}$ at a range of oxygen concentrations (Fig. 4B). Under these conditions, $C_E^{PCr/ATP}$ rose above −0.1 at an [$O_2$] of 5% saturating.

We simulated the direct effect of changes in intracellular oxygen concentration on PCr/ATP, in the absence of changes in any enzyme/process activity. Fig. 4C shows that cell ATP was defended well even at very low oxygen concentrations. However, cell PCr decreased moderately with falling [$O_2$] at higher oxygen concentrations, and then fell rapidly at oxygen concentrations of below approximately 10% saturating. As one can see, an appropriate decrease in [$O_2$] is able to reduce PCr/ATP to any desired level. Because severe hypoxia or even local anoxia seems likely to occur in many heart disorders, this mechanism seems to account at least for a significant part of the discussed effect.

## Discussion

The ratio of myocardial phosphocreatine to ATP has both scientific and clinical relevance.[7,9,10,20] Given that (i) PCr/ATP reflects the balance of energy provision and utilization in the heart, (ii) oxidative phosphorylation is the principal energy supply pathway in the heart and (iii) mitochondrial dysfunction and/or lowered oxygen availability is a common finding in a number of common environmental and clinical conditions, we used a computer model of oxidative phosphorylation to analyze the sensitivity of myocardial PCr/ATP to changes in $PO_2$ and mitochondrial pathway and enzyme activity. In normoxia, the activity of individual respiratory chain complexes needed to be significantly impaired (to around one fifth normal) before there was a substantial impact on myocardial PCr/ATP. However, PCr/ATP was much lower even at normal enzyme activity–and far more sensitive to changes in respiratory chain enzyme activity–in hypoxia. Mitochondrial proton leak had little effect on PCr/ATP in normoxia. However, a combination of increased uncoupling and hypoxia led to uncoupling having a substantial degree of control over PCr/ATP. In addition, hypoxia alone had a pronounced effect on PCr/ATP. Of all the enzymes studied, PCr/ATP was most sensitive to changes in the activity of the adenine nucleotide translocase (ANT) in normoxia and cytochrome oxidase in hypoxia.

Investigators working in the area of myocardial energetics have uncovered a range of mitochondrial abnormalities in obesity, type 2 diabetes, heart failure, and in response to environmental hypoxia. These include blunted activity of the respiratory chain complexes,[21–26] reduced activity of the $F_1 - F_O$ ATP-synthase[22] and increased respiratory uncoupling.[26–29] For example, Marin-Garcia and co-workers reported a reduction in Complex III activity to around 20% compared with controls in a canine model of volume-overload heart failure.[22] In the same experiment, Complex V (ATP-synthase) activity was reduced to 30% *vs.* controls. In our simulations, the control coefficients of both these enzymes over myocardial PCr/ATP climbed sharply as enzyme activity fell below approximately 25% normal (at saturating [$O_2$] or under physiological normoxia) (Fig. 2A–D). This threshold was significantly higher under cell hypoxia, and as high as 50% in the case of cytochrome oxidase (Fig. 2D). It follows that if our simulations were accurate then the respiratory chain abnormalities reported by Marin-Garcia *et al.* would be sufficient to cause a substantial reduction in myocardial PCr/ATP (from 1.9 to approximately 1.5 in response to the Complex III deficiency alone at saturating [$O_2$], see Fig. 2A). However, it must be borne in mind that respiratory chain dysfunction differs qualitatively depending on the nature of the disease. For example, those with idiopathic dilated cardiomyopathy have significantly higher respiratory chain activity than those with ischemic heart disease,[24] perhaps unsurprisingly. We also simulated the effect of reduced substrate dehydrogenation and, consequently, NADH delivery on myocardial PCr/ATP. Our simulations showed that under saturating [$O_2$] the control coefficient of substrate dehydrogenation was 0.06 (Table 1) and, as in many other cases, climbed sharply when substrate dehydrogenation was limited to below approximately one fifth normal (Fig. 2B). However, under

conditions of hypoxia, substrate dehydrogenation exerted substantial control over myocardial PCr/ATP (see Table 1, Fig. 2E and Fig. 2F) and had one of the highest control coefficients observed ($C_E^{PCr/ATP} = 0.16$).

Given that a reduced myocardial PCr/ATP ratio has been observed in both ischemic heart disease, hypoxia and type 2 diabetes (where coronary atherosclerosis is common[30]), we simulated the effect of changes in extracellular [$O_2$] in the absence of changes in any mitochondrial enzymes or pathways. Lowered [$O_2$] decreases PCr/ATP in the following way: $O_2$ is one of the substrates of cytochrome oxidase and a decrease in its concentration must be compensated by a decrease in $\Delta p$ and increase in the reduction level of cytochrome c in order to keep the flux as constant as possible (although the compensation is never perfect[31,32]). The lowered $\Delta p$ decreases (through slowing down ATP synthase) the mitochondrial ATP/ADP. This in turn decreases (through slowing down ANT) the cytosolic ATP/ADP (because of the very high ATP/ADP ratio ATP is essentially constant and mostly ADP changes). This leads (through the equilibrium of creatine kinase) to a decrease of PCr/Cr (both PCr and Cr change significantly, because their concentrations are comparable). As a result, PCr/ATP decreases.

We found that PCr/ATP was strongly dependent on myocardial oxygenation once [$O_2$] dropped below approximately 10% saturating (Fig. 4C), bearing in mind that severe hypoxia could produce substantially lower cell [$O_2$] than this. Therefore in environmental hypoxia, type 2 diabetes and ischemic heart disease, hypoxia alone may be a substantial driver of reduced myocardial PCr/ATP. Indeed our simulations agreed exceptionally well with data previously acquired from the hearts of sheep exposed to progressive hypoxia (compare, for example, Fig. 9 in ref. 1 with Fig. 4C). It could be argued that the oxygen concentrations chosen by us for normoxia (24 μM) and hypoxia (2.4 μM) are slightly too high. However, we preferred to have some 'safety margin' and not exaggerate by using potentially unphysiologically low concentrations (of course, lower concentrations would only strengthen the effects observed in our simulations). For clarity, the effect of the whole range of oxygen concentration on PCr/ATP is shown in Fig. 4C

Under conditions of very low oxygen concentration or very low activity of the respiratory chain complexes the ATP synthase can work in the reverse direction, *i.e.* it hydrolyses ATP and builds up the protonmotive force ($\Delta p$). Within our model, this results directly from its kinetic description (see Korzeniewski *et al.*, 2005 and the Supplementary Materials):

$$\nu_{SN} = k_{SN}\frac{\gamma - 1}{\gamma + 1} \qquad (2)$$

where $\nu_{SN}$ is the rate of ATP synthase, $k_{SN} = 117\,706$ M min$^{-1}$ is its rate constant, $\gamma = 10^{\Delta G_{SN}/Z}$ and $\Delta G_{SN} = n_A * \Delta p - \Delta G_P$ is the thermodynamic span of ATP synthase ($\Delta G_P$ is the intramitochondrial phosphorylation potential proportional to matrix log (ATP/(ADP*$P_i$)) and $n_A = 2.5$ is the stoichiometry of protons needed for synthesis of one ATP molecule). If $\Delta G_{SN} > 0$ and therefore $\gamma > 1$, ATP synthase works in the forward direction (ATP synthesis); if $\Delta G_{SN} = 0$ and therefore $\gamma = 1$, there is zero net flux through ATP synthase; if $\Delta G_{SN} < 0$ and therefore $\gamma < 1$, ATP synthase works in the backward direction (ATP hydrolysis). Therefore, our kinetic description of ATP synthase reflects the full reversibility of this enzyme.

The model used in the present study did not explicitly involve the PCr/Cr shuttle.[13] Of course, we are aware of the existence and importance of this shuttle. However, we also believe that: (1) creatine kinase is very quick and works close to thermodynamic equilibrium, especially in steady-states, and (2) the diffusion of PCr and Cr is also very quick and therefore there are no significant gradients of PCr and Cr (and, consequently, of ADP and ATP). Thus the simple near-equilibrium description of creatine kinase we use (*cf.* ref. 14) is kinetically completely equivalent to a more complicated description explicitly involving the entire PCr/Cr shuttle. The possibility of such simplifications emphasizes the usefulness and advantage of simple models – it is much easier to understand intuitively the CK equilibrium, than the kinetic functioning of the whole PCr/Cr shuttle, and there is no need to include the full comprehensive description of this shuttle, because this would not have any influence on the behaviour of the whole system.

Relatively recently, the hypothesis has been put forward that increased mitochondrial proton leak contributes to the energy deficit seen in obesity,[29] diabetes,[4] heart failure[27,28,33,34] and after acute hypoxic exposure.[20] Our simulations suggest that this would not be the case in physiological normoxia (Fig. 4A and B); nor would it appear to be the case under hypoxia, on the condition that leak activities are near normal (Fig. 4B, solid line). This finding may have an important implication. Current thinking posits that mitochondrial proton leak, far from being a maladaptive process, is an important mechanism for controlling mitochondrial reactive oxygen species production.[35] Our simulations suggest that regulated proton leak could be employed at a wide range of cell [$O_2$] without substantially hindering cell energetics. However, our simulations also showed that if proton leak is raised several-fold in the presence of reduced cell [$O_2$] then it does begin to exert substantial control over myocardial PCr/ATP (Fig. 4B, dashed line). Thus mitochondrial uncoupling may contribute to reduced myocardial PCr/ATP in conditions where cell [$O_2$] is lowered such as acute hypoxia, coronary artery disease or in cases where extensive cardiac remodelling has led to impaired $O_2$ delivery or diffusion.[36,37] Our simulations also suggest that those cells with increased rates of uncoupling would be more susceptible to energy depletion when subjected to hypoxia, whether pathological or environmental. Our findings also suggest a novel way to experimentally test the hypotheses regarding increased mitochondrial uncoupling in working heart – by experimentally progressively reducing inspired oxygen tension, subjects with increased myocardial mitochondrial uncoupling should display a greater reduction in PCr/ATP at higher inspired $PO_2$ than controls.

Transgenic mice lacking the gene for ANT in striated muscle have, among other serious pathologies, hypertrophic cardiomyopathy.[38] Furthermore, increased activity or expression of ANT rescues cardiac function both in pressure-overload hypertrophy[39] and diabetic cardiomyopathy.[40] We therefore investigated the effect of changes in ANT and phosphate transporter activity on myocardial PCr/ATP. Although the

 

phosphate transporter did not have a substantial effect, ANT had the highest control coefficient of any enzyme or pathway studied, at 0.14 (Table 1). Experimental support for our findings comes from a model of viral myocarditis in guinea pigs, in which the animals produced inhibitory antibodies against their own ANT. Animals that tested positive for autoantibodies had significantly lower myocardial PCr/ATP than virus-positive/autoantibody-negative controls.[41] Our simulations suggest that more experimental work is required to study the effect of altered myocardial ANT function in human cardiac failure.

In many cases our simulations could produce PCr/ATP ratios less than 1.0. Yet in reality ratios as low as this are extremely rare. There is a report in the literature of a patient with dilated cardiomyopathy whose myocardial PCr/ATP ratio was less than 1.0[7]; this patient died a week later. On this basis it would appear reasonable to suggest that myocardial PCr/ATP ratios less than 1 are not compatible with life. Although substantial changes in enzyme activity are required to produced PCr/ATP $< 1.0$ in isolation, when combined with hypoxia the situation is different. For example, under hypoxia a reduction in the activity of Compex IV of less than 50% was sufficient to reduce PCr/ATP below this critical threshold, suggesting that a combination of hypoxia and mitochondrial dysfunction is sufficient to cause death.

Our discussion has centered on myocardial PCr/ATP as a dependent variable. However, a reduction in PCr/ATP and concomitant increase in Pi/PCr leads to an increase in free ADP. An increase in free ADP causes diastolic dysfunction and an increase in left ventricular end-diastolic pressure in the working heart,[9] possibly by slowing the rate of cross bridge cycling.[10] Thus a reduction in PCr/ATP may have not only direct prognostic and diagnostic utility, it may be an important driver of cardiac dysfunction in itself. This suggests that the reductions in PCr/ATP that we describe here are clinically important not only because a loss of ATP homeostasis ultimately leads to cell death. Rather it may be that any reduction in myocardial PCr/ATP has a direct impact on cardiac function, *via* impaired relaxation in diastole.

The model used in the present study is relatively simple when compared with some other computer models of heart energetics used to model hypoxia (*e.g.* ref. 42–44). However, increased complexity does not necessarily result in improved realism. Simple models are much easier to understand and verify and contain a smaller number of adjustable, free parameter values. In more complex models, the large number of estimated (yet unknown) parameters can easily be adjusted so that the model mimics a limited set of experimental data with no improvement in predictive ability. Despite not being parameter-fitted to a particular set of data, our model showed excellent qualitative and quantitative agreement with published experimental results (Fig. 5).

Additionally, detailed description is frequently simply unnecessary. For instance, contrary to some other models,[42–44] our model treats the entire hydrogen supply box (TCA cycle, glycolysis and so on) as one 'black box' block and describes it by one simple kinetic equation. However, this is completely sufficient in theoretical studies focused on particular oxidative phosphorylation complexes and their interaction (through cytochrome oxidase) with oxygen. The hydrogen supply block has a low flux control coefficient, and therefore its detailed kinetics are of minor importance in this instance. What was crucial in the present study were the flux control coefficients and entire threshold curves (dependences of flux through the system on complex activities) for particular complexes defined within Metabolic Control Analysis (MCA). Another crucial property relevant for the present studies was the dependence of system variables ($VO_2$, ATP/ADP/$P_i$, $\Delta p$, reduction level of NAD and cytochrome c) on oxygen concentration ($[O_2]$). Our model is the only one that has been tested for these system properties,[31,32,45] and therefore it was perfectly suited for the computer simulations carried out in the present paper. Of course, one should always bear in mind that computer models are at best only rough approximations of reality with a limited range of applicability.

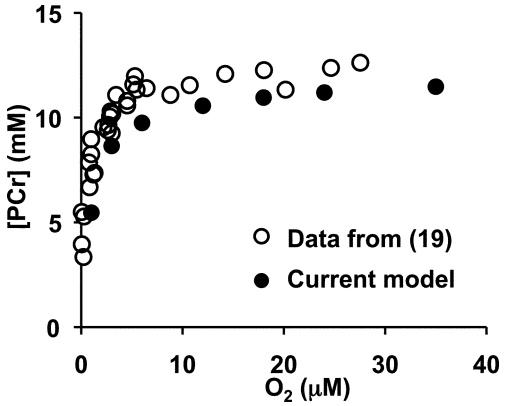


**Fig. 5** The effect of changes in $[O_2]$ on myocellular [PCr]: a comparison between the results of the simulations presented in the present paper and experimental data from Kreutzer and Jue.[19]

## Conclusions

We used a computer model of oxidative phosphorylation, coupled with creatine kinase, to study the effects of hypoxia and mitochondrial dysfunction on myocardial PCr/ATP. In physiological normoxia, all oxidative phosphorylation complexes, NADH supply and proton leak had degrees of control over PCr/ATP of the same order of magnitude. Hypoxia significantly increased this degree of control for elements of the system. In particular, proton leak had a relatively high degree of control over PCr/ATP in hypoxia, supporting earlier hypotheses regarding the importance of myocardial proton leak in modulating the energy status of the ischemic or hypoxic heart. Nevertheless, hypoxia alone seems to be a very efficient reducer of PCr/ATP. Our simulations support three important ideas: First, that mitochondrial abnormalities are able to contribute significantly to (or even, when great enough, be the only reason for) the blunted PCr/ATP observed in heart failure; second, that hypoxia and mitochondrial dysfunction interact in important ways to determine the energy status of the failing heart; and third, that hypoxia alone is able to decrease PCr/ATP considerably.

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