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Integrated Functions of Cardiac Energetics, Mechanics, and Purine Nucleotide Metabolism

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Abstract

Purine nucleotides play central roles in energy metabolism in the heart. Most fundamentally, the free energy of hydrolysis of the adenine nucleotide adenosine triphosphate (ATP) provides the thermodynamic driving force for numerous cellular processes including the actin-myosin crossbridge cycle. Perturbations to ATP supply and/or demand in the myocardium lead to changes in the homeostatic balance between purine nucleotide synthesis, degradation, and salvage, potentially affecting myocardial energetics and, consequently, myocardial mechanics. Indeed, both acute myocardial ischemia and decompensatory remodeling of the myocardium in heart failure are associated with depletion of myocardial adenine nucleotides and with impaired myocardial mechanical function. Yet there remain gaps in the understanding of mechanistic links between adenine nucleotide degradation and contractile dysfunction in heart disease. The scope of this article is to: (1.) review current knowledge of the pathways of purine nucleotide depletion and salvage in acute ischemia and in chronic heart disease; (2.) review hypothesized mechanisms linking myocardial mechanics and energetics with myocardial adenine nucleotide regulation; and (3.) highlight potential targets for treating myocardial metabolic and mechanical dysfunction associated with these pathways. It is hypothesized that an imbalance in the degradation, salvage, and synthesis of adenine nucleotides leads to a net loss of adenine nucleotides in both acute ischemia and under chronic high-demand conditions associated with the development of heart failure. This reduction in adenine nucleotide levels results in reduced myocardial ATP and increased myocardial inorganic phosphate. Both of these changes have the potential to directly impact tension development and mechanical work at the cellular level.

Introduction

Overview of primary roles of purine nucleotides in myocardial energy metabolism

Purine compounds are centrally involved in cellular energy metabolism (Figure 1). The primary currency of cellular energy metabolism, adenosine triphosphate (ATP), is made up of an adenine base, ribose sugar, and a triphosphate group. Numerous critical cellular

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processes are made thermodynamically favorable by coupling of the processes to the hydrolysis of ATP, to form adenosine diphosphate (ADP) and inorganic phosphate (Pi). For example, in the cardiomyocyte, hydrolysis of ATP drives the actin-myosin crossbridge cycle underlying myocardial tension development and mechanical work. Similarly, ATP hydrolysis represents the thermochemical driving force for ion homeostasis, biosynthetic processes, and cellular signaling. In the heart, synthesis of ATP from its hydrolysis products is primarily accomplished via mitochondrial oxidative phosphorylation. Through continuous hydrolysis and synthesis, the human heart resynthesizes its entire ATP pool several times per minute under basal conditions [226]. ADP may be further hydrolyzed to adenosine monophosphate (AMP), which is maintained in the myocardium at concentrations on the order of 1 μM—orders of magnitude lower than that of ATP or ADP [28]. As illustrated in Figure 1, AMP may be converted to several downstream products, adenosine, inosine monophosphate (IMP) and hypoxanthine, and resynthesized via several salvage pathways from these metabolites. In addition, purines are synthesized *de novo* from pentose sugars supplied via the pentose phosphate branch of glycolysis. Under steady state conditions fluxes through the pathways of purine nucleotide synthesis, degradation, and salvage, illustrated in Figure 1, are maintained in balance.

Physiological and pathophysiological perturbations to this balance can result in changes to the concentrations of metabolites in these pathways. During exercise in skeletal muscle, acute increases in AMP can cause a temporary shift of the adenine phosphate nucleotides ATP, ADP, and AMP into IMP. This temporary shift is thought to facilitate the maintenance of cellular ATP hydrolysis potential in exercise. During recovery the adenine nucleotide pool is replenished via the reactions of the purine nucleotide cycle (Figure 1, reactions 2, 3, and 4). The potential role of the purine nucleotide cycle is less well studied in the myocardium than in skeletal muscle. In myocardial ischemia, oxygen-dependent ATP supply (oxidative phosphorylation) is pathologically impaired, resulting in an acute imbalance of supply and demand in the working heart. This imbalance leads to drastic degradation and depletion of adenine nucleotides in the myocardium during acute ischemia. Chronic shifts in the balance between synthesis, degradation, and salvage can also result in chronic pathological reductions in the adenine nucleotide pool. Chronic degradation and depletion of the adenine nucleotide pool is associated with energetic and mechanical dysfunction in the diseased heart. Mechanisms governing depletion of adenine nucleotides in heart disease, and mechanisms linking adenine nucleotide balance, cellular energetics, and myocardial mechanics have been hypothesized and are reviewed herein.

The purine metabolites involved in these pathways are also involved in signaling pathways within the cardiomyocyte and the myocardium. Chronic or acute increases in intracellular AMP and ADP allosterically activates AMP-activated-protein-kinase (AMPK) [118, 151], which activates mitochondrial metabolism and the uptake of glucose and fatty acids [118, 151]. Adenosine, an intermediate of the AMP degradation pathway permeates out of the cardiomyocyte and targets the A_1 and A_2 adenosine receptors, both of which modulate adenylyl cyclase [94]. Adenosine binding to the A_2 receptor expressed on vascular smooth muscle cells leads to increases in cyclic AMP, which causes smooth muscle relaxation [94]. Thus, pathological degradation of adenine nucleotides in ischemia/hypoxia can lead to the release of a vasodilatory signal to increase blood flow and oxygen delivery.

Purine nucleotide cycle

The purine nucleotide cycle (PNC) consists of three reactions converting AMP to IMP, IMP to adenylosuccinate, and adenylosuccinate back to AMP (reactions 2–4 in Figure 1). In the first step of the cycle, AMP is deaminated by AMP deaminase (AMPD) into IMP, releasing NH_3. IMP and aspartate are converted into adenylosuccinate via the adenylsuccinate synthetase reaction, a reaction that requires hydrolysis of GTP to be thermodynamically favorable under cellular conditions. The final reaction of the cycle is catalyzed by adenylosuccinate lyase, which converts adenylosuccinate into AMP and fumarate.

Adenine degradation pathways

AMP is degraded either by conversion to IMP via AMP deaminase (reaction 2 in Figure 1) or by dephosphorylation to adenosine via cytosolic 5’-nucleotidases. Both of these degradation pathways converge in the production of inosine, which is produced from IMP by 5’-nucleotidases, and from adenosine by adenosine deaminase. Further degradation of inosine ultimately yields the waste product uric acid. (Guanine monophosphate (GMP) can also enter this pathway via GMP reductase, not shown in Figure 1). The 5’-nucleotidase reactions remove the phosphate group from purine monophosphates while subsequent degradation steps remove the ribose sugar group to yield hypoxanthine and oxygenate the purine group (reaction catalyzed by xanthine oxidase) to yield xanthine and ultimately uric acid.

Adenine salvage pathways

Both AMP and GMP may be synthesized via two main purine salvage routes, via which AMP and GMP are resynthesized from intermediates of the degradation pathway. Both hypoxanthine and guanine are recycled by the enzyme hypoxanthine-guanine phosphoribosyltransferase (HGRPT) into IMP and GMP, respectively. (GMP- and guanine-linked reactions are not illustrated in Figure 1.) Both of the reactions catalyzed by HGRPT use 5-phosphoriboyl-1-pyrophosphate (PRPP), a product of the pentose phosphate pathway, as a sugar phosphate donor. Adenine nucleotide is synthesized from IMP via the purine nucleotide cycle. Adenosine is phosphorylated to AMP via adenosine kinase, a step requiring the transfer of a phosphate group from ATP to ADP. AMP can also be produced from adenosine by a two-step reaction, involving the removal of the pentose sugar to yield adenine, and the addition of a pentose sugar phosphate group from PRPP (reactions 13 & 14 in Figure 1). Thus, these salvage pathways require either the transfer of a phosphate from ATP to adenosine or the addition of a pentose phosphate sugar from PRPP to adenine or hypoxanthine.

De novo synthesis of adenine nucleotides

In *de novo* purine biosynthesis, pentose phosphate sugars are derived from the pentose phosphate offshoot of glycolysis, and the purine ring is synthesized with nitrogen contributed from amino acids. The sequence of eleven reactions, from PRPP to IMP (reactions 21–31 in Figure 1) requires the hydrolysis of five ATP to ADP, and is thus more energetically demanding than the salvage pathways.

Purine *de novo* synthesis is regulated by feedback inhibition at multiple steps in the pathway. The *de novo* synthesis pathway is upregulated in conditions associated with chronic depletion of adenine nucleotides, such as myocardial hypertrophy [236]. The metabolites AMP, ADP, GMP, guanine diphosphate (GDP), and IMP all act as allosteric regulators of the enzymes in the synthesis pathway [162]. Both ADP and GDP inhibit PRPP formation by PRPP synthase (reactions 16 & 20 in Figure 1) by binding to an allosteric site on the enzyme [162]. Thus, production of PRPP, a crucial precursor for purine synthesis, is inhibited when purine levels, as reflected in ADP or GDP concentrations, are relatively high. The first step of *de novo* synthesis, glutamine-PRPP amidotransferase (reaction 21 Figure 1) is inhibited at high concentrations of AMP, GMP, and IMP [162]. The formation of adenine nucleotide from IMP is facilitated by the PNC reactions adenylosuccinate synthetase and adenylosuccinate lyase. Adenylosuccinate synthetase is allosterically inhibited by AMP and GDP [98, 162].

Like AMP, 5-Aminoimidazole-4-carboxamide ribonucleotide (AICAR), an intermediate of the *de novo* synthesis pathway, can also activate AMPK [118]. Thus, activation of AMPK by AICAR may be an important alternate route to activate AMPK in conditions of depleted adenine nucleotides in which the *de novo* synthesis pathway is upregulated.

An et al. showed that six enzymes in the *de novo* synthesis pathway combine to form a *purinosome*, a multi-enzyme complex [7]. The three enzymes that form the core of the purinosome are PRPP amidotransferase, trifunctional purine biosynthetic protein adenosine-3, and N-formylglycinamidine ribonucleotide. The outer enzymes are adenylosuccinate lyase, multifunctional protein ADE2, and the bifunctional purine biosynthesis protein PURH. Adenylsuccinate synthetase (ADSS) is a component of this purinosome complex, suggesting that the complex specifically serves to synthesize adenine nucleotides via the *de novo* purine synthesis pathway. Furthermore, French et al. observed that in HeLa cells *purinosomes* are colocalized with the mitochondria, suggesting a direct link between adenine nucleotide synthesis and mitochondrial energetics [70].

Basal myocardial purine nucleotide de novo synthesis, salvage, and degradation rates

Under basal conditions adenine nucleotide degradation, synthesis, and salvage processes are in continuous and balanced operation. One way to quantify flux through each pathway is to use isotopic labeling of the metabolites utilized in these pathways.

Zimmer et al. examined *de novo* purine synthesis in the isolated perfused rat heart as well as in the rat heart *in situ* [235]. They provided ^{14}C-labeled glycine, a metabolite used in step 2 of *de novo* synthesis, in the *ex vivo* perfusate or injected into the jugular vein *in vivo*, to determine the rate of incorporation into adenine nucleotides. A faster rate of synthesis of 0.14 nmol · min^{-1} · g dry weight tissue^{-1} was found *in vivo* compared with a rate of 0.020 nmol · min^{-1} · g dry weight tissue^{-1} observed in the isolated perfused heart [235]. Dow et al. measured the rate of ^{14}C-glycine uptake to estimate the rate of purine *de novo* synthesis rate in mature rat cardiomyocytes and reported a rate 0.049 nmol · min^{-1} · g wet weight tissue^{-1}, which corresponds to 0.20 nmol · min^{-1} · g dry weight tissue^{-1} [55]. Thus, estimated rates of purine *de novo* synthesis range from 0.005 to 0.049 nmol · min^{-1} · g wet weight tissue^{-1}, or 0.020 to 0.20 nmol · min^{-1} · g dry weight tissue^{-1}, in the heart [55, 199, 235].

Namm et al. introduced labeled adenosine, adenine, inosine, and hypoxanthine at various concentrations into the perfusate in an isolated heart preparation to estimate salvage rates *ex vivo* [158]. At perfusate concentrations of 0.050 μM, all the species were taken up to synthesize purine nucleotides at approximately equal rates of ~0.050 nmol · min^{-1} · g dry weight $tissue^{-1}$ [158]. When concentrations were increased, it was found that the heart preferentially takes up adenosine compared with adenine, inosine, and hypoxanthine. This preference is apparent when adenosine was incorporated at a rate of 0.50 nmol · min^{-1} · g dry weight $tissue^{-1}$ while inosine and hypoxanthine were taken up at rates of around 0.14 nmol · min^{-1} · g dry weight $tissue^{-1}$ when these substrates were supplied at concentrations of 1.0 μM [158]. Rates of inosine and hypoxanthine incorporation attain values of approximately 0.63 nmol · min^{-1} · g dry weight $tissue^{-1}$ when supplied at concentrations of 5.0 μM [158]. A similar incorporation rate of 0.45 nmol · min^{-1} · g dry weight $tissue^{-1}$ was reported for isolated rat hearts perfused with 20 μM ^{14}C-labeled hypoxanthine or inosine [87]. Reibel and Rovetto reported that a peak rate of adenosine salvage in the isolated rat heart of 45 nmol · min^{-1} · g dry weight $tissue^{-1}$ could be achieved with adenosine perfusate concentrations of 50 μM [189]. A myocardial preference for uptake and salvage of adenosine versus hypoxanthine and inosine into adenine nucleotides has also been observed in cultured cardiomyocytes [237].

Thus, overall, the estimated salvage rates range from 0.050 to 0.63 nmol · min^{-1} · g dry weight $tissue^{-1}$ with physiologically reasonable concentrations of substrates, while the maximum estimated rate of *de novo* synthesis *in vivo* is approximately 0.20 nmol · min^{-1} · g dry weight $tissue^{-1}$. The net purine release (including adenosine, inosine, hypoxanthine, xanthine, and uric acid) from the non-working perfused isolated rat heart is reported to be approximately 20 nmol · min^{-1} · g dry weight $tissue^{-1}$ [87]—a value markedly higher than the estimated maximal *de novo* synthesis and salvage rates combined. This drastic discrepancy is likely a reflection of regional hypoxia in the isolated buffer perfused heart [23, 196].

Estimated maximal reaction rates (estimated V_{max} values under saturating substrate conditions) for the enzymes of the degradation, PNC, and salvage pathways in rat cardiomyocytes are listed in Table 1. Estimated maximal reaction rates are obtained from Brown et al. and Bowditch et al. [32, 33] while assuming 30 mg of wet tissue weight is equal to 10^6 cardiomyocytes [54]. The reported V_{max} values exceed the estimated basal rates of *de novo* synthesis and salvage, indicating a substantial capacity to increase flux beyond the basal rates in these pathways. The maximal flux from adenylosuccinate to AMP (Figure 1, reaction 4), the final step in generating adenine nucleotide either through the PNC or through *de novo* purine synthesis is estimated to be 2.0 nmol · min^{-1} · g dry weight $tissue^{-1}$. This value exceeds the rate of salvage observed when degradation products are supplied at physiologically reasonable concentrations of 5.0 μM and less. Yet under certain circumstances the overall salvage rate may be limited by the V_{max} of adenylosuccinate synthetase [33].

Adenine Nucleotides and Myocardial Energetics—Theoretical Basis

ATP supply and demand in the working heart

Hydrolysis of ATP provides chemical free energy to drive the molecular processes underlying cardiac pumping and cellular homeostasis (Figure 1). Phosphorylation of ADP to resynthesize ATP is, in turn, driven by the oxidation of a variety of primary carbon substrates (i.e., fats and carbohydrates) in cardiomyocytes. Processes such as cross-bridge cycling require that ATP be synthesized (and ADP and inorganic phosphate (Pi) consumed) at sufficient concentrations such that normal functions are not kinetically or thermodynamically impaired. Under basal working conditions the concentration of ATP in the cytosol of ventricular myocytes is approximately 8.0 mM [16, 226]. That is, specifically 8.0 millimoles of ATP per liter of cytosolic water. At a basal level of ATP demand, with matched ATP hydrolysis and synthesis rates of approximately 0.50 millimole of ATP per liter cytosol water per second (0.50 mM $\cdot$ sec^{-1}), the cytosolic ATP/ADP ratio is approximately 100, with [ADP] $\approx$ 80 μM [226]. The other hydrolysis product, Pi is estimated to be present at a concentration of approximately [Pi] = 0.50 mM at basal work rates in the myocardium of large mammals [75]. During maximal exercise, when the rate of ATP hydrolysis exceeds 1.0 mM $\cdot$ sec^{-1}, the concentration of Pi is estimated to increase several fold to 2.0 mM or more, while myocardial ATP/ADP ratio and ADP concentration remain relatively constant [75]. With these phosphate metabolite concentrations, the free energy at which ATP is synthesized by mitochondria and delivered to the cytosol, calculated as

$$\Delta_r G_{ATP} = \Delta_r G_r^0 + R\mathrm{T}\ln\frac{[\mathrm{ADP}][\mathrm{Pi}]}{[\mathrm{ATP}]} = -35\ \mathrm{kJ\ mol}^{-1} + R\mathrm{T}\ln\frac{[\mathrm{ADP}][\mathrm{Pi}]}{[\mathrm{ATP}]},$$

is estimated to decrease in magnitude from approximately −64 kJ $\cdot$ mol^{-1} under basal conditions to −58 kJ $\cdot$ mol^{-1} in exercise conditions.

Respiratory control in striated muscle

Respiratory control is the process by which the rate of oxygen-dependent ATP synthesis (oxidative phosphorylation) is regulated. Synthesis of ATP via mitochondrial oxidative phosphorylation is regulated via feedback of ATP hydrolysis products, which serve as substrates for ATP synthesis [19–21, 195, 221, 226]. This feedback regulation can be quantified in terms of the dependence of oxidative ATP synthesis rate on the concentrations of the substrates ADP and Pi. The dependence of the ATP synthesis rate on substrate concentrations is governed by the constants, K_{ADP} and K_{Pi}, the half-saturation values for ADP and Pi. These values are observed to be approximately K_{ADP} = 30 μM and K_{Pi} = 1.0 mM from *in vitro* experiments using suspensions of purified mitochondria [19]. These half-saturation values quantify apparent Michaelis-Menten dependences of the rate of ATP synthesis on substrate concentrations. Specifically, under a condition where inorganic phosphate concentration is not limiting, and physiological carbon substrates and oxygen are in abundance, mitochondrial ATP synthesis occurs at half its maximal rate when [ADP] is approximately equal to the K_{ADP} value of 30 μM. Similarly, when ADP is not limiting, ATP

synthesis occurs at half its maximal rate when [Pi] is approximately equal to the K_{Pi} value of 1.0 mM.

Based on these dependencies a simple phenomenological description of respiratory control *in vivo* may be constructed:

$$\frac{V}{V_{max}} = \frac{1}{\left(1+\frac{30\ \mu M}{[ADP]}\right)\left(1+\frac{1\ mM}{[Pi]}\right)} = \frac{1}{1+\frac{30\ \mu M}{[ADP]}+\frac{1\ mM}{[Pi]}+\frac{(30\ \mu M)(1\ mM)}{[ADP][Pi]}} \quad (1)$$

where V is the rate of ATP synthesis and V_{max} is the maximal rate of ATP synthesis. This expression adopts different reduced forms in skeletal muscle versus myocardium. In skeletal muscle the total phosphate pool is relatively large, resulting in high levels of Pi, ranging from a few millimolar under resting conditions, to tens of millimolar in exercise [128, 210]. Under conditions where [Pi] >> 1.0 mM in skeletal muscle, Equation (1) reduces to

$$\frac{V}{V_{max}} \approx \frac{1}{1+\frac{30\ \mu M}{[ADP]}} \quad (2)$$

and respiration is primarily controlled by [ADP]. This theoretical model was first shown to effectively explain respiratory control *in vivo* in human skeletal muscle in the 1980's [40]. During exercise, increases in ATP hydrolysis lead to increases in [ADP], which acts to increase oxidative ATP synthesis rate. Although the primary controller of oxidative phosphorylation in skeletal muscle is understood to be [ADP], subsequent studies have demonstrated that the simple mathematical model of respiratory control of Equation (2) fails to match data on the relationship between oxidative ATP synthesis rate and [ADP] at the lowest levels of metabolic demand [110], when V_{max} may be less than 1%. Various modifications to the model have been proposed to account for function at varying demand levels and in different muscle types [44, 72, 110, 128].

In contrast to skeletal muscle, the myocardium operates at much lower levels of Pi and, consequently, much higher levels of ADP. When [ADP] >> 30 μM, valid for cardiac muscle under physiological conditions, Equation (1) reduces to

$$\frac{V}{V_{max}} \approx \frac{1}{1+\frac{1\ mM}{[Pi]}} \quad (3)$$

and respiration is primarily controlled by Pi [20, 226]. This simple relationship between respiratory rate and Pi level and the underlying feedback-mediated mechanism, has been shown to be valid *in vitro* and in animal studies [19, 21, 226, 227]. Specifically, in animal studies employing invasive instrumentation for *in vivo* ^{31}phosphorous magnetic resonance spectroscopy (^{31}P-MRS), myocardial Pi levels have been observed to rise above the limit of

detection of approximately 1.0 mM when myocardial work is stimulated with pacing and/or catecholamine administration [226]. In those studies, the theoretical predicted relationship between Pi and respiratory control *in vivo* is observed. In a human study using the latest 7-Tesla technology for non-invasive myocardial ^{31}P-MRS, the variability and uncertainty of *in vivo* myocardial phosphate measurements substantially exceed the magnitude of predicted values of myocardial Pi concentration and of differences in phosphate concentrations at rest versus physiological stress conditions [10].

Regardless of the lack of precise quantitative data from the human myocardium to test this model in humans [16], the simple expression of Equation (3), and more generally that of Equation (1), represents an effective working description of respiratory control in the myocardium *in vivo*, validated in numerous *in vivo* animal studies. Moreover, it gives a theoretical framework to interpret how changes in purine nucleotide levels associated with acute and chronic disease affect respiratory control.

Effects of adenine nucleotide and phosphate metabolite pools on cardiac energetics and respiratory control in vivo

In diseased states the relationships between cardiac work rate and concentrations of phosphate metabolites—including ADP and Pi—is altered compared to normal. The creatine phosphate (CrP)/ATP ratio is diminished compared to normal in patients with aortic valve disease [45] and ATP concentration in myocardium is lower in dilated cardiomyopathy heart failure patients than in healthy subjects [202]. Although heart failure is a complex syndrome which can arise due to a variety of pathophysiological abnormalities, alterations to the myocardial energetics state (e.g., ATP hydrolysis potential, CrP/creatine ratio) are well established hallmarks of heart failure irrespective of etiology [25, 86, 165].

Wu et al. [227] showed that a primary driver of these observed changes in energetic state of the myocardium in a canine model of heart failure is the depletion of cytosolic pools of adenine nucleotides, total phosphate, and total creatine that occurs with the progression of decompensatory myocardial remodeling. Analyzing the effects of these metabolite pool levels on respiratory control *in vivo*, Wu et al. showed that observed reductions in myocardial creatine phosphate to ATP ratio (CrP/ATP) and increases in Pi in the failing myocardium can be explained by the respiratory control feedback system operating in the context of altered metabolite pools. Although the analysis of Wu et al. used a detailed computer model describing mitochondrial substrate oxidation and oxidative phosphorylation, the phenomenon of increasing Pi with decreasing adenine nucleotide pools can be understood in terms of the simple model of Equation (1): a reduction in total adenine nucleotide (TAN) levels results in reduction of both [ATP] and [ADP]. At a given level of ATP demand, reductions in [ADP] require increases in [Pi] to match supply to demand. Thus, changes to the adenine nucleotide levels in the myocardium act through the mechanism of respiratory feedback control to effect changes in the myocardial energetic status. Namely, a reduction in TAN level brings about, not just reductions in ATP and ADP, but also compensatory increases in Pi. This theoretical prediction that reductions in the TAN pool in heart failure will result in elevated [Pi] has been validated by *in vivo* observations on hypertrophic cardiomyopathy patients [215].

Predictions associated with the simple control model of Equation (1) are shown in Figure 2 to illustrate how a reduction in the TAN pool leads to an increase in myocardial [Pi]. Here we invoke a set of well validated assumptions: (1.): Cytoplasmic [ATP] is set to 7.5 mM and remains constant at all work levels, (2.): Cytoplasmic [ADP] is much lower than [ATP] or [CrP], and (3.): Cytoplasmic [ADP] can be calculated on the basis of creatine kinase equilibrium. As a result of assumptions 1 and 2, the sum [Pi] + [CrP] remains effectively constant across different workloads. Setting resting [CrP] = 15 mM (to give CrP/ATP = 2), and assuming that [Pi] is approximately 0.50 mM at the basal work state, we have the relationship [Pi] + [ATP] + [CrP] = 23 mM. Thus, as [Pi] varies, [CrP] can be calculated [CrP] = 15.5 – [Pi]. Putting these assumptions together we have a simple model for the steady-state relationships between myocardial [Pi], [ADP], and V/V_{max}:

$$[\mathrm{CrP}] = 15.5\ \mathrm{mM} - [\mathrm{Pi}]$$
$$[\mathrm{ADP}] = \frac{[\mathrm{ATP}]([\mathrm{CrP}] - \mathrm{Cr}_0)}{K_{CK}[\mathrm{CrP}]} \quad (4)$$

where $\mathrm{Cr}_0 = 35$ mM is the total creatine concentration and $K_{Ck} = 134$ is the apparent equilibrium constant for creatine kinase. Computing [CrP] and [ADP] as functions of [Pi] using Equation (4) and calculating V/V_{max} as a function of [ADP] and [Pi], we obtain the relationships plotted in Figure 2 for the normal case (black curves). The ratio V/V_{max} is plotted in Figure 2 over the range of 0.16 to 0.56, corresponding to a 3.5-fold range in ATP demand. (Note that the parameter V_{max} in this analysis corresponds to the maximal mitochondrial ATP synthesis rate, which is stimulated *in vitro* by ADP and Pi concentrations that are supraphysiological. Thus V/V_{max}does not reach the value of 1 *in vivo*.)

The open circles in Figure 2 represent mean values from *in vivo* ^{31}P spectroscopy measurements from canine myocardium from Zhang et al. [15, 80, 170, 232, 233]. In these studies the signal from [Pi] is not detected until it exceeds a value of approximately 10% of the basal CrP level, or approximately 1.5 mM. While there is a high degree of uncertainty in the data, both the simple mathematical analysis and data indicate that [Pi] increases from submillimolar levels under basal conditions to approximately 2–3 mM at high work conditions.

Mathematical predictions associated with a 50% reduction in the TAN pool are indicated by the red curves in Figure 2. As described above, a reduction in TAN level leads to a reduction in [ADP], which is associated with an increase in myocardial [Pi] needed to maintain respiratory control. Thus, as TAN levels drop in disease, [ADP] becomes more limiting than in the normal physiological condition, while [Pi] concentrations are higher and less limiting. As a result, respiratory control with reduced TAN is shifted from being mediated primarily by Pi to being mediated more by ADP than it is under normal healthy levels of TAN.

The theoretical framework of Wu et al. has also been used to explore how the changes in metabolic pools that occur with aging in humans impact myocardial metabolism and cardiac energetics. Gao et al. [75] parameterized an age-structured population model of myocardial energy metabolism based on *in vivo* resting-state ^{31}P-MR spectroscopy and

MRI data on female subjects from Köstler et al. [125] and Jakovljevic et al. [105]. The identified model was used to predict the relationship between phosphate metabolite levels and myocardial work rate as a function of age in the population. In addition, based on the assumption that the oxygen cost of stroke work is constant, the model was used to predict maximum left ventricular cardiac power output. Their analysis predicts that from age 20 to 80: (1.) changes in metabolite pools that occur with aging impair the myocardial capacity to synthesize ATP at physiological free energy levels; and (2.) resulting changes to myocardial energetics contribute to reductions in maximal left ventricular power output with aging. Furthermore, this analysis suggests that depletion of adenine nucleotide pools may affect myocardial energetics and mechanical-energetic coupling in normal aging in a way similar to that associated with heart failure.

Effects of adenine nucleotide and phosphate metabolite pools on mechanical power generation

Several studies have shown that impaired energetic state (delineated by a decreased CrP/ATP ratio assessed *in vivo* by ^{31}P MRS) is associated with dilated myopathy and mortality [31, 49]. These observations beg the central question: Do the metabolic changes associated with heart failure affect the kinetic function of the cross-bridge in a way that causes cellular contractile function impairment and, therefore, impacts whole-heart pump function?

Tension development and mechanical work in striated muscle are driven by ATP-, ADP- and Pi-dependent interactions between actin and myosin filaments in the sarcomere. Specifically, the cross-bridge cycle of attachment of myosin heads to actin filaments, generation of contractile force, and unattachment to complete the cycle involves binding and hydrolysis of ATP and unbinding of hydrolysis products ADP and Pi. It is not unexpected that tension development and power output are influenced by the concentrations of these metabolites. In permeabilized muscle fiber preparations, where concentrations of ATP, ADP, and Pi are experimentally controlled, the rate of tension development and maximal sliding velocities are lowered when ATP concentration is reduced to subphysiological levels [46]. Similarly, developed tension in cardiac muscle decreases when inorganic phosphate concentration is increased from zero to a few millimolar. Recent observations reveal that while the maximal force and velocity are sensitive to changes in ATP concentration only in the submillimolar concentration range, muscle power generation, which occurs at submaximal velocity and force, is sensitive to changes in ATP concentration in range of 5–10 mM [22]. Thus, in *in vitro* permeabilized muscle preparations the changes in ATP and Pi concentrations that are associated with heart failure are observed to reduce tension development and power output. Computer models that integrate the effects of metabolite levels on muscle dynamics have been developed to simulate and analyze how *in vivo* myocardial energetics-mechanics coupling may be altered in disease compared to healthy control metabolic conditions [139, 148, 211, 212].

Responses in Adenine Nucleotides to Acute Changes in Metabolic Supply and Demand

Acute perturbations to either metabolic ATP supply or cellular ATP demand in the myocardium necessarily result in acute changes in levels in the metabolites in the pathways illustrated in Figure 1. When demand in the myocardium is increased, ADP levels increase moderately (Figure 2). However, because AMP concentration, governed by the adenylate kinase reaction (reaction 1 in Figure 1), varies with the square of the ADP concentrations, a moderate increase in ADP can result in a more substantial increase in AMP. Specifically, the 30–40% increase in myocardial ADP concentration that is predicted to be associated with a transition from rest to exercise may be associated with a doubling of myocardial AMP concentration, potentially leading to an increase in purine degradation through pathways downstream of AMP production, as well as an increase in AMPK-mediated signaling. In addition, AMP allosterically stimulates numerous enzymes, including those of glycolysis.

In a situation, such as acute ischemia, where oxidative ATP production is severely limited, the high metabolic demand of the myocardium leads to a rapid depletion of ATP, and production of ADP, AMP, and other downstream purine degradation products, leading to a net depletion of adenine nucleotides. However, details underlying the processes that drive depletion are lacking. The relative contributions of different pathways of degradation and depletion during ischemia/hypoxia and during reperfusion, and the mechanisms governing these pathways, are not clearly established. Moreover, the purine nucleotide cycle (PNC), a potential route of nucleotide salvage following ischemia and reperfusion, produces intermediates of the tricarboxylic acid (TCA) cycle.

As such, the metabolic pathways of the TCA cycle, glycolysis and the pentose phosphate pathway, and those of purine degradation and salvage all interoperate in a way that is governed by the balance (or potential imbalance) between ATP supply and demand.

Regulation of energy metabolism by adenine nucleotides

In transitioning from basal to a high-work exercise state in human myocardium, ATP demand increases by a factor of roughly three [74]. This three-fold range of ATP hydrolysis and supply is reflected in a three-fold range of myocardial oxygen consumption and myocardial perfusion [163]. While adenine nucleotides, specifically ATP, are the primary source of chemical energy used to drive processes such as mechanical power generation and ion homeostasis, adenine nucleotides also serve as major signaling molecules governing metabolic transitions between different demand levels [12, 13, 76]. Concentrations of ATP, ADP, and AMP have regulatory effects on enzymes in central metabolic pathways including glycolysis and the TCA cycle. ATP and ADP are obvious candidates for regulatory molecules due to their direct use or production in many enzymatic reactions. However, as described above, both ATP and ADP are held at relatively stable concentrations across the physiological range of ATP demand in the myocardium. Thus, AMP is expected to play a more important regulatory role than ATP or ADP under physiological conditions.

The concentration of AMP in the myocardium is governed primarily by the adenylate kinase reaction:

$$2\ \mathrm{ADP} \leftrightharpoons \mathrm{ATP} + \mathrm{AMP}$$

(reaction 1 in Figure 1) in which one phosphate is transferred from one ADP to another, yielding one AMP and one ATP. This reaction is maintained near equilibrium under physiological conditions [35, 191]. Therefore, since ATP concentration is maintained at a nearly constant value [AMP] varies approximately with $[ADP]^2$, and relatively small changes in [ADP] result in relatively greater changes in [AMP]. AMP plays numerous roles in allosterically regulating enzymes involved in energy metabolism and in activating AMPK. The multiple roles of AMPK-mediated signaling in striated muscle metabolism are reviewed elsewhere [115]. In addition, AMP represents the entry point for purine nucleotide degradation and depletion pathways as well as the end-product of the salvage pathways illustrated in Figure 1. Thus, via mass action, increases in AMP concentration will lead to increased depletion of the adenine nucleotide pool and, potentially, to changes in myocardial ATP and ADP concentrations.

Examples of the numerous mechanisms via which AMP affects energy metabolism include the effect of AMPK-mediated inhibition of acetyl-CoA (acetyl-Coenzyme A) carboxylase [194], an enzyme that catalyzes the production of malonyl-CoA needed in fatty acid synthesis and storage. Malonyl-CoA also inhibits mitochondrial fatty acid uptake and β-oxidation. Thus, increases in AMP concentration, associated with a high-demand work state, inhibit fatty acid synthesis and storage and stimulate the production of acetyl-CoA to fuel the TCA cycle. Another key metabolic enzyme regulated by AMP is phosphofructokinase (PFK), a critical branching point for the use of glucose in either glycolysis or fatty acid synthesis. PFK is activated by AMP, potentially facilitating an up-regulation of glycolysis and glycolytic production of pyruvate in a high-demand state compared with basal state [12].

Function of the purine nucleotide cycle during changes in ATP supply and demand

The kinetics and function of the purine nucleotide cycle (PNC) are better understood in skeletal muscle than they are in the myocardium. However, investigation of the operation of the PNC in skeletal muscle may give insights into how this system may operate in the heart. Lowenstein hypothesized three main roles of the PNC in muscle energetics: (1.) regulating relative levels of ATP, ADP, and AMP during changes in work state; (2.) driving anaplerotic entry of malate derived from amino acids into the TCA cycle; and (3.) regulation of glycolysis via modulation of AMP concentration [141]. The first mechanism hypothesized by Lowenstein acts through the combination of the adenylate kinase and AMP deaminase (AMPD) reactions (Figure 3, reactions 1 and 2). During exercise, when ADP concentrations increase—particularly in skeletal muscle—consumption of AMP by AMPD helps provide the driving force for the adenylate kinase reaction to generate ATP, consume ADP, and maintain a relatively high ATP/ADP concentration ratio. The AMPD reaction also generates ammonia (Figure 3, reaction 2), which, like AMP, stimulates PFK activity, potentially helping to stimulate glycolysis in exercise [2].

Key studies in skeletal muscle energetics have assessed ammonia, inosine monophosphate (IMP), adenylosuccinate, and adenine nucleotide concentrations in muscle extracts and in intact muscle. Tornheim and Lowenstein used a cell extract from rat hindlimb muscle to study the kinetics of the PNC *in vitro* [213]. They observed that under basal conditions, where ATP hydrolysis is minimal, the majority of the adenine nucleotide pool remained in the form ATP, with ADP phosphorylation maintained by the creatine kinase reaction [213]. At physiological levels of ATP hydrolysis potential and with excess aspartate available, IMP added to the system is nearly completely converted to adenine nucleotides—a situation reflecting resting basal conditions. On the other hand, when ATP hydrolysis was stimulated (via the addition of hexokinase and 2-deoxyglucose), leading to adenylosuccinate synthetase inhibition by GDP, the entire adenine nucleotide pool is converted into IMP. This study demonstrates that under conditions that simulate exercise, IMP is readily synthesized from adenine nucleotides in skeletal muscle extracts and AMP, ADP, and ATP can readily be regenerated from IMP at a physiological phosphorylation potential.

Measurements of intermediates of the PNC and related metabolites in exercising intact muscle provide further insights into the operation of this system *in vivo*. Goodman and Lowenstein assayed IMP, adenylosuccinate, AMP, ADP, ATP, creatine phosphate, inosine, and hypoxanthine in rat hindlimb muscle (primarily gastrocnemius and posteroinferior thigh muscle) undergoing isometric contractions [81]. They observed that, as expected, ATP progressively decreased while ADP, AMP, and IMP rose during graded increases in stimulation intensity. The majority of reductions in total adenine nucleotides (TAN) were accounted for in increases in IMP, with smaller contributions from increases in inosine and hypoxanthine concentrations. These observations support the hypothesis that generation of IMP during exercise helps ameliorate the exercise-induced build-up of AMP and ADP, helping to maintain cellular ATP/ADP ratio.

A critical corollary to this hypothesis is that the exercise-associated depletion of adenine nucleotides to form IMP is temporary and that adenine nucleotides are replenished via the PNC during recovery. Indeed, Goodman and Lowenstein found that while the TAN pool was depleted by 20% during 15 minutes of stimulation, after 15 minutes of recovery it returned to 90% of the resting control value [81]. Adenylosuccinate was detectable immediately after exercise and during the first 7.5 minutes of the recovery period. While the majority of the TAN pool lost during stimulation is accounted for in increases in IMP, adenylosuccinate, inosine, and hypoxanthine, approximately 10% was unaccounted for, potentially due to the release of inosine and hypoxanthine from myocytes or further degradation of hypoxanthine to xanthine and uric acid. Thus, it is possible that the PNC alone is not able to fully restore adenine nucleotides depleted during exercise and that the salvage and synthesis pathways contribute as well.

The second role of the PNC hypothesized by Lowenstein is grounded in the fact that the complete turnover of the PNC is associated with the conversion of aspartate into fumarate, a TCA cycle intermediate (Figure 3, reactions 3 and 4). Fumarate generated by the PNC may be converted to malate by cytosolic fumarase, which can then be transported into mitochondria. Via this mechanism, the PNC represents a potential route for anaplerotic entry of substrates derived from amino acids into the TCA cycle. In support of this

hypothesis, rat hindlimb muscle stimulated for 15 minutes, mimicking exercise, showed an increase of over 200% in overall concentration of TCA cycle intermediates compared to the basal condition [11]. During the stimulation period, increases in fumarate and malate concentration precede increases in other intermediates. Furthermore, the addition of hadacidin, an inhibitor of adenylosuccinate synthetase (Figure 3, reaction 3), markedly lowers the rise in fumarate and malate during stimulation [11, 150]. In similar experiments using AICAriboside, which is converted to 5-Aminoimidazole-4-carboxamide (AICAR) in cells, an inhibitor of adenylosuccinate lyase (Figure 3, reaction 4), Flanagan et al. showed that inhibition of the PNC in rat gastrocnemius muscle reduces production of malate, lowers ATP, and results in substantially lower TAN pool levels following stimulation [68]. From these observations it is concluded that inhibition of adenylosuccinate lyase not only inhibits the formation of fumarate, but also inhibits a continuous salvage of adenine nucleotides that are shuttled through the PNC during exercise. Consequently, adenine nucleotides are pushed into degradation pathways. In sum, these observations suggest that the PNC provides an anaplerotic source of TCA cycle intermediates in exercising skeletal muscle.

Compared to the literature on skeletal muscle there have been fewer investigations of the PNC in the myocardium at rest and during exercise. A study using cultured primary rat cardiomyocytes and rat skeletal muscle myotubes assessed purine nucleotide metabolism using isotopically labeled formate, hypoxanthine, adenine, and adenosine [237]. In cell extracts, they found significantly lower rates of AMPD activity in cardiomyocytes when compared to skeletal muscle myotubes. However, cardiomyocyte salvage of hypoxanthine to generate purine nucleotides was still observed at rates similar to those seen in skeletal muscle. Therefore, Zoref-Shani et al. concluded that while the enzymatic activity for the conversion of AMP to IMP is relatively low in cardiomyocytes, the basal rate of turnover of the second and third reactions in the PNC (Figure 3, reactions 3 and 4) may be similar to that of skeletal muscle [237]. On the other hand, Taegtmeyer observed, using an isolated working rat heart preparation, no differences in adenine nucleotide levels, ammonia production, aspartate use, or malate concentration at different work rates [207]. Based on these observations, it may be concluded that the PNC does not turnover at appreciably different rates in resting versus exercising myocardium. However, these observations do not suggest that there is no flux through the PNC in the myocardium or that this flux does not change under any conditions. The PNC could play a critical role in the metabolic response to pathological conditions that cause an imbalance between ATP supply and demand resulting in high cellular concentrations of AMP.

Acute myocardial ischemia and reperfusion

Ischemia occurs in the myocardium when blood flow through coronary arteries is restricted or blocked, and oxygen supply to the working myocardium is impaired. Due to continuous contractile function, a significant reduction in perfusion leads to an acute imbalance between ATP supply and demand because mitochondrial ATP production (oxidative phosphorylation) is substantially diminished under conditions where oxygen is limiting. In a timescale of minutes, ischemic/hypoxic conditions lead to a collapse of myocardial ATP concentration, and a buildup of ATP hydrolysis products ADP and inorganic phosphate (Pi) [216]. Extended periods of ischemia/hypoxia ultimately lead to irreversible tissue damage and

cell death. While skeletal muscle may undergo 4–6 hours of ischemia before showing signs of irreversible damage, irreversible damage to the myocardium is apparent after less than 30 minutes of ischemia [79, 97, 111, 112].

A major pathological metabolic consequence of myocardial ischemia/hypoxia is an acute reduction in the TAN pool. Figure 4 shows data on adenosine phosphate metabolites (ATP, ADP, and AMP) and degradation products (adenosine, inosine, hypoxanthine, and xanthine) in the isolated ischemic rat myocardium (from [218]). Under initial basal conditions the metabolites of purine degradation pathways are maintained at myocardial concentrations that are orders of magnitude smaller than the TAN pool concentration of roughly 10 mM. However, during ischemia when ATP levels drop, degradation intermediates build up to millimolar concentrations. During reperfusion, these intermediate may wash out of the myocardium resulting in net depletion of the adenine pools. Martin et al. compared metabolic changes following cold and warm cardiac ischemia in mice, pig, and human hearts to determine the protective effects of cooling tissues during organ transplantation [146]. During warm ischemia, in all species, the sum of ATP and ADP concentration declined over 50% while AMP concentration increased five-fold in the first 12 minutes. It took four hours to attain equivalent changes during cold ischemia. Figure 5 shows data from Kroll et al. on myocardial phosphocreatine (creatine phosphate (CrP), denoted PCr in the figure) and ATP in the isolated rabbit heart during an initial baseline, 45 minutes of severe underperfusion, and reperfusion [126]. These experiments demonstrate that a substantial proportion of the TAN pool may be degraded during ischemia and washed out of the myocardium, resulting in an incomplete recovery in ATP concentration following reperfusion. Indeed, following an acute bout of ischemia followed by reperfusion, myocardial adenine nucleotides have been shown to remain at lower than physiological concentrations for days after reperfusion [123, 190].

Measuring rates of purine degradation in baseline, ischemic, and reperfused isolated rat hearts, Harmsen et al. observed a six-fold increase during ischemia and a nine-fold increase during reperfusion compared with baseline [87]. Moreover, they found that under basal conditions, the primary metabolite released from the myocardium of the perfused heart is uric acid (64%) with lower proportions of adenosine, inosine, hypoxanthine, and xanthine present in the effluent. Following ischemia, the rates of release of adenosine, inosine, hypoxanthine, and xanthine increase and uric acid no longer made up the majority of purine degradation metabolites in the effluent [146]. Furthermore, there is evidence that an elevated rate of adenine nucleotide *de novo* synthesis is induced by ischemia in isolated rat hearts [235]. However, the observed rate is too slow to counter the loss of adenine nucleotides seen during ischemia and reperfusion. Thus, salvage pathways represent a more important source for replenishment of the TAN pool during acute ischemia-reperfusion.

Kloner et al. carried out *in situ* occlusion of the left anterior descending artery in dogs followed by 3 days of reperfusion [123]. During 15 minutes of ischemia, concentrations of ATP in the myocardium dropped almost 50% while CrP decreased by 86%. After 90 minutes of reperfusion, ATP rose to 67% of nonischemic levels and CrP was completely restored. Yet even after 72 hours of reperfusion, myocardial ATP had still not been fully restored and remained at 78% of the initial nonischemic concentration. Kloner et al. also measured left

ventricular end-diastolic and end-systolic myocyte lengths to assess contractile function in the canine heart during ischemia as well as at 90 minutes and 72 hours of recovery [123]. As expected, active shortening in the ischemic myocardium was markedly reduced. Shortening was restored upon reperfusion to 12% and 58% of preischemic values after 90 minutes and 72 hours of recovery, respectively. While this study did not investigate a direct link between impaired contractile function and depletion of adenine nucleotides, the relationship between these phenomena suggests that reduced adenine nucleotide level is a contributing factor to diminished left ventricular active shortening following ischemia and reperfusion. Furthermore, these results suggest that an incomplete restoration of the TAN pool even after 72 hours of recovery may contribute to the incomplete restoration of contractile function.

In contrast to a coronary occlusion or ligation model in which a region of myocardium that is supplied by a specific branch of the coronary tree is made ischemic, an isolated working heart preparation allows for the exposure of the myocardium to global ischemia and hypoxia. Pasque et al. demonstrated that left ventricular work decreases commensurately with ATP depletion in the isolated working rat heart during ischemia, and that both myocardial ATP concentration and work rate recover incompletely during reperfusion [178]. They also showed that ATP depletion and impairment of contractile function were ameliorated by infusion of D-ribose, hypothesized to elevate myocardial 5-phosphoriboyl-1-pyrophosphate (PRPP) (the sugar phosphate donor used in purine salvage). Similarly, using a working rabbit heart preparation, Kroll et al. measured developed left ventricular pressure during severe underperfusion and observed a severe reduction in contractile function associated with reduced myocardial ATP, pH, and increased Pi [126]. Similar to the observed incomplete recovery in ATP concentration, left ventricular systolic pressure did not recover to baseline values following reperfusion. These studies suggest the loss of adenine nucleotides associated with acute ischemia plays a significant role causing contractile dysfunction after reperfusion.

In fact, it has been proposed that the degree to which ATP and the TAN pool have declined at the time of reperfusion determines the degree of ventricular recovery after reperfusion [97, 188]. This hypothesis is consistent with the theoretical analysis of myocardial energetics presented above. Moreover, a significant correlation has been observed between ATP concentration and mean ventricular power during reperfusion [188]. For example, Reibel and Rovetto demonstrated in the isolated working rat heart that once ATP dropped below 65% of preischemic levels, recovery of aortic output could not be achieved at an afterload of 60 mmHg during reperfusion. In a similar study Humphrey et al. showed that ATP above 1.0 μmol · g tissue^{-1} (32% of normoxic concentrations) was needed to reestablish basal cardiac output following ischemia and reperfusion [97]. Further evidence supporting the dependance of ventricular function on myocardial adenine nucleotide levels comes from experiments that used various strategies to prevent ATP loss or increase rates of salvage in ischemia-reperfusion. Perfusing the isolated rat heart with D-ribose (needed for synthesis of PRPP used in salvage and *de novo* synthesis—Figure 1 reactions 11, 14, and 21) accelerates purine salvage rates as well as heightens functional recovery by 15% after 15 minutes of reperfusion [87, 178]. (In Pasque et al. [178], functional recovery was represented as the percent recovery of minute work (cardiac output × systolic pressure) to preischemic values.) In both the studies of Harmsen et al. and Pasque et al., adenine nucleotide loss was reduced

in hearts supplied with D-ribose compared with untreated hearts [87, 178]. Another way to prevent ATP loss is to decrease its use and inhibit degradation. Isolated rat hearts perfused with low calcium buffer, adenosine, and erythro-9-(2-hydroxy-3-nonly)adenine (EHNA) (an inhibitor of adenosine deaminase—Figure 1, reaction 10) show a 100% recovery of aortic output after 30 minutes of ischemia followed by 60 minutes of reperfusion [97]. Calcium depletion decreases myosin ATPase activation, while adenosine and EHNA inhibit purine degradation. None of these interventions alone were able to restore cardiac function during reperfusion. The combination of the three yielded the best recovery of aortic output.

Despite this experimental evidence and theoretical foundation, the hypothesis that depletion of total adenine nucleotide and ATP concentrations in ischemia drives deterioration of mechanical function in myocardial ischemia and reperfusion has been challenged. Alternate hypotheses have been derived from studies showing correlations between postischemic contractile failure and increases in phosphomonoesters [109], glycolytic products [161], Pi [93, 129], and decreases in CrP [208]. Of course, ischemia-induced changes in concentrations of these compounds are linked to changes in adenine nucleotide levels. Observed increases in Pi, for example, are expected to be a direct consequence of reductions in the TAN pool, as detailed above. Hoerter et al. designed an experiment to acutely reduce myocardial ATP and CrP without causing increases in cellular Pi levels [93]. In these experiments, hearts were perfused with 2-deoxyglucose which is phosphorylated by hexokinase, resulting in significantly lowered Pi concentration. Because the 2-deoxyglucose-6-phosphate is not further processed by glycolysis, the addition of deoxyglucose results in sequestering of cellular stores of phosphate into 2-deoxyglucose-6-phosphate and diminishes ATP and CrP without a rise in Pi. In these experiments, Hoerter et al. demonstrated that normoxic isolated rat hearts were able to sustain 65% of cardiac work output seen before treatment while ATP and CrP were decreased by >90% and 85%, respectively [93]. Thus, they conclude that the rise in Pi is a major driver of mechanical dysfunction during pathological imbalances between ATP supply and demand. The potential link between elevated Pi and mechanical dysfunction is supported by theoretical studies [22, 139, 148, 211, 212], as is the direct link between both chronic and acute depletion of adenine nucleotides and increases in Pi levels, as detailed above.

Yet, regardless of reductions in TAN and ATP levels, other factors are known to occur during ischemia-reperfusion that contribute to cell injury and death and, therefore, influence the contractile function of the myocardium. Reactive oxygen species (ROS) and associated oxidative damage accumulates in the myocardium during reperfusion. Major sources of ROS include xanthine oxidase [61] and mitochondrial complex I [137], both of which are potentially linked to the purine degradation and salvage pathways. The role of xanthine oxidase is most immediately apparent—as this enzyme catalyzes the last two steps in purine degradation (Figure 1, reaction 7 and 8) and exists in two forms: xanthine dehydrogenase and xanthine oxidase. Cells synthesize this enzyme in the dehydrogenase form and it has been hypothesized its converted to the oxidase form via proteolytic cleavage by a calcium-activated protease [61]. Under basal oxygenated conditions the enzyme is maintained predominantly (~90%) in the dehydrogenase form [39, 61]. Under ischemic conditions in the myocardium, the percentage of the enzyme in the oxidase form rises to 30% [39]. Given that during acute ischemia-reperfusion the rate of release and degradation of purines from

the myocardium is an order of magnitude higher than it is during basal conditions, the production of ROS by this pathway is likely elevated during reperfusion.

Complex I mediated generation of ROS during reperfusion is associated with the accumulation of NADH and succinate during ischemia. If reperfusion follows ischemia, the rapid oxidation of these accumulated species in the mitochondria is a source of pathologically elevated ROS production [41]. Since the major route of succinate accumulation during ischemic/hypoxic conditions is the reduction of fumarate by succinate dehydrogenase [41], a source of fumarate is required to sustain accumulation of succinate. One potential source of fumarate during ischemia and reperfusion is the turnover of the PNC. Since the adenylosuccinate synthetase reaction (Figure 3, reaction 3) is driven by hydrolysis of GTP, production of fumarate through the PNC during ischemia may not remain thermodynamically favorable for prolonged periods of ischemia. In addition, if fumarate derived from the PNC during reperfusion provides an anaplerotic supply of mitochondrial malate, this pathway may directly impact mitochondrial redox state and ROS generation by complex I.

Myocardial Adenine Nucleotide Metabolism and Cardiac Mechanics in Chronic Heart Failure

Chronic depletion of adenine nucleotides in the failing myocardium

One of the hallmarks of heart failure, regardless of etiology, is metabolic dysfunction, broadly apparent in altered substrate utilization and diminished ATP concentration. Studies have shown that a key contributor to the failing heart's metabolic profile is a depletion of adenine nucleotide levels in the myocardium of failing hearts compared with that of normal healthy hearts. Specifically, numerous studies have reported reductions in myocardial ATP content in heart failure/cardiomyopathy patients, and correlations between ventricular functional metrics (e.g., ejection fraction and end-diastolic filling pressure) and myocardial ATP content [18, 214]. Starling et al. measured ATP and total adenine nucleotides (TAN) in left- and right-ventricular biopsies obtained from heart failure patients and right-ventricular samples from patients without systolic or diastolic failure [202]. They found a decline in both ATP and TAN pool levels in heart failure compared to healthy donors, as well as a strong negative correlation between myocardial ATP concentration and pulmonary capillary wedge pressure. Using ^{31}P spectroscopy to quantify phosphate metabolites *in vivo*, Okada et al. [171] reported ATP levels in the myocardium in hypertrophic cardiomyopathy are 36–50% lower than in healthy subjects. Beer et al. [25] reported myocardial ATP levels in patients with dilated cardiomyopathy are 35% lower than in healthy subjects. Kostler et al. [125] reported a substantial reduction in ATP levels in the myocardium with aging. Studies of various animal models of cardiac decompensation and failure report depleted adenine nucleotide levels in diseased hearts compared to controls. Progressive loss of both ATP and TAN levels are observed in canine pacing-induced models of cardiac decompensation and failure [153, 198]. Lopez et al. observed similar reductions in myocardial adenine nucleotide levels in a chronic aortic constriction rat model of heart failure compared with the sham control [139].

Metabolomic profiling of myocardium and plasma from heart failure patients reveals patterns of changes in the pathways illustrated in Figure 1 that are broadly associated with depletion of purine synthesis intermediates and increases in degradation products [67, 223]. Broadly, adenine nucleotide degradation products have been found to be increased in plasma in atrial fibrillation [234], rheumatic heart disease [48], ischemic heart disease [64], hypertrophic cardiomyopathy [102], heart failure [154], and following myocardial infarction [133]. Several recent studies on animal models reveal further insights into the broad metabolomic changes in the purine synthesis and degradation pathways associated with heart failure. Metabolomic profiling in the rat transverse aortic constriction model, Lopez et al. [139] found that depletion of the total adenine pool is associated with increases in hypoxanthine, xanthine, adenosine, adenine, and uric acid, and an upregulation of the AMPD3 isoform of AMP deaminase (catalyzing reaction 2 in Figure 1).

In sum, observations on adenine nucleotide metabolites in heart failure reveal increases in degradation and net reductions in adenine nucleotide levels in diseased compared with heathy myocardium. Moreover, in both animal models and patients, the degree of depletion has been shown to be correlated with the degree of mechanical dysfunction [18, 139, 202, 214].

A potential mechanism driving adenine nucleotide depletion in chronic heart disease

Pathological hypertrophic cardiac remodeling and decompensation follows from conditions in which the myocardium operates in a chronically high-demand state: hypertension, aortic stenosis, and valvular insufficiencies all represent pathological conditions that increase myocardial work, and thus increase oxygen and ATP demand. Myocardial ATP and oxygen demand in the dilated heart is elevated due to elevated myocardial work compared with the healthy normal heart. Similarly, certain mutations in myofilament proteins associated with hypertrophic remodeling have been shown to be associated with reductions in contraction efficiency, resulting in a chronic high-demand state [175, 225].

In each of these conditions, myocardial ATP demand is chronically elevated. Analogous to the response to acute supply-demand mismatch such as following ischemia, when ATP demand increases, AMP concentrations increase (Figure 2), shifting the balance between degradation and salvage pathways toward increased degradation. Under conditions of chronically elevated ATP demand, adenine nucleotide levels may be depleted until a new balance between degradation, salvage, and synthesis is achieved in which the TAN level is reduced to a non-physiological level. Thus, it is possible that, while acute ischemia leads to rapid depletion of adenine nucleotides due to a drastic reduction in ATP supply, a chronic increase in ATP demand can yield similar reductions in TAN over much longer time scales. If this hypothetical mechanism contributes to the energetic phenotype of the failing heart, then inhibition of adenine nucleotide degradation and depletion may lead to preservation of the TAN pool in pathological cardiac remodeling and improve myocardial function.

Theoretical link between adenine nucleotide depletion and mechanical dysfunction in heart failure

To test the hypothesis that the myocardial capacity to maintain ATP, ADP, and inorganic phosphate (Pi) at physiological levels determines the maximum capacity of the myocardium to do mechanical work, Gao et al. simulated how the changes in metabolic pools that occur with aging impact myocardial metabolism and cardiac power output [75]. They identified an age-structured population model of myocardial energy metabolism based on *in vivo* resting-state ^{31}P-MR spectroscopy and MRI data on female subjects and used the identified model of to predict maximum left ventricular power output, assuming that the oxygen demand per beat is proportional to stroke work. Model simulations predicted that median maximum left ventricular power output drops from approximately four watts at age 20 to less than three watts at age 80, in agreement with clinical observations [105, 160]. Gao et al. concluded that changes in the myocardial TAN pool that occurs with aging impair the myocardial capacity to maintain ATP and its hydrolysis products ADP and Pi at appropriate concentrations to support contractile function. Furthermore, their results suggest that there are fundamental similarities in the mechanisms impeding myocardial energetics and mechanical-energetic coupling in normal aging and heart failure.

Tewari et al. [211, 212] used simulations of rat myocardial mechanics to explore causal links between metabolic and mechanical function. Their simulations predicted that the changes in metabolite pools observed in hypertrophy and decompensation directly contribute to systolic dysfunction. Lopez et al. [139] assessed the potential causality of the link between cardiac mechanics and depletion of the TAN pool in a pressure-overload model of cardiac hypertrophy, decompensation, and failure in rats, revealing a tight link between depletion of TAN in the myocardium and degradation of systolic function. The potential causality of this association was investigated using a multi-scale model to simulate cellular energetics, muscle mechanics, and whole-heart pumping [139, 148]. Taken together, these experimental and theoretical studies in rodent models predict that (1.) reductions in TAN that occur with heart failure causally contribute to systolic dysfunction, and (2.) restoration of metabolic state will improve mechanical function, suggesting a therapeutic potential.

Cardiovascular Pharmacology and Enzymopathies of Purine Regulation

Pharmacology of the purine nucleotide cycle and purine synthesis, degradation, and salvage pathways

Purine pharmacology is a broad and diverse field with compounds targeting purine metabolism including chemotherapeutics, anti-malarial drugs, herbicides, and vasodilators [14, 119, 136, 156]. A wide range of compounds targeting the degradation, synthesis, and salvage pathways of Figure 1 have been identified using natural substrate substitutions, molecular dynamics, high throughput small molecule screening strategies, and the isolation of various naturally occurring antibiotics [157, 187, 193]. Here, we aim to synthesize a concise summary of the clinical utility of purine pharmacology in human purine metabolism, with a specific focus on potential impacts on cardiovascular function and disease.

Table 2 summarizes the targets of and mechanisms associated with key compounds affecting the pathways shown in Figure 1, along with descriptions of known effects on cardiovascular function. This list was compiled by listing the United States Food and Drug Administration (FDA) approved inhibitors for each target where available. Where no FDA approved inhibitor exists, inhibitors with reported use in humans were prioritized. Molecular inhibitors of *de novo* synthesis span every enzyme in the pathway. While numerous inhibitors exist, only 7 of the 14 *de novo* synthesis inhibitors in Table 1 are FDA approved for use in humans. Broadly speaking, these inhibitors lead to immunosuppression because lymphocytes lack the ability to use purine salvage pathways. As a result, inhibition of *de novo* synthesis causes decreased mitosis rates of activated T and B cells [166]. Salvage inhibitors are less numerous than *de novo* synthesis inhibitors. However, 6-mercaptopurine (6-MP), a competitive inhibitor of hypoxanthine-guanine phosphoribosyltransferase (HGPRT) (Figure 1, reaction 11) that decreases the salvage of guanine and hypoxanthine to GMP and inosine monophosphate (IMP), is widely used. Adenosine kinase inhibitors cause severe nervous system toxicity, with brain microhemorrhage foci occurring in both rats and dogs with numerous adenosine kinase inhibitors- likely because neurons require robust salvage to function [106]. Purine nucleotide cycle inhibitors, aside from 6-MP, are not used in humans. Lastly, purine degradation inhibitors include drugs ranging from toxic chemotherapeutic drugs to medications used to treat gout. The primary mode of action of this class of chemotherapeutics involves the buildup of toxic intermediates of purine degradation, such as deoxyadenosine [82]. Xanthine oxidase (XO) inhibitors are used primarily as anti-gout medications by reducing the conversion of hypoxanthine to uric acid.

Because decreases in the total adenine nucleotide (TAN) pool cause impairment of cardiac contractility, one might expect inhibitors of *de novo* synthesis, salvage, and the purine nucleotide cycle to be detrimental to cardiac function, and inhibitors of degradation to be cardioprotective. However, such associations have generally not been observed. Inhibitors of purine metabolism, aside from XO inhibitors, may induce dose-limiting toxicities to immune cells and the nervous system before they can observably alter cardiovascular metabolism. However, in patients where these drugs are necessary, such as with organ transplantation and cancer treatment, cardiovascular effects have been noted.

For example, a clinical trial evaluated outcomes associated with mycophenolic acid (MMF) versus azathioprine (AZA, an immunosuppressive prodrug of 6-MP) for immunosuppression in heart transplantation [124]. MMF is a selective inhibitor of the *de novo* synthesis of guanine nucleotides, whereas AZA is a nonselective inhibitor, altering rates of *de novo* synthesis, salvage, and the purine nucleotide cycle. Kobashigawa et al. [124] report a 0.66 relative risk of rejection with hemodynamic compromise in the MMF group relative to the AZA group, potentially consistent with the interpretation that inhibition of adenine nucleotide salvage impairs myocardial contractile function. However, while use of AZA in heart transplant leads to worse outcomes, there is evidence that AZA improves outcomes in treating pediatric dilated cardiomyopathies with active myocarditis [36]. Patients treated with AZA in comparison with prednisone, the conventional therapy, saw improvements in many hemodynamic parameters including ejection fraction, cardiac index, left ventricular end-diastolic diameter, and mean pulmonary wedge pressure. These results are interpreted

to be due to enhanced immunosuppressive effects of AZA over prednisone, and not cardiac metabolic differences in the AZA group [36]. Sirolimus, an mTOR (mammalian target of rapamycin) inhibitor used in heart transplantation, has been shown to improve both cardiac allograft vasculopathy and diastolic function compared to other immunosuppressive strategies [6, 183]. However, it is difficult to say whether these effects are related to effects on the one-carbon metabolic pathway that generates 10-formyltetrahydrofolate (N^{10}-Formyl-THF), a precursor for *de novo* synthesis (Figure 1), or through one of the other modes of action attributed to mTOR inhibitors.

The effects of the XO inhibitor allopurinol on cardiovascular physiology and disease have been extensively studied. Allopurinol, while initially designed as a chemotherapeutic, is primarily used for managing gout, and with over three million estimated active US users [1] represents an optimal medication to observe real world differences in cardiovascular outcomes with a purine altering drug. Allopurinol was first found to have cardiovascular benefits in gout patients before more recently being used in cardiovascular trials with non-hyperuricemic patients. Gout patients receiving allopurinol have been shown to have a reduced relative risk of myocardial infarction (0.47 relative risk compared to patients not receiving an XO inhibitor) by a recent meta-analysis [219]. In cardiovascular patients (with and without gout), allopurinol has been shown to have beneficial effects, although not in overall morbidity or mortality [142, 168, 184]. Cappola et al. [37] observed that allopurinol improves myocardial efficiency ($\Delta P/\Delta t_{max}/M\dot{V}o2$ and $SW/M\dot{V}o2$) in patients with idiopathic dilated cardiomyopathy. Another study, looking at patients with documented coronary artery disease and effort-induced angina, showed significant increases in exercise time prior to ST depression (an electrocardiographic marker of insufficient myocardial ATP supply [38]) with allopurinol vs placebo [168]. And, while not statistically significant, allopurinol has been shown in percutaneous coronary intervention (PCI) to lower levels of both creatine kinase-MB (CKMB) and troponin T compared to placebo [4]. One human study aimed at elucidating the mechanism of allopurinol's cardiovascular benefit showed that allopurinol reduces vascular tissue oxidative stress and improves endothelial dysfunction in patients with documented coronary artery disease [184]. Another study focused on mechanism of benefit found that allopurinol improves intracellular concentration of CrP, increases CrP/ATP ratio, and decreases cytosolic ADP concentrations, leading to an increased magnitude of the free energy of ATP hydrolysis [91]. Overall, the findings that allopurinol, an inhibitor of purine degradation, improves myocardial energetics, in combination with the findings of Cappola et al. [37], that allopurinol improves myocardial efficiency, support the hypothesis that perturbations to TAN level play a direct role in modulating cardiac function in disease, and that the purine degradation pathway may represent a viable target for improving cardiac energetics in heart disease.

While several other compounds listed in Table 2 have noted beneficial cardiac effects, many are also associated with significant cardiotoxicities. Methotrexate (MTX), for example, is associated with multiple case reports of arrythmia, angina, and sudden cardiac death, however the specific role of MTX in these cases is undocumented [78, 180]. Another drug with noted cardiovascular toxicities, Pentostatin, or 2'-deoxycoformycin, is associated with a reported 3–10% incidence rate of significant cardiovascular events [82, 174]. Pentostatin is a potent inhibitor of adenosine deaminase (Figure 1, reaction 10), and its

administration results in significant increases in deoxy-ATP, with concomitant decreases in ATP. Pemetrexed [172], Azaserine [59], Fludarabin-Melphalan [217], and Sirolimus [117] each have limited case report(s) of severe cardiotoxicity.

Enzymopathies of the purine nucleotide cycle, and purine synthesis, degradation, and salvage pathways

Genetic disruptions to enzymes of purine metabolism tend to fall in three distinct levels of severity. Enzymopathies with a moderate severity phenotype tend to cause myopathy, urinary calculi, and/or gout. A higher severity category is associated with severe intellectual disability and/or severe immunosuppression. Enzymopathies with the most extreme phenotype are not compatible with life. The first description of an inborn error of purine metabolism, xanthinuria, was likely by Marcet in 1817 [143]. However, this condition was not fully described until much later in 1954 [52]. In the years since, thousands of studies on enzymopathies in these purine pathways have been published. For simplicity's sake, Table 3 summarizes the predominate phenotypes of each enzyme's homozygous recessive related disease. Associated cardiovascular effects are summarized below.

Enzymopathies in the moderate severity category include APRT deficiency, ADSSL1 deficiency, AMP deaminase (AMPD) deficiency, and type 1 xanthinuria. Enzymopathies in the higher severity phenotype include PRS deficiency, PRS superactivity, AICA-ribosiduria, Lesch-Nyhan syndrome (HGPRT deficiency), adenosine kinase deficiency, ADS/SAICAR lyase deficiency, cytosolic 5'-nucleotidase superactivity, adenosine deaminase deficiency, and PNP deficiency. Enzymopathies in the most severe category include PAICS deficiency, guanase (guanine deaminase) deficiency, and ITPase deficiency. Similar to pharmacological inhibitors of purine metabolism, many of these enzymopathies cause significant immunosuppression and neurologic toxicities. As with many vital enzymes in human biology, numerous isoforms exist for many of the enzyme catalyzing the reactions illustrated in Figure 1. A genetic defect in one isoform of one purine metabolism enzyme often causes no known notable cardiovascular effect, as reported in Table 3, potentially due to sufficient genetic redundancy in these critical pathways. As noted by Dewulf et al. [53], as of July 2022, no patient had been reported with any enzymatic defect of any of the first five steps of *de novo* purine synthesis. Mutations that could lead to significant cardiovascular toxicities, such as to any *de novo* synthesis enzyme without effective redundancy, likely occur within biology, however, are likely incompatible with human life.

ITPase deficiency is one such example of an enzymopathy of purine metabolism that results in significant reductions in life expectancy. While relatively minor ITPase deficiencies that may cause adverse reactions to certain pharmacological compounds have been noted in the general population, individuals with profound deficiencies—with less than approximately 5% baseline normal ITPase activity as measured in patient fibroblasts and erythrocytes—develop profound dilated cardiomyopathies, leading to death in numerous patients [85, 120]. Gaunase deficiency has also been reported to cause rapid death within days of birth [127]. The mechanism of injury other than profound hypoxia was not noted. PAICS deficiency has also been reported to be fatal within days of birth by progressive hypotension and hypoxia before total cardiorespiratory failure [179]. While the mechanism for PAICS deficiency

leading to death remains unresolved, in two reported instances of this deficiency, patients had skin fibroblasts with the inability to form *purinosomes* [179]. Other deficiencies as listed in Table 3 have been shown to cause muscle wasting (ADS/SAICAR lyase deficiency [104]), exercise intolerance (Arts syndrome [181]), and distal myopathy (ADSSL1 deficiency [176]), however the exact mechanisms affecting skeletal muscle or cardiac muscle are not resolved. Various other deficiencies listed in Table 3 are associated with congenital cardiovascular structural defects. However, in these cases it is difficult to infer causation, as many of these patients are offspring of consanguinity, leading to relatively high amounts of homozygosity affecting numerous biochemical pathways [84].

AMP deaminase 1 (AMPD1) deficiency, also known as myoadenylate deaminase deficiency (MADD), is the most commonly occurring genetic deficiency in human skeletal muscle [169]. Musculoskeletal effects associated with AMPD1 deficiency are more thoroughly described than for any of the deficiencies described above [65]. The decreased conversion of AMP to IMP seen in AMPD1 deficiency leads to exercise intolerance, and with excessive exercise, muscle cramping, a phenotype consistent with the loss of function of the PNC in contributing to the maintenance of ATP hydrolysis potential in exercise. Patients with AMPD1 deficiency are also at increased risk of malignant hyperthermia [66].

Yet, despite the association with myopathy symptoms, it has been observed that the nonfunctional C34T allele of AMPD1 is cardioprotective [8, 138, 230]. Deficiency of AMP deaminase was first described by Engel et al. [60] with regard to a hypokalemic periodic paralysis patient, with no reference to a cardiovascular phenotype. The first reference to a cardiovascular phenotype in AMPD1 deficiency is associated with an inheritable fatal cardiomyopathy, which was described as an autosomal dominant disease [197]. However, a search of the subsequent literature reveals no other reports showing an association between AMPD1 deficiency and an inheritable cardiomyopathy. Much of literature regarding cardiovascular function in AMPD1 deficiency reports beneficial correlation between the C34T genotype and outcomes in heart failure [138], coronary artery disease [8], ischemic cardiomyopathy [230], and heart transplantation [228]. Loh et al. [138] conducted the first major study of AMPD1 genotype in heart disease. They genotyped 132 sequential advanced congestive heart failure (CHF) patients and retrospectively determined their first hospitalization for CHF and incidence of cardiac transplantation or death. They found approximately 16% of patients with at least one copy of the mutant AMPD1 allele. While no differences were noted in ejection fraction, cardiac index, VO2 max, or a number of other cardiovascular measurements, the AMDP1 (−/+)/(−/−) group survived on average 4.4 years longer without cardiac transplantation or death than individuals homozygous for the wild type allele, as shown in Figure 6. Another study similarly found that individuals with coronary artery disease and at least one mutant AMPD1 allele had a 4.4% chance of cardiovascular death, whereas individuals homozygous for the wild type allele had an 11.9% chance of cardiovascular death over an average course of 3.5 years [8]. One hypothesis for the mechanism of this benefit is that increased levels of plasma adenosine, derived from skeletal muscle, lead to ischemic preconditioning of the myocardium, thereby showing an increased benefit in patients with ischemic cardiomyopathies [230]. Indeed, presence of at least one mutant AMPD1 allele was a statistically significant predictor of transplant-free cardiovascular survival in patients with ischemic left ventricular dysfunction, whereas in

nonischemic patients, this association was not found [230]. The findings of two other studies focusing on AMPD1's role in ischemia are not consistent with a cardioprotective role for AMPD1 deficiency. Collins et al. found that the AMPD1 C34T genotype is not a predictor of survival in post-myocardial infarction patients [43]. Similarly, Andreassi et al. showed that the C34T genotype is not associated with improved outcomes after coronary revascularization (percutaneous transluminal coronary angioplasty and coronary artery bypass graft) [9].

Studies of AMPD1 deficiency in the context of heart transplantation reveal a potential role for this deficiency that is independent from its impact on skeletal muscle metabolism. Yacoub et al. [228] showed that donor hearts selected for transplantation had a significantly higher rate of C34T mutation in the AMPD1 gene than in donor hearts that were not used for transplant. Since screening of potential donor hearts uses a set of metrics that does not include AMPD1 genotype, this result may suggest a beneficial effect of C34T genotype on cardio-preservation that is independent of the effects of this genotype in other tissues. However, a related study of the relationship between heart recipient survival and the AMPD1 genotype of the donor heart revealed that recipients of C34T genotype containing donor hearts had worse overall 1-year survival after transplantation, likely due to an increased incidence of early graft dysfunction [206]. Thus, interpreting these findings from heart transplant studies is not straightforward. Nor do these studies rule out a contributing role for AMPD1 deficiency in skeletal muscle affecting cardiovascular function.

Discussion in the literature of AMPD as a therapeutic target in cardiovascular disease largely focuses on potential effects on adenosine production and AMPK activity [231], and less on a potential to improve preservation of total adenine nucleotide levels by slowing adenine nucleotide degradation [114]. In light of the synthesis of the roles purine nucleotide regulation has in cardiac energetics and mechanics assembled herein, targeting purine degradation pathways to preserve adenine nucleotide pools in heart disease is suggested as a viable target for therapy development.

Conclusions and Perspectives

Both acute myocardial ischemia and decompensatory remodeling of the heart are associated with depletion of myocardial adenine nucleotides and with impaired myocardial mechanical function. While the mechanisms underlying purine nucleotide depletion in acute ischemia are better understood than those driving depletion in chronic heart disease, a number of important questions remain regarding the major biochemical processes driving myocardial metabolism in ischemia and reperfusion. In particular, testing and refining hypotheses on how adenine degradation and salvage pathways interact with production and consumption of intermediates of the tricarboxylic acid cycle and other metabolic substrates in the myocardium during ischemia are important areas for current research.

It has been known for almost a century that dysfunction in energy metabolism plays a role in the etiology of heart failure [47, 89, 100, 164]. And it has been known for decades that a depletion of adenine nucleotides is a defining feature of myocardial metabolic dysfunction in heart failure. Yet next to nothing is firmly established about the mechanisms driving these

changes. We propose that just as an imbalance in the degradation, salvage, and *de novo* synthesis of adenine nucleotides leads to a net loss of adenine nucleotides in ischemia, a chronic shift in the balance between these processes is driven by a chronic high-demand state in the development of heart failure.

Regardless of the underlying causes, in acute or chronic conditions, depletion of adenine nucleotide levels is associated with diminished myocardial mechanical function. Recent theoretical and experimental studies reviewed herein support the hypothesis that the reduction in adenine nucleotides seen in both chronic and acute disease conditions leads to reduced myocardial ATP and increased myocardial inorganic phosphate. Both of these changes have the potential to directly impact tension development and mechanical work at the cellular level. Exploration of these hypothesized mechanisms linking myocardial mechanics and energetics with myocardial adenine nucleotide regulation promise to yield insights into unexplored/unexploited treatment targets for myocardial metabolic and mechanical dysfunction.

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Major Teaching Points

1. The adenine nucleotides adenosine triphosphate (ATP), adenosine diphosphate (ADP), and adenosine monophosphate (AMP) play central roles in energy metabolism.
2. The total adenine nucleotide (TAN) pool concentration in the myocardium is maintained via a dynamic balance between purine *de novo* synthesis, degradation, and salvage pathways.
    a. Degradation of adenine nucleotides is governed by the balance of ATP synthesis and ATP demand in the myocardium.
    b. Under high ATP-demand (high-work/exercise) or impaired ATP-supply conditions (ischemia/hypoxia), increased AMP production leads to increased purine degradation flux.
3. The TAN pool can be substantially reduced in heart disease compared with normal physiological levels.
    a. In acute ischemia the TAN pool is depleted through a rapid increase in flux through purine degradation pathways, and permeation of degradation products out of cardiomyocytes into the interstitium.
    b. In chronic heart failure, reduction of the TAN pool is hypothesized to be a consequence of chronic ischemia and/or elevated ATP demand, chronically shifting the balance between purine synthesis, degradation, and salvage pathways.
    c. Reductions in the TAN pool concentration are associated with increases in myocardial inorganic phosphate concentration.
4. Pathophysiological reductions in the TAN pool, whether associated with chronic disease, or acute ischemia, are associated with myocardial mechanical dysfunction. It is hypothesized that this dysfunction is caused in part by the effects of both a reduction in myocardial ATP and an increase in myocardial inorganic phosphate, on the force generating actin-myosin crossbridge cycle.

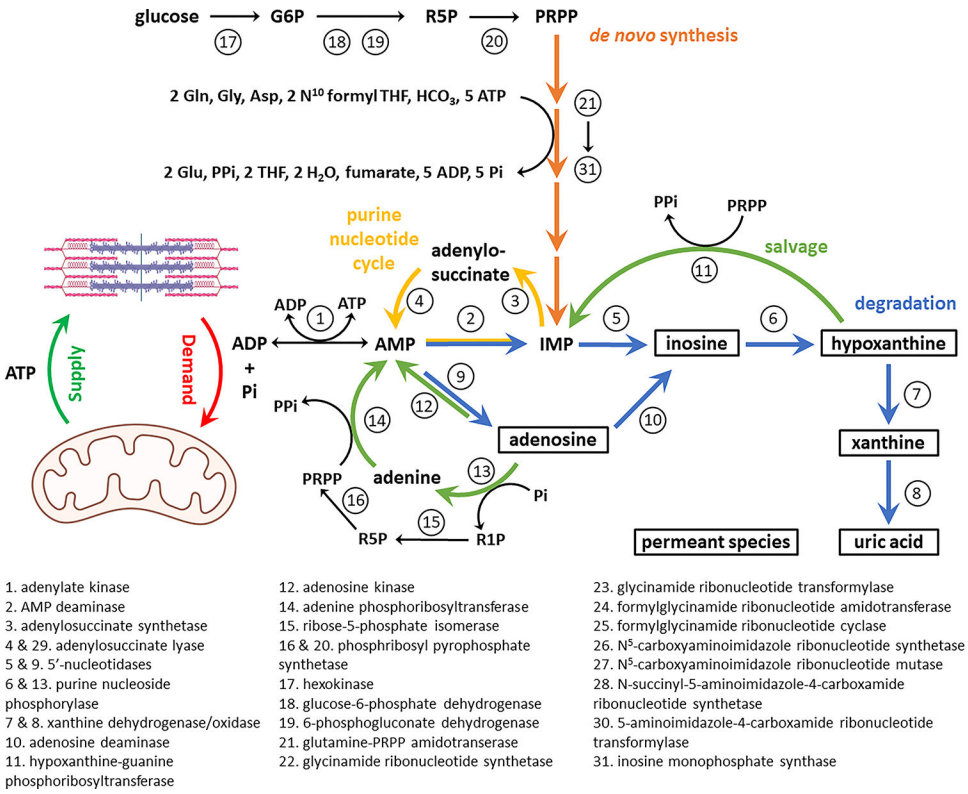


Figure 1:

Pathways of purine nucleotide *de novo* synthesis, degradation, and salvage. The enzymes associated with the reactions in this pathway are annotated below the diagram. Highly permeant products of purine degradation, adenosine, inosine, hypoxanthine, xanthine, and uric acid are indicated by boxes. Mitochondria and sarcomere diagrams obtained from Biorender. Abbreviations: adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), inorganic phosphate (Pi), inosine monophosphate (IMP), pyrophosphate (PPi), phosphoribosyl pyrophosphate (PRPP), ribose-1-phosphate (R1P), ribose-5-phosphate (R5P), glucose-6-phosphate (G6P), glutamine (Gln), glycine (Gly), aspartate (Asp), tetrahydrofolate (THF), bicarbonate (HCO_3), glutamate (Glu).

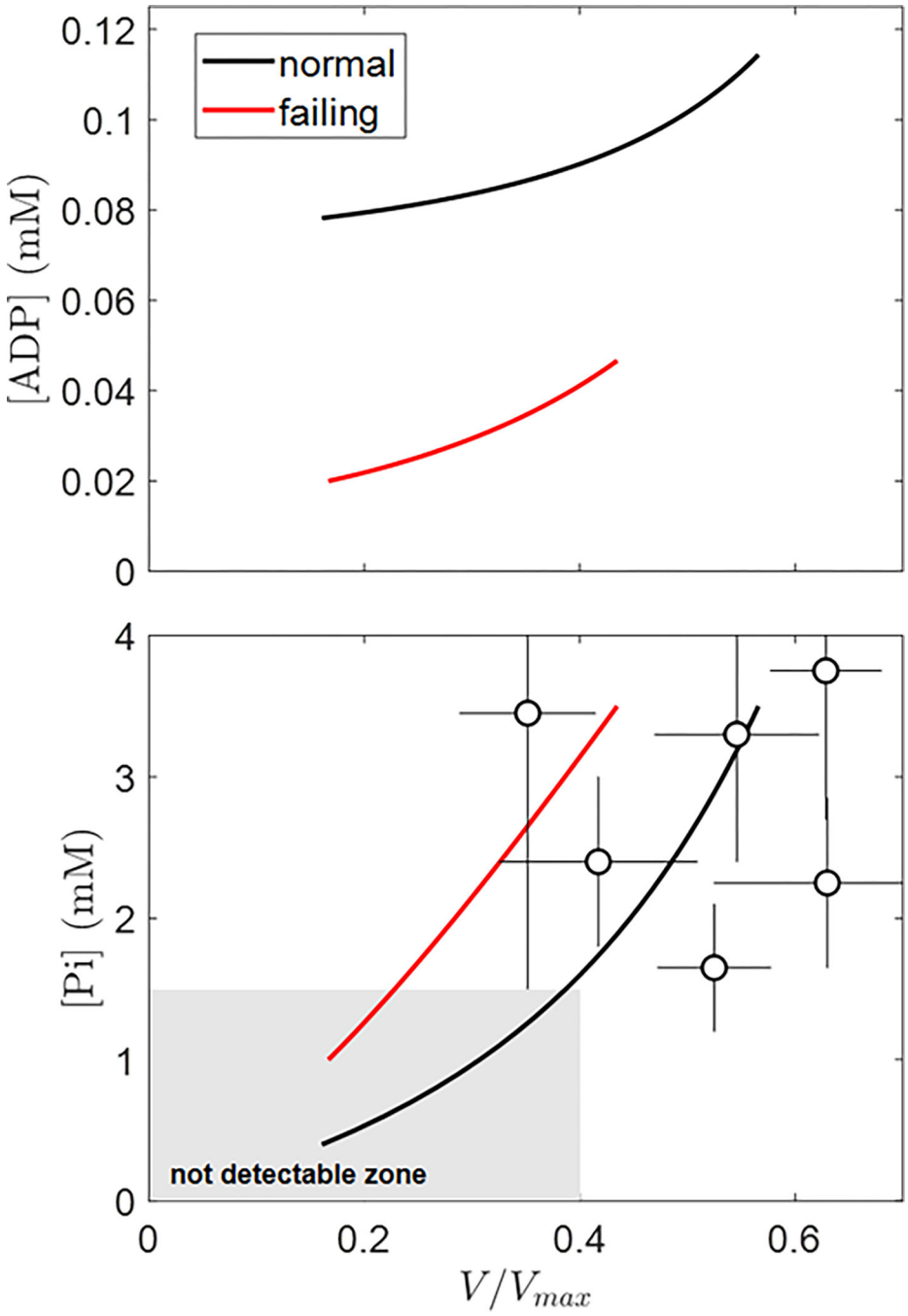
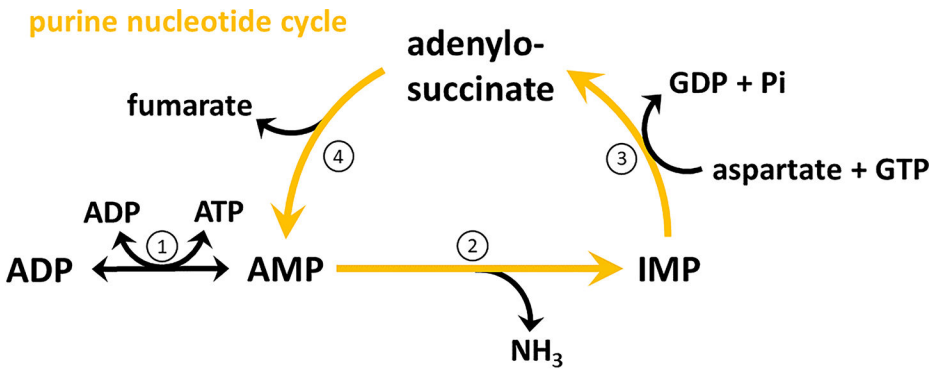


Figure 2:

Myocardial respiratory control *in vivo*. Relationships between ATP demand level V/V_{max} and myocardial [ADP] and [Pi] predicted by Equation (1) are plotted following the simple analysis described in the text. The black lines (normal case) are associated with the concentration pool values indicated in the text. The red lines (failing case) are associated with a 50% reduction in TAN level from the control value.



1. adenylate kinase
2. AMP deaminase
3. adenylosuccinate synthetase
4. adenylosuccinate lyase

Figure 3:

Detailed pathway illustration fo the purine nucleotide cycle. The purine nucleotide cycle (PNC) encompases reactions 2, 3, and 4 from Figure 1. Reaction 3, adenylosuccinate synthetase, consumes asparate and is thermodynamically driven by GTP hydrolysis. Reaction 4, adenylosuccinate lyase, generates fumarate.

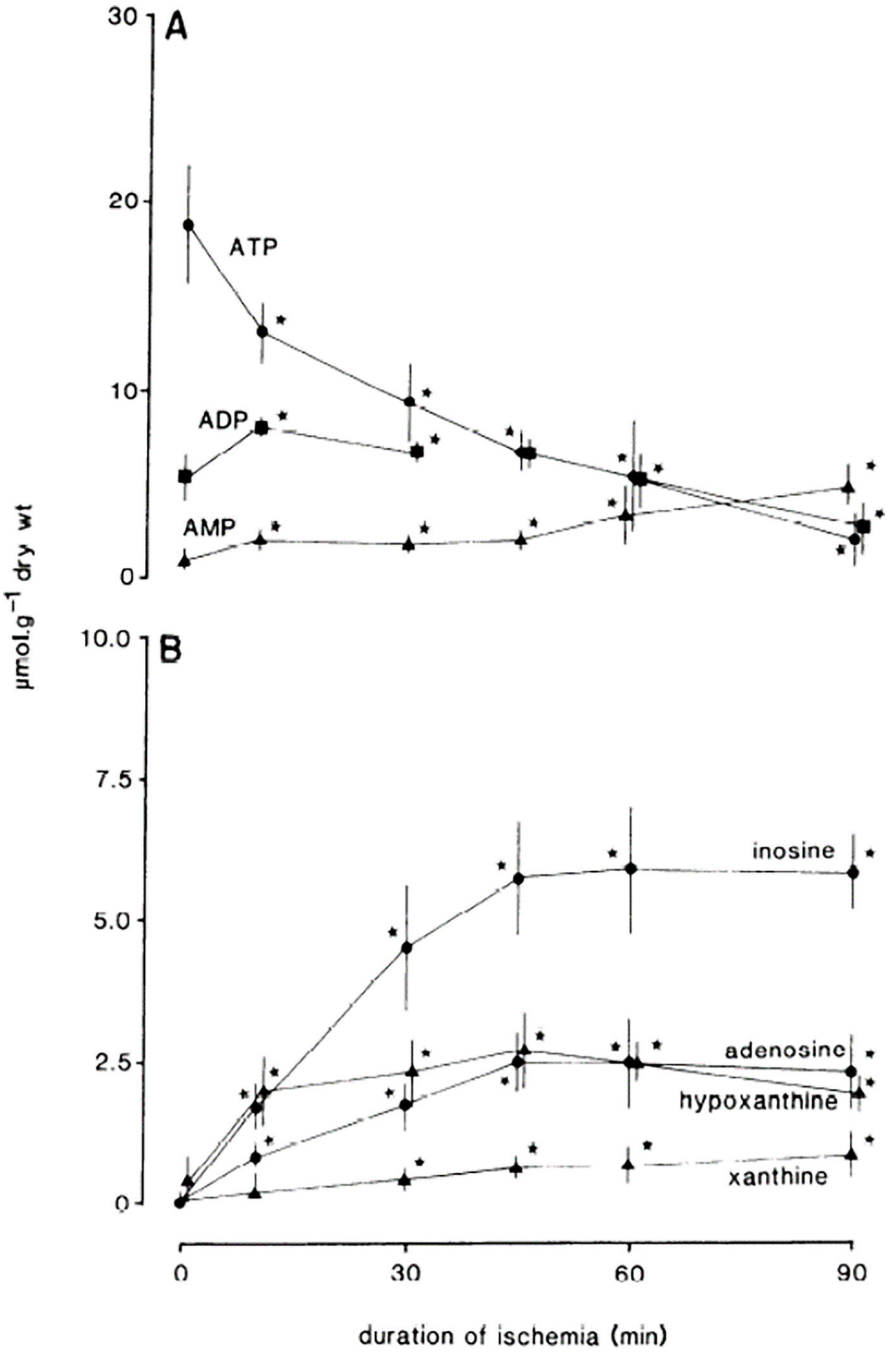


Figure 4:

Depletion of adenine nucleotides and buildup of metabolites of purine degradation during no-flow ischemia in the rat myocardium. The time course of concentrations of adenine nucleotide depletion (A) and downstream products of AMP degradation (B) were measured under no-flow ischemia by Van Bilsen et al. [218]. The major degradation products are inosine, adenosine, and hypoxanthine. Further degradation of hypoxanthine to xanthine and uric acid is inhibited by lack of oxygen. Figure reprinted with permission.

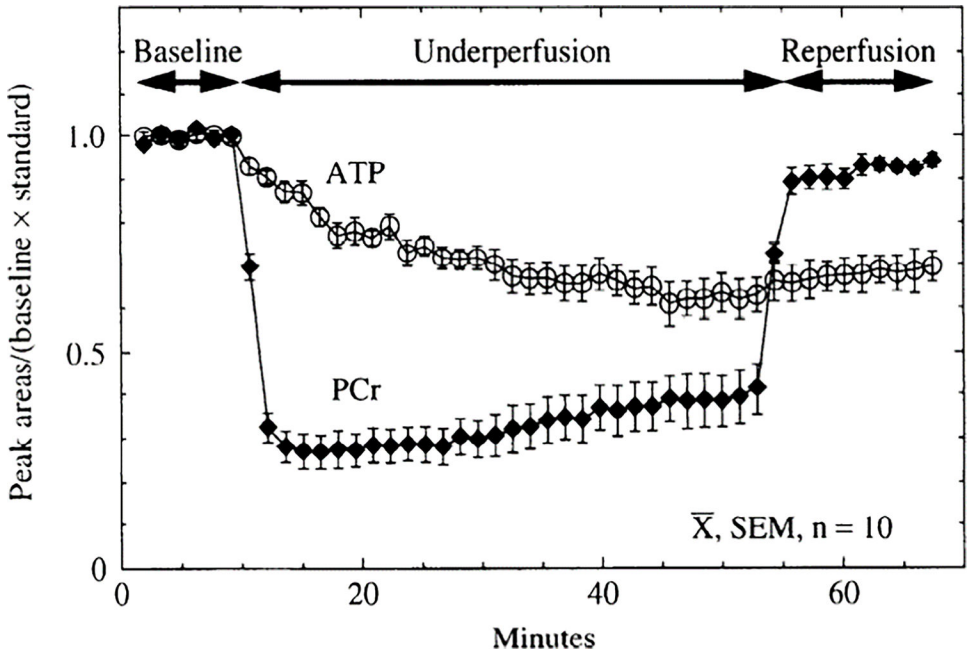


Figure 5:

Depletion of ATP during severe underperfusion and reperfusion. Relative levels of ATP and creatine phosphate (CrP, denoted PCr in the figure) were measured by ^{31}phosphate-NMR in isolated rabbit heart [126]. During underperfusion, the ATP hydrolysis potential is rapidly depleted, as reflected in the PCr concentration. The PCr and ATP hydrolysis potential recover to basal levels following reperfusion while the ATP concentration does not. Figure reprinted with permission.

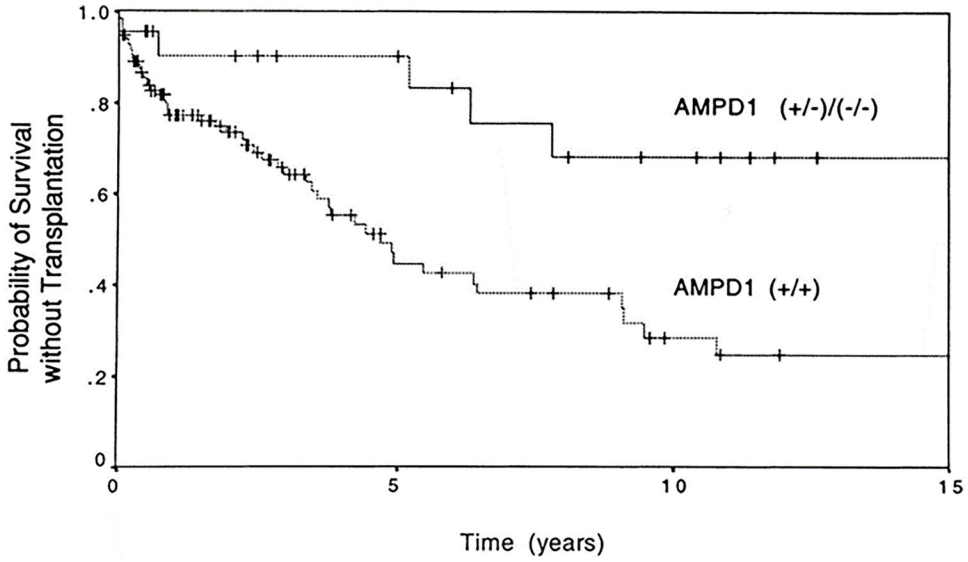


Figure 6:

Survival curves for congestive heart failure (CHF) patients with and without at least one copy of the mutant AMPD1 allele. From Loh et al. [138], the probability of survival without transplant is plotted as a function of years following first hospitalization for CHF. Figure reprinted with permission.

Table 1:

The maximal rate of reaction (V_{max}) of enzymes in the degradation pathway, the purine nucleotide cycle, and the salvage pathway have been measured in cell homogenate from isolated adult rat cardiomyocytes kept at 37°C and at pH 7[32, 33].

Enzyme	Reaction in Figure 1	V_{max} ± SD (μmol · min^{-1} · g wet weight tissue^{-1})	Reference
Degradation pathway			
5'nucleotidase	5,9	2.3 ±0.34	[32]
adenosine deaminase	10	0.48 ± 0.12	[32]
purine nucleoside phosphorylase	6,13	0.053 ± 0.021	[32]
Purine nucleotide cycle			
AMP deaminase	2	0.98 ±0.07	[32]
adenylosuccinate synthetase	3	0.002 ± 0.001	[33]
adenylosuccinate lyase	4	0.49 ± 0.02	[33]
Salvage pathway			
hypoxanthine-guanine phosphoribosyltransferase	11	0.32 ± 0.03	[33]
adenine phosphoribosyltransferase	14	0.27 ± 0.30	[33]
purine nucleoside phosphorylase	6,13	0.053 ± 0.021	[32]

Table 2:

Pharmacological inhibitors of purine metabolism.

Target (Fig. 1 reaction #)	Name	Cardiovascular Effect	Reference
Inhibitors of enzymes of de novo purine synthesis			
PRPP Amidotransferase (21):	6-MP	-See 6-MP below.	[14, 36]
GAR transformylase (23):	Pemetrexed	-No reported cardiovascular effects with wide use, aside from one case report of dilated cardiomyopathy.	[14, 172]
FGAR Amidotransferase (24):	Azaserine, & DON	-As a class, has no reported cardiovascular toxicities in phase I and II studies, aside from one case report of acute cardiogenic shock with Azaserine.	[59, 130] [56, 130, 182]
FGAM Cyclase (25):	NCI_121957	-Not currently used *in vivo.*	[187]
PAICS (28):	MRT00252040	-Not currently used *in vivo.*	[96]
AICAR Transformylase (30):	MTX, & Cpd14	-See MTX below. -Not currently used *in vivo.*	[14] [201]
IMP Dehydrogenase:	6-MP, Ribavirin, MMF, Mizoribine, & Sappanone A	-See 6-MP below. -Has well known cardiovascular toxicity and black box warning of “worsening of cardiac disease and…fatal and nonfatal myocardial infarctions”. However, this is assumed to be related to decreases in hemoglobin and/or co-administered compounds. -No reported cardiovascular toxicities with wide use. In cardiac transplant specifically, MMF has been shown to reduce relative risk of rejection with hemodynamic compromise. -No reported cardiovascular toxicities in post marketing surveillance. -Not currently used in human trials (IMPDH2 specific inhibitor).	[14, 36] [26, 134, 155] [5, 57, 124, 152] [83, 229] [135]
GMP Synthetase:	Angustmycin A, & Mizoribine	-Not currently used in human trials. -See Mizoribine above.	[99] [88]
DHFR:	MTX	-Has well known but rare association ventricular arrhythmias.	[14, 78, 140, 180]
mTOR:	Sirolimus	-No reported cardiovascular toxicities with wide use, aside from one case report of reversible cardiomegaly. In cardiac transplantation specifically, sirolimus has been shown to improve cardiac allograft vasculopathy and diastolic function.	[6, 27, 117, 183]
Inhibitors of purine salvage pathways			
HGPRT (11):	6-MP	-No reported cardiovascular toxicities with wide use. Azathioprine, its active metabolite, has been shown to accelerate beneficial cardiac remodeling in children with dilated cardiomyopathy and active myocarditis.	[14, 36]
Adenosine Kinase (12):	GP-3269, 5-Iodotubercidin, & ABT-702	-As a class, has no reported cardiovascular toxicities in early clinical studies.	[62] [90] [106, 107]
Inhibitors of the purine nucleotide cycle			
AMP Deaminase (2):	Coformycin, & Compound 3	-Not currently used in human trials. -Not currently used in human trials.	[157] [3]

Target (Fig. 1 reaction #)	Name	Cardiovascular Effect	Reference
ADS Synthetase (3):	6-MP,	-See 6-MP above.	[14, 36]
	Alanosine,	-No reported cardiovascular toxicities in phase I and II studies.	[108, 122]
	& Hadacidin	-No reported cardiovascular toxicities in preliminary studies.	[58, 116]
Inhibitors of purine degradation			
Cytosolic 5'-Nucleotidase II (5):	Fludarabine	-Has reported but rare incidence of cardiovascular toxicity when given in combination with melphalan.	[42, 217]
Cytosolic 5'-Nucleotidase I (9):	5-Eddu	-Not currently used in human trials.	[77]
Purine nucleoside phosphorylase (PNP) (6 & 13):	Forodesine,	-No reported cardiovascular toxicities in phase I and II studies.	[121, 147]
	& Ulodesine	-No reported cardiovascular toxicities in phase I studies.	[17, 63]
Xanthine Oxidase (7 & 8):	Allopurinol,	-No reported cardiovascular toxicities with wide use. Allopurinol has been shown to improve myocardial energetics and efficiency, with conflicting reports as to whether a mortality benefit exists. -No reported cardiovascular toxicities with wide use.	[50, 219, 224]
	Febuxostat, & Topiroxostat	-No reported cardiovascular toxicities with limited use.	[24, 209, 224] [95, 149]
Adenosine Deaminase (10):	Pentostatin,	-Has well-known cardiovascular toxicity with CKMB elevations seen in 3–10% of patients and rare reports of new onset heart failure.	[82, 174]
	& Coformycin	-See Coformycin above.	[157]
Guanase:	Azepinomycin	-Not currently used in human trials.	[101]

Table 3:

Enzymopathies of purine metabolism.

Diseases (Fig. 1 enzyme #)	Description:	Reference
Enzymopathies of de novo purine synthesis		
PRS Dysfunction (16 & 20): (Arts Syndrome, DFN2, & CMTX5)	- PRPS1 gene mutation results in reduced purine synthesis from both the *de novo* pathway and salvage pathway due to decreased amounts of the precursor molecule PRPP. This results in near absent levels of hypoxanthine and uric acid, as well as the many disease phenotypes, including but not limited to blindness, hearing impairment, neuropathies, intellectual disability, and muscle weakness. Exercise intolerance has also been reported in sporadic cases, which may improve with SAM supplementation. *There are no specific reports of cardiac involvement.*	[51, 73, 131, 167, 181, 205]
PRS Superactivity (16 & 20):	-Has multiple severities as enzyme activities increase, PRPP levels rise, and flux through the purine synthesis pathways increase. Patients with the most severe phenotype often have hearing impairment, intellectual disability, and hypotonia, as well as the biochemical derangements hyperuricemia and hyperuricosuria. *There are no specific reports of cardiac involvement.*	[200]
PAICS Deficiency (28):	-Results in multiple congenital malformations and early neonatal death. *Echocardiography of affected patients showed no structural abnormalities.*	[179]
AICA-ribosiduria (30 & 31):	-Attributed to ATIC Deficiency. The accumulation of AICA-riboside and other purine synthesis intermediates leads to profound intellectual disability, early onset epilepsy, and significant hypotonia. It has been associated with multiple cardiovascular structural defects.	[113, 144, 185]
Enzymopathies of purine salvage pathways		
Adenosine Kinase Deficiency (12):	-Results in a lack of adenosine salvage. This leads to excess levels of adenosine, methenamine, and other intermediates of the homocysteine-methionine cycle. These biochemical derangements lead to developmental delay, hypotonia, epilepsy, and hepatopathy. It has been associated with an increased risk of cardiovascular structural defects.	[29, 203]
APRT Deficiency (14):	-Leads to an accumulation of 2,8-dihydroxyadenine due to a lack of adenine salvage. *No extrarenal symptoms of APRT deficiency have been reported.*	[30, 220]
HGPRT Deficiency: (Lesch-Nyhan Syndrome)	-Associated with significant reductions in guanine and hypoxanthine salvage, as well as increased levels of hypoxanthine, xanthine, and uric acid. These biochemical derangements lead to severe intellectual disability and self-injurious behavior. *There are no specific reports of cardiac involvement.*	[71]
Enzymopathies of the purine nucleotide cycle		
AMP Deaminase Deficiency (MADD) (2):	-Results in decreased conversion of AMP to IMP. This leads to exercise intolerance, muscle cramping, and an increased risk of malignant hyperthermia. Interestingly, numerous academic groups have implicated that both heterozygous and homozygous AMP deaminase deficiencies are associated with improved survival in heart failure patients and improvements in heart transplantation.	[8, 43, 65, 138, 186, 230]
ADSSL1 Deficiency (3):	-Results in decreased conversion of IMP to Adenylosuccinate. This primarily leads to facial weakness and a distal myopathy. *Echocardiography of affected patients showed no structural abnormalities.*	[176, 177, 204]
ADS/SAICAR Lyase Deficiency (4):	-Results in an accumulation of succinyladenosine and SAICA-riboside. This leads to intellectual disability, epilepsy, and muscle wasting. *Echocardiography of affected patients showed no structural abnormalities.*	[34, 103, 104, 159]
Enzymopathies of purine degradation		
Cytosolic 5′-Nucleotidase Superactivity:	-Increased catabolism of nucleotides by 5' nucleotidase has been shown to increase overall salvage, and decreases *de novo* synthesis, leading to hypouricosuria and decreases in PRPP levels. This causes intellectual disability, epilepsy, and frequent infections. The 5'-nucleotidase isoform responsible is not currently known. *There are no specific reports of cardiac involvement.*	[173, 192]

Diseases (Fig. 1 enzyme #)	Description:	Reference
PNP Deficiency (6 & 13): (PNPD & Nezelof Syndrome)	-Limits conversion of inosine and guanosine to hypoxanthine and guanine. This leads to both intellectual disability and recurrent infections mediated by T cell deficiencies. *There are no specific reports of cardiac involvement.*	[145, 222]
Type I Xanthinuria (7 & 8):	-Also known as xanthine oxidase deficiency, this results in the accumulation of xanthine. This accumulation leads to xanthine kidney stones and increased risk of renal failure. *There are no specific reports of cardiac involvement.*	[132]
Adenosine Deaminase Deficiency (10):	-Limits conversion of adenosine and deoxyadenosine to inosine and deoxyinosine, and as a result leads to the accumulation of dATP and s-adenosylhomocysteine. This causes both intellectual disability and severe combined immunodeficiency. *There are no specific reports of cardiac involvement.*	[69, 92]
Guanase Deficiency:	-There are limited case reports of guanase deficiency, however, it appears to be fatal in the neonatal timeframe, resulting in limited phenotyping. *There are no specific reports of cardiac involvement.*	[127]
ITPase Deficiancy:	-ITPase deficiency results in the accumulation of ITP and dITP in the cell. This results in early infantile encephalopathy, and in some reported cases, an infantile onset dilated cardiomyopathy.	[85, 120]