JEPonline

Creatine and Exercise – Strong Evidence for Stronger Heart Muscle?

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ABSTRACT

Webster I, Huisamen B, Du Toit EF. Creatine and Exercise – Strong Evidence for Stronger Muscles? **JEPonline** 2011;14(5):85-108. There has been a dramatic increase in the use of dietary creatine supplementation among sports men and women, and by clinicians as a therapeutic agent in muscular and neurological diseases. The effects on skeletal muscles have been documented and reviewed extensively. However, this review looks at another important muscle – the heart – and both the advantages and disadvantages to creatine supplementation, exercise, and the combination. The proposed mechanisms of each are examined and explained.

Key Words: Cardioprotection, Ischemia, Reperfusion Injury

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TABLE OF CONTENTS

INTRODUCTION

In 1992, the American Heart Association [39] declared that physical inactivity is an independent risk factor for the development of coronary artery disease (CAD), highlighting what a large role physical activity plays in procuring health and physiological harmony. For decades exercise has been described as both a preventative measure and a prophylactic for many diseases and ailments. This is especially relevant in cardiovascular disease prevention and treatment. The beneficial cardiovascular effects of regular exercise were documented as early as 1960 [114], which was followed by many studies that support the initial research findings [42,56,98,152].

Creatine supplementation has been used for years by sportsmen and women as a legal and natural aid to enhance endurance, power, and decrease recovery time. It is advertised on numerous websites as the safe and easy way to improve athletic performance and increase muscle mass. Creatine monohydrate, creatine phosphate, and creatine ethyl esters are all forms of creatine that are taken by athletes and body builders to enhance exercise performance [30,132]. Irrespective of which form of creatine athletes use, the results all seem to favor increased muscle power [63], decreased recovery time, and increased time to fatigue [116]. Although the focus has been on the impact of

creatine on skeletal muscle, this review investigates the effects of creation supplementation primarily on another vitally important muscle - the heart.

EXERCISE
Beneficial Effects of Exercise
Exercise from early on in life has been seen to be beneficial to the myocardium [121], and has been found to prolong life expectancy and quality of life [89]. Exercise also protects against death from CAD and other causes [123]. An increase in physical activity, albeit moderate, can decrease the chances of a myocardial infarction (MI) and may accelerate recovery after an MI [78]. Animal and human studies also indicate that regular exercise decreases myocardial ischemia and reperfusion injury [12,100]. In normal subjects, regular exercise or training results in enhanced body sensitivity to insulin [70]. This has implications for diabetic and insulin sensitive people, where increased physical activity is beneficial in counteracting a high-fat diet-induced insulin resistance [72] as well as delaying the onset of non insulin-dependent diabetes mellitus (type 2 diabetes) or even preventing the disease.

Other risk factors for coronary heart disease include body weight, body mass index (BMI), cholesterol, LDL cholesterol, and triacylglycerols; all are decreased with an exercise regime [109], as is the progression of atherosclerosis [75]. In addition, physical training improves cardiac function as evidenced by an increase in left ventricular end-diastolic volume, stroke volume, and ejection fraction. Eccentric hypertrophy is due to hypertrophic growth of the walls of a hollow organ, especially the heart, in which the overall size and volume are enlarged [25]. This hypertrophy is associated with an improved left ventricular systolic and diastolic function rather than fibrosis which would be expected to compromise mechanical function [95].

Regular exercise also results in weight loss, and thus helps to decrease blood pressure resulting in reduced hypertension in both men and women [7,118]. Hemodynamic changes in response to exercise can also decrease the chance of ischemic heart disease by reducing platelet aggregation and increased fibrinolytic activity [146].

Detrimental Effects of Exercise
Article titles such as "Runners who don`t train well can have a marathon of miseries" [40] and "Ironman athletes put hearts at risk of fatal damage, experts warn" [122], imply that exercise is not necessarily as infallible as it is made out to be. Thompson et al. [134] suggest that exercise is not always beneficial as forceful activity can also acutely and rapidly increase the risk of sudden cardiac death or myocardial infarction in susceptible persons. Exercise is a stressor, and although prolonged exposure to moderate episodes may precondition the heart and protect it, the question of "how much is too much" is a relevant concern [43,74]. Cardiac hypertrophy and associated alterations in the structural properties of the microvasculature have been seen with chronic strenuous exercise [86]. Similarly, alterations in the structure and function of the sarcoplasmic reticulum with acute strenuous exercise have been observed [20]. For example, acute strenuous exercise has been linked to depression in the rate of Ca^{2+} uptake, a diminished Ca^{2+} release, and an increase in the intracellular free Ca^{2+} concentration, which in turn could activate proteolytic pathways.

There is also evidence for a simultaneous activation of the coagulation, fibrinolysis, and complement system as well as for a release of histamine after a short maximal intensity exercise regime [35]. Short-term, high-intensity exercise can lead to significant and prolonged dysfunction of the mitochondrial energy status of peripheral blood leucocytes, and an increased predisposition to apoptosis and raised pro-inflammatory mediators [137]. This could in turn lead to CAD [33]. These results suggest an immunosuppressive effect of excessive exhaustive exercise training [58].

Mechanisms of Exercise Induced Cardiac Protection
As expressed in the preceding sections of this review, exercise training has been shown to not only protect the heart against ischemia and reperfusion induced damage, but also has the known benefit of decreasing the risk of CAD and myocardial infarction. There are two mechanisms thought to induce protection. First, by decreasing many of the causes of ischemia, that is, by reducing risk factors for coronary artery disease (such as blood pressure, cholesterol, risk of atherosclerosis), coronary blood flow is adequate to maintain myocardial integrity. Second, although not fully understood, intrinsic cardioprotective mechanisms such as exercise induced increases in coronary circulation, increases in heat shock protein expression (HSPs) in the heart, increases in myocardial antioxidant levels, and improved function of the sarcolemma K_{ATP} channels are implicated in the protection from CAD. In the following section we will briefly discuss each of these exercise induced changes and the implications of these changes on the ischemic/reperfused heart.

Sheer Stress and Vascular Remodeling
Exercise increases oxygen demand of working skeletal muscles, which leads to an increase in cardiac output and blood flow through the vasculature [75]. Shear stress, the stress placed on the vascular wall by the circulating blood, increases during exercise and elevates free radical production in endothelial cells, up-regulates protective antioxidant enzymes and heat-shock proteins and down-regulates pro-apoptotic factors [90]. Exercise also activates endothelial- and inducible-nitric oxide synthase (eNOS and iNOS) that leads to greater nitric oxide (NO) availability [28]. Nitric oxide contributes to vessel homeostasis by inhibiting vascular smooth muscle contraction thus inducing blood vessel dilation, platelet aggregation, and leukocyte adhesion to the endothelium.

Long term chronic exercise training can result in angiogenesis and arteriogenesis in the heart [147] and skeletal muscle [48]. Both adaptations result in an increase in blood flow and an improved blood flow capacity to the vasculature and muscle [82]. See Figure 1.
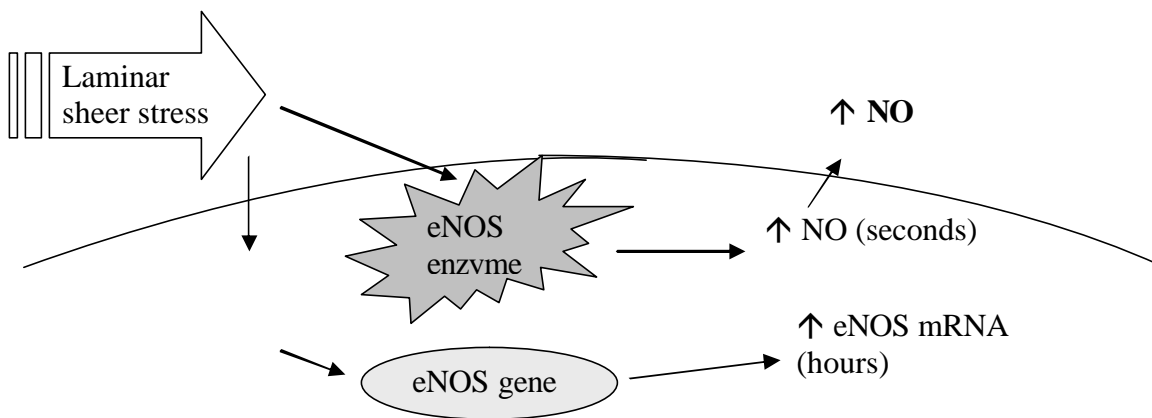
Figure 1: Shear stress induced NO production by vascular endothelial cells.

Heat Shock Proteins
Heat shock proteins (HSP) are a class of functionally related proteins whose expression is increased when cells are exposed to stress (such as with increased temperature, ischemia, and exercise). They reduce apoptotic and necrotic cell death by antagonizing apoptosis inducing factors (e.g., caspases [115] or by enhancing the activity of mitochondrial complexes I-V) [124]. HSP70's role in exercise

induced cardioprotection has been studied and shown to be effective in protecting the myocardium from ischemic injury [10,50].

Antioxidants

Increased reactive oxygen species (ROS) production by the mitochondria during reperfusion is at least in part responsible for injury. Antioxidants stop the reactions by removing free radicals, and inhibit oxidation reactions by being oxidized themselves [127]. An increase in antioxidants thus helps scavenge the ROS. Enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). Important nonenzymatic antioxidants include reduced glutathione and vitamins E and C [111].

Although there are reports suggesting that GPx and CAT activity increases with exercise [59], there are also reports that suggest the contrary [29]. MnSOD is however the antioxidant which has been shown to be increased with exercise [41].   But despite this association it has not been established whether this antioxidant is essential for cardioprotection [80]. See Figure 2.
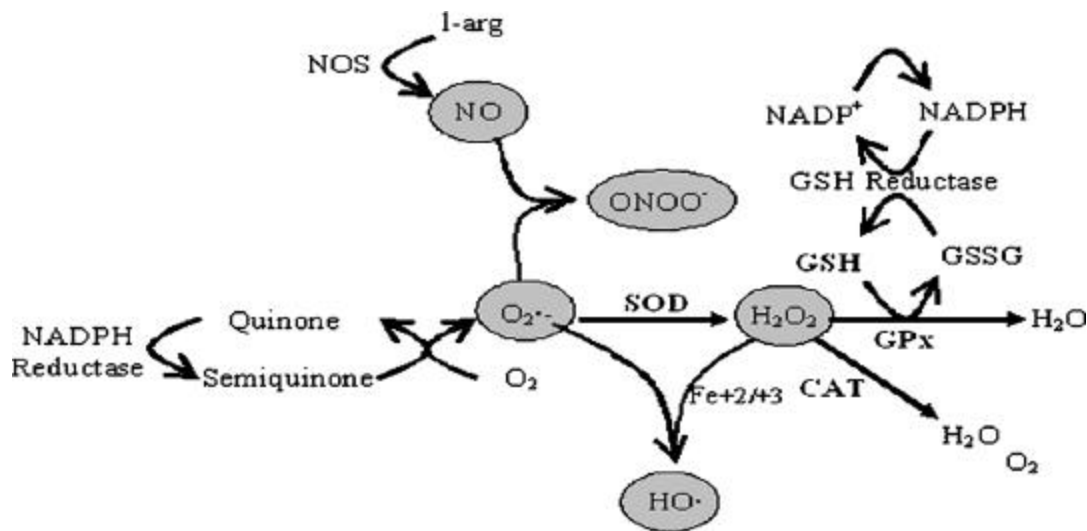
Figure 2: Pathways of major cellular oxidant formation and endogenous antioxidant action. Species noted in gray circles represent some of the reactive oxygen and nitrogen species capable of mediating damage to cellular protein, lipid, and DNA. GSH, reduced glutathione; GSSG, oxidized glutathione; NADP+, Nicotinamide adenine dinucleotide: NADPH, reduced Nicotinamide adenine dinucleotide: GPx, glutathione peroxidase : SOD, superoxide dismutase;H_2O_2, hydrogen peroxide; H_2O, water; NO, nitric oxide; NOS, nitric oxide synthase; O_2, oxygen; O_2-, superoxide; ONOO-, peroxynitrite. Adapted from Snow and Murphy [8].

K_{ATP} *Channels*

The ATP-sensitive potassium channel (K_{ATP}) is normally inhibited by intracellular ATP and opens during periods of energy depletion [101]. K_{ATP} channels are known to exist in the sarcolemmal membrane as well as the mitochondrial membrane of cardiomyocytes. There is evidence both for [27] and against [16] a role for the mitochondrial channels' in cardioprotection. It has been shown to be a mediator of cardioprotection induced by preconditioning either by ischemia [18], pharmacological manipulation [34] or exercise [16].

Although sarcolemmal K_{ATP} channel activation in the ischemic myocardium is critically important for cell survival and protection of function, its electrophysiological effects include shortening of the action potential duration and the refractory period. These effects are potentially proarrhythmic and can promote the development of lethal arrhythmias, [64]. Consequently, the inhibition of sarcolemmal K_{ATP} channels in ischemic myocardial cells can prevent lethal ventricular arrhythmias and sudden cardiac death [37,138], implicating increased K_{ATP} opening in sudden cardiac death associated with exercise. The opening of the mitochondrial K_{ATP} channels has also been implicated in improved calcium handling by the cell, reduced mitochondrial matrix swelling, increased oxidative metabolism, and decreased release of ROS by the mitochondria during preconditioning [47,102]. However, Brown et al. [16] have shown that the mitochondrial K_{ATP} channels are not an essential mediator in exercised induced cardioprotection but rather the sarcolemmal K_{ATP} channels that were infarct sparing after regional ischemia.

Mitochondria
The mitochondria are the powerhouses of the cell. During exercise, when the energy demand of the myocardium increases substantially, the mitochondria's ATP output is increased to meet the demand. Besides ATP synthesis, mitochondria also play a significant role in osmotic regulation, pH control, signal transduction, and calcium homeostasis [14,21].

Exercise training has been shown to improve mitochondrial efficiency of oxidative phosphorylation by increasing the removal of ROS and decreasing free radical production in skeletal muscle [126]. Bo et al. [8] showed that exercise training also increases mitochondrial ATP synthetase activity, ADP to oxygen consumption (P/O) ratio, respiratory control ratio (RCI), and MnSOD activity in cardiac muscle. Ascensao and colleagues [4] showed that endurance training decreased heart mitochondrial susceptibility to MPTP opening. However, not all studies have shown that exercise benefits the mitochondrion. Leucocyte mitochondria show a lowered energy status and a higher incidence of apoptosis during high intensity training [58].

Pro-Survival Pathways
Exercise training activates components of the RISK pathway. Exercise training has been shown both to increase PKB/Akt phosphorylation in the hearts of spontaneously hypertensive rats [76] and normalize the PKB/Akt phosphorylation in the myocardium of Zucker diabetic rats [77]. Increased PKB/Akt signaling would also be expected to increase Glut4 translocation for increased glucose uptake and usage [145]. Cardioprotection via the pro-survival pathways is emphasized by the findings of Siu et al. [2004] and Quindry et al. [113] who found that exercise training decreased the extent of apoptosis in cardiac and skeletal muscle.

Iemitsu et al. [60] concluded that exercise training activated multiple mitogen activated protein kinase (MAPKs: ERK, JNK, and p38) pathways in the heart. P38-MAPK is important in many biological processes including cell growth, differentiation, myocyte hypertrophy, and apoptosis [6,144], but it has been implicated as a mediator of ischemic injury [26]. P38-MAPK activation has been seen to gradually decline with the development of exercise-induced cardiac hypertrophy after approximately 12 weeks [60].

AMPK
AMP-activated protein kinase (AMPK) plays a key role in the regulation of fuel supply and energy-balance. AMPK is generally inactive under normal conditions, but it is activated in response to hormonal signals and stressors such as strenuous exercise, anoxia, and ischemia that increase the AMP/ATP ratio. Once active, muscle AMPK enhances both the uptake and oxidative metabolism of fatty acids, glucose transport, and glycolysis [3]. AMPK enhances glucose uptake via activation of

GLUT4 translocation, fatty acid oxidation via acetyl-CoA carboxylase [51], and glycolysis by inhibiting glycogen synthase [49]. AMPK is activated during exercise [23,24]. However it has also recently been shown that although AMPK is activated by exercise, the alpha2 isoform of AMPK seems to not be essential for glucose uptake in exercising, AMPK deficient mice [87].

CREATINE

The heart is an aerobic or oxygen consuming organ and, therefore, relies almost exclusively on the oxidation of substrates for creation of energy. It can only withstand oxygen deprivation for a short while and still have enough energy to function normally. Thus, in a steady state, determination of the rate of myocardial oxygen consumption provides an accurate measure of its total metabolism. When the supply cannot meet the demand, an energy imbalance ensues. The principle behind creatine supplementation is to provide limitless energy.

$$PCr + ADP \Leftrightarrow Cr + ATP$$
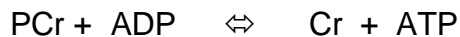
The bidirectional phosphocreatine shuttle highlighted above [8], catalyzed by creatine kinase (CK), prompted the use of creatine supplementation that has been predominant in the last decade. Phosphocreatine is particularly important in muscle [67], sperm [79], and nerve tissues that are subjected to fluctuations in energy demand. With the high delivery of phosphocreatine to the muscle after supplementation, driving the constant restoration of ATP supply, energy supply is expected to be indefatigable [148].

Sources of Creatine
Creatine is a non-essential amino acid which is derived from both the diet and synthesized de novo from arginine and glycine by glycine amidinotransferase (AGAT) and guanidinoacetate methyltransferase (GAMT) [142]. This synthesis takes place mostly in the liver and pancreas and to a lesser extent in the brain and testes [13,97]. Creatine is non-enzymatically broken down into creatinine and excreted by the kidneys in urine [11]. The rate at which creatine is degraded is 1.6% which equates to 2 gm per day. This amount needs to be replenished either by endogenous synthesis or by dietary intake [55]. About half of this (±1 gm per day) is provided by the diet, from sources such as meat and fish and the remainder is synthesized endogenously [57]. However, an increase in serum levels of creatine as a result of supplementation results in a decrease in AGAT enzyme activity, enzyme level, and mRNA expression in rat kidney [94], thus producing less creatine [36].

Creatine Absorption
The ingestion of a carbohydrate containing solution (e.g., fruit juice) aids in the absorption of creatine from the gut, and may increase total creatine in the muscle by up to 60% [46]. However, while insulin and insulin secretion stimulating food appears to enhance muscle uptake of creatine, high carbohydrate meals may slow the absorption of creatine from the intestine [91].

Cellular Creatine Uptake and Storage
Skeletal muscle is the tissue in which most (approximately 95%) of the body's creatine is stored. The remaining 5% is stored in the heart, brain, and testes [129]. Generally, creatine is transported in the blood from areas of production (liver, kidney, and pancreas) to tissues requiring it (skeletal and heart muscle, brain, and testes). Also, the brain and testes produce their own creatine. Creatine is then taken up into cells by a special creatine transporter called the CreaT, which is located on the cell membrane [143].

Over 90% of cellular creatine uptake occurs via the Na^+/Cl^- CreaT, against a large concentration gradient [84]. The extracellular creatine content regulates the transport of creatine into cells [85]. CreaT content is reduced in heart failure [99]. This may contribute to the depletion of intracellular creatine compounds and thus to the reduced energy reserve in the failing myocardium. This discovery has clinical implications, suggesting that the CreaT is a target for therapeutic studies.

Beneficial Effects of Creatine Supplementation

Building body bulk [151], increased muscle power and strength [30,132], increased endurance [83], increased muscle glycogen accumulation [103,139] for increased energy storage and utilization capacity, decreased lactate production [22] and decreased inflammation and muscle soreness [125] are all associated with creatine supplementation.

Clinical Use of Creatine

Not only does creatine have ergogenic effects, but it has also been used as a prophylactic in many muscular and neurological diseases. Since the decrease in cellular creatine is a possible reason for muscle weakness and atrophy and disturbances in cellular homeostasis in diseased states, the normalization of creatine in the cells with supplementation may be a reason for its effectiveness in relieving the effects in these circumstances [150]. Studies investigating the effects of creatine supplementation on muscular dystrophies have shown the efficacy of creatine to alleviate the clinical symptoms of the disease [38,69]. Creatine supplementation in heart failure patients also increases skeletal muscle's performance. This is possibly due to an increase in muscle creatine [45].

In mitochondrial encephalopathy, lactic acidosis disease (MELAS) creatine supplementation completely abolished the symptoms after 4 weeks [5], and in Parkinson's disease creatine supplementation enhances the benefits of weight training [52]. Also, creatine supplementation has positive effects on bone structure and function [2]. Recent work has eluded to the fact that creatine supplementation may help improve insulin sensitivity in type 2 diabetes [105]. Interestingly, creatine has been found to increase antioxidants in skin, and can be protective against UV and other environmental damage [81].

Many positive effects have been documented with the use of creatine as a supplement, both in the healthy and the diseased state. However, care should be taken because the effect of creatine loading on skeletal muscle ergogenics may be negated by the intake of caffeine [140].

Detrimental Effects of Creatine Supplementation

Despite the positive observations detailed above, not all the evidence in the literature is encouraging. There have been reports of adverse effects of creatine supplementation. It has been found to bring about gastrointestinal stress and diarrhea [108]. Short-term, high-dose oral creatine supplementation increases the production and thus the excretion of potential cytotoxic compounds, methylamine, and formaldehyde, but does not have any detrimental effects on kidney permeability [110]. In addition, creatine supplementation exacerbates the allergic response of the lungs in mice [141].

Creatine supplementation has also been associated with atrial fibrillation and a rapid heart rate in a 30 yr old man who was admitted to the emergency room [66]. Clinicians could not find any reason for his condition, and when his medical history was examined it was revealed that he had been using creatine as a supplement. He was treated with anticoagulants. His heart rate stabilized and he was sent home 24 hrs later with no obvious adverse consequences.

Despite the negative effects of creatine, there are also studies that show no effect of creatine on endurance, power or recovery. Herda et al. [53] found that creatine supplementation did not increase

power output or muscle endurance. No increased power output or performance was found in tennis players [103]. Also, 7 days of creatine supplementation did not influence cardiac resistance to oxidative stress, alter heart rate or oxygen uptake responses to exercise in cyclists [68]. Similarly, 28 days of creatine supplementation did not improve sprint performance in endurance cycling [54].

Proposed Mechanisms of Creatine Induced Cardiac Protection
ATP is created in the mitochondrion by oxidative phosphorylation, and this ATP is then stored in the form of phosphocreatine (PCr) in the cytosol. In the inner membrane space in the mitochondrion a phosphate group is transferred from ATP to Cr, forming ADP and PCr. This reaction is catalyzed by the mitochondrial CK isoform (MiCK). PCr leaves the intermembrane space by diffusion and reaches the cytosol where it is used by cytosolic myofibrillar creatine kinases (MMCK) for the rephosphorylation of cytosolic ADP into creatine and ATP for use by ATPases for energy in cytosolic reactions. Such transfer of energy has been termed the phosphocreatine shuttle [8]. This also ensures that there is never an accumulation of ATP in the mitochondria, thus ensuring a gradient in the mitochondria for continuing ATP production [128]. This increased intracellular creatine potentially acts as a store of phosphate groups to be used during ATP synthesis as energy for the cell. See Figure 3.
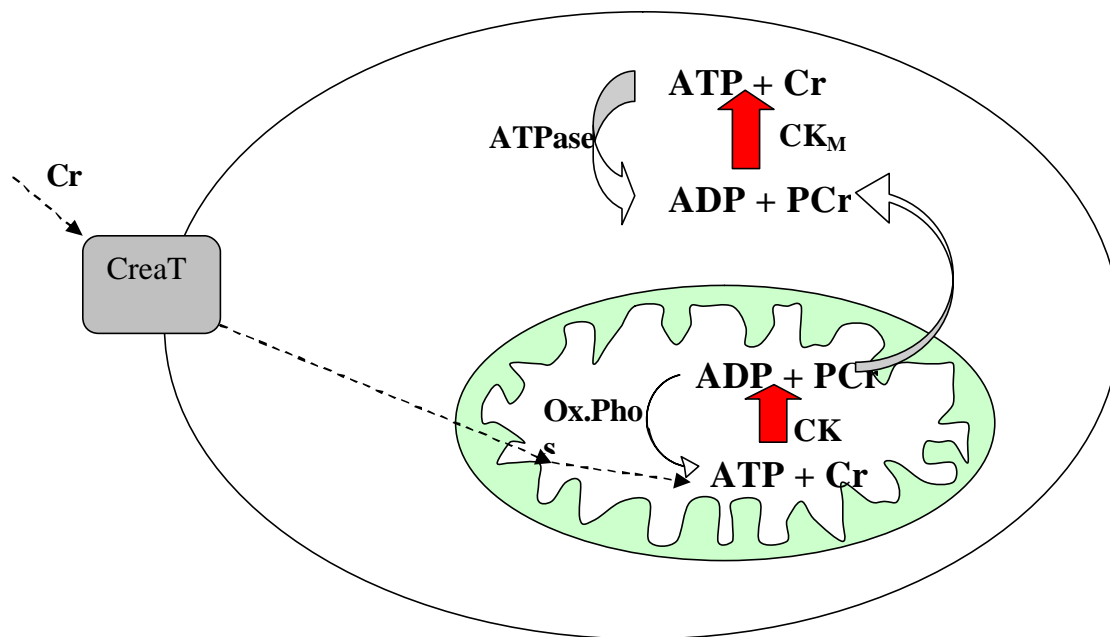

Figure 3. Schematic representation of the phosphocreatine shuttle model, adapted from [71]. During oxidative phosphorylation (OxPhos), ADP is converted to ATP when phosphocreatine In the mitochondrial intermembrane space, ATP donates a phosphate group to Cr and produces PCr. This reaction is controlled by mitochondrial creatine kinase (MiCK). ADP is released into the cytosol where myofibrillar creatine kinase (MMCK) produces ATP and Cr from ADP and PCr, and ATPase controls the reverse reaction.

Brzezinska and colleagues [17] concluded that dietary Cr increased cardiac muscle high energy phosphate reserves and its oxidative potential in the rat model after 7 days of supplementation. Creatine supplementation has been shown to increase cardiac creatine reserves only slightly since initial total creatine concentrations are high [62]. They also showed that a minimum of 2 weeks of supplementation was required to raise muscle creatine levels. However, Boehm et al. [9] showed that there was no difference in total creatine transporter levels in cardiac muscle from rats after 6 weeks of

creatine supplementation, nor was there an increase in PCr or Cr in the heart tissue. McClung et al. [92] reported similar results after 3 weeks of supplementation. They found that although the Cr content of the heart tissue increased, total Cr (TCr), which is the sum of both Cr and PCr, did not increase.

These conflicting results may be a consequence of the animal model used, animal housing conditions, or the dosage of creatine used. In addition, the duration of study, the method of sacrifice and tissue extraction may have played a role. Then, too, there are other considerations such as the manner of feeding may also be a factor (e.g., intubation tube/ oral gavage).

In brain tissue from rats, creatine administration stops the inhibition of the Na^+,K^+ ATPase pump in a model of metabolic disease [119]. Under basal conditions the Na^+/K^+ ATPase pump uses ATP to remove Na^+ and increase intracellular K^+, thus maintaining ion homeostasis in the cellular environment. During ischemia, the Na^+/H^+ exchanger becomes activated in response to intracellular acidosis [106] which causes protons to leave the cell down its concentration gradient. The resulting influx of Na^+, occurring as a result of a reduction in ATP and thus a reduction of Na^+/K^+ pump activity, causes the intracellular accumulation of Na^+. The pump is inhibited and thus the membrane potential is negatively affected.

Zhu et al. [153] reported that creatine supplementation reduced caspase-induced cell death cascades. Caspases are signalling proteins in cells inducing apoptosis, or programmed cell death, and they are termed "executioner proteins." The cascade includes signalling molecules which activate post transcriptional changes in effector caspases which, then, cause apoptosis in the cell. Active caspase-3 and cytochrome c were found to decrease in neurons after creatine supplementation.

The expression of the insulin dependent glucose transporter, GLUT4, increases in rat and human skeletal muscle with creatine supplementation [65,104], as does AMP-activated protein-kinase (AMPK) phosphorylation [22]. AMPK is involved in the regulation of fuel supply and energy-generating pathways in response to the metabolic needs of the organism. It is activated in response to hormonal signals and stressors such as strenuous exercise, anoxia, and ischemia which increase the AMP/ATP ratio. Ceddia and Sweeney [23] have also observed decreased lactate production and increased glucose oxidation with creatine supplementation. Other researchers have also shown increased glycogen storage in skeletal muscles in humans during creatine supplementation [31,120], thus promoting the storage of energy reserves for use when required.

From the above research findings it would seem that creatine supplementation increases both the energy reserves and the mobilization of energy reserves in the heart and, therefore, would leave it better prepared to withstand an ischemic event. Increased phosphate for regeneration of ATP stores, increased glycogen for energy, as well as increased GLUT4 for glucose uptake and glycolysis during an ischemic event would be expected to afford protection against ischemia and reperfusion injury.

Creatine Supplementation and Exercise in Laboratory Studies
Effects of Exercise and Creatine on Infarct Size
Reduction in infarct size with exercise was reported as far back as the 1970s [93]. Infarct size was reduced in exercised rats that were subjected to 48 hrs of *in vivo* coronary artery occlusion. This benefit may have been partly related to increased myocardial vascularity that was observed [93]. Melling et al. [96] found decreased infarct size in the ex vivo heart excised after 24 hrs of acute exercise (60 min of treadmill running) and subjected to regional ischemia, with an increase in HSP70, possibly providing the protection. Brown et al. [15] showed decreased cardiac infarct sizes after

treadmill run training in rats trained for a 1 hr a day for 20 weeks. Exercise training also induced a reduction in infarct size *in vivo* in rats subjected to an 8-week swimming regime 3 hrs per day, 5 days per week [152].

Although De Waard and Duncker [32] reported that exercise in mice using voluntary wheel running for 8 weeks reduced post-MI mortality and reduced LV dysfunction, it did not reduce infarct size. In fact, the thickness and area of infarct worsened in the exercise trained group. Infarct size was reduced in the brain after 3 weeks of creatine supplementation in an induced stroke model in mice [112]. This was independent of levels of Cr, PCr or ATP, which were found to be unaltered in the brain tissue. The same group found that life-long creatine administration failed to decrease infarct size in the brain after an induced stroke, suggesting that adaptive mechanisms could occur which compromise the beneficial effects of creatine. Data from Rawson et al [117] implied that oral creatine supplementation does not reduce skeletal muscle damage or improve functional recovery after hypoxic resistance exercise. These data illustrate that information on the effect of creatine supplementation and exercise in regards to infarct size in various organs are contradictory, some showing protection while others failed to show such benefits.

Effects of Exercise and Creatine on Post Ischemic Cardiac Function

Zhang et al. [152] found that after 8 weeks of free loading swim training 3 hrs per day, 5 days per week, left ventricular systolic pressure (LVSP) improved in rats subjected to regional ischemia *in vivo*. Demirel et al. [29] found an improved myocardial LVDP and rate pressure product (RPP) recovery after 5 days of treadmill exercise training for 20 min per day. This was associated with an increased HSP72 expression and antioxidant enzyme activity, showing beneficial effects of short term exercise. Lennon et al. [80] found that moderate (55% VO_2 max) and high intensity treadmill training provided protection against 20 min of global ischemia as reflected by enhanced recovery of cardiac output (Q) and cardiac work, while RPP recovery, heart rate and coronary flow were no different from controls. Burelle et al. [19] also found that treadmill training for 10 weeks (4 days per week) protected isolated hearts against reperfusion injury when using Q as the end point. They found the hearts from exercise trained animals had higher glucose and palmitate oxidation rates before and after ischemia and lower glycolysis rates at these times.

Cardioprotection against ischemia and reperfusion damage was seen in hearts from exercised rats in males but not females [136]. The female's hearts displayed better recovery of LVDP than the hearts of males, but not better than their control, post-ischemic values. It was postulated that the female heart was possibly already maximally protected by estrogen and could, therefore, not be further protected by exercise training. Starnes et al. [130] found low intensity training (55-60% VO_2 max) did not improve cardiac recovery of heart work after 20 min global ischemia and reperfusion. Brown et al. [15] found no LVDP or CF differences under baseline conditions, and although LVDP was greater immediately after ischemia in trained hearts, LVDP had decreased to values comparable to those of control hearts by the end of reperfusion. One study by Mancardi et al. [88] has shown that stressful forced exercise using treadmill training is detrimental to the ischemic heart, increasing infarct size and decreasing LVDP recoveries in the heart.

Many of the studies that have documented cardioprotection with exercise training have used different end points to assess reperfusion myocardial viability. These end points include coronary flow (CF), active tension, LVDP recovery, Q, cardiac work recovery, and infarct size) [16,78,118,152]. This is possibly because these groups looked at the effects of regional [16,152] or low flow ischemia [78,118] on these parameters, while no study has been documented on total global ischemia. The exercise models used were also different. Zhang et al. [152] used swim training similar to our model and

Brown et al. [16], Reger et al. [118] and Le Page et al. [78] used treadmill training. These differences in model may have led to different results.

Bowles and Starnes [12] and Lennon et al. [80] looked at Q and cardiac work recovery and found that it was increased. However, considering that Q is a function of both aortic output (AO) and coronary flow (CF), the increased Q may have been due to an increase in CF without an increase in AO. Myocardial function (pressure and stroke work) was preserved by creatine infusion in a model of coronary artery bypass grafting. Creatine infusion for 10 min during CAL and 10 min of reperfusion increased myocardial cellular ATP levels during ischemia and reperfusion in the treated animals [149]. Creatine supplementation in cardioplegic solution during heart surgery also resulted in better post surgery left ventricular work [133].

Interestingly, Thorelius [135] showed that creatine phosphate in a cardioplegic solution led to better stroke work after aortic valve surgery even though no increases in myocardial ATP or PCr levels were observed. Yet, creatine supplementation (1% body weight in powdered rat chow) for 21 days did not provide cardioprotection during global ischemia (which was induced until ATP was completely depleted in the heart) in rats [107]. Here the Langendorff perfusion apparatus was used and mechanical functional measured was HR multiplied by systolic pressure. The time taken to restore function to normal after ischemia was similar in untreated and creatine supplemented hearts.

Effects of Exercise and Creatine on Biochemical markers
A combination of swim training and creatine supplementation for 2 months in hypertensive rats increased mitochondrial creatine kinase (CK $_{Mi}$) expression [44]. Increased CK $_{Mi}$ expression in the myocardium is characteristically associated with hypertrophy. Hypertrophy in hypertensive hearts is associated with increased risk of cardiac death that is not characteristic of exercised hearts. Pressure overload and coronary artery disease both caused increased CK expression in a study by Ingwall and co-workers [61]. This anomaly has not been addressed in either study.

In a study by McClung et al. [92], chronic exercise stress in rats induced a significant decrease in cardiac-muscle total RNA. A loss of cardiac RNA results in a decrease in muscle protein which is detrimental to mechanical function of the heart. Creatine supplementation, in conjunction with the same exercise stress, corrected this attenuation and resulted in values of RNA that were comparable to those of control animals.

CONCLUSION

The majority of scientific evidence supports a positive outcome after the use of creatine and exercise. However, there is also evidence suggesting that the "positive outcome" is not always the case. This conflicting information in the literature supports the need for more research to be done before this supplement can be regarded as safe or marketed as an effective treatment for clinical conditions and a miracle supplement for sportsmen and women.

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REFERENCES

1. Andres RH, Ducray AD, Schlattner U, Wallimann T, Widmer HR. Functions and effects of creatine in the central nervous system. *Brain Res Bull* 2008;76(4):329-43.

2. Antolic A, Roy BD, Tarnopolsky MA, Zernicke RF, Wohl GR, Shaughnessy SG, Bourgeois JM. Creatine monohydrate increases bone mineral density in young Sprague-Dawley rats. *Med Sci Sports Exerc* 2007;39(5):816-820.

3. Arad M, Seidman CE, Seidman JG. AMP-activated protein kinase in the heart: Role during health and disease. *Circ Res* 2007;100(4):474-488.

4. Ascensao A, Magalhaes J, Soares JM, Ferreira R, Neuparth MJ, Marques F, Oliveira PJ, Duarte JA. Moderate endurance training prevents doxorubicin-induced in vivo mitochondriopathy and reduces the development of cardiac apoptosis. *Am J Physiol Heart Circ Physiol* 2005;H722–731.

5. Barisic N, Bernert G, Ipsiroglu O, Stromberger C, Müller T, Gruber S, Prayer D, Moser E, Bittner RE, Stöckler-Ipsiroglu S. Effects of oral creatine supplementation in a patient with MELAS phenotype and associated nephropathy. *Neuropediatrics* 2002;33(3):157-161.

6. Bassi R, Heads R, Marber MS, Clark JE. Targeting p38-MAPK in the ischemic heart: Kill or cure? *Curr Opin Pharmacol* 2008;8(2):141-146.

7. Bessman SP, Geiger PJ. Transport of energy in muscle: The phosphocreatine shuttle. *Science* 1981; 211:448-452.

8. Blumenthal JA, Sherwood A, Gullette EC, Babyak M, Waugh R, Georgiades A, Craighead LW, Tweedy D, Feinglos M, Appelbaum M, Hayano J, Hinderliter A. Exercise and weight loss reduce blood pressure in men and women with mild hypertension: effects on cardiovascular, metabolic, and hemodynamic functioning. *Arch Intern Med* 2000;160(13):1947-1958.

9. Bo H, Jiang N, Ma G, Qu J, Zhang G, Cao D, Wen L, Liu S, Ji LL, Zhang Y. Regulation of mitochondrial uncoupling respiration during exercise in rat heart: role of reactive oxygen species (ROS) and uncoupling protein 2. *Free Radic Biol Med* 2008;44(7):1373-1381.

10. Boehm E, Chan S, Monfared M, Wallimann T, Clarke K, Neubauer S. Creatine transporter activity and content in the rat heart supplemented by and depleted of creatine. *Am J Physiol Endocrinol Metab* 2003;284: E399–E406.

11. Boluyt MO, Brevick JL, Rogers DS, Randall MJ, Scalia AF, Li ZB. Changes in the rat heart proteome induced by exercise training: Increased abundance of heat shock protein hsp20. *Proteomics* 2006;6:3154–3169.

12. Borsook H, Dubnoff JW. The hydrolysis of phosphocreatine and the origin of urinary creatinine. *J Biol Chem* 1947;168:493–510.

13. Bowles DK, Starnes JW. Exercise training improves metabolic response after ischemia in isolated working rat heart. *J Appl Physiol* 1994;76:1608–1614.

14. Braissant O, Henry H, Loup M, Eilers B, Bachmann C. Endogenous synthesis and transport of creatine in the rat brain: An *in situ* hybridization study. *Mol Brain Res* 2001;86:193–201.

15. Brookes PS, Yoon Y, Robotham JL, Anders MW, Sheu SS. Calcium, ATP, and ROS: A mitochondrial love–hate triangle. *Am J Physiol Cell Physiol* 2004;4:C817–833.

16. Brown DA, Jew KN, Sparagna GC, Musch TI, Moore RL. Exercise training preserves coronary flow and reduces infarct size after ischemia-reperfusion in rat heart. *J Appl Physiol* 2003; 95: 2510–2518.

17. Brown DA, Chicco AJ, Jew KN, Johnson MS, Lynch JM, Watson PA, Moore RL. Cardioprotection afforded by chronic exercise is mediated by the sarcolemmal, and not the mitochondrial, isoform of the KATP channel in the rat. *J Physiol* 2005;569(3):913-924.

18. Brzezinska Z, Nazar K, Kaciuba-Uscilko H, Falecka-Wieczorek I, Wójcik-Ziólkowska E. Effect of a short-term dietary creatine supplementation on high-energy phosphates in the rat myocardium. *J Physiol Pharmacol* 1998;49(4):591-595.

19. Budas GR, Jovanovic S, Crawford RM, Jovanovic A. Hypoxia-induced preconditioning in adult stimulated cardiomyocytes is mediated by the opening and trafficking of sarcolemmal KATP channels. *FASEB J* 2004;18(9):1046-1048.

20. Burelle Y, Wambolt RB, Grist M, Parsons HL, Chow JCF, Antler C, Bonen A, Keller A, Dunaway GA, Popov KM, Hochachka PW, Allard MF. Regular exercise is associated with a protective metabolic phenotype in the rat heart. *Am J Physiol Heart Circ Physiol* 2004; 287: H1055–H1063.

21. Byrd SK. Alterations in the sarcoplasmic reticulum: a possible link to exercise-induced muscle damage. *Med Sci Sports Exerc* 1992;24(5):531-536.

22. Cadenas E. Mitochondrial free radical production and cell signaling. *Mol Aspects Med* 2004;1–2:17–26.

23. Ceddia RB, Sweeney G. Creatine supplementation increases glucose oxidation and AMPK phosphorylation and reduces lactate production in L6 rat skeletal muscle cells. *J Physiol* 2004; 555(2):409-421.

24. Chen ZP, McConell GK, Michell BJ, Snow RJ, Canny BJ, Kemp BE. AMPK signaling in contracting human skeletal muscle: acetyl-CoA carboxylase and NO synthase phosphorylation. *Am J Physiol Endocrinol Metab* 2000;279: E1202–E1206.

25. Chen ZP, Stephens TJ, Murthy S, Canny BJ, Hargreaves M, Witters LA, Kemp BE, McConell GK. Effect of exercise intensity on skeletal muscle AMPK signaling in humans. *Diabetes* 2003;52: 2205–2212.

26. Cox ML, Bennett JB, Dudley GA. Exercise training induced alterations of cardiac morphology. *J Appl Physiol* 1986;61(3):926-931.

27. Da Silva R, Grampp T, Pasch T, Schaub MC, Zaugg M. Differential activation of mitogen-activated protein kinases in ischemic and anesthetic preconditioning. *Anesthesiology* 2004;100(1):59-69.

28. Das B, Sarkar C. Cardiomyocyte mitochondrial KATP channels participate in the antiarrhythmic and antiinfarct effects of KATP activators during ischemia and reperfusion in an intact anaesthetized rabbit model. *Polish J Pharmacol* 2003;55:771–786.

29. Davis ME, Cai H, Drummond GR, Harrison DG. Shear stress regulates endothelial nitric oxide synthase expression through c-Src divergent signaling pathways. *Circ Res* 2001;89:1073-1080.

30. Demirel HA, Powers SK, Zergeroglu MA, Shanely RA, Hamilton K, Coombes J, Naito H. Short term exercise improves myocardial tolerance to in vivo ischemia/ reperfusion in the rat. *J. Appl. Physiol* 2001;91:2205–2212.

31. Dempsey RL, Mazzone MF, Meurer LN. Does oral creatine supplementation improve strength? *J Fam Pract* 2002;51(1):945-951.

32. Derave W, Eijnde BO, Verbessem P, Ramaekers M, Van Leemputte M, Richter EA, Hespel P. Combined creatine and protein supplementation in conjunction with resistance training promotes muscle GLUT-4 content and glucose tolerance in humans. *J Appl Physiol* 2003;94(5):1910-1916.

33. De Waard MC, Duncker DJ. Prior exercise improves survival, infarct healing, and left ventricular function after myocardial infarction. *J Appl Physiol* 2009;107(3):928-36.

34. Diamant M, Tushuizen ME, Sturk A, Nieuwland R. Cellular microparticles: New players in the field of vascular disease? *Eur J Clin Invest* 2004;34(6):392-401.

35. Du Toit EF, Genis A, Opie LH, Pollesello P, Lochner A. A role for the RISK pathway and K(ATP) channels in pre- and post-conditioning induced by levosimendan in the isolated guinea pig heart. *Br J Pharmacol* 2008;154(1):41-50.

36. Dufaux B, Order U, Liesen H. Effect of a short maximal physical exercise on coagulation, fibrinolysis, and complement system. *Int J Sports Med* 1991;12(1):S38-42.

37. Edison EE, Brosnan ME, Meyer C, Brosnan JT. Creatine synthesis: production of guanidinoacetate by the rat and human kidney in vivo. *Am J Physiol Renal Physiol* 2007;293:1799-1804.

38. Englert, HC, Heitsch H, Gerlach U, Knieps S. Blockers of the ATP sensitive potassium channel SUR2A/Kir6.2: a new approach to prevent sudden cardiac death. *Curr Med Chem Cardiovasc Hematol Agents* 2003;1:253–271.

39. Felber S, Skladal D, Wyss M, Kremser C, Koller A, Sperl W. Oral creatine supplementation in Duchenne muscular dystrophy: a clinical and 31P magnetic resonance spectroscopy study. *Neurol Res* 2000;22(2):145-150.

40. Fletcher GF, Blair SN, Blumenthal J, Casperson C, Chaitman B, Epstein S, Falls H, Froelicher ES, Froelicher VF, Pina IL. Benefits and recommendations for physical activity programs for all Americans: a statement for health professionals by the committee on exercise and cardiac rehabilitation of the Council on Clinical Cardiology, American Heart Association. *Circulation* 1992;86:340–344.

41. Foreman J. *The Boston Globe* (Boston, MA). 17 April 2006.

42. French JP, Hamilton KL, Quindry JC, Lee Y, Upchurch PA, Powers SK. Exercise-induced protection against myocardial apoptosis and necrosis: MnSOD, calcium-handling proteins, and calpain. *FASEB J* 2008;22: 2862–2871.

43. Froelicher VF. Does exercise protect from coronary artery disease? *Adv Cardiol* 1980;27:237-242.

44. George K, Shave R, Warburton D, Scharhag J, Whyte G. Exercise and the heart: Can you have too much of a good thing? *Med Sci Sports Ex* 2008;40(8):1390-1392.

45. Golden AL, Bright JM, Lawler JE. Changes in creatine kinase expression induced by exercise in borderline hypertensive rat hearts. *Clin Exp Hypertens* 1994;16(5):577-593.

46. Gordon A, Hultman E, Kaijser L, Kristjansson S, Rolf CJ, Nyquist O, Sylvén C. Creatine supplementation in chronic heart failure increases skeletal muscle creatine phosphate and muscle performance. *Cardiovasc Res* 1995;30(3):413-418.

47. Green AL, Hultman E, Macdonald IA, Sewell DA, Greenhaff PL. Carbohydrate ingestion augments skeletal muscle creatine accumulation during creatine supplementation in humans. *Am J Physiol* 1996;271(5 Pt 1):E821-826.

48. Gross GJ, Peart JN. KATP channels and myocardial preconditioning: An update. *Am J Physiol Heart Circ Physiol* 2003;285(3):H921-30.

49. Gute D, Fraga C, Laughlin MH, Amann JF. Regional changes in capillary supply in skeletal muscle of high-intensity endurance-trained rats. *J Appl Physiol* 1996;81(2):619-626.

50. Halse R, Fryer LG, McCormack JG, Carling D, Yeaman SJ. Regulation of glycogen synthase by glucose and glycogen: A possible role for AMP-activated protein kinase. *Diabetes* 2003;52(1):9-15.

51. Hamilton KL, Staib JL, Phillips T, Hess A, Lennon SL, Powers SK. Exercise, antioxidants, and HSP72: Protection against myocardial ischemia/reperfusion. *Free Rad Biol & Med* 2003;34(7):800–809.

52. Hardie DG, Carling D. The AMP-activated protein kinase--fuel gauge of the mammalian cell? *Eur J Biochem* 1997;246(2):259-273.

53. Hass CJ, Collins MA, Juncos JL. Resistance training with creatine monohydrate improves upper-body strength in patients with Parkinson disease: a randomized trial. *Neurorehabil Neural Repair* 2007;21(2):107-115.

54. Herda TJ, Beck TW, Ryan ED, Smith AE, Walter AA, Hartman MJ, Stout JR, Cramer JT. Effects of creatine monohydrate and polyethylene glycosylated creatine supplementation on muscular strength, endurance, and power output. *J Strength Cond Res* 2009;23(3):818-26.

55. Hickner RC, Dyck DJ, Sklar J, Hatley H, Byrd P. Effect of 28 days of creatine ingestion on muscle metabolism and performance of a simulated cycling road race. *J Int Soc Sports Nutr* 2010;7;7:26.

56. Hoberman HD, Sims EAH, Peters JH. Creatine and creatinine metabolism in the normal male adult studied with the aid of isotopic nitrogen. *J Biol Chem* 1948;172: 45–58.

57. Holloszy JO, Skinner JS, Barry AJ, Cureton TK. effect of physical conditioning on cardiovascular function. A Ballistocardiographic Study. *Am J Cardiol* 1964;14:761-769.

58. Hoogwerf BJ, Laine DC, Greene E. Urine C-peptide and creatinine (Jaffe method) excretion in healthy young adults on varied diets: Sustained effects of varied carbohydrate, protein, and meat content. *Am J Clin Nutr* 1986;43:350–360.

59. Hsu TG, Hsu KM, Kong CW, Lu FJ, Cheng H, Tsai K. Leukocyte mitochondria alterations after aerobic exercise in trained human subjects. *Med Sci Sports Exerc* 2002;34(3):438-442.

60. Husain K, Somani SM. Interaction of exercise and adenosine receptor agonist and antagonist on rat heart antioxidant defense system. *Mol Cell Biochem* 2005;270:209–214.

61. Iemitsu M, Maeda S, Jesmin S, Otsuki T, Miyauchi T. Exercise training improves aging-induced downregulation of VEGF angiogenic signaling cascade in hearts. *Am J Physiol Heart Circ Physiol* 2006;291(3):H1290-298.

62. Ingwall JS, Kramer MF, Fifer MA, Lorell BH, Shemin R, Grossman W, Allen PD. The creatine kinase system in normal and diseased human myocardium. *N Engl J Med* 1985; 313(17):1050-4.

63. Ipsiroglu OS, Stromberger C, Ilas J, Höger H, Mühl A, Stöckler-Ipsiroglu S. Changes of tissue creatine concentrations upon oral supplementation of creatine-monohydrate in various animal species. *Life Sci* 69(15):1805-1815.

64. Izquierdo M, Ibañez J, González-Badillo JJ, Gorostiaga EM. Effects of creatine supplementation on muscle power, endurance, and sprint performance. *Med Sci Sports Exerc* 2002;34(2):332-343.

65. Janse MJ, Wit AL. Electrophysiological mechanisms of ventricular arrhythmias resulting from myocardial ischemia and infarction. *Physiol Rev* 1989;69: 1049–1169.

66. Ju JS, Smith JL, Oppelt PJ, Fisher JS. Creatine feeding increases GLUT4 expression in rat skeletal muscle. ***Am J Physiol Endocrinol Metab*** 2005;288(2):E347-352.

67. Kammer RT. Lone atrial fibrillation associated with creatine monohydrate supplementation. ***Pharmacotherapy*** 2005;25(5):762-764.

68. Kapelko VI, Kupriyanov V, Novikova N.A, Lakomkin VL, Steinschneider AY, Severina MY, Veksler VI, Saks VA. The cardiac contractile failure induced by chronic creatine and phosphocreatine deficiency. ***J Mol Cell Cardiol*** 1988;20(6) 465-479.

69. Kingsley M, Cunningham D, Mason L, Kilduff LP, McEneny J (2009). Role of creatine supplementation on exercise-induced cardiovascular function and oxidative stress. **Oxid Med Cell Longev** 2(4):247-254.

70. Kley RA, Vorgerd M, Tarnopolsky MA. Creatine for treating muscle disorders. ***Cochrane Database Syst Rev*** 2007;24(1):CD004760.

71. Koivisto VA, Sherwin RS. Exercise in diabetes: Therapeutic implications. ***Postgrad Med*** 1979 Nov; 66(5):87-91, 94-96.

72. Kongas O, van Beek JHGM. Creatine Kinase in energy metabolic signaling in muscle. ***Nature Precedings*** : doi:10.1038/npre.2007.1317.1

73. Kraegen EW, Storlien LH, Jenkins AB, James DE. Chronic exercise compensates for insulin resistance induced by a high-fat diet in rats. ***Am J Physiol*** 1989;256(2:1):E242-249.

74. Kramsch DM., Aspen AJ, Abramowitz BM, Kreimendahl T, Wood WB. Reduction of coronary atherosclerosis by moderate conditioning exercise in monkeys on an atherogenic diet. ***N Engl J Med*** 1981;305:1483–1489.

75. La Gerche A, Prior DL. Exercise—Is it possible to have too much of a good thing? ***Heart Lung Circ*** 2007;16(3):S102-104.

76. Laaksonen MS, Kalliokoski KK, Luotolahti M, Kemppainen J, Teräs M, Kyröläinen H, Nuutila P, Knuuti J. Myocardial perfusion during exercise in endurance-trained and untrained humans. ***Am J Physiol Regul Integr Comp Physiol*** 2007;293(2):R837-843.

77. Lajoie C, Calderone A, Béliveau L Exercise training enhanced the expression of myocardial proteins related to cell protection in spontaneously hypertensive rats**. *Pflugers Arch*** 2004;449(1):26-32.

78. Lajoie C, Calderone A, Trudeau F, Lavoie N, Massicotte G, Gagnon S, Béliveau L. Exercise training attenuated the PKB and GSK-3 dephosphorylation in the myocardium of ZDF rats. ***J Appl Physiol*** 2004;96(5):1606-1612.

79. Le Page C, Noirez P, Courty J, Riou B, Swynghed B, Besse S. Exercise training improves functional post-ischemic recovery in senescent heart**. *Exper Gerontol*** 2009;44:177–182.

80. Lee HJ, Fillers WS, Iyengar MR. Phosphocreatine, an intracellular high-energy compound, is found in the extracellular fluid of the seminal vesicles in mice and rats. *Proc Natl Acad Sci USA* 1988;85(19):7265-7269.

81. Lennon SL, Quindry JC, Hamilton KL, French JP, Hughes J, Mehta JL, Powers SK. Elevated MnSOD is not required for exercise-induced cardioprotection against myocardial stunning. *Am J Physiol Heart Circ Physiol* 2004;287:H975–H980.

82. Lenz H, Schmidt M, Welge V, Schlattner U, Wallimann T, Elsässer HP, Wittern KP, Wenck H, Stäb F, Blatt T. The creatine kinase system in human skin: Protective effects of creatine against oxidative and UV damage in vitro and in vivo. *J Invest Dermatol* 2005;124(2):443-452.

83. Leung FP, Yung LM, Laher I, Yao X, Chen ZY, Huang Y. Exercise, vascular wall and cardiovascular disease. *Sports Med* 2008;38(12):1009-1024.

84. Little JP, Forbes SC, Candow DG, Cornish SM, Chilibeck PD. Creatine, arginine, alpha-ketoglutarate, amino acids and medium chain triglycerides and endurance performance. *Int J Sport Nutr Exerc Metab* 2008;18(5):493-508.

85. Loike JD, Somes M, Silverstein SC: Creatine uptake, metabolism, and efflux in human monocytes and macrophages. *Am J Physiol* 1986;251:C128–C135.

86. Loike JD, Zalutsky DL, Kaback E, Miranda AF, Silverstein SC. Extracellular creatine regulates creatine transport in rat and human muscle cells. *Proc Natl Acad Sci USA* 1988;85(3):807-811.

87. Loud AV, Beghi C, Olivetti G, Anversa P. Morphometry of right and left ventricular myocardium after strenuous exercise in preconditioned rats. *Lab Invest* 1984;51(1):104-111.

88. Maarbjerg SJ, Jorgensen SB, Rose AJ, Jeppesen J, Jensen TE, Treebak JT, Birk JB, Schjerling P, Wojtaszewski JF, Richter EA. Genetic impairment of {alpha}2-AMPK signaling does not reduce muscle glucose uptake during treadmill exercise in mice. *Am J Physiol Endocrinol Metab* 2009;297(4):E924-934.

89. Mancardi D, Tullio F, Crisafulli A, Rastaldo R, Folino A, Penna C, Pagliaro P. Omega 3 has a beneficial effect on ischemia/ reperfusion injury, but cannot reverse the effect of stressful forced exercise. *Nutr Metab Cardiovasc Dis* 2009;19(1):20-26.

90. Marom-Klibansky R, Drory Y. Physical activity for the elderly. *Harefuah* 2002;141(7):646-50, 665, 664.

91. Marsh SA, Coombes JS. Exercise and the endothelial cell. *International J Cardiol* 2005;99:165–169.

92. McCall W, Persky AM. Pharmacokinetics of creatine. *Subcell Biochem* 2007;46:261-273.

93. McClung JM, Hand GA, Davis JM, Carson JA. Effect of creatine supplementation on cardiac muscle of exercise stressed rats. *Eur J Appl Physiol* 2003;89(1):26-33.

94. McElroy CL, Gissen SA, Fishbein MC. Exercise-induced reduction in myocardial infarct size after coronary artery occlusion in the rat. *Circulation* 1978;57(5):958-962.

95. McGuire DM, Gross MD, Van Pilsum JF, Towle HC. Repression of rat kidney L-arginine:glycine amidinotransferase synthesis by creatine at a pretranslational level. *J Biol Chem* 1984;259:12034–12038.

96. McMullen JR, Jennings GL. Differences between pathological and physiological cardiac hypertrophy: novel therapeutic strategies to treat heart failure. *Clin Exp Pharmacol Physiol* 2007;34:255–262.

97. Melling CW, Thorp J, Thorp DB, Milne KJ, Noble EG. Myocardial Hsp70 phosphorylation and PKC-mediated cardioprotection following exercise. *Cell Stress and Chaperones* 2009;14:141–150.

98. Moore NP. The distribution, metabolism and function of creatine in the male mammalian reproductive tract: A review. *Int J Androl* 2000;23: 4–12.

99. Morris JN, Hardman AE. Walking to Health. *Sports Med* 1997;23(5):306-332.

100. Neubauer S, Remkes H, Spindler M, Horn M, Wiesmann F, Prestle J, Walzel B, Ertl G, Wallimann T. Downregulation of the Na(+)-creatine cotransporter in failing human myocardium and in experimental heart failure. *Circulation* 1999;100(18):1847-1850.

101. Niederberger M, Mlczoch J, Kühn P, Sperlich S. Exercise therapy in coronary disease. An overall practical model for long-term rehabilitation. *Acta Med Austriaca* 1977;4(1):20-24.

102. Noma A. ATP-regulated K1 channels in cardiac muscle. **Nature** 1983;305:147–148.

103. O'Rourke B. Myocardial KATP channels in preconditioning. *Circ Res* 2000;87;845-855.

104. Op't Eijnde B, Vergauwen L, Hespel P. Creatine loading does not impact on stroke performance in tennis. *Int J Sports Med* 2001;22(1):76-80.

105. Op't Eijnde B, Ursø B, Richter EA, Greenhaff PL, Hespel P. Effect of oral creatine supplementation on human muscle GLUT4 protein content after immobilization. *Diabetes* 2001;50:18–23.

106. Op't Eijnde B, Jijakli H, Hespel P, Malaisse WJ. Creatine supplementation increases soleus muscle creatine content and lowers the insulinogenic index in an animal model of inherited type 2 diabetes. *Int J Mol Med* 2006;17(6):1077-1084.

107. Opie LH. Myocardial metabolism and heart disease. *Jpn Circ J* 1978;42(11):1223-1247.

108. Osbakken M, Ito K, Zhang D, Ponomarenko I, Ivanics T, Jahngen EG, Cohn M. Creatine and cyclocreatine effects on ischemic myocardium: 31P nuclear magnetic resonance evaluation of intact heart. *Cardiology* 1992;80(3-4):184-195.

109. Ostojic SM, Ahmetovic Z. Gastrointestinal distress after creatine supplementation in athletes: are side effects dose dependent? *Res Sports Med* 2008;16(1):15-22.

110. Ponjee GA, Janssen EM, Hermans J, Wersch JW. Regular physical activity and changes in risk factors for coronary heart disease: a nine months prospective study. **Eur J Clin Chem Clin Biochem** 1996;34(6):477-483.

111. Poortmans JR, Kumps A, Duez P, Fofonka A, Carpentier A, Francaux M. Effect of oral creatine supplementation on urinary methylamine, formaldehyde, and formate. **Med Sci Sports Exerc** 2005;37(10):1717-1720.

112. Powers SK, Hamilton K. Antioxidants and exercise. **Clin Sports Med.** 1999;18(3):525-536.

113. Prass K, Royl G, Lindauer U, Freyer D, Megow D, Dirnagl U, Stöckler-Ipsiroglu G, Wallimann T, Priller J. Improved reperfusion and neuroprotection by creatine in a mouse model of stroke. **J Cereb Blood Flow Metab** 2007;27(3):452-429.

114. Quindry JC, French J, Hamilton K, Lee Y, Mehta JL, Powers S. Exercise training provides cardioprotection against ischemia–reperfusion induced apoptosis in young and old animals. **Exp Gerontol** 2005;40(5):416-425.

115. Raab W. Metabolic protection and reconditioning of the heart muscle through abitual physical exercise. **Ann Intern Med** 1960;53:87-105.

116. Ravagnan L, Gurbuxani S, Susin SA, Maisse C, Daugas E, Zamzami N, Mak T, Jaattela M, Penninger JM, Garrido C, Kroemer G. Heat-shock protein 70 antagonizes apoptosis-inducing factor. **Nat Cell Biol** 2001;3:839–843.

117. Rawson ES, Wehnert ML, Clarkson PM. Effects of 30 days of creatine ingestion in older men. **Eur J Appl Physiol Occup Physiol** 1999;80(2):139-44.

118. Rawson ES, Conti MP, Miles MP. Creatine Supplementation Does Not Reduce Muscle Damage or Enhance Recovery From Resistance Exercise. **J Strength & Cond Res** 2007; 21(4):1002-1324.

119. Reger PO, Barbe MF, Amin M, Renna BF, Hewston LA, MacDonnell SM, Houser SR, Libonati JR. Myocardial hypoperfusion/reperfusion tolerance with exercise training in hypertension. **J Appl Physiol** 2006;100(2):541-547.

120. Ribeiroa CAJ, Leipnitza G, Amarala AU, de Bortolic G, Seminottia B, Wajnera M. Creatine administration prevents Na+,K+-ATPase inhibition induced by intracerebroventricular administration of isovaleric acid in cerebral cortex of young rats. **Brain Res** 2009; 262:81 –88.

121. Robinson TM, Sewell DA, Hultman E, Greenhaff PL. Role of submaximal exercise in promoting creatine and glycogen accumulation in human skeletal muscle. **J Appl Physiol** 1999; 87:598-604.

122. Rockstein M, Lopez T, Chesky JA.Effects of exercise on the biochemical aging of mammalian myocardium. II. Creatine phosphokinase. **Mech Ageing Dev** 1981;16(4):379-383.

123. Rose D. Ironman athletes put hearts at risk of fatal damage, experts warn. **The Times** 22 January 2007.

124. Rosengren A, Wilhelmsen L. Physical activity protects against coronary death and deaths from all causes in middled aged men. Evidence from a 20 year follow-up of the primary prevention study in Giiteborg. *AEP* 1997;7(1):69-75.

125. Sammut IA, Jayakumar J, Latif N, Rothery S, Severs NJ, Smolenski RT, Bates TE, Yacoub MH. Heat stress contributes to the enhancement of cardiac mitochondrial complex activity. *Am J Pathol* 2001;158:1821–1831.

126. Santos RV, Bassit RA, Caperuto EC, Costa Rosa LF. The effect of creatine supplementation upon inflammatory and muscle soreness markers after a 30 km race. *Life Sci* 2004;75(16):1917-1924.

127. Servais S, Couturier K, Koubi H, Rouanet JL, Desplanches D, Sornay-Mayet MH, Sempore B, Lavoie JM, Favier R. Effect of voluntary exercise on H_2O_2 release by subsarcolemmal and intermyofibrillar mitochondria. *Free Radic Biol Med* 2003;35(1):24-32

128. Sies H. Oxidative stress: Oxidants and antioxidants. *Exp Physiol* 1997;82(2):291-5.

129. Siu PM, Bryner RW, Martyn JK, Alway SE. Apoptotic adaptations from exercise training in skeletal and cardiac muscles. *FASEB J* 2004;18(10):1150-2.

130. Soboll S, Conrad A, Eistert A, Herick K, Krämer R. Uptake of creatine phosphate into heart mitochondria: a leak in the creatine shuttle. *Biochim Biophys Acta* 1997;1320(1):27-33.

131. Snow J, Murphy R. Creatine and the creatine transporter: A review. *Molec & Cell Biochem* 2001;224:169–181.

132. Starnes JW, Taylor RP, Ciccolo JT. Habitual low-intensity exercise does not protect against myocardial dysfunction after ischemia in rats. *Eur J Cardiovasc Prev Rehabil* 2005;12(2):169-174.

133. Starnes JW, Taylor RP. Exercise-induced cardioprotection: Endogenous mechanisms. *Med Sci Sports Exerc* 2007 Sep; 39(9):1537-1543.

134. Stone MH, Sanborn K, Smith LL, O'Bryant HS, Hoke T, Utter AC, Johnson RL, Boros R, Hruby J, Pierce KC, Stone ME, Garner B. Effects of in-season (5 weeks) creatine and pyruvate supplementation on anaerobic performance and body composition in American football players. *Int J Sport Nutr* 1999;9(2):146-165.

135. Thelin S, Hultman J, Ronquist G, Hansson HE. Metabolic and functional effects of creatine phosphate in cardioplegic solution. Studies on rat hearts during and after normothermic ischemia. *Scand J Thorac Cardiovasc Surg* 1987;21(1):39-45.

136. Thompson PD, Franklin BA, Balady GJ, Blair SN, Corrado D, Estes NA, Fulton JE, Gordon NF, Haskell WL, Link MS, Maron BJ, Mittleman MA, Pelliccia A, Wenger NK, Willich SN, Costa F. American Heart Association Council on Nutrition, Physical Activity, and Metabolism; American Heart Association Council on Clinical Cardiology; American College of Sports Medicine. Exercise and acute cardiovascular events placing the risks into perspective: a scientific

statement from the American Heart Association Council on Nutrition, Physical Activity, and Metabolism and the Council on Clinical Cardiology. *Circulation* 2007;115(17):2358-2368.

137. Thorelius J, Thelin S, Ronquist G, Halden E, Hansson HE. Biochemical and functional effects of creatine phosphate in cardioplegic solution during aortic valve surgery--a clinical study. *Thorac Cardiovasc Surg* 1992;40(1):10-13.

138. Thorp DB, Haist JV, Leppard J, Milne KJ, Karmazyn M, Noble EG. Exercise training improves myocardial tolerance to ischemia in male but not in female rats. *Am J Physiol Regul Integr Comp Physiol* 2007;293(1):R363-371.

139. Tuan TC, Hsu TG, Fong MC, Hsu CF, Tsai KK, Lee CY, Kong CW. Deleterious effects of short-term, high-intensity exercise on immune function: Evidence from leucocyte mitochondrial alterations and apoptosis. *Br J Sports Med* 2008;42(1):11-15.

140. Vajda S, Baczkó I, Leprán I. Selective cardiac plasma-membrane KATP channel inhibition is defibrillatory and improves survival during acute myocardial ischemia and reperfusion *Eur J Pharmacology* 2007;577:115–123.

141. Van Loon LJ, Murphy R, Oosterlaar AM, Cameron-Smith D, Hargreaves M, Wagenmakers AJ, Snow R. Creatine supplementation increases glycogen storage but not GLUT-4 expression in human skeletal muscle. *Clin Sci (London)* 2004;106(1):99-106.

142. Vandenberghe K, Gillis N, Van Leemputte M, Van Hecke P, Vanstapel F, Hespel PJ. Caffeine counteracts the ergogenic action of muscle creatine loading. *Appl Physiol* 1996;80(2):452-457.

143. Vieira RP, Duarte AC, Claudino RC, Perini A, Santos AB, Moriya HT, Arantes-Costa FM, Martins MA, Carvalho CR, Dolhnikoff M. Creatine supplementation exacerbates allergic lung inflammation and airway remodeling in mice. *Am J Respir Cell Mol Biol.* 2007;37(6):660-667.

144. Walker JB. Creatine: Biosynthesis, regulation, and function. *Adv Enzymol* 1979;50:177–252.

145. Wallimann T, Schlattner U, Guerrero L, Dolder M: The phosphocreatine circuit and creatine supplementation, both come of age! In: *Guanidino Compounds* Blackwell Science, 1994;117–129.

146. Wang Y, Huang S, Sah VP, Ross J Jr, Brown JH, Han J, Chien KR. Cardiac muscle cell hypertrophy and apoptosis induced by distinct members of the p38 mitogen-activated protein kinase family. *J Biol Chem* 1998;273(4):2161-2168.

147. Wang Q, Somwar R, Bilan PJ, Liu Z, Jin J, Woodgett JR, Klip A. Protein kinase B/Akt participates in GLUT4 translocation by insulin in L6 myoblasts. *Mol Cell Biol* 1999;19(6):4008-4018.

148. Watts EJ. Haemostatic changes in long-distant runners and their relevance to the prevention of ischemic heart disease. *Blood Coagul Fibrinolysis* 1991;2(2):221-225.

149. White FC, Bloor CM, McKirnan MD, Carroll SM. Exercise training in swine promotes growth of arteriolar bed and capillary angiogenesis in heart. *J Appl Physiol* 1998;85(3):1160-1168.

150. Williams MH, Kreider RB, Branch JD. ***Creatine: The Power Supplement.*** Illustrated Edition. *Human Kinetics*. 1999; p22.

151. Woo YJ, Grand TJ, Zentko S, Cohen JE, Hsu V, Atluri P, Berry MF, Taylor MD, Moise MA, Fisher O, Kolakowski S. Creatine phosphate administration preserves myocardial function in a model of off-pump coronary revascularization. ***J Cardiovasc Surg*** 2005; 46(3):297-305.

152. Wyss M, Felber S, Skladal D, Koller A, Kremser C, Sperl W. The therapeutic potential of oral creatine supplementation in muscle disease. ***Med Hypotheses*** 1998;51(4):333-336.

153. Yolek JS, Duncan ND, Mazzetti SA, Staron RS, Putukian M, Gómez AL, Pearson DR, Fink WJ, Kraemer WJ. Performance and muscle fiber adaptation to creatine supplementation and heavy resistance training. ***Med Sci Sports Exerc*** 1999;31(8):1147-1156.

154. Zhang KR, Liu HT, Zhang HF, Zhang QJ, Li QX, Yu QJ, Guo WY, Wang HC, Gao F. Long-term aerobic exercise protects the heart against ischemia/ reperfusion injury via PI3 kinase-dependent and Akt-mediated mechanism. ***Apoptosis*** 2007;12:1579–1588.

155. Zhu S, Li M, Figueroa BE, Liu A, Stavrovskaya IG, Pasinelli P, Beal MF, Brown RH Jr, Kristal BS, Ferrante RJ, Friedlander RM. Prophylactic creatine administration mediates neuroprotection in cerebral ischemia in mice. ***J Neurosci*** 2004;24(26):5909-5912.

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