Creatine kinase and blood pressure: Clinical and therapeutic implications
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Chapter 1

Introduction and outline of the thesis
**INTRODUCTION**

Coupling of intracellular ATP-producing and consuming processes that are spatially separated is essential to the bioenergetics of living organisms. The creatine kinase (CK; ATP:creatine N-phosphoryl transferase) system is thought to play a key role in the intracellular energy homeostasis. The CK-system couples cellular ATP-producing with ATP-consuming processes, by catalyzing the reversible transfer of a high-energy phosphate moiety (Pi) between creatine and ADP, via the reaction:

\[
\text{MgADP + CrP + H}^+ \leftrightarrow \text{MgATP + Cr.}
\]

CK is specifically located at subcellular energy producing compartments, including the mitochondrion and near glycolytic enzymes, as well as energy consuming compartments, such as Na\(^+\)/K\(^+\)-ATPase and Ca\(^{2+}\)-ATPase at cellular membranes and myosin light chain kinase and myosin ATPase at the contractile proteins. Due to this specific localization, ATP generated by glycolysis and oxidative phosphorylation, is shuttled as phosphocreatine to subcellular locations of ATP utilization, where ATP is regenerated (Figure).\(^1\)-\(^3\)

The CK-system is of particular importance in tissues that display high and variable rates of ATP turnover, including skeletal muscle, the cardiovascular system, brain, and the kidney.\(^4\)-\(^6\) In these tissues the enzyme provides ATP for muscle contraction and ion transport. There is a widespread interindividual variability in activity of the enzyme. Relatively high tissue and serum CK activity occurs commonly in the population, but is typically found in men, obese, and black people from African descent. The high CK state is a generalized condition with morphological and functional effects on different organ systems.\(^5\),\(^7\)

High CK activity was previously postulated as a genetic factor that could explain the higher blood pressures found in black people, a population subgroup with a greater prevalence of hypertension and its complications. Pressor responses were proposed to be enhanced via increased ATP availability for cardiovascular contractility, renal sodium retention, and capillary rarefaction of skeletal muscle.\(^4\) In line with this, population studies showed that serum CK activity was associated with blood pressure, independent of age, sex, BMI, and ethnicity.\(^8\),\(^9\) In addition, subjects with high CK activity were reported to display increased vascular contractility.\(^10\) However, it remains to be studied whether high CK activity explains the greater tendency for renal salt retention in black people. Furthermore, high tissue CK activity may be involved in the higher prevalence of other conditions that frequently coexist with hypertension in this population subgroup, such as obesity and uterine fibroids.
Hypertension affects more than a quarter of the adult population worldwide, nearing 1 billion people, and is the leading risk factor for cardiovascular morbidity and mortality.\textsuperscript{11-13} Therefore, research on biological pathways leading to hypertension and its unequal distribution among population subgroups is needed. We will focus on a genetically determined high CK phenotype with effects on skeletal muscle, heart, blood vessels, and the kidney, in relation to hypertension and other clinical conditions that increase hypertension risk.

**Figure. The creatine kinase system.**

The creatine kinase (CK) system shuttles ATP, generated by oxidative phosphorylation in the mitochondrion or by glycolysis, as phosphocreatine (CrP) to sites of ATP utilization, including Na\textsuperscript{+}/K\textsuperscript{+}-ATPase, Ca\textsuperscript{2+}-ATPase, and myosin-ATPase, where ATP is regenerated. CM, cellular membrane; MEM, mitochondrial outer membrane; MIM, mitochondrial inner membrane; Matrix, mitochondrial matrix; Cr, creatine; CT, creatine transporter; CK\textsubscript{cyt}, the cytosolic isoform of CK; CK\textsubscript{mi}, the mitochondrial isoform of CK; SER, sarcoplasmatic reticulum.
Vascular system and smooth muscle

An elevated arterial blood pressure is achieved either by constriction of arterioles causing diminished volume capacity or by fluid overload exceeding the capacity of the arterial tree, both resulting in increased pressure against the arterial wall.\textsuperscript{14} In hypertensive patients, the increased pressure is predominantly the result of increased total peripheral resistance of blood vessels, determined by the amount of vasoconstriction of small arteries and arterioles, or “resistance sized arteries”. These vessels are characterized by the presence of myogenic tone, i.e. their intrinsic ability to contract in response to a sudden increase of transmural pressure. This myogenic tone becomes more vigorous as vessel size decreases.\textsuperscript{15} In these arteries, CK is tightly bound near vascular smooth muscle contractile proteins, including myosin ATPase and myosin light chain kinase, where the enzyme provides ATP for smooth muscle contraction.\textsuperscript{1} In addition, high activity of the enzyme is thought to keep ADP levels near the contractile proteins low. Smooth muscle contraction consists of a fast, force generating component at high energy costs, and a slow tonic maintenance of tension at low energy costs which is thought to depend on the ability to have attached but dephosphorylated crossbridges. For this maintenance ADP is required. If ADP at the contractile proteins does not achieve the required level, excessive shortening may occur before crossbridge formation, leading to increased vasoconstriction.\textsuperscript{4} In accordance, microvascular contractility was shown to decrease with inhibition of intravascular CK.\textsuperscript{10}

In chronic hypertension vascular tone is only a short-term modulator, while structural adaptation of resistance vessels is an obligatory requirement for elevated blood pressure to be maintained for a long time. With sustained hypertension vascular smooth muscle hypertrophy leads to an increase in wall thickness and narrower lumen. As CK activity has been reported to be upregulated in trophic responses of vascular tissue to meet the increased energetic demands, high CK activity may enhance smooth muscle proliferation in hypertension.\textsuperscript{16,17}

Smooth muscle proliferation and remodeling is known to cause several other clinical conditions, including uterine fibroids, the most common pelvic benign neoplasm. Hypertension and uterine fibroids are more frequently diagnosed in black and obese women.\textsuperscript{19} As CK stimulates growth responses as well as vascular contractility, it may be hypothesized that high CK predisposes to the development of both conditions.
Skeletal Muscle
The vascular peripheral resistance is partly dependent on the morphologic characteristics of skeletal muscle. Muscle is a heterogenous tissue comprising fibers which vary in their metabolic and contractile nature and which occur in varying proportions in individual muscles. Muscle fibers are classified on the basis of these properties into two major 'types'; type I and type II. Highest CK activity of all tissues is found in type II fibers. These fibers are typically fit for burst exercise with a fast time to peak tension, with CK as the main ATP buffer. Cytosolic CK is tightly coupled to anaerobic glycolysis, whereas mitochondrial fatty acid oxidation capacity and glucose uptake are limited, rendering them relatively insulin resistant. In addition, high CK activity in these fibers is associated with capillary rarefaction and relatively high vascular resistance. In contrast, type I or "slow twitch" fibers have a slow time to peak tension, rich in mitochondria, derive ATP mainly from oxidation of fatty acids, and have a high glucose uptake, rendering them relatively fatigue-resistant. These fibers are densely vascularised.

In line with the morphological and metabolic characteristics of skeletal muscle fibers, high CK activity, as in type II fibers, may contribute to increased peripheral resistance and higher blood pressures. Furthermore, the tight coupling of CK with anaerobic glycolysis, may limit the capacity of muscle to oxidize fatty acids and glucose, leading to storage as lipid instead of utilization. Therefore, the high CK phenotype might be hypertension and obesity prone.

Heart
The heart contains 20-40% of skeletal muscle CK activity. To maintain an adequate cardiac output, the myocardium consumes more energy than any other organ. Because the amount of ATP is small (10 mM, enough for only a few beats) compared with the demand (10,000 times greater), the myocardial cell must continually re-synthesize ATP to maintain cardiac pump function. In the heart, the CK-system is of particular importance to maintain local ATP levels constant and contribute to myocardial contractile capacity. Myofibrillar CK, functionally coupled to myosin ATPase, maintains high ATP/ADP ratios and limits the rate of ADP release, which prevents a decline in maximum shortening velocity of the myofibrils. The importance of the CK-system in the myocardium is illustrated by the finding that CK activity and other components of the CK-system are reduced in the failing heart, and that intervention in the CK-system is studied as treatment for patients with heart failure.
Kidney

The cardiac output largely dependent on sodium and volume homeostasis, with the kidney as the major regulator. It is long known that sodium plays a major role in the regulation of blood pressure. However, there is a wide interindividual variability in renal sodium handling and the effect on blood pressure.\textsuperscript{24}

The amount of sodium excreted by the kidneys depends on the balance between filtration by the glomeruli and reabsorption in the tubuli. After filtration more than 99% of the filtered sodium is reabsorbed. This process is achieved by tight cooperation of exchangers, transporters, and ion channels in the nephron.\textsuperscript{25} Proximal tubule sodium handling accounts for 60-70% of reabsorption of all filtered sodium, 20 to 30% of the filtered load is absorbed in the thick ascending loop of Henle, and 5 to 10% in the distal tubule.\textsuperscript{26} Importantly, in all parts of the nephron, Na\textsuperscript{+}/K\textsuperscript{+}-ATPase resides at the basolateral surface, where it provides the force for the vectorial transport of sodium from the tubular lumen to the blood compartment, by coupling hydrolysis of ATP to the active exchange of three intracellular Na\textsuperscript{+} ions for two K\textsuperscript{+} ions.\textsuperscript{26} In the kidney, CK is functionally coupled to renal Na\textsuperscript{+}/K\textsuperscript{+}-ATPase and the ATP produced by colocalized CK is preferentially used for the high and fluctuating ATP demand of sodium transport across the tubular epithelial cells.\textsuperscript{27-29} Thus, high CK activity in the kidney tubule cells may lead to increased availability of ATP for the active process of sodium reabsorption. This may underlie the reduced ability to excrete sodium and the greater prevalence of sodium-sensitive hypertension in black people.\textsuperscript{30}

Evolutionary viewpoint

Humans as a genus appeared in the African savanna about 2 million years ago. The rapid expansion of brain size starting at that time is thought to be associated with upregulation of the CK-system in the brain, in order to adapt to the increasing metabolic demands.\textsuperscript{31,32} In the context of human evolution, the high CK phenotype was likely exceptionally efficient for the hunter-gatherer ancestors: high cardiovascular contractility and the ability to retain sodium in the kidney would help to maintain adequate blood pressures in times when sodium and volume depletion by heat exhaustion was a daily threat (the ancestors survived on a diet with only a fraction of the salt that we consume). Furthermore, the predominance of fast type II fibers in skeletal muscle would enhance the capacity for short bursts of running for survival and facilitate optimal storage of carbohydrates as lipid in times of food shortage. However, evolution has transformed the human environment, including the rapid transformation of human lifestyles from
small food-foraging societies to large and economically complex states in less than 5000 years with increased access to high-caloric and sodium rich food and lack of physical activity. Some of us may then be unfortunate and inherit a predisposition to conditions that in our modern society have harmful effects.33

**Outline of the Thesis**

This thesis consists of two parts. **PART I** focuses on CK in the kidney and resistance arteries, as a sustained elevation of blood pressure is only achieved by excess renal sodium retention or general vasoconstriction. Furthermore, associations between CK, hypertension, and other clinical conditions that frequently coexist with hypertension are studied. In **Chapter 2** we assessed whether subjects with high CK activity display enhanced sodium retention. In **Chapter 3** it is studied whether transcriptional activity of CK in resistance sized arteries correlates with blood pressure. In **Chapter 4** the association of CK activity with obesity, a risk factor for hypertension and, is addressed. In search for other risk factors we assessed whether the prevalence of hypertension is increased in women with uterine leiomyomata in **Chapter 5**.

In **PART II** we focus on the CK-system as a possible therapeutic target in hypertension and cardiovascular disease and on the effect of CK inhibition in tissues with high energy demands. If high CK activity promotes sodium retention and vascular contractility, hypertension may be more difficult to treat in subjects with high CK. Therefore, we assessed in **Chapter 6** whether serum CK is associated with hypertension treatment failure in the general population. As the CK-system is thought to be involved in hypertension and cardiovascular disease, we questioned what was known regarding therapeutic intervention in the CK-system. Thus, in **Chapter 7** we systematically searched the literature for the existing evidence on interference in the CK-system in hypertension and cardiovascular disease. In **Chapter 8** we performed a systematic review on the effect of beta-guanidinopropionic acid, an inhibitor of the flux through the CK reaction, on function and tissues with high energy demands in **Chapter 8**. Finally, in **Chapter 9A** we assessed the effect of inhibition of the CK-system with beta-guanidinopropionic acid on blood pressure and properties of resistance arteries in hypertensive animals, whereas **Chapter 9B** describes a protocol for the first-in-man study with this compound.
REFERENCES