Chapter 10

Summary and discussion
SUMMARY

An introduction for this thesis is provided in Chapter 1. The creatine kinase system is thought to play a key role in the intracellular energy homeostasis, by coupling of cellular ATP-producing with ATP-consuming processes. High activity of the enzyme was previously linked to hypertension. In this chapter we first discuss the pathways via which generalized high CK activity, with effects on skeletal muscle, heart, blood vessels, and the kidney may increase hypertension risk. In addition, we hypothesize that high activity of the enzyme may be involved in the pathogenesis of other clinical conditions that frequently coexist with hypertension.

PART I: CLINICAL CONDITIONS

The studies in the first part of this thesis mainly focus on associations between CK, blood pressure, and other clinical conditions that increase hypertension risk.

In Chapter 2 we assessed whether subjects with high serum CK activity display reduced urinary sodium excretion, as a reduced ability to excrete sodium may lead to higher blood pressures. In the kidney, sodium reabsorption is primarily driven by basolateral Na⁺/K⁺-ATPase, which exchanges intracellular sodium for potassium. The ATP for this highly energy demanding process is provided by colocalized CK. Therefore, high serum CK activity, as an indirect measure of renal CK, may be associated with reduced sodium excretion after a salt load, via increased availability of ATP for sodium reabsorption. In healthy male participants, younger than 50 years, with normotension or untreated hypertension, on 7 days of low sodium (<50 mmol/d) followed by 3 days of high sodium (>200 mmol/d), we examined differences in 24-h urine sodium excretion after a high salt diet between participants with low and high CK activity. We showed that serum CK was negatively correlated with 24-h urinary sodium excretion after a high salt diet; a correlation coefficient of -0.41 (95% CI, -0.62 to -0.16). Sodium excretion was 416.6(23.6) mmol/day in the lowest compared to 257.9(31.3) mmol/day in the highest CK tertile (p<0.001). Under the assumption that serum CK reflects tissue CK, the reduced sodium excretion with high serum CK activity may imply that CK has a contribution to sodium reabsorption in the renal tubules. Further studies should focus on the role of CK in the kidney in renal sodium handling.

The association of CK with blood pressure was also proposed to be the result of high CK activity in resistance arteries, where it regenerates ATP for vascular contractility.
Chapter 3 describes the results of a study on the possible association between human microvascular CK and blood pressure. Resistance-sized arteries from omental fat donated by consecutive women during uterine fibroid surgery were isolated. Microvascular CK isoenzyme mRNA was assessed using quantitative real-time PCR. The study shows that normalized CKB copy numbers, ranging between 5.18 and 24.43, were strongly correlated with blood pressure, with correlation coefficients for systolic and diastolic blood pressure of 0.64 (95% CI, 0.14 to 0.88) and 0.88 (0.64 to 0.96) respectively. This is the first evidence for an association between human microvascular CK gene expression and blood pressure that adds to the existing evidence on the potential role of CK in the enhancement of vascular contractility and pressor responses.

Hypertension occurs more frequently in obese people and high tissue and serum CK activity are more common in this group. We proposed that high CK activity in skeletal muscle could be involved in the pathogenesis of hypertension as well as obesity. In skeletal muscle highest CK activity is found in fast type II fibers. These fibers are particularly fit for short-term high-intensity exercise fuelled by CK and anaerobic glycolysis, with a low capacity for uptake and oxidation of fatty acids and glucose. Consequently, high skeletal muscle CK activity, as with a predominance of type II fibers, may promote storage of fatty acids and glucose as lipid in adipose tissue rather than uptake and oxidation in skeletal muscle, leading to obesity. Therefore, as a first assessment of a possible link between CK and obesity, we studied in Chapter 4 whether serum CK activity is associated with body mass index (BMI) in the general population. We analyzed a cross-sectional multi-ethnic population sample of 1444 subjects, aged 35 to 60. In multivariable linear regression analysis we showed that log serum CK is the main predictor of BMI, with an increase in BMI of 3.1 kg/m² (95% CI, 1.8 to 4.3 kg/m²) per log CK increase after adjustment for age, sex, ethnicity, educational level, and serum creatinine as a measure of muscle mass. Under the assumption that serum CK activity reflects tissue CK activity, the findings of this study may suggest that high CK activity in skeletal muscle increases obesity risk. As it is known that high CK activity in skeletal muscle is associated with capillary rarefaction, leading to increased peripheral resistance and higher blood pressures, the high CK phenotype might be hypertension and obesity prone. Further studies are needed to assess whether high CK in obesity is an epiphenomenon, or part of a causal pathway leading to obesity.

Several previous reports suggest that hypertension and obesity share a common association with uterine fibroids, the most common pelvic benign neoplasm. Although women are at greater risk of premature cardiovascular death than men, with
hypertension as a main risk factor, risk factors for hypertension in women are relatively understudied. Therefore, Chapter 5 describes the results of a retrospective cohort study on the prevalence of hypertension in women admitted for surgery for fibroids. We included 241 women with uterine fibroids (126 black), 308 women who underwent surgery for other gynaecological reasons (37 black), and 606 population controls (360 black), with a mean age of 43.4 (SD6.6), 41.3 (11.2), and 45.0 (6.6) y respectively, and a mean BMI of 27.4 (5.3), 25.5 (5.4), and 28.0 (5.6) kg/m². High blood pressure was found in 43.6, 28.6, and 24.3% of the women with fibroids, other surgery, and population controls respectively (p<0.001 for comparisons between women with fibroids and controls). Women with fibroids were more likely to have high blood pressure after adjustment for age, BMI, and ethnicity with an odds ratio of 2.7 (95% CI, 1.9 to 3.9). The most common hypothesis forwarded for the association between uterine fibroids and hypertension is that they share a common growth pathophysiology of smooth vascular and smooth uterine muscle. As CK is known to provide ATP for smooth muscle growth, high CK activity may stimulate growth responses of uterine and vascular smooth muscle, leading to a predisposition for hypertension and uterine fibroids. Furthermore, the high CK phenotype could explain the common association of fibroids and hypertension with obesity. Further research is needed to establish the role of CK as a common growth promoting mediator in the pathways leading to hypertension and uterine fibroids.

PART II: THERAPEUTIC IMPLICATIONS

In this part we focused on the CK-system as a possible therapeutic target in hypertension and cardiovascular disease and the effect of CK inhibition on 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 investigated in Chapter 6 whether serum CK activity after rest is associated with failure of hypertension treatment in the general population. We analyzed a multi-ethnic sample of the general population (N=1444), aged 34-60y. Hypertension prevalence was respectively 26.8, 30.8, and 41.2% for the lowest (<88 IU/L) through the highest population CK tertile (>145 IU/L); (p<0.001). Treatment failed in 72.9% of subjects within the highest CK tertile vs 46.7% with low CK (p=0.004). In logistic regression analysis, CK was the main predictor of treatment failure (adjusted OR 3.7; 95% CI, 1.2 to 10.9), independent of age, sex, BMI, fasting glucose, ethnicity, and education level.
Further study should focus on prospective analysis of this association, in order to assess causal inferences and whether CK could serve as a biomarker for difficult to treat hypertension.

In Chapter 7 we systematically reviewed the evidence regarding the effectiveness of interventions directly targeting the CK system as compared to placebo in adult patients with essential hypertension or cardiovascular disease. We searched MEDLINE, EMBASE, LILACS, and the Cochrane Controlled Trials Register without language restriction and included only randomized controlled trials comparing creatine or creatine analogues with placebo in patients with essential hypertension, heart failure, or myocardial infarction. The outcomes assessed were death, total myocardial infarction, hospitalizations for congestive heart failure, change in ejection fraction, and changes in diastolic or systolic blood pressure. Full reports or abstracts from 1164 papers yielded 11 trials in 1474 patients with heart failure, acute myocardial infarction, or ischemic heart disease. The drugs used were either creatine, phosphocreatine, or phosphocreatinine. In patients with heart failure no study showed a clear effect on ejection fraction. In patients with acute myocardial infarction, two out of four trials reported mortality outcomes, with no significant effect of creatine or creatine analogues (RR 0.73, 95% CI, 0.22 to 2.45). The main effect of the interventions seems to be on improvement of dysrhythmia, although there is some evidence that dyspnoea might improve in patients with heart failure. In conclusion, it is not clear whether intervention in the CK-system has an effect on mortality, progression of myocardial infarction, ejection fraction, or blood pressure in patients with hypertension and cardiovascular disease.

In Chapter 8, we performed a systematic review on the effect of the CK inhibitor beta-guanidinopropionic acid (βGPA) on function and morphology of tissues with high energy demands. βGPA attenuates the flux through the CK reaction by competitive inhibition of cellular creatine uptake. This amino acid is marked as safe for human use, but the effects and side effects are not clear. We searched the electronic databases Pubmed, EMBASE, the Cochrane Library, and LILACS from their inception through March 2011. We retrieved 131 publications, mainly considering the effect of chronic oral administration of βGPA (0.5 to 3.5%) on skeletal muscle, the cardiovascular system, and brain tissue in animals. βGPA decreased intracellular creatine and phosphocreatine in all tissues studied. In skeletal muscle, this effect induced a shift from glycolytic to oxidative metabolism, increased cellular glucose uptake, and increased fatigue tolerance. In heart tissue this shift to mitochondrial metabolism was less pronounced. Myocardial contractility was modestly reduced, including a decreased ventricular
developed pressure, albeit with unchanged cardiac output. In brain tissue adaptations in energy metabolism resulted in enhanced ATP stability and survival during hypoxia. In conclusion, chronic βGPA increases fatigue tolerance of skeletal muscle and survival during ischaemia in animal studies, with modestly reduced myocardial contractility. The findings of this review show that CK is important in cellular energy metabolism but not indispensible.

As high CK activity has been linked to higher blood pressures, inhibition of the CK-system may have a blood pressure lowering effect. Therefore, we investigated in Chapter 9A whether treatment with βGPA reduces blood pressure in the spontaneously hypertensive rat, an animal model for essential hypertension, with known high CK activity in heart and blood vessels prior to the development of hypertension. Male, 16-weeks-old spontaneously hypertensive rats (N=16) were assigned to a standard diet with or without βGPA. Blood pressure was measured weekly by the non-invasive tail-cuff method. After 4 weeks the effect on vasodilatory responses of mesenteric arteries was assessed in a wire myograph. Treatment with βGPA significantly reduced systolic and diastolic blood pressure compared to controls, by 42.7 (5.5) (p<0.001) and 35.3 (4.8) mm Hg (p=0.004). The vasodilatory response to the CK-inhibitor dinitrofluorobenzene was enhanced by 82.2% after treatment with βGPA (p=0.008). Moreover, incubation of isolated rat mesenteric arteries with 150 mg βGPA induced a 25.7(4.4)% vasodilation, demonstrating a decreased vascular contraction potential by CK inhibition. To our knowledge, we are the first to show that CK inhibition with βGPA reduces blood pressure. The results suggest that inhibition of the CK-system may be a promising new target for antihypertensive treatment. Therefore, we developed a protocol for the first-in-man study in healthy men with βGPA compared to its analogue creatine and placebo in Chapter 9B. The main outcomes will be tolerability, and changes in hemodynamic and biochemical parameters, including blood pressure and serum CK activity. A dose of 100 mg was calculated from animal studies as the human equivalent dose. This study is expected to show that there are no serious side effects with this low dose of βGPA.

DISCUSSION

Hypertension affects nearly 1 billion people worldwide and is one the most powerful contributors to cardiovascular morbidity and mortality. The prevalence of hypertension and its associated morbidity is higher in black people of African descent, but, despite extensive research, it is not fully explained why those ethnic differences exist. Thirteen
years ago it was proposed that higher CK activity in cardiovascular muscle and other tissues with high energy demands in black people could be a genetic factor contributing to the excess burden of hypertension in this group. Subsequently, a considerable amount of evidence substantiating this hypothesis was gathered. The general aim of this thesis was to build further evidence for an association between CK and pressor responses. Furthermore, our aim was to assess whether high tissue CK activity contributes to the greater occurrence of other conditions that frequently coexist with hypertension in people of African descent, such as obesity and conditions with smooth muscle proliferation involved.

**Clinical conditions**

As an elevated arterial blood pressure is achieved either by constriction of arterioles causing diminished volume capacity or through sodium retention causing fluid overload, we have further explored the role of CK in these two pathways. First, we have shown that subjects with high CK activity significantly excrete less sodium after a high salt diet. Under the assumption that serum CK reflects tissue CK, this finding may imply that high CK activity promotes sodium reabsorption in the renal tubules. Second, in line with the potential role of CK in the enhancement of vascular contractility, it was shown that transcriptional activity of the enzyme in isolated human resistance arteries strongly correlates with blood pressure. These findings may suggest that a genetically determined high CK phenotype, including high CK in the kidney and vasculature, contributes to the greater susceptibility of black people for renal sodium retention and attenuated vasodilatory responses. However, several issues need further exploration. First, the association of CK with renal sodium handling should be studied in more detail. Although serum CK is known to reflect tissue CK, it is not clear whether this is an accurate reflection of CK activity in the kidney. Direct assessment of CKB isoenzyme levels in the kidney in relation to sodium handling should provide a better insight into this relation. Second, regarding vascular CK, it is not completely clear whether upregulation of CK at the transcriptional level is associated with increased protein expression and whether this upregulation is a consequence of the hypertensive state. However, taken all the evidence together, including the strong association of CK mRNA with blood pressure, increased vascular contractility with high CK in resistance arteries as previously reported, high CK activity in heart and aorta prior to the development of hypertension in rodents, and the reduced ability to excrete sodium, a genetically programmed high CK phenotype is likely to have a contribution to vascular contractility.
and sodium retention leading to higher blood pressures. Subsequently, the hypertensive state may lead to upregulation of CK transcriptional activity to meet the increased cardiovascular energy demand, which is substantiated by rodent studies showing increased transcriptional activity and protein expression of the enzyme in conditions of ventricular pressure overload.\textsuperscript{10} Clearly, further study is needed to unravel the relation between CK mRNA, CK protein expression, and hypertension.

As a first indication of a possible link between CK and obesity, we showed that serum CK activity is associated with body mass index, which is in line with the thrifty nature of skeletal muscle containing high CK activity. Furthermore, we reported that hypertension occurs more frequently in women with uterine fibroids and proposed that CK could be a common growth prone mediator in the proliferation of uterine and vascular smooth muscle. These findings suggest that a genetically determined high CK phenotype, including high CK activity in skeletal muscle, heart, the kidney, and smooth muscle of the vasculature and other organs, may provide a link between hypertension and other conditions that frequently coexist. However, it is clear that the association between CK and obesity needs further exploration. Furthermore, assessment of CK in uterine and fibroid tissue in relation to blood pressure may provide further insights in the involvement of CK in the pathophysiology of fibroids and hypertension.

**Implications for therapy**

Successful treatment of hypertension is difficult despite the availability of several classes of antihypertensive drugs. Treatment fails in nearly half of hypertensive patients, including many patients with uncomplicated hypertension.\textsuperscript{2,11} We have reported that serum CK was the main and independent predictor of antihypertensive treatment failure in a cross-sectional population study. Obviously, prospective analysis is needed before causal inferences can be made. However, as current antihypertensive agents are far from satisfactory, especially in population subgroups with high CK activity, and CK is known to provide ATP to subcellular ATPases affecting blood pressure as the final intracellular step before pressor responses occur, drugs targeting the CK system may be effective for the treatment of hypertension. We have described the promising results of our study in hypertensive animals. Inhibition of the CK-system with βGPA significantly reduced systolic and diastolic blood pressure compared to controls, as well as CK-dependent microvascular contractility. Importantly, we have reported in our systematic review that chronic βGPA treatment had minor side effects in animals, showing that CK is important in cellular energy metabolism but not indispensable. Therefore, inhibition the CK-system may be a novel target for antihypertensive treatment. However, before
this can be implemented in clinical practice, several issues need to be addressed. First, the effect of a longer duration of βGPA treatment as well as different doses in hypertensive animals is not yet studied. Further rodent studies should focus on these aspects of βGPA treatment. Second, research should be extrapolated to humans. Our first-in-man study with βGPA should make clear whether βGPA is tolerated in healthy men and what the effects are on biochemical and hemodynamic parameters. If βGPA is well tolerated, as expected, the next step may then be the assessment of the blood pressure lowering efficacy of βGPA in hypertensive patients.

Figure 2. Clinical implications of the high creatine kinase phenotype.

This figure depicts the proposed mechanism through which the high creatine kinase (CK) phenotype with high activities in skeletal muscle, heart, kidney, and smooth muscle, may lead to hypertension. In the kidney, high CK activity may lead to increased sodium retention through increased ATP availability for Na⁺/K⁺-ATPase, leading to a higher cardiac output. In the cardiovascular system, high CK activity is thought to provide ATP to enzymes involved in contractile responses, including myosin ATPase, Ca²⁺-ATPase, and myosin light chain kinase (MLCK), leading to increased peripheral resistance of blood vessels. Furthermore, CK may promote vascular and uterine smooth muscle proliferation, explaining the common occurrence of uterine fibroids and hypertension. The vicious circle between hypertension and vascular smooth muscle hypertrophy is not depicted. Finally, in skeletal muscle coupling of CK to anaerobic glycolysis is associated with limited mitochondrial fatty acid and glucose oxidation capacity, promoting obesity. In addition, high CK activity in these fibers is associated with capillary rarefaction and increased peripheral resistance.
CONCLUSION

In conclusion, generalized high CK activity could be a plausible biological factor that explains the higher blood pressures, as the result of increased vascular tone and enhanced sodium retention, as well as the greater occurrence of obesity and hypertrophic conditions in black people (Figure). However, it should be noted that these associations are thought to be independent of ethnicity. It could be questioned whether the existing evidence proves that high CK activity can cause hypertension. As suggested in the introduction of this thesis, in the context of human evolution, the high CK phenotype could have been particularly beneficial for our ancestors: high cardiovascular contractility, the ability to retain sodium in the kidneys, and the enhanced capacity of skeletal muscle for short bursts of running and storage of carbohydrates as lipid may have increased survival rates without causing hypertension. Importantly, environmental changes only started 5000 years ago, with increasing access to high-caloric and sodium-rich food. This short time interval makes it plausible that some of us may still be genetically programmed for survival in a different environment, leading to a predisposition for hypertension and obesity in times of salt and food abundance and lack of physical activity.

The evidence in this thesis on the relation between CK and blood pressure includes association studies. An association confirms that two conditions coexist and temporality is not shown. However, the hypothesis was not refuted in those studies. On the contrary, we have shown that CK is associated with sodium excretion and have reported a strong association of vascular CK with blood pressure. Notably, we have shown that hypertension can be treated by inhibition of the CK-system, which strongly contributes to the evidence for a causal role of CK in the development of hypertension. Thus, although proving causality is difficult, the findings in this thesis call for further exploration of the pathways via which CK affects blood pressure. Eventually, this may lead to recognition of the high CK state as a condition that increases hypertension risk.
References