Creatine Kinase Activity Is Associated With Blood Pressure

Lizzy M. Brewster, MD; Gideon Mairuhu, MD; Navin R. Bindraban, MD; Richard P. Koopmans, MD, PhD; Joseph F. Clark, PhD; Gert A. van Montfrans, MD, PhD

Background—We previously hypothesized that high activity of creatine kinase, the central regulatory enzyme of energy metabolism, facilitates the development of high blood pressure. Creatine kinase rapidly provides adenosine triphosphate to highly energy-demanding processes, including cardiovascular contraction, and antagonizes nitric oxide–mediated functions. Relatively high activity of the enzyme, particularly in resistance arteries, is thought to enhance pressor responses and increase blood pressure. Tissue creatine kinase activity is reported to be high in black people, a population subgroup with greater hypertension risk; the proposed effects of high creatine kinase activity, however, are not “race dependent.” We therefore assessed whether creatine kinase is associated with blood pressure in a multiethnic population.

Methods and Results—We analyzed a stratified random sample of the population of Amsterdam, the Netherlands, consisting of 1444 citizens (503 white European, 292 South Asian, 580 black, and 69 of other ethnicity) aged 34 to 60 years. We used linear regression analysis to investigate the association between blood pressure and normal serum creatine kinase after rest, as a substitute measure of tissue activity. Creatine kinase was independently associated with blood pressure, with an increase in systolic and diastolic pressure, respectively, of 8.0 (95% CI, 3.3 to 12.7) and 4.7 (95% CI, 1.9 to 7.5) mm Hg per log creatine kinase increase after adjustment for age, sex, body mass index, and ethnicity.

Conclusions—Creatine kinase is associated with blood pressure. Further studies are needed to explore the nature of this association, including how variation in cardiovascular creatine kinase activity may affect pressor responses. (Circulation. 2006;114:2034-2039.)

Key Words: creatine kinase ▪ blood pressure ▪ metabolism ▪ muscle, smooth ▪ nitric oxide

Black people of West African descent are known to have high tissue1 and serum2 creatine kinase (CK) activities. We previously hypothesized that this high activity is a genetic factor that increases hypertension risk in this population subgroup.3 CK catalyzes the reversible transfer of the high-energy phosphate moiety (P) between creatine and adenosine diphosphate (ADP): MgADP + P ⇌ MgATP + Creatine. The rate of transfer of the phosphoryl group from creatine phosphate to ADP by CK is greater than the maximum rate of adenosine triphosphate (ATP) generation by oxidative phosphorylation, and this ensures rapid resynthesis of ATP.4–7

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The enzyme is abundantly expressed in both the mitochondrion and the cytosol. At the mitochondrial site, it facilitates the formation of creatine phosphate, which is transported by CK to subcellular locations of high-energy demands. Here, CK is tightly bound in the immediate proximity of ATP-utilizing enzymes such as Na+/K⁺-ATPase and Ca²⁺-ATPase at membranes and myosin light chain kinase and myosin ATPase at the contractile proteins, where it rapidly provides ATP to these enzymes. CK thus fuels highly energy-demanding processes such as sodium retention, cardiovascular contractility, and remodeling of arteries.5–11 Relatively high activity of the enzyme, particularly in resistance arteries, is thought to enhance pressure responses and lead to higher blood pressure levels.5

Variation in CK activity may help explain differences in blood pressure levels between black and white people,3 but the proposed mechanism of enhanced pressor responses with high tissue CK acts independently of ethnicity or “race.” Because we aim to identify biological variations that are relevant for disease across different population subgroups,12–15 we assessed, in a multiethnic population, whether variation in CK activity is associated with blood pressure levels independent of ethnicity.

Methods

Study Population

The study population was selected from participants in the “SUrinamese in the Netherlands: Study on ETHnicity and health” (SUNSET) Study.15 Suriname is a former colony of the Netherlands on the east coast of South America, with a population consisting mainly of descendants of Africans and South Asians. Many Surinamese migrated to the Nether-
land in the past decades. This group is known to have a high risk of development of cardiovascular disease,\textsuperscript{15} which prompted our SUNSET study. The institutional review committee approved the study, and participants gave written informed consent. The methods of the SUNSET study have been described elsewhere.\textsuperscript{15}

In brief, the registration offices of the selected municipalities in Amsterdam provided a stratified random population sample of 3000 noninstitutionalized persons (1000 white European and 2000 Surinamese-Dutch persons) aged 34 to 60 years, living in Amsterdam. Cardiovascular risk factors, socioeconomic status, and self-defined ethnicity were assessed through a questionnaire. We instructed participants to abstain from heavy exercise for 3 days before visiting our hospital for a physical examination. Walking, driving a car, and normal daily activities were allowed. Physical examination included height, weight, and blood pressure levels. Blood pressure was measured with an Omron M4 oscillometric device (Omron Healthcare Europe BV, Hoofddorp, the Netherlands) in a quiet room with the subject seated. An appropriately adjusted cuff size was used on the nondominant arm, which was supported at heart level. To account for blood pressure variability, blood pressure was calculated as the mean of the first 2 consecutive readings, with a maximum of 5 mm Hg difference, as recommended by the Dutch Institute for Healthcare Improvement.\textsuperscript{16} This method results in lower blood pressure readings with smaller SDs.\textsuperscript{17} Laboratory studies included serum CK activity estimated with automated analyzers (Roche/Hitachi Systems, Roche Diagnostics, Indianapolis, Ind) according to procedures recommended by the International Federation of Clinical Chemistry.\textsuperscript{18}

**Outcome Measure**

The primary outcome of the present study was the association between normal serum CK activities after 3 days of rest and blood pressure levels after adjustment for age, sex, body mass index (BMI), and ethnicity.

**Statistical Analysis**

To calculate the sample size, we used previously reported differences in serum CK activity and blood pressure between white women (to represent those with low CK) and black men (to represent those with high CK) of approximately 250 IU/L for CK and 10 mm Hg and 5 mm Hg for SBP and DBP, respectively. We calculated that 765 persons needed to enter the study to detect this association with a 2-tailed $\alpha = 0.05$ and $1−\beta = 0.80$. Statistical analyses were performed with the SPSS statistical software package for Windows (Microsoft, Redmond, Wash), version 14.0 (SPSS Inc, Chicago, Ill).

Because the CK distribution was expected to be extremely skewed to the right, we planned to exclude outliers and establish the empirical 97.5 percentile point of CK, to discard values that were abnormally high.\textsuperscript{20} If the data were skewed to a significant degree after this procedure,\textsuperscript{20} a logarithmic transformation to base 10 would be performed to achieve a more symmetrical distribution.

We first assessed whether blood pressure levels were different among CK tertiles using the nonparametric Kruskal-Wallis test. We further calculated 1-tailed Spearman correlations for SBP and DBP levels versus CK activity, age, BMI, sex, ethnicity, and education level. Finally, univariable and multivariable regression models were used to explore the relation between blood pressure levels and CK activity (and other variables that significantly correlated with blood pressure) with forced and blockwise entries. We initially entered the predictors of blood pressure and CK together in the model (forced entry). Thereafter, we considered the established predictors of blood pressure together in a blockwise approach and evaluated whether CK had significant additional predictive value. Furthermore, we verified whether the assumptions of the linear regression model, including normality, linearity, and homoscedasticity, were met.

We excluded subjects without data on blood pressure levels or CK activity and those with controlled hypertension (SBP <140 mm Hg and DBP <90 mm Hg with antihypertensive drug therapy) from the analysis of the association between blood pressure and CK. We planned to statistically correct the results for white, South Asian, and black people only because we expected to include small numbers of people of other ethnicity. Finally, we assessed whether altering the inclusion criteria had a major effect on the results of the analyses. We reanalyzed the data with several exclusions: we excluded participants who were using statins because these drugs may cause an increase in CK activity; we excluded those with uncontrolled hypertension because the proposed association between CK and blood pressure might have been diluted if antihypertensive drugs were taken; and we excluded those with renal failure because this condition has been associated with higher serum CK activity.\textsuperscript{21} Where applicable, we considered a probability value of <0.05 to be significant. Data in parentheses are 95% confidence intervals unless otherwise specified.

The authors had full access to the data and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

**Results**

We gathered data on serum CK, blood pressure, and ethnicity in 1444 subjects. Table 1 summarizes the characteristics of the participants. Crude CK activity ranged from 14 to 5783 IU/L (median 111 IU/L), with a distribution highly skewed to the right as expected (z score for skewness 228.5). After the exclusion of 3 outliers and 36 participants with CK activities above the 97.5 percentile, the data were still skewed to a

**TABLE 1. Baseline Characteristics of Included Participants**

<table>
<thead>
<tr>
<th>Participants</th>
<th>Total* (n=1444)</th>
<th>White (n=503)</th>
<th>South Asian (n=292)</th>
<th>Black (n=580)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male, %</td>
<td>41.2</td>
<td>49.9</td>
<td>46.2</td>
<td>32.1</td>
</tr>
<tr>
<td>Age, y†</td>
<td>45.4 (6.6)</td>
<td>47.8 (6.8)</td>
<td>44.7 (6.6)</td>
<td>43.7 (5.9)</td>
</tr>
<tr>
<td>BMI, kg/m²‡</td>
<td>27.3 (5.2)</td>
<td>26.1 (4.7)</td>
<td>27.0 (5.2)</td>
<td>28.4 (5.3)</td>
</tr>
<tr>
<td>CK, IU/L‡</td>
<td>111 (76–172)</td>
<td>88 (64–127)</td>
<td>105 (75–164)</td>
<td>149 (99–228)</td>
</tr>
<tr>
<td>SBP, mm Hgt</td>
<td>126.3 (19.9)</td>
<td>123.8 (19.5)</td>
<td>126.9 (19.0)</td>
<td>128.3 (20.1)</td>
</tr>
<tr>
<td>DBP, mm Hgt‡</td>
<td>81.9 (11.6)</td>
<td>79.3 (11.4)</td>
<td>83.0 (10.7)</td>
<td>83.8 (11.8)</td>
</tr>
<tr>
<td>Hypertensive, %</td>
<td>32.8</td>
<td>25.8</td>
<td>34.2</td>
<td>38.4</td>
</tr>
<tr>
<td>Treated, %§</td>
<td>33.6</td>
<td>23.8</td>
<td>45.0</td>
<td>33.6</td>
</tr>
<tr>
<td>Adequately treated, %</td>
<td>39.6 (13.3)</td>
<td>45.2 (10.8)</td>
<td>40.0 (18.0)</td>
<td>38.7 (13.0)</td>
</tr>
</tbody>
</table>
significant degree (z score for skewness 22.3). Subsequent log transformation of the data reduced the non-Gaussian distribution characteristics of positive skewness to a z score of 1.5.

For the association between CK and blood pressure as a continuous outcome, 1343 subjects were analyzed, because we excluded 39 participants with extreme values and 62 of the remaining subjects with controlled hypertension (a total number of 101 participants). Crude mean blood pressure levels differed significantly across tertiles of log CK (n = 447, 444, and 452, respectively), with a mean SBP (SD) for the first through the third tertile of 122.5 (20.9) versus 125.8 (20.9) versus 128.5 (20.9) mm Hg and a mean DBP (SD) of 79.2 (11.7) versus 81.7 (11.4) versus 84.8 (12.0) mm Hg (P < 0.001 for differences between CK tertiles for both SBP and DBP; Figure 1, in which ± 2 SEMs are plotted, not SDs). There was no significant difference in smoking status or fasting glucose between groups. Correlation coefficients for blood pressure levels and CK activity and other variables that significantly correlated with blood pressure are shown in Table 2. In the univariable analysis, blood pressure increase per log CK increase was 13.94 (9.56 to 18.33) mm Hg for SBP and 9.32 (6.76 to 11.89) mm Hg for DBP. When we adjusted for age, sex, BMI, and ethnicity in linear regression analysis, the difference in SBP and DBP was, respectively, 7.98 (3.27 to 12.68) and 4.69 (1.88 to 7.50) mm Hg/log CK increase (Table 2) when forced entry was used. We subsequently considered the established predictors of blood pressure (age, gender, BMI, and ethnicity) together in a blockwise approach and evaluated whether CK had significant additional predictive value. The order of variable entry had no impact on the outcomes. The assumptions of the linear regression model, including normality, linearity, and homoscedasticity, were met. The interaction between ethnicity and log CK was not significant (P = 0.2 for SBP and 0.9 for DBP). Finally, we reanalyzed the data for the main outcome with the exclusion of participants using statins (n = 32), those with uncontrolled hypertension (n = 94), and participants with a serum creatinine concentration >110 μmol/L, the upper reference limit at our hospital (n = 31), and found no changes in the significant association between CK activity and blood pressure.

Discussion

The main finding in the present study’s population sample was that blood pressure levels increased with each log CK tertile (7.98 [3.27 to 12.68] for SBP and 4.69 [1.88 to 7.50] mm Hg for DBP) after adjustment for age, sex, BMI, and ethnicity. There is no evidence that CK clearance is altered in those with higher blood pressure levels or that circulating CK is derived from the luminal surface of vascular endothelial cells, but higher blood pressure levels may have caused cardiovascular muscle damage and increased serum CK activity. We did not assess isoenzymes in the present study, but a normal CK isoenzyme spectrum was reported in subjects with relatively high serum CK activity and uncomplicated hypertension. In addition, normal isoenzymes were found in apparently healthy black people with high serum CK activities, as well as in other healthy population subgroups. We therefore consider it unlikely that a major portion of the people from a random population sample, with normal blood pressures and normal CK activities, would have cardiovascular damage to the extent that it would lead to abnormal CK isoenzyme distribution.

A common cause of elevated serum CK activity levels is exercise. The resting period of 3 days should have substantially reduced the effect of exercise on serum CK, but CK activity can be elevated up to 3 weeks after eccentric muscular activity (in which muscle contracts and lengthens at the same time). None of the participants reported that they had been involved in such vigorous exercise. We cannot exclude the possibility that high-normal serum CK activities in nonblack participants in particular reflected subclinical muscle damage due to exercise; however, this would actually have led to an underestimation of the association between blood pressure and serum CK activity in the present study.

![Figure 1. Crude SBP and DBP according to CK tertiles. I–III are the first through the third tertile of log serum CK (IU/L), with 447, 444, and 452 subjects, respectively. Values are mean ± 2 SEM. Both SBP and DBP differed significantly among CK tertiles (P < 0.001, Kruskal-Wallis test).](image-url)
Another possible explanation for the association found between blood pressure and serum CK activity at rest is that in the absence of overt tissue damage or dysfunction, serum CK activity reflects tissue activity. Serum CK activity is the result of tissue CK concentration,\textsuperscript{28,29} the release of CK from tissues,\textsuperscript{28} lymphatic flow,\textsuperscript{30} and CK clearance by the liver.\textsuperscript{29,31} Normal tissue loses a small fraction of cytosolic CK into the interstitial space, as was shown in \textsuperscript{31}P nuclear magnetic resonance spectroscopy studies.\textsuperscript{28} In physiological and pathological states, release from tissue is proportional to tissue CK activity.\textsuperscript{28,29} Intersitial CK is subsequently transported through lymphatic vessels into the bloodstream.\textsuperscript{30} Consequently, differences in tissue CK activity found in healthy population subgroups\textsuperscript{1} are detected in serum\textsuperscript{2} as well. Healthy black people are reported to have approximately twice the CK activity of white people in skeletal muscle\textsuperscript{1} and approximately twice the activity in serum,\textsuperscript{2} with normal isoenzyme patterns.\textsuperscript{23,26} The proportionate release of tissue CK is also apparent in the rare condition of human CK deficiency, in which tissue CK activity is very low compared with that of control subjects.\textsuperscript{32–34} Serum CK activity in this condition is comparatively low, even after frank tissue damage.\textsuperscript{32–34} The molecular basis of the coordinated expression of different isoenzymes at specific subcellular sites in specific tissues and of the large differences in activity found between human subgroups is unknown.\textsuperscript{1–6,35}

High tissue CK activity, whether constitutive, induced, or both, may rather directly enhance contractile responses by enhancing cellular energy and contractile reserve.\textsuperscript{5} CK acts as an energy transducer at the cardiovascular contractile proteins, supplying ATP for the contractile process.\textsuperscript{3–7,11} The activity of the enzyme is reported to be higher in the myocardiun and aorta of relatively young spontaneously hypertensive rats than in controls and is also higher in animal models of acute pressure overload of the left ventricle.\textsuperscript{3,11,36,37} This high CK activity may alter local ADP levels at the contractile proteins and contribute to the enhanced contractility and myosin ATPase activity.\textsuperscript{3,38} On the other hand, in heart failure in humans and in animals, including older spontaneously hypertensive rats, low total CK activity, a reduced proportion of total CK activity represented by mitochondrial CK and CK-MM, and accumulation of CK-MB with a reduction in the flux through the CK reaction are typical findings.\textsuperscript{39–41} This provides evidence that the level of CK activity may modulate the function and dysfunction of cardiovascular tissue.

Particularly in resistance arteries, a small increase in CK activity may markedly increase contractility, with a potentially large impact on blood pressure levels.\textsuperscript{3} Calcium-dependent, RhoA/Rho kinase and nitric oxide (NO)–guanosine 3’,5’-cyclic monophosphate pathways, the main intracellular effectors of blood pressure–regulating systems in vascular smooth muscle, converge on metabolic processes fueled by CK (Figure 2).\textsuperscript{3–6,38,42–46} Vascular smooth muscle contraction is thought to consist of a fast, force-generating component at relatively high energy costs and a slow, tonic maintenance of tension,\textsuperscript{47} thought to depend on the ability to have attached but dephosphorylated cross-bridges (latch bridges),\textsuperscript{47,48} for which ADP is required.\textsuperscript{49} The latch bridge is formed when a phosphorylated myosin head is dephosphorylated while attached to actin. The dephosphorylation renders the myosin a less active ATPase, which therefore is less able to hydrolyze ATP and is relatively unable to cycle. In vascular smooth muscle, the role of the latch bridge is to maintain a constant myogenic tension at a constant length with low energy cost. Therefore, at a constant blood pressure and blood volume, arteries can maintain vasogenic tone with low energy costs.\textsuperscript{47–49} Vascular CK activity is relatively low.\textsuperscript{6} Greater CK activity could bind more ADP and increase the rate of the conversion of ADP to ATP, which could reduce the relative levels of local ADP at the contractile proteins.\textsuperscript{3,6} If ADP levels at the contractile proteins do not achieve the level required for dephosphorylated latch bridges, then the smooth muscle tension response could be altered, leading to excessive shortening before latch formation, which would increase contractility.\textsuperscript{3} A rapid cross-bridge cycling rate would be maintained because of lower ADP, and actual relaxation could be attenuated because of competition between detaching and rapidly reattaching cross-bridges.\textsuperscript{3}

In addition to a direct effect on contractility, high CK activity may inhibit NO-dependent functions, in part by reducing the bioavailability of L-arginine. CK and NO are antagonizing systems: CK enhances ATP buffer capacity and contractile reserve, as well as growth responses and salt retention, whereas NO inhibits these functions.\textsuperscript{2–11,43–46} The higher demand for creatine that accompanies high CK activity\textsuperscript{50} may decrease the availability of L-arginine and reduce the rate of NO synthesis. Creatine and NO are both synthesized from L-arginine, but creatine synthesis, which occurs in the kidney and the liver, demands nearly 10 times the flux of plasma L-arginine represented by NO synthesis.\textsuperscript{43} Despite intracellular L-arginine concentrations that should saturate

![Figure 2. CK and the main regulatory pathways of vascular smooth muscle contraction. NO, RhoA/Rho kinase, and calcium-dependent pathways are intracellular effectors of blood pressure–regulating systems that converge on metabolic processes fueled by CK.\textsuperscript{3–6,38,42–46} CK is colocalized with Ca\textsuperscript{2+}–ATPase and myosin ATPase, and evidence suggests the enzyme is also colocalized with myosin light chain kinase (MLCK), to rapidly supply these enzymes with ATP using creatine phosphate.\textsuperscript{3–6} High CK activity, particularly at myosin ATPase in resistance arteries,\textsuperscript{38} could directly enhance pressor responses, with a great impact on blood pressure.\textsuperscript{3} SER indicates sarcoplasmic reticulum; MLCP, myosin light chain phosphatase.](https://circ.ahajournals.org/doi/10.1161/01.HYP.0000184760.61983.51)
endothelial NO synthase, the rate of NO synthesis is limited by the rate of endothelial L-arginine uptake.\textsuperscript{43,51}

High CK activity, low NO bioavailability, and attenuated NO-mediated responses occur more often in black people.\textsuperscript{1–3,14,52–54}

There is great interest in identifying the operative biochemical pathways that underlie variations in bioavailability of NO.\textsuperscript{14,52–54} Given the existing evidence on the synthesis of creatine, which requires a major portion of the available L-arginine,\textsuperscript{43} and the greater concentration of creatine with higher CK activity,\textsuperscript{51} high tissue CK activity may be one factor that reduces NO bioavailability, by reducing L-arginine available for NO synthesis. The observation that black people were found to respond with greater endothelium-dependent vasodilation with L-arginine supplementation than white people\textsuperscript{54} is in line with our hypothesis.

No dramatic difference in cardiovascular function is necessary for the proposed alterations in blood pressure with high CK activity. Even a small increase in the contractility of vascular smooth muscle could have a profound effect on resistance of flow and hence arterial pressure. As expressed in the Poiseuille-Hagen formula, blood flow and resistance in vivo are markedly affected by small changes in the caliber of the vessel. We believe that high CK activity may be quite beneficial for rapid and dynamic energy demand. Contraction and relaxation are active, dynamic processes. The speed of relaxation and contraction is just as important as the tension achieved. The present results would support a role for the CK system in modulating the rate of the tension change. Thus, increased CK activity in muscle tissue might lead to hyperdynamic activity. Hyperdynamic activity in the vascular smooth muscle of a conductance artery might cause the arterial contraction rate to increase disproportionately and decrease compliance and lumen diameters. It is proposed, therefore, that high CK activity could increase vascular smooth muscle dynamic activity and cause, or contribute to, higher blood pressure levels because of the more narrowed lumen diameters that could result.\textsuperscript{3}

In conclusion, we observed that CK activity at rest is independently associated with blood pressure levels on a population level. The presented data appear to be in line with our hypothesis that high-normal serum CK activity at rest in otherwise healthy people reflects high tissue CK activity, which enhances ATP buffer capacity for pressor responses.\textsuperscript{3}

We cannot exclude the possibility that subclinical cardiovascular damage contributed to the results; however, we consider this option unlikely, because normotensive and hypertensive people with relatively high CK activities have been shown to have normal isoenzyme patterns.\textsuperscript{23,24,26} Our observations in the present study may help explain blood pressure differences between population subgroups on the basis of CK activity. Further study is needed to directly assess the effect of variations in tissue CK activity on ATP buffer capacity and NO-mediated functions, as involved in sodium retention, cardiovascular contractility, and remodeling of arteries.

Disclosures

None.

References


Hypertension may be linked to energy metabolism. The creation and maintenance of blood pressure is a highly energy-demanding process that requires heart and vascular work. Consequently, subjects with high tissue creatine kinase (CK) activity may have greater adenosine triphosphate–buffering capacity for pressor responses. CK is a central regulatory enzyme of energy metabolism. The enzyme buffers and transports high-energy phosphate moieties derived from glycolysis and oxidative phosphorylation to subcellular locations of high energy demands, where it rapidly generates adenosine triphosphate in situ. Black people of sub-Saharan African descent are known to have relatively high tissue CK activity, and we previously hypothesized that this may increase hypertension risk. We now report an association between serum CK at rest, as a measure of tissue CK, and blood pressure in a random population sample of black, white, and South Asian people. Whereas high CK activity was more commonly found in black people, the association was independent of age, sex, body mass index, and ethnicity. It has been firmly established that low flow through the CK reaction is related to muscle hypofunction. The present data suggest that cardiovascular function may be altered with relatively high activity of the enzyme, leading to increased hypertension risk. The global burden of cardiovascular disease is high, and despite adequate medical care, ethnic disparities in the incidence and control of hypertension remain apparent. This study may provide a new perspective on these disparities, attending to the question of whether energy metabolism matters in hypertensive disease.
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