Creatine kinase and blood pressure: Clinical and therapeutic implications

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Creatine kinase predicts obesity
in the general population

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Chapter 4

ABSTRACT

Background There are ample data on environmental factors that affect obesity, but biological factors are understudied. We focus on creatine kinase, the central regulatory enzyme of cellular energy metabolism. Creatine kinase activity is typically high in skeletal muscle type II fibers. There is evidence that predominance of type II, insulin resistant fibers is associated with obesity and weight gain after overfeeding. Therefore, we assessed whether serum creatine kinase after rest is independently associated with obesity in the general population.

Method We analyzed a stratified sample of the multi-ethnic population of Amsterdam, the Netherlands, consisting of 1444 citizens aged 34 to 60 years. We used linear regression analysis to assess the independent association between serum creatine kinase and body mass index.

Results Mean body mass index increased from the first through the third creatine kinase tertile, with values of respectively 26.4 (5.1), 27.2 (5.2), and 27.9 (5.0) kg/m². Creatine kinase was independently associated with body mass index in multivariable regression analysis, with an increase in body mass index of 3.1 (95% CI: 1.8 to 4.3) kg/m²/log creatine kinase increase, after adjustment for age, sex, ethnicity, educational level, and serum creatinine, as a surrogate measure of muscle mass.

Discussion Creatine kinase is strongly and independently associated with body mass index. As creatine kinase has been previously linked to hypertension, the high creatine kinase phenotype might be hypertension and obesity prone. Further studies are needed to assess whether high creatine kinase in obesity is an epiphenomenon, or part of a causal pathway leading to obesity.
BACKGROUND

Obesity has reached epidemic proportions globally. The age-adjusted prevalence is reported to range from 6 to 36% in Western countries. Obesity is part of the metabolic syndrome and a risk factor for hypertension, diabetes mellitus, and cardiovascular disease.1-3

Individuals differ in their susceptibility to gain weight.4 Although there are ample reports on environmental factors leading to obesity, there is relative lack of research on biological factors related to this condition.5-7 In this study we focus on the association of obesity with the enzyme creatine kinase (CK).

CK plays a key role in cellular energy metabolism. By catalyzing the reversible transfer of high energy phosphates from phosphocreatine to ADP generating ATP, via the reaction: MgADP + CrP + H+ ↔ MgATP + Cr, the enzyme rapidly provides ATP for cellular ATPases.8-10 In skeletal muscle, high activity of the enzyme is typically found in type II fibers. Type II fibers are primarily fit for burst exercise, with cytosolic creatine kinase as the main ATP buffer.11 In these fibers, cytosolic CK is tightly coupled to glycolysis, whereas mitochondrial fatty acid oxidation capacity and glucose uptake are limited, rendering these fibers relatively insulin resistant.8,12-14 In line with this, previous studies have shown an association between predominance of fast, type II skeletal muscle fibers and weight gain.15-19 Overfeeding of healthy young men with relatively higher type II fiber proportions induced a greater gain of fat mass compared to men with higher type I fiber proportions.17

In the absence of overt tissue damage, serum CK activity after three days of rest is thought to mainly reflect skeletal muscle cytosolic CK activity, which is predominantly derived from type II fibers.20-23 Therefore, we assessed whether serum CK is associated with obesity in the general population.

METHOD

Study population

For this study, we used the dataset as described previously of 1444 non-institutionalized persons (including 503 white European and 672 Surinamese-Dutch persons, and 269 with “other ethnicity”) aged 34 to 60 years, living in Amsterdam.21 The Medical Ethnics committee of the Academic Medical Center, Amsterdam, the Netherlands, approved the study and this study was conducted in accord with the ethical principles
of the Declaration of Helsinki. Cardiovascular risk factors, the use of antihypertensive drugs, socioeconomic status, and self-defined ethnicity were assessed through a questionnaire. We instructed participants to abstain from heavy exercise for three 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). Body mass index (BMI) was calculated as weight (kg) divided by the height (rounded to the nearest centimeter) squared. Laboratory studies included serum CK activity after 3 days of rest and serum creatinine, estimated with automated analyzers (Roche/Hitachi Systems, Roche Diagnostics, Indianapolis, Ind), according to procedures recommended by the International Federation of Clinical Chemistry.21,24

**Primary outcome measure**

The primary outcome of this study was the association between serum CK activity after 3 days of rest and BMI after adjustment for age, sex, ethnicity, educational level and serum creatinine, after the exclusion of subjects with renal failure (creatinine >110 μmol/L) as this is proportional to muscle mass.25,26

**Statistical analyses**

To calculate the sample size for the association between serum CK and BMI, we used previously reported data on the correlation of the type II fiber area of the human musculus vastus lateralis and BMI \( R=0.18 \).19 As these fibers are the main source of serum CK, we assumed a similar correlation coefficient between CK and BMI, and calculated that 414 persons needed to enter the study to detect this association with 2-tailed \( \alpha=0.05 \) and \( 1-\beta=0.80 \).

**Data analyses**

Because the CK distribution was extremely skewed to the right, 24 outliers were excluded, using the empirical 97.5 percentile point of CK. Furthermore, logarithmic transformation to base 10 resulted in a more symmetrical distribution, as described previously.21 We first estimated differences in BMI across CK tertiles with One-way ANOVA. Thereafter, we calculated 1-tailed Spearman correlations for BMI versus CK activity, and age, sex, ethnicity, educational level, and creatinine. We used univariable and multivariable regression models to assess whether serum CK had predictive value on
Creatine kinase predicts obesity in the general population

BMI, independent of other established predictors. We verified whether the assumptions of the linear regression model, including normality, linearity, and homoscedasticity were met. Data in parentheses are 95% confidence intervals unless otherwise specified. Statistical analyses were performed with the SPSS statistical software package for Windows, version 19.0 (SPSS Inc, Chicago).

Table 1. Baseline characteristics of the participants.

<table>
<thead>
<tr>
<th>Participants (n)</th>
<th>Total (1342)*</th>
<th>White (487)</th>
<th>South Asian (267)</th>
<th>Black (522)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men (%)</td>
<td>40.9</td>
<td>49.5</td>
<td>46.4</td>
<td>31.0</td>
</tr>
<tr>
<td>Age (y)†</td>
<td>45.4 (6.6)</td>
<td>47.8 (6.8)</td>
<td>44.6 (6.6)</td>
<td>43.7 (5.8)</td>
</tr>
<tr>
<td>BMI (kg/m²)†</td>
<td>27.2 (5.1)</td>
<td>26.1 (4.7)</td>
<td>26.7 (4.9)</td>
<td>28.3 (5.3)</td>
</tr>
<tr>
<td>≥ 25.0 (%)</td>
<td>61.9</td>
<td>52.4</td>
<td>61.8</td>
<td>69.9</td>
</tr>
<tr>
<td>≥ 30.0 (%)</td>
<td>24.0</td>
<td>14.8</td>
<td>19.1</td>
<td>34.5</td>
</tr>
<tr>
<td>≥ 35.0 (%)</td>
<td>7.8</td>
<td>4.9</td>
<td>4.5</td>
<td>12.1</td>
</tr>
<tr>
<td>CK (IU/L)‡</td>
<td>108 (76 to 167)</td>
<td>88 (64 to 127)</td>
<td>102 (75 to 160)</td>
<td>145 (98 to 213)</td>
</tr>
<tr>
<td>Creatinine (μmol/L)†</td>
<td>74.4 (29.6)</td>
<td>73.8 (20.3)</td>
<td>71.2 (16.8)</td>
<td>76.4 (41.0)</td>
</tr>
<tr>
<td>SBP (mm Hg)†</td>
<td>126.3 (20.3)</td>
<td>123.8 (19.8)</td>
<td>127.4 (19.5)</td>
<td>128.3 (20.7)</td>
</tr>
<tr>
<td>DBP (mm Hg)‡</td>
<td>81.9 (11.9)</td>
<td>79.3 (11.5)</td>
<td>83.3 (11.0)</td>
<td>83.8 (12.2)</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>29.9</td>
<td>23.8</td>
<td>30.3</td>
<td>35.4</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)†</td>
<td>5.4 (1.1)</td>
<td>5.7 (1.0)</td>
<td>5.4 (1.0)</td>
<td>5.1 (1.0)</td>
</tr>
<tr>
<td>Glucose (mmol/L)†</td>
<td>5.7 (1.7)</td>
<td>5.5 (1.2)</td>
<td>6.3 (2.3)</td>
<td>5.6 (1.8)</td>
</tr>
</tbody>
</table>

BMI, body mass index; SBP, DBP, systolic/diastolic blood pressure; Hypertension, systolic / diastolic blood pressure ≥140/90 mm Hg and/or treated; CK, creatine kinase; *Includes participants with “other” ethnicity; † Mean (SD); ‡ Median (IQR); § High glucose in South Asians as previously reported.43

RESULTS

The dataset contained 1444 subjects.17 Crude CK activity ranged from 14 to 5783 IU/L (median 111 IU/L). Mean BMI was 27.2 kg/m² (SD 5.1). Exclusion of outliers and log transformation of the data as described in the method section reduced the non-Gaussian distribution characteristics of positive skewness to a z-score of 1.5.21 We also excluded 1 subject without data on BMI and 62 subjects with controlled hypertension. The latter are thought to have lower CK activities compared to uncontrolled hypertension.21 Hence, 1342 subjects were analyzed. The characteristics of the analyzed subjects are listed in
Table 1. Mean BMI increased from the first through the third log CK tertile, with values of resp. 26.4 (5.1), 27.2 (5.2), and 27.9 (5.0) kg/m² (p<0.05 for tertile I vs II and III, p=0.51 for tertile II vs III). Correlation coefficients for CK and other parameters that correlated significantly with BMI are shown in Table 2. In univariable analysis, BMI increase per log CK increase was 2.57 kg/m² (95% CI: 1.46 to 3.69). When we adjusted for age, sex, ethnicity, educational level, and serum creatinine in linear regression analysis, CK was the main predictor of BMI with a beta coefficient of 3.06 (95% CI: 1.79 to 4.33) (Table 2). The assumptions of the linear regression model, including normality, linearity, and homoscedasticity, and no collinearity were met. In particular, the correlation between CK and creatinine was low (R 0.38). Finally, the sequence of variable entry had no impact on the outcomes (data not shown).

Table 2. Correlation coefficients and linear models for BMI.

<table>
<thead>
<tr>
<th></th>
<th>Correlation Coefficients*</th>
<th>Multivariable Regression β (95% CI)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>18.2</td>
<td></td>
</tr>
<tr>
<td>Log CK</td>
<td>0.15</td>
<td>3.06 (1.79 to 4.33)</td>
</tr>
<tr>
<td>Sex‡</td>
<td>0.16</td>
<td>1.98 (1.36 to 2.60)</td>
</tr>
<tr>
<td>Educational level§</td>
<td>0.09</td>
<td>1.52 (0.89 to 2.15)</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.11</td>
<td>0.12 (0.08 to 0.16)</td>
</tr>
<tr>
<td>Creatinine**</td>
<td>0.08</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

* One-tailed Spearman rank correlation; P<0.01 for all variables. Creatinine, a non-enzymatic metabolite derived from creatine in skeletal muscle and therefore a substitute measure of muscle mass, was not significant in univariable and multivariable regression analysis. †The β-coefficients are for 1-unit increase in the continuous variables age, BMI, and log CK; ‡With higher BMI in women; §With higher BMI at a lower level of education; ¶Black vs non-Black people; **With higher BMI at lower serum creatinine levels.
Creatine kinase predicts obesity in the general population

In skeletal muscle, creatine kinase (CK) buffers and transports ATP produced by anaerobic glycolysis and mitochondrial oxidative phosphorylation to sites of energy consumption, including myofibrils (myosin-ATPase) and membrane ion pumps (Ca$^{2+}$-ATPase). Based on the existing evidence we propose that the CK-system has a key regulatory function in processes that direct the entry and oxidative catabolism of glucose and fatty acids in muscle cells. Type II fibers display high cytosolic CK activity, closely coupled to glycolysis, while the mitochondrial capacity for oxidation of glucose and fatty acids is relatively low. Thus, high cytosolic CK activity may be associated with limited cellular glucose and fatty acid uptake and utilization, and predispose to the storage of lipid as fat tissue. In contrast, type I fibers contain less cytosolic CK activity, relatively more mitochondrial CK activity, and a greater capacity for oxidation of glucose and fatty acids in the mitochondrion.

**Figure. Creatine kinase in skeletal muscle energy metabolism.**

Cr, creatine; CT, creatine transporter; FA, fatty acid; FT, fatty acid transporter; GLUT4, insulin dependent glucose transporter protein-4; SER, sarcoplasmatic reticulum; TAG, triacylglycerol; DAG, diacylglycerol; MIM, mitochondrial inner membrane; matrix, mitochondrial matrix. In skeletal muscle, creatine kinase (CK) buffers and transports ATP produced by anaerobic glycolysis and mitochondrial oxidative phosphorylation to sites of energy consumption, including myofibrils (myosin-ATPase) and membrane ion pumps (Ca$^{2+}$-ATPase). Based on the existing evidence we propose that the CK-system has a key regulatory function in processes that direct the entry and oxidative catabolism of glucose and fatty acids in muscle cells. Type II fibers display high cytosolic CK activity, closely coupled to glycolysis, while the mitochondrial capacity for oxidation of glucose and fatty acids is relatively low. Thus, high cytosolic CK activity may be associated with limited cellular glucose and fatty acid uptake and utilization, and predispose to the storage of lipid as fat tissue. In contrast, type I fibers contain less cytosolic CK activity, relatively more mitochondrial CK activity, and a greater capacity for oxidation of glucose and fatty acids in the mitochondrion.
Table 3. Characteristics of skeletal muscle fiber types.

<table>
<thead>
<tr>
<th>Type II</th>
<th>Type I</th>
</tr>
</thead>
<tbody>
<tr>
<td>High CK</td>
<td>Low CK</td>
</tr>
<tr>
<td>Predominantly glycolytic</td>
<td>Predominantly oxidative</td>
</tr>
<tr>
<td>Mitochondria poor</td>
<td>Mitochondria rich</td>
</tr>
<tr>
<td>Capillary rarefaction</td>
<td>High density of capillaries</td>
</tr>
<tr>
<td>Anaerobic</td>
<td>Aerobic</td>
</tr>
<tr>
<td>Burst exercise</td>
<td>Endurance capacity</td>
</tr>
<tr>
<td>Low GLUT-4 expression</td>
<td>Higher GLUT-4 expression</td>
</tr>
<tr>
<td>Insulin Resistant</td>
<td>Insulin sensitive</td>
</tr>
<tr>
<td>Less glucose uptake</td>
<td>High glucose uptake</td>
</tr>
<tr>
<td>Glucose and fatty acid stored as lipid</td>
<td>Glucose and fatty acid utilisation</td>
</tr>
<tr>
<td>Obesity prone</td>
<td>Lean</td>
</tr>
<tr>
<td>Hypertension prone</td>
<td>Normotension</td>
</tr>
</tbody>
</table>

CK, creatine kinase; GLUT-4, insulin-dependent glucose transporter protein 4.

DISCUSSION

The main finding of this study is that serum CK is strongly associated with BMI in a random population sample. This association was independent of age, sex, ethnicity, and serum creatinine, as a measure of muscle mass.

Serum CK activity is thought to reflect CK activity from striated skeletal muscle, in particular high CK type II fibers (Table 3).20-23 These fibers are well known to be associated with obesity and weight gain. As these fibers tend to be glycolytic and insulin resistant with less capacity for oxidation of fatty acids compared to type I fibers, this is thought to lead to storage of fatty acids as lipid instead of utilization.17-19,27

The association of CK and fiber type distribution with obesity may be explained by the central regulatory function of the enzyme in key metabolic processes that direct the entry and oxidative catabolism of glucose and lipids in skeletal muscle fibers (Figure). 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 adenosine diphosphate, via the reaction:

\[ \text{MgADP} + \text{CrP} + \text{H}^+ \leftrightarrow \text{MgATP} + \text{Cr}. \]
ATP generated by glycolysis and oxidative phosphorylation, is transported as phosphocreatine to subcellular locations of ATP utilization, such as myofibrills and membrane ion pumps, where ATP is regenerated.\textsuperscript{9,10,13,28} In type II fibers, high (cytosolic) CK activity, functionally coupled to glycolysis, ensures rapid resynthesis of ATP for burst contractions.\textsuperscript{14} On the other hand, the mitochondrial content and vascularisation is low in these fibers, with low expression of the insulin dependent transporter protein GLUT-4. This is associated with limited capacity for uptake and oxidation of fatty acids and glucose.\textsuperscript{9,13,29,30} Therefore, high skeletal muscle CK activity and type II fiber predominance may promote storage fatty acids and glucose as lipid in adipose tissue rather than uptake and oxidation in skeletal muscle, which may lead to obesity. In agreement with this, it was shown that skeletal muscle CK activity was 25.7% higher in obese subjects.\textsuperscript{31} In addition, CK activity of type Ila fibers of healthy young men was reported to correlate negatively with metabolic rate.\textsuperscript{17} In contrast, type I fibers contain less CK, are more reliant on mitochondrial metabolism, contain more capillaries, and are well fit for endurance exercise. These fibers utilize fatty acids as the major energy source, with a higher glucose uptake (Table 3), leading to less storage of fatty acids as lipid.\textsuperscript{27} In support of this, animal studies showed that inhibition of the CK-system lead to a shift from type II to type I fiber predominance, mitochondrial proliferation, increased oxidative enzyme activities, and 10% weight loss, increased GLUT-4 protein content, and improved glucose tolerance.\textsuperscript{32-35}

One strength of this study is the multiethnic sample with people of European, South Asian, and African ancestry. High skeletal muscle and serum CK activities are found in black people,\textsuperscript{36-38} a population subgroup with a higher risk for obesity compared to white people.\textsuperscript{39-41} However, the association of CK with BMI was independent of ethnicity in our analyses. This implies that CK may have potential use as a biomarker for obesity risk beyond skin color or ethnicity.

Second, we standardised for exercise. Serum CK activity is elevated up to 3 days with regular exercise, and up to a week after strenuous eccentric exercise, where the muscle lengthens and contracts at the same time against an external load. This leads to disruption of muscle fibers, and highly elevated serum CK, to up to 10.000 IU/L during a week or longer.\textsuperscript{42} However, none of the participants stated to be involved in such eccentric exercise. Still, we cannot exclude an exercise induced component in the CK values, but this would have led to an underestimation of the association between CK and BMI.
Limitations include the cross-sectional design of this study, which implicates causal inferences cannot be made. Furthermore, we cannot completely exclude increasing muscle mass with increasing BMI levels in this population. However, this is unlikely, as we found no significant association between serum creatinine within the normal range (excluding subjects with kidney failure), as a substitute measure of muscle mass, and BMI in our population.

In summary, we showed that serum CK activity is independently associated with obesity in a large population sample. As CK has been previously linked to hypertension, the high CK phenotype might be hypertension and obesity prone, as the muscle characteristics that underlie high serum CK, with predominance of high CK type II muscle fibers, include low vascularisation and low capacity for glucose and fatty acid oxidation, leading to higher peripheral resistance and insulin resistance. Further studies are needed to assess whether high CK in obesity is an epiphenomenon, or part of a causal pathway leading to obesity and to assess the role of CK and energy metabolism in the main risk factors for cardiovascular disease.
REFERENCES

Chapter 4


