Serum creatine kinase activity is not a reliable marker for muscle damage in conditions associated with low extracellular glutathione concentration

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Creatine kinase (CK, EC 2.7.3.2) assays usually contain thiol-reducing compounds to restore the enzyme activity. In this study, we investigated the effect of endogenous extracellular glutathione on serum CK activity. We examined CK activity and glutathione concentrations in serum from 200 healthy subjects (107 males, 93 females) and 38 patients with multiple organ failure, muscle wasting, and low serum CK activity (<50 U/L) (24 males, 14 females). Muscle damage was further evaluated using serum myoglobin concentrations and aldolase activity. In the overall group, serum glutathione concentrations correlated with serum CK activity (r = 0.791) but not with myoglobin concentrations and aldolase activity. In patients with multiple organ failure, low serum CK activities were accompanied by extremely low serum glutathione concentrations (<0.5 μmol/L, P < 0.001). Endogenous glutathione can be regarded as a CK-preserving agent during the lifetime of the enzyme in the circulation (22 h on average). Serum CK activity should be interpreted with caution in patients with liver disease and multiple organ failure. In these conditions, the loss of CK activity due to extracellular glutathione depletion cannot be restored by the presence of thiol-reducing compounds in the CK assays.

Materials and Methods

SUBJECTS AND PATIENTS

Blood was sampled by venipuncture, allowed to clot, and centrifuged (1000g, 10 min, room temperature). The supernatant serum was collected for analysis. Serum was obtained from 200 healthy sedentary blood donors (107 males, 93 females; ages 30 ± 7 years) without recent skeletal muscle trauma (within 2 months before the sampling). Concomitantly, 38 intensive care patients (24 males, 14 females; ages 57 ± 15 years) with low serum CK activity (<50 U/L) suffering from multiple organ failure...
(impaired renal and liver function and muscle wasting in the presence of inflammation) were studied. This study was approved by the ethical committee of the University Hospital of Ghent.

BIOCHEMICAL ASSAYS
The catalytic activity of CK in serum was determined at 37 °C according to the IFCC method (7) on a HITACHI 747 analyzer using CK-NAC reagents (Boehringer Mannheim). The total glutathione concentration in serum was measured according to the method described by Griffith (8), which was performed on a HITACHI 911 analyzer (Boehringer Mannheim) according to the manufacturer’s instructions. All necessary reagents were purchased from Sigma Chemicals, including glutathione (reduced form), β-NADPH-tetrasodium salt, Ellman’s reagent [5,5’-dithiobis(2-nitrobenzoic acid)], and glutathione reductase (EC 1.6.4.2; type III from baker’s yeast). The serum myoglobin concentration was measured by fixed-time immunonephelometry on a BN II nephelometer using the N Latex Myoglobin kit (Behringwerke AG) (9). The aldolase activity in serum was determined at 37 °C on a HITACHI 911 analyzer using commercial reagents (Boehringer Mannheim) (10).

STABILITY OF CK IN VITRO
Serum was obtained from healthy young men and pooled (initial CK activity, 121 U/L; glutathione concentration, 2.95 μmol/L). Glutathione (reduced form) was added to the serum pool to obtain final concentrations of 2.95, 4.95, and 6.95 μmol/L. A serum pool with a low glutathione concentration (<0.5 μmol/L; initial CK activity, 133 U/L) was studied as well. In another low glutathione pool (initial CK activity, 171 U/L), supplementation with reduced glutathione (final concentration, 3.0 μmol/L) and oxidized glutathione (Sigma Chemicals; final concentrations, 1.5 and 3.0 μmol/L) was examined. The catalytic activity of CK was monitored during incubation of the various serum pools at 37 °C for 48 h.

REVERSIBILITY OF THIOL OXIDATION
Because addition of thiol-reducing agents to the serum before analysis might restore CK activity (11, 12), the potential reversibility of thiol oxidation was investigated by adding fresh aqueous solutions of NAC (final concentration, 10 mmol/L), β-mercaptoethanol (final concentration, 280 mmol/L), and reduced glutathione (final concentration, 10 mmol/L) to the serum 30 min before CK assay. NAC and β-mercaptoethanol were purchased from Fluka Chemie AG. The addition of these agents produced minimal sample dilution (<1%). These sample pretreatments were performed in two sera from healthy controls, two sera from intensive care patients, and in pooled sera with low (<0.5 μmol/L) and physiological (3.15 μmol/L) glutathione concentrations after incubation for 48 h at 37 °C in vitro. Results were expressed as the percentage change of CK activity of the untreated sera.

STATISTICS
Results were expressed as median and interquartile ranges. Comparison of data between patients and healthy subjects was performed using the Mann–Whitney U-test. Correlations of serum glutathione with other parameters were examined using regression analysis. Statistical significance was considered as P < 0.05.

Results
SERUM GLUTATHIONE AND MUSCLE PARAMETERS
The data on glutathione, CK, aldolase, and myoglobin in healthy subjects and in intensive care patients suffering from multiple organ failure are summarized in Table 1. In the patients, serum CK activity was low (P <0.001) despite increased values found for the other muscle markers, myoglobin and aldolase. Similarly, serum glutathione concentrations were lower in intensive care patients than in healthy subjects (P <0.001). Serum myoglobin concentrations and aldolase activities were significantly higher in intensive care patients, as expected for multiple-organ failure and muscle wasting. We observed no gender-related difference in the total serum glutathione concentration. The low serum glutathione and CK activity in multiple-organ-failure patients were present in both sexes.

RELATIONSHIP BETWEEN SERUM CK AND GLUTATHIONE
In the overall study population (controls and patients), a significant correlation was observed between the serum CK activity (y, U/L) and the serum glutathione concentration (x, μmol/L): y = 32.28x + 5.85, r = 0.791; Sdw = 30.04 (Fig. 1). The regression equations differed between

| Table 1. Serum glutathione and muscle markers in healthy controls and in patients with multiple organ failure. |
|-------------------------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Controls                                        | Patients                        |                                  |
| Males                                           | Females                        | Males                           | Females                        |
| n                                               | 107                            | 93                              | 24                             | 14                             |
| Glutathione, μmol/L                            | 3.02 (2.66–3.50)a              | 2.88 (2.49–3.40)                | 0.20 (0.15–0.43)b               | 0.36 (0.10–0.98)b               |
| CK, U/L                                         | 118 (96–156)                   | 78 (61–103)                     | 12 (10–34)c                     | 34 (24–47)c                     |
| Myoglobin, μg/L                                 | 14.8 (11.8–22.9)               | 10.0 (7.1–13.8)                 | 18.6 (16.2–52.9)c               | 18.8 (12.1–60.8)b               |
| Aldolase, U/L                                   | 4.1 (3.0–5.3)                  | 2.9 (2.2–3.6)                   | 5.4 (4.8–5.9)d                  | 5.3 (4.1–8.1)d                  |

a Median (interquartile range)

P <0.001, b P <0.005, and c P <0.05 versus controls (Mann–Whitney U-test).
controls ($y = 41.97x - 24.35, r = 0.655$) and intensive care patients ($y = 31.03x - 0.43, r = 0.663$). In contrast, no significant correlation was observed between the serum glutathione concentration and the serum myoglobin concentration or serum aldolase activity.

**SERUM CK STABILITY IN VITRO**

The in vitro stability of serum CK in the presence of various extracellular glutathione concentrations is shown in Fig. 2. The rate of loss of CK activity depended on the serum glutathione concentration. In the sample with a physiological serum glutathione concentration (2.95 μmol/L), CK activity was reduced by 30% after 30 h incubation. The stability of the enzyme was higher in the presence of supraphysiological amounts of glutathione: these samples showed a residual CK activity of >90% during the same incubation period. In the pool with the lowest glutathione concentration (0.38 μmol/L), CK has the highest rate of activity loss. After 30 h (corresponding with two plasma half-lives of the enzyme activity), the serum CK activity in the latter sample was already reduced by 70%. Addition of oxidized glutathione to a low glutathione pool (from intensive care patients) did not change the stability of CK (residual activity <30% after 30 h), whereas addition of reduced glutathione to the same pool produced a residual CK activity of 55% after 30 h incubation.

**REVERSIBILITY OF THIOL OXIATION**

Addition of thiol-reducing compounds (NAC, β-mercaptoethanol, or glutathione) to serum 30 min before analysis did not change the measured CK activity in healthy controls (increase of original activity of the untreated sera <2%). Similarly, this sample pretreatment did not restore
the low CK activity in sera from intensive care patients (<5% increase). The in vitro loss of CK activity in the presence of low and physiological glutathione concentrations for 48 h could not be reversed by this additional sample treatment before analysis (<4% increase of CK activity of untreated sera).

**Discussion**

In the present study, we have demonstrated that the serum CK activity is dependent on the extracellular glutathione concentration. This is not the case with the other biochemical muscle markers, aldolase and myoglobin. Furthermore, low serum CK activities are observed in intensive care patients with low extracellular glutathione concentrations. In these patients, the depletion of the extracellular glutathione pools is one of the CK-modifying factors in serum that is responsible for the low serum CK activities.

In contrast to CK, the serum myoglobin concentration and aldolase activity were increased in critically ill patients. Although muscle wasting was evident in these patients, increases of myoglobin concentrations partially reflect the decreased renal function in patients with multiple organ failure. Aldolase is a less specific marker for muscle injury than CK because it is also present in other tissues (13).

Addition of thiol-containing components such as NAC to the reaction mixture of CK assays is commonly used to restore the serum CK activity (1). Addition of thiols during sample storage has also been found to have a restoring effect on CK activity. However, we found that the effect of major in vivo glutathione depletion on CK activity cannot be restored by the incorporation of reactivating compounds in the CK assays. Even addition of thiol-reducing agents directly to serum before analysis does not restore CK activity.

In vivo extracellular glutathione is partly reduced, partly oxidized (14). The in vitro long-term incubation experiments performed in this study confirm the observation that endogenous reduced glutathione protects against the aging of CK in biological fluids. In contrast, oxidized glutathione did not protect against the loss of CK activity in vitro.

Because CK is mainly metabolized by the liver macrophages (15), the plasma half-life of CK increases in severe liver insufficiency. Consequently, an accumulation of CK activity in plasma is to be expected in this case. Therefore, the finding of low serum CK activity in multiple organ failure and liver insufficiency is paradoxical but can be explained by oxidative damage of the enzyme produced by extracellular glutathione depletion. Low extracellular glutathione concentrations have been associated with liver disease and increased oxidative stress. Patients suffering from liver cirrhosis show lower extracellular glutathione concentrations (16). Certain drugs (17) (e.g., paracetamol and isoniazid) can deplete the extracellular glutathione concentration, as do aging and fasting (18).

Previous occasional findings of unexpected low serum CK concentrations after proven myocardial infarction (19–20) and in patients suffering from infective endocarditis (21), sepsis (5, 6), connective tissue disease (22), prolonged illness (23), severe liver disease (24–26), and metastatic disease (12) are also in agreement with the present findings. These conditions may lead to an underestimation of the myocardial infarct size when serum CK activity measurements are used (27). Consequently, methods for infarct-sizing based on the determination of serum CK cannot be recommended in conditions associated with low serum glutathione concentrations. In these situations, methods based on other biochemical markers such as myoglobin (28) (in the absence of renal failure) should be preferred to assess the myocardial infarct size. Because the muscle cell can also produce glutathione (29), intracellular glutathione depletion (increased oxidative stress in the myocyte) may decrease the CK activity before the enzyme is released into the circulation.

In addition to glutathione depletion, other causes for low CK activity have been described. In multiple organ failure, tissue factors, such as lysosomal enzymes, that are able to deactivate CK are released into the circulation (30). Treatment with certain drugs also has been associated with a low serum CK activity (31–34). Furthermore, the absence of physical exercise in the (immobilized) critically ill patients contributes to low CK activity in serum.

In conclusion, attention should be paid to the effect of endogenous glutathione when interpreting serum CK activity, especially in clinical conditions associated with low extracellular glutathione concentrations. This in vivo effect cannot be restored by the in vitro addition of reactivating sulfhydryl compounds to the reagents for CK determination or directly to the serum before analysis.

**References**


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