SKELETAL MUSCLE AND BLOOD OXIDATIVE STRESS IN A CHRONIC KIDNEY DISEASE ANIMAL MODEL

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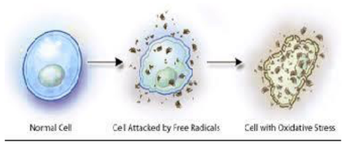
OBJECTIVES

Chronic kidney disease (CKD) leads to muscle atrophy, metabolic disorders, diminished exercise capacity and fatigue[1]. Complex mechanisms causing dysfunction have been proposed, and oxidative stress may be implicated.

The presence of oxidative stress in CKD is evidenced by an overabundance of lipid, carbohydrate and protein oxidation products in blood and skeletal muscle of patients [2].



To avoid a variety of confounding factors in human patients (years in dialysis, comorbidities, pharmaceuticals, gender, nutritional status, etc.) we employ an animal model mimicking renal failure to investigate the effects of uremia on redox study.

The aim of this study was to evaluate the effects of uremia on skeletal muscle (psoas, soleus) and blood redox status in a rabbit model of renal insufficiency

METHODS

New Zealand rabbits were used. Surgery (sham-operation, N=3 or 5/6 partial nephrectomy, N=6) and euthanasia protocols approved by the ethic committee of the University of Thessaly.

Blood samples (serum, plasma, red blood cell lysate), psoas and soleus muscle samples were harvested, frozen under liquid nitrogen, and stored at -80° C until analyzed.

Protein Carbonyls (PC), Catalase activity (CAT), Thiobarbituric Acid Reactive Substances (TBARS), Reduced Glutathione (GSH), Oxidized Glutathione (GSSG) and GSH/GSSG Ratio, Glutathione Reductase Activity, Uric Acid and Glucose were evaluated in the blood derivatives and muscle homogenates.

Non-parametric analysis methods were used (Mann-Whitney U test for means comparisons). Associations between parameters were assessed using the Spearman's rank correlation test. Significance level was set at $p<0.05$.

RESULTS

PC concentration was significantly higher in uremic psoas compared to control ($p<0.05$) and significantly higher in uremic soleus compared to control ($p<0.05$) (Figure 1).

Moreover GR (Glutathione Reductase) activity tended to be higher in uremic psoas compared to control ($p=0.053$). No other significant differences were observed.

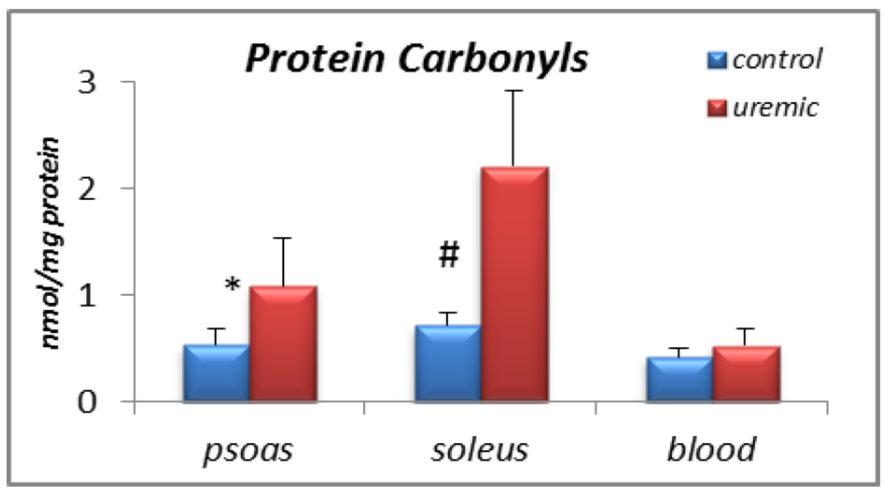
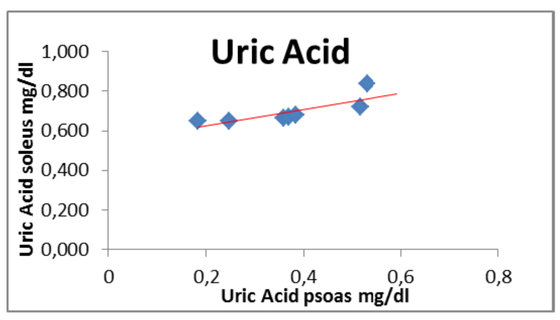


Figure 1. Protein Carbonyls (PC) concentration in skeletal muscle homogenates (psoas, soleus) and blood of control and uremic rabbits (Mean+SD).
* depicts statistically significant differences between control and uremic group.

Regarding blood analysis, CAT activity in control group was significantly higher compared to uremic group ($p<0.05$) (Figure 2A). TBARS concentration tended to be higher in uremic group compared to control ($p=0.05$) (Figure 2B).

We examined, in the pool of samples, the possible association between blood and either of the two muscle types or between the muscle types for the examined indices. Apart from Glucose (blood and psoas levels, Fig 3) and TBARS (blood and soleus levels, rho=-0.72, $p<0.05$) we found no other relationship between blood and muscle levels. Likewise we found no correlations between psoas and soleus levels with the exception of Uric Acid (Fig. 3), and PC concentration (rho=0.86, $p<0.05$).



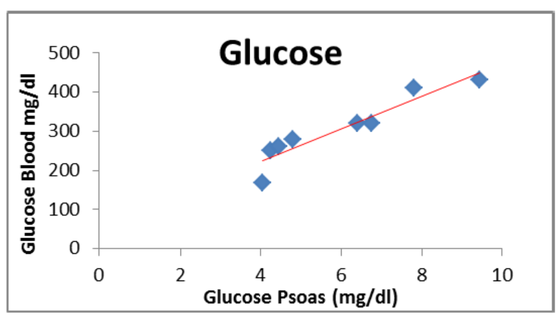
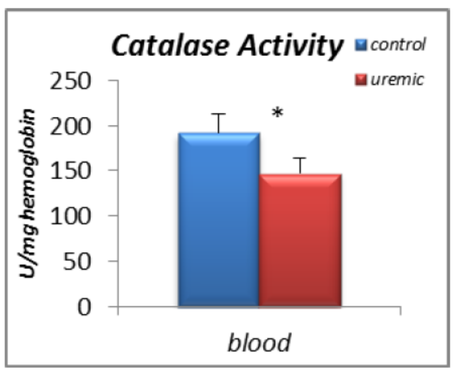


Figure 3. Association of selected muscle or blood parameters. Top: correlation of Uric Acid concentration between soleus and psoas skeletal muscle (rho=0.75, $p<0.05$). Bottom: correlation of Glucose concentration between blood and psoas skeletal muscle (rho=0.79, $p<0.05$).



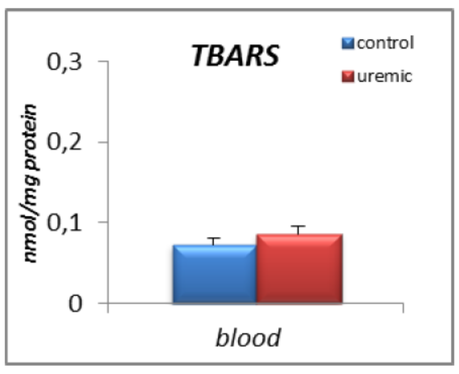


Figure 2. Left: Catalase activity, Right: TBARS (Mean+SD). * depicts statistically significant differences between control and uremic group.

DISCUSSION & CONCLUSIONS

Oxidative modification of proteins leads to alterations of amino acid sequences and rapid degradation. Carbonyl formation is considered as an early marker for protein oxidation. Recent studies have shown that plasma protein carbonyl levels are increased in uremia[4]. Moreover, skeletal muscle contains high levels of myofibrillar proteins, being susceptible to free radical oxidation. In the present study we found that there was evidence for oxidative damage to proteins in the blood and the skeletal muscle of uremic animals.

Our results indicated that CAT activity was suppressed in the erythrocytes of the uremic group. It is proposed that reduced antioxidant defense mechanisms in the erythrocytes is one of the most important factors leading to peroxidation in the membrane lipid structure of the erythrocytes and thereby to hemolysis and anemia in the patients with CKD[5].

TBARS levels in uremic blood revealed a tendency of oxidative modification of lipids in uremia.

Interestingly blood levels of most redox and metabolic indices examined did not reflect muscle concentrations. This should be taken into account when interpreting the literature.

Our findings reveal a generalized increase in oxidative stress which can make it a pathological element of interest in uremia, possibly explaining in part the observed muscle dysfunction in renal patients. Further efforts should be made in order to achieve a better understanding of its causes and to identify new tools effective in its clinical management.

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Acknowledgments. *This research has in part been co-financed by the European Union (European Social Fund - ESF) and Greek national funds through the Operational Program "Educational and Lifelong Learning" of the National Strategic Reference Framework (NSRF) - Research Funding Program: Thales (MuscleFun Project-MIS 377260) Investing in knowledge society through the European Social Fund.*

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Co-financed by Greece and the European Union