



Changes of para-, meta- and ortho-tyrosine over time in burned patients

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ABSTRACT

Purpose: Serious burn injury leads to oxidative stress resulting in production of meta- and ortho-tyrosine, while para-tyrosine is the physiological isoform. Our aim was to investigate the metabolism of these tyrosine isoforms following major burn injury.

Methods: Fifteen patients requiring intensive care were followed for 5 consecutive days after major burn injury. Serum and urine concentrations of para-, meta-, and ortho-tyrosine were measured with high performance liquid chromatography. Fifteen healthy matching individuals were invited as control group.

Results: Median serum concentration of normal isoform para-tyrosine decreased in burned patients between days 2 and 5 ($p < 0.01$). Mean meta- and ortho-tyrosine levels were significantly higher in patients compared to controls in the same time period ($p < 0.05$). Renal excretion of para-tyrosine increased significantly in our observation period ($p < 0.01$).

Conclusions: Pathologic isoforms of tyrosine accumulate in serum meanwhile the level of normal isoform decreases possibly due to belated enhanced renal excretion or, to decreased synthesis after major burn injury.

1. Introduction

Reactive oxygen species (ROS) production leading to damage to vital structures, including lipids, amino acids, proteins, nucleic acids of cells and mitochondria, as well as inflammatory cell activation and endothelial dysfunction is a result of oxidative stress (OS) following burn injury (Bauer and Bauer, 1999; Gallery, 2011). (Fig. 1)

Oxidative stress may lead to the development of multiple organ failure in burned patients (Kehrer, 1993). Inflammation and tissue hypoxia are key inducers of ROS production in critically ill patients, while generated ROS may exert parenchymal renal damage, microvascular and functional deterioration in a vicious circle (Heyman et al., 2011). ROS production following NADPH oxidase activation can induce circulatory dysfunction and capillary bed plugging (Tyml, 2011). As a direct measurement of ROS on the site of production is complicated, the quantitative measurement of OS-derived products including lipid peroxidation markers, or decrease of endogenous antioxidants may be helpful. (Fig. 1) Commonly measured lipid peroxidation markers are malondialdehyde (MDA), oxidized phospholipids and oxidized low-density lipoprotein. (Ho et al., 2013). Higher levels of lipid

peroxidation products including conjugated dienes (CD) and MDA levels and the ongoing oxidant changes after burn injury may explain organ dysfunction and additional sepsis. (Demling and Lalonde, 1990) Additionally, myeloperoxidase (MPO) activity may reflect inflammation-related OS.

Our previous studies revealed increased MDA levels, MPO activity, increased inducible ROS production from white blood cells and shorter lag phase of ROS production in burn injury patients. This increase was marked on the 2nd and 3rd study days following thermal injury (Foldi et al., 2009; Csontos et al., 2012).

Reactive oxygen and nitrogen species can also lead to the production of abnormal amino acids. Normal physiological enzymatic processes produce para-tyrosine (p-Tyr) from L-phenylalanine (Phe) predominantly in the kidney due to the activation of enzyme phenylalanine (4)-hydroxylase (PAH) enzyme. Besides the common p-Tyr, also meta- and ortho-tyrosine (m- and o-Tyr) can be formed from Phe in the excess of hydroxyl free radicals (HO)[•] (Stadtman and Berlett, 1991; Galano and Cruz-Torres, 2008), therefore, one may recognize m- and o-Tyr as OS products. (Fig. 2)

Several former studies proved elevated levels of m- and o-Tyr in case

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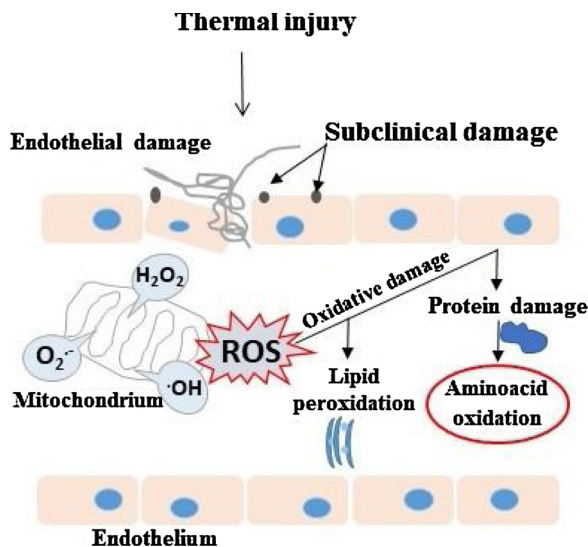


Fig. 1. Development of amino acid oxidation due to endothelial damage, inflammatory cytokine release and ROS production caused by thermal injury.

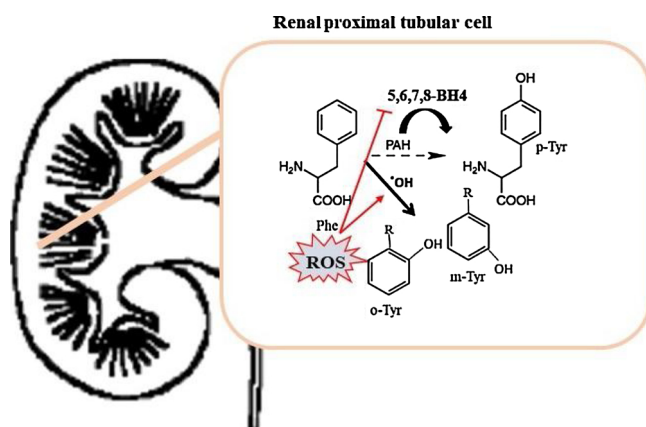


Fig. 2. Production of different tyrosine isoforms from phenylalanine due to phenylalanine-hydroxylase enzyme and oxidative stress (mainly hydroxyl free radicals) ROS inhibit the cofactor 5,6,7,8-BH4 and induce the production of pathological tyrosine isoforms. Abbreviations: Phe- phenylalanine, p-Tyr-para-tyrosine, m-Tyr-meta-tyrosine, o-Tyr-ortho-tyrosine, 5,6,7,8-tetrahydrobiopterin (5,6,7,8-BH4) Phenylalanine-hydroxylase enzyme-PAH, Hydroxyl radical- OH, Reactive oxygen species- ROS.

of tissue damage caused by OS, as well as their correlation with different OS markers (Lubec et al., 1996; Dandona et al., 2001; Jorres et al., 2000). A recent study by Mikolás and associates discussed the potential role of m- and o-Tyr in the development of erythropoietin resistance (Mikolás et al., 2014).

However abnormalities of Phe and Tyrosine kinetics are better known, according to our current knowledge no data exists regarding the time course of different tyrosine (Tyr) isoforms after burn injury. It comes natural to investigate the connection between Tyr isoforms and Injury Severity Indexes like APACHE II (Acute Physiology And Chronic Health Evaluation) which was designed as a mortality prediction tool but was not intended to influence the medical management of patients during their ICU stay, SAPS II (Simplified Acute Physiology Score), which is a severity score and mortality estimation tool developed from a large sample of medical and surgical patients in North America and Europe based on 17 factors, and ABSI (Abbreviated Burn Severity Index) which is a five-variable scale to help assess burn severity. The variables considered are (1) sex, (2) age, (3) presence of inhalation injury, (4) presence of a full-thickness burn, and (5) percentage of total

body surface area burned. The aim of our study was to assess the time course of known Tyr isoforms in blood and urine samples of burned patients.

2. Methods

2.1. Subjects and study design

The study protocol was designed in accordance to the ethical guidelines of 2003 Declaration of Helsinki, and authorized by the local ethics committee with 4422 reference number. Patients or their next of kin provided written informed consent to gather, summarize and analyse their data and samples for investigation. Healthy, age and gender matched individuals were invited as controls. A five-day study interval was chosen based on the results of earlier studies. We hypothesized that this wide time frame would allow the detection of both the ascending and descending phase of oxidative stress response (Lubec et al., 1996; Dandona et al., 2001; Jorres et al., 2000).

2.2. Patients care

Patients were treated in uniform way. Fluid resuscitation was guided by the central venous oxygen saturation (ScVO₂). We aimed to maintain ScVO₂ > 65 % (Csontos et al., 2008). Invasive transcatheter pulmonary haemodynamic monitoring (PiCCO, Pulsion Medical Systems, Munich, Germany) was started if the initial resuscitation goal was not achieved within 4 h or Total Body Surface Area (TBSA) was > 40 % (Csontos et al., 2008; Foldi et al., 2010). Wound excision and grafting were started within 72 h and enteral feeding was commenced on the first day after injury when haemodynamic stability had been reached. Twenty to thirty percent of burned surface was excised and grafted in one sitting. Operations were repeated every 3–4 days. In case of suspected long-term ventilation, tracheostomy was performed before the first grafting. If inhalation injury was suspected (facial burn, soot in the throat etc.) bronchoscopy was carried out for verification.

2.3. Inclusion and exclusion criteria

Inclusion criteria were burn injury affecting more than 15 % of the body surface and admission to our ward within 6 h after injury. Exclusion criteria were electrical injury, presence of any obvious bacterial infection on admission, extreme burn severity (> 80 % total burnt surface area), previously documented chronic left heart or renal insufficiency, age less than 18 years or documented previous medication (e.g. chronic use of corticosteroid medication) or treatment (e.g. radio-, chemotherapy) affecting the inflammatory response of the body to burns, and known malignant disease and anuria during the study period.

2.4. Sample collection

Blood samples were obtained from central venous catheter while peripheral blood samples were gathered from healthy individuals. Urine was collected for 24 h in both groups. Blood was centrifuged to obtain serum. Serum and urine samples were stored at –80 °C until further examinations. Blood samples were drawn on admission (day 1) and on the four consecutive days (days 2–5) at seven o'clock a.m. before operations or painful dressing changes. Serum and urinary creatinine levels were also part of the routine measured parameters. Samples were examined at the Institute of Laboratory Medicine, University of Pécs.

2.5. Tyrosine measurement

One hundred and twenty-five µl of Trichloro-acetic acid (TCA) was added to 500 µl serum (/urine), then samples were incubated on ice for 30 min. Centrifugation was used to separate precipitate. Supernatant

was filtered by a syringe filter (0.2 µm) (Millipore, Billerica, MA, USA). Serum and urinary concentrations of para-, meta-, ortho-tyrosine and phenylalanine were determined as described earlier, using reverse phase-high-performance liquid chromatography (HPLC) (Shimadzu USA Manufacturing INC) (C18 silica column, 250 × 4 mm) with fluorescence detection (λEX = 275 nm; λEM = 305 nm for the tyrosines and λEX = 258 nm; λEM = 288 nm for phenylalanine) (Toth et al., 2011). For better peak resolution, a 4-fold dilution with TCA was applied just before the HPLC measurement. External standard was used to calculate concentrations. Calibration curves for p-, m-, o-Tyr and Phe were established in the range 3.125–25 µM; 7.8125–500 nM; 7.8125–500 nM and 4.687–37.5 µM, respectively. In case of a value exceeding these ranges, further dilution was applied.

2.6. Statistical analysis

Statistical Package for the Social Sciences (SPSS) software, version 20.0 (IBM Corporation, USA) was used for statistical analysis. Data were expressed as median and inter-quartile range (IQR (standard 25th–75th percentile) as distribution was not normal according to Kolmogorov-Smirnov test. Pairwise Kruskal-Wallis test was carried out with Dunn-Bonferroni post-hoc adjustment for interday analysis. Values of $p < 0.05$ were considered significant. The minimal number of patients was determined on the basis of our previous studies (Foldi et al., 2009; Csontos et al., 2012).

3. Results

3.1. Demographic and patients data

Fifteen patients and 15 healthy individuals were included into the study. Twelve patients survived the whole study period, two patients died on day 4 and one on day 5. Three patients had inhalation injury detected. However, the leading causes of death were cardiac and respiratory failure during the trial period. Further 3 patients died of later septic complications during Intensive Care Unit (ICU) stay after the 5 days of observation period. Although non-survivors were notably older and TBSA was higher, the difference between survivors and non-survivors was not statistically significant. We calculated the 24 h mean norepinephrine requirement (µg/kg/24 h) of the patients who needed the treatment. Demographic and patient data are summarized in Table 1.

Table 1

Demographic and Clinical Data of burned Patients on day 1, represented with median and 25–75 percentiles.

	Median (25–75th)
Age	68 (54–81)
TBSA (%)	25 (20–35)
ABSI	7 (5–8)
APACHE II	11 (8–16,5)
SAPS II	28 (24–39)
Serum Creatinine (µmol/L)	62 (53–80)
BUN (mmol/L)	458 (3,26–6,9)
UO (24 h) (ml)	2490 (1663–3346)
p-tyrosine (µmol/L)	369 (30,8–44,6)
m-tyrosine (nmol/L)	129 (6,57–21,49)
o-tyrosine (nmol/L)	212 (11,26–37,4)
phenylalanine (µmol/L)	5029 (33,4–67,44)

Abbreviations: IQR- Interquartile Range, TBSA – Total Body Surface Area, ABSI- Abbreviated Burn Severity Index, APACHE II- Acute Physiology and Chronic Health Evaluation II, SAPS II- Simplified Acute Physiology Score II, BUN-blood urea nitrogen, UO-Urine Output, tyr- tyrosine.

3.2. Serum tyrosine and phenylalanine levels

Serum p-Tyr levels showed significant decreasing tendency ($p < 0.035$). There was no statistical difference between healthy controls and burned patients on admission, but the p-Tyr levels were significantly lower in burned patients on days 2 ($p < 0.001$), 3 ($p < 0.001$), 4 ($p = 0.001$), and 5 ($p < 0.001$) compared to controls (Fig. 3A).

Serum m-Tyr levels did not show significant tendency through the observation period. Its levels were similar in burned patients compared to healthy controls on day 1, then significantly elevated on days 2 ($p = 0.024$), 3 ($p = 0.002$) and 4 ($p = 0.036$), eventually turned back to a level similar to controls on day 5 (Fig. 3B). A similar trend was observed in case of serum o-Tyr but with significant increasing tendency through the observation period ($p = 0.026$). Compared to controls its levels were significantly higher on days 2 ($p = 0.024$), 3 ($p = 0.003$), 4 ($p = 0.002$). On day 5 a significant difference was no more found ($p = 0.450$) (Fig. 3C).

Phe levels did not show any significant tendency ($p = 0.250$), and its levels were higher in burned patients compared to healthy volunteers, but it was significant on day 2 ($p = 0.045$) only (Fig. 3D).

3.3. Urinary tyrosine excretions

Urinary p-Tyr excretion showed a significant increasing tendency ($p = 0.013$). Comparing patients and controls there was no significant difference from days 1 to 3, then became significantly higher in patients on days 4 ($p = 0.004$) and 5 ($p < 0.001$) (Fig. 4A).

m-Tyr excretion did not show any tendency during the observation period. Its levels did not differ significantly in patients compared to controls from days 1 to 4. However, a significantly higher level was found in patients on day 5 ($p = 0.041$) (Fig. 4B).

o-Tyr excretion did not show any tendency during the study. Higher but not significant o-Tyr excretion was found in patients on all days (Fig. 4C).

3.4. Clinical parameters

Urine output (UO) showed an increasing tendency ($p < 0.001$). Comparing to day 1, its volume was not significantly higher on days 2 and 3, but it became significantly higher on days 4 ($p = 0.002$) and 5 ($p < 0.001$) (Fig. 5A).

Serum creatinine levels of the patients were in the normal range during the whole study period (Fig. 5B).

3.5. Correlations with injury severity and clinical markers

Serum Phe levels did not show any correlation with TBSA ($r = 0.173$, $p = 0.149$) or serum albumin levels ($r = 0.009$, $p = 0.960$) but positive correlation was found with ABSI ($r = 0.538$, $p = 0.038$) and APACHE II ($r = 0.385$, $p = 0.001$). Similarly, serum p-Tyr showed significant positive correlation with ABSI ($r = 0.527$, $p = 0.043$), APACHE II ($r = 0.271$, $p = 0.022$), also with serum Phe levels ($r = 0.442$, $p = 0.001$) and norepinephrine requirement ($r = 0.515$; $p = 0.05$). Norepinephrine requirement showed a positive correlation with ABSI ($r = 0.667$; $p = 0.007$).

3.6. Data results comparing clinical outcomes

P-tyr levels of non-survivors were not significantly higher than that of survivors, however, m-, and o-tyr excretions were significantly higher in non-survivors on day 3. $p = 0.004$ ABSI and SAPS II was significantly higher in non-survivors $p = 0.012$ and 0.019 . Figure not shown

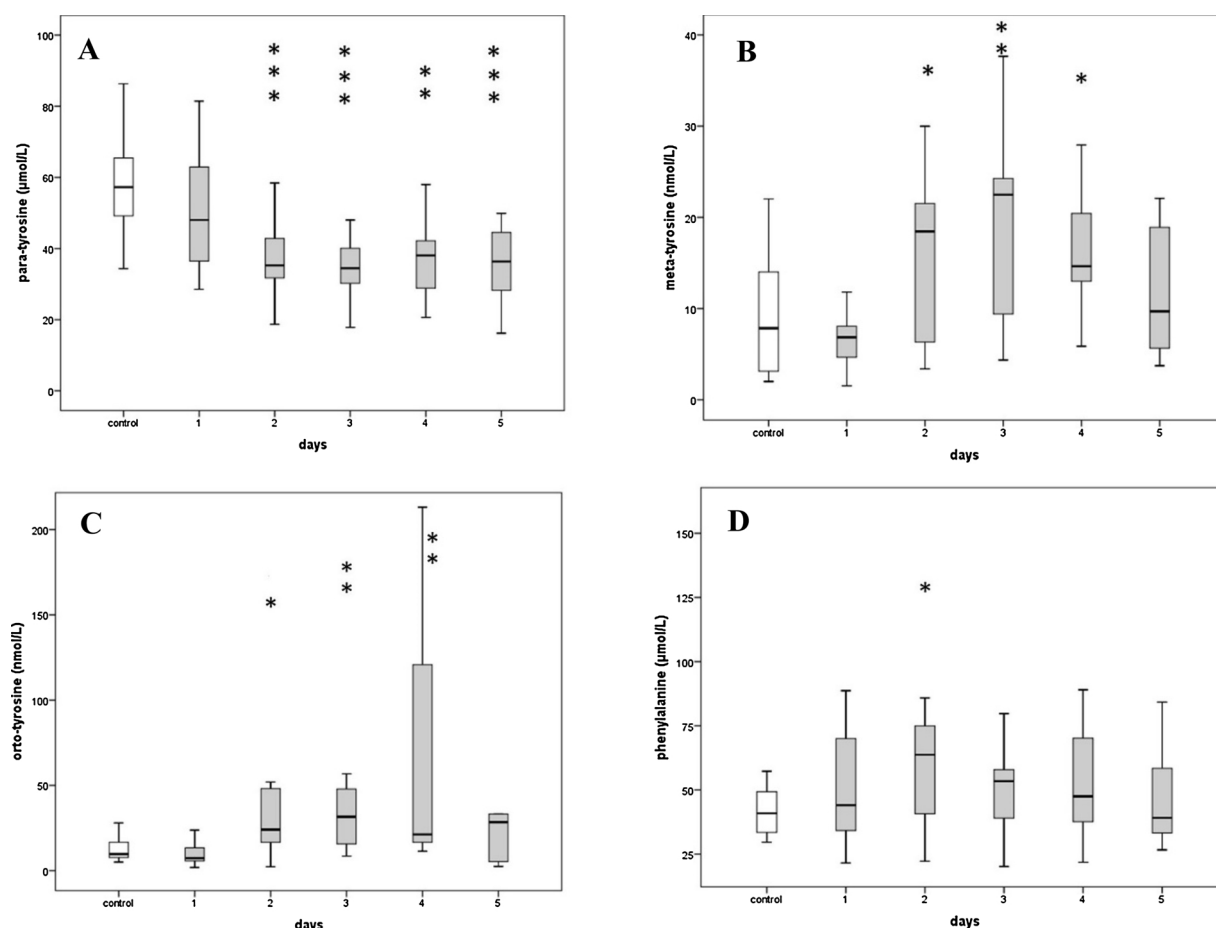


Fig. 3. Serum para-tyrosine (A), meta-tyrosine (B) ortho-tyrosine and phenylalanine (D) levels in burns and controls. Data are expressed as 95 % confidence interval, median and interquartile range. *Symbols indicate group differences between burns and controls * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

4. Discussion

Earlier studies examined serum total Tyr levels in burn injury and higher serum Phe/Tyr levels were hypothesized to be associated with reduced survival (Chang et al., 1983; Herndon et al., 1978).

According to our knowledge this is the first study examining the levels of different Tyr isoforms in burns. Serum p-Tyr course expressed a clearly different trend compared to m-Tyr and o-Tyr levels. A possible cause of the described difference is the two pathways of Tyr synthesis, as p-Tyr may be produced enzymatically in physiological conditions mainly in the kidneys, as well as to a much lower extent in OS, while m- and o-Tyr are formed non-enzymatically in OS only.

The observed decrease of p-Tyr in serum may be due to i) lowered levels of precursor molecules, ii) increased renal excretion or iii) decreased renal synthesis. According to our data, serum Phe levels were not lower in patients compared to controls, in fact, it was even significantly higher on day 2. In previous studies the levels of most amino acids dropped but Herndon and associates found elevated levels of Phe in septic and non-septic burned patients similarly to Rath and associates (Herndon et al., 1978; Rath et al., 1987). Coudray-Lucas and associates found elevated Phe levels on the seventh day after injury (Coudray-Lucas et al., 1985). The study of Cynober and associates showed elevated Phe levels from day 4 which remained elevated until day 28 (Cynober et al., 1982 Sep). In contrast to these data in our study the highest Phe level was found on the second day after injury. These data support our hypothesis that not lower levels of the precursor Phe are the underlying cause of the decreased p-Tyr levels.

p-Tyr-excretion was significantly higher in patients on days 4-5 only when serum p-Tyr levels were slightly increasing. It does not support

the role of increased renal loss in the observed lower p-Tyr levels. Therefore, one feasible hypothesis is the decreased synthesis. The underlying cause of that may be tubular injury or decreased PAH-activity. It is known that in burn injury the existing ROS (eg. hydroxyl free radicals) can destroy PAH cofactor 5,6,7,8-tetrahydrobiopterin (5,6,7,8-BH4) which can impair PAH activity as well and eventually can cause decreased conversion of Phe to Tyr. (Herndon et al., 1978; Ploder et al., 2008).

Burn is associated with hypovolemia in the first 48 h following injury and it together with the developing inflammation may also impair renal function thus decreasing renal synthesis of p-Tyr (Csontos et al., 2008; Lavrentieva et al., 2013; Sanchez-Sanchez et al., 2013). The normal serum creatinine levels during the study do not seem to support the presence of severe glomerular injury. However, moderate changes in tubular function may also occur without a significant rise in serum creatinine. Based on the above mentioned facts, probably both pathogenic ways may play a potential role, and upon the available data, we cannot provide a relative contribution of the two processes.

Serum m- and o-Tyr levels (biomarkers of hydroxyl free radical-related oxidative damage) were elevated in burn patients. These elevations were most marked on day 3, which is in concordance with former findings on elevated MDA levels and increased ROS synthesis (Lubec et al., 1996; Dandona et al., 2001). Burn injury-induced oxidative stress has already been described in several other articles (Ritter et al., 2003; Pintauro et al., 2000). The similar trend of m- and o-Tyr and other OS markers supports our hypothesis that serum m- and o-Tyr levels can reflect the OS after burn injury. An alternative explanation for the increase of serum levels of m- and o-Tyr would be a decreased renal clearance of the abnormal amino acids, however, urinary excretion of

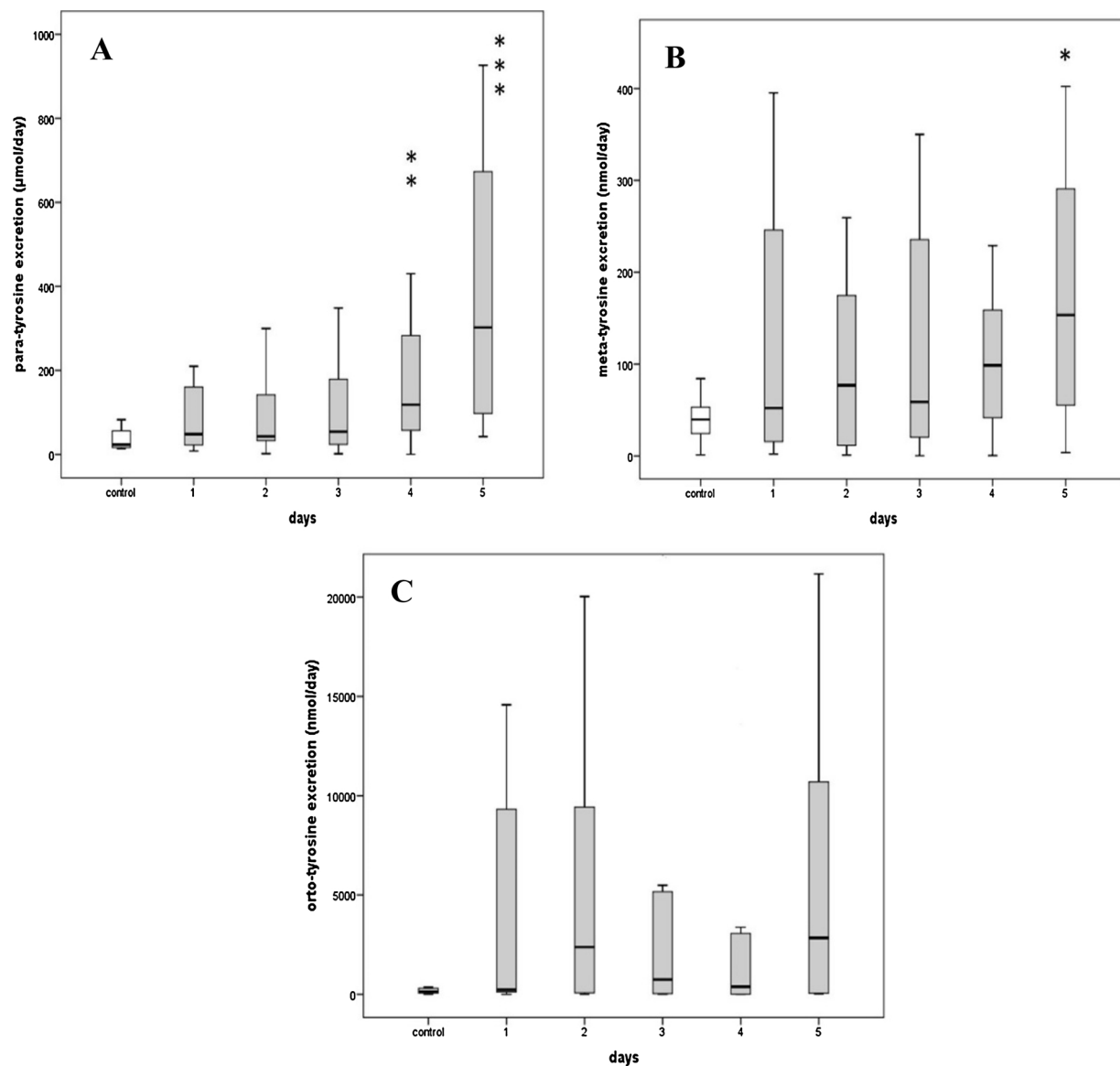


Fig. 4. para-tyrosine (A), meta-tyrosine (B) and ortho-tyrosine (C) excretion in burns and controls. Data are expressed as 95 % confidence interval, median and interquartile range. *Symbols indicate group differences between burns and controls * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

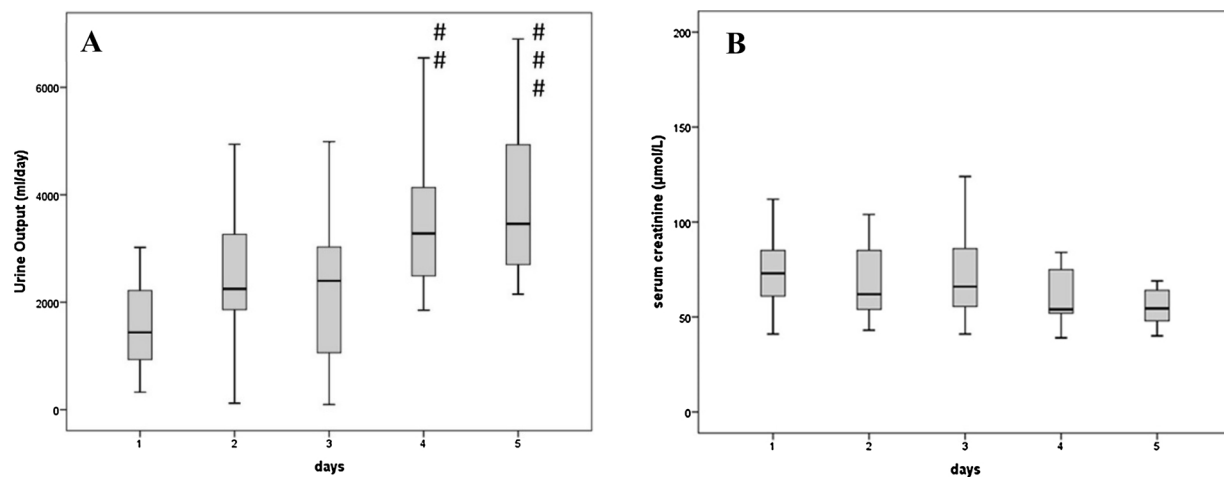


Fig. 5. 24 -hs urine output (A) and serum creatinine (B) levels in burns. Data are expressed as 95 % confidence interval, median and interquartile range. *Symbols indicate intra-group differences compared to day 1 ## $p < 0.01$, ### $p < 0.001$.

the pathologic Tyr isoforms was not decreased as compared to controls, thus this possibility can be rejected. The existing correlation with ABSI and APACHE II suggest that serum Phe levels can reflect the severity of injury.

The positive correlation between p-Tyr and injury severity indexes is interesting because p-Tyr is decreased compared to controls. It might be explained by disturbed DOPA, dopamine, epinephrine and norepinephrine synthesis from p-Tyr in more seriously injured patients. This hypothesis is supported by the positive correlation of norepinephrine requirement and ABSI, as well as its correlation with serum p-Tyr.

Concluding increased m- and o-Tyr levels can reflect the OS after burn injury similarly to the decreased p-Tyr levels.

5. Limitation of the study

Although it has been calculated on the basis of our previous studies, we regard our study population small. We did not performed measurement of other oxidative stress markers in this study, therefore we could only compare the kinetic of tyrosine isoforms with each other and time course of oxidative stress markers measured in our previous studies. The underlying mechanism behind the correlation between serum p-Tyr and norepinephrine requirement raises decreased tyrosine hydroxylase activity, or an influential role of intravenously administered norepinephrine due to a negative feedback on tyrosine hydroxylase activity? Moreover, to detect subclinical renal injury the measurement of more sensitive markers e.g. Neutrophil-gelatinase-activated lipocalin (NGAL), cystatin C would have been beneficial as well as para-hydroxyphenylacetic acid (pHPA), 3-nitro-4-hydroxyphenylacetic acid or 3-nitrotyrosine.

Disclosure

The authors declare that there is no conflict of interest regarding the publication of this paper.

The manuscript has been published only as an oral presentation in the 30th ESICM Annual Congress. Wien, September 23–27, 2017, and is not being considered for publication elsewhere.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.imbio.2020.151917>.

References

- Bauer, V., Bauer, F., 1999. Reactive oxygen species as mediators of tissue protection and injury. *Gen. Physiol. Biophys.* 18, 7–14.
- Chang, X.J., Yang, C.C., Hsu, W.S., et al., 1983. Serum and erythrocyte amino acid pattern: studies on major burn cases. *Burns Incl. Therm. Inj.* 9, 240–248.
- Coudray-Lucas, C., Cynober, L., Lioret, N., et al., 1985. Origins of hyperphenylalaninemia in burn patients. *Clin. Nutr.* 4 (August (3)), 179–183.
- Csontos, C., Foldi, V., Fischer, T., et al., 2008. Arterial thermodilution in burn patients suggests a more rapid fluid administration during early resuscitation. *Acta Anaesthesiol. Scand.* 52 (6), 742–749.
- Csontos, C., Rezman, B., Foldi, V., Bogar, L., Drenkovics, L., Röth, E., Weber, G., Lantos, J., 2012. Effect of N-acetylcysteine treatment on oxidative stress and inflammation after burn injury. *Burns* 38, 428–437.
- Cynober, L., Dinh, F.N., Blonde, F., et al., 1982 Sep. Plasma and urinary amino acid pattern in severe burn patients-evolution throughout the healing period. *Am. J. Clin. Nutr.* 36 (Sep (3)), 416–425.
- Dandona, P., Mohanty, P., Hamouda, W., et al., 2001. Inhibitory effect of two day fast on reactive oxygen species (ROS) generation by leukocytes and plasma ortho-tyrosine, meta-tyrosine concentrations. *J. Clin. Endocrinol. Metabol.* 86, 2899–2902.
- Demling, R.H., Lalonde, C., 1990. Systemic lipid peroxidation and inflammation induced by thermal injury persists into the post-resuscitation period. *J. Trauma* 30 (Jan (1)), 69–74.
- Foldi, V., Csontos, C., Bogar, L., Roth, E., Lantos, J., 2009. Effects of fluid resuscitation methods on burn trauma induced oxidative stress. *J. Burn. Care Res.* 30 (6), 957–966.
- Foldi, V., Lantos, J., Bogar, L., et al., 2010. Effects of fluid resuscitation methods on the pro- and anti-inflammatory cytokines and expression of adhesion molecules after burn injury. *J. Burn Care Res.* 31 (3), 480–491.
- Galano, A., Cruz-Torres, A., 2008. HO radical reactions with phenylalanine in free and peptide forms. *Org. Biomol. Chem.* 6, 732–738.
- Gallery, H.F., 2011. Oxidative stress and mitochondrial dysfunction in sepsis. *Br. J. Anaesth.* 107, 57–64.
- Herndon, D.N., Wilmore, D.W., Mason, A.D., et al., 1978. Abnormalities of phenylalanine and tyrosine kinetics. *Arch. Surg.* 113, 133–135.
- Heyman, S.N., Rosen, S., Rosenberger, C., 2011. A role for oxidative stress. *Contrib. Nephrol.* 174, 138–148.
- Ho, E., Galougahi, K.K., Liu, C.C., et al., 2013. Biological markers of oxidative stress: applications to cardiovascular research and practice. *Redox Biol.* 1, 483–491.
- Jorres, R.A., Holz, O., Zachgo, W., et al., 2000. The effect of repeated ozone exposures on inflammatory markers in bronchoalveolar lavage fluid and mucosal biopsies. *Am. J. Respir. Crit. Care Med.* 161, 1855–1861.
- Kehrer, J.P., 1993. Free radicals as mediators of tissue injury and disease. *Crit. Rev. Toxicol.* 23, 21–48.
- Lavrentieva, A., Kontakiotis, T., Kaimakamis, E., et al., 2013. Evaluation of arterial waveform derived variables for an assessment of volume resuscitation in mechanically ventilated burn patients. *Burns* 39, 249–254.
- Lubec, B., Hain, M., Denk, W., et al., 1996. Brain lipid peroxidation and hydroxyl radical attack following the intravenous infusion of hydrogen peroxide in an infant. *Free Radic. Biol. Med.* 21, 219–223.
- Mikolás, E., Kun, S., Laczy, B., et al., 2014. Incorporation of ortho- and meta-tyrosine onto cellular proteins leads to erythropoietin-resistance in an erythroid cell line. *Kidney Blood Pressure Res.* 38, 217–225.
- Pintaudi, A.M., Tesoriere, L., D'Arpa, N., et al., 2000. Oxidative stress after moderate to extensive burning in humans. *Free Radic. Res.* 33, 139–146.
- Ploder, M., Neutrauer, G., Spittler, A., et al., 2008. Serum phenylalanine in patients post trauma and with sepsis correlate to neopterin concentrations. *Amino Acids* 35, 303–307.
- Rath, T., Roth, E., Keidl, R., et al., 1987. Phenylalanine: total amino acid ratio in 45 burn patients. *Scand. J. Plast. Reconstr. Surg. Hand Surg.* 21 (3), 297–300.
- Ritter, C., Andrades, M., Guerreiro, M., et al., 2003. Plasma oxidative parameters and mortality in patients with severe burn injury. *Intensive Care Med.* 29, 1380–1383.
- Sanchez-Sanchez, M., Garcia-de-Lorenzo, A., Lopez, T., et al., 2013. A protocol for resuscitation of severe burn patients guided by Transpulmonary thermodilution and lactate levels. *Crit. Care* 17, R176.
- Stadtman, E.R., Berlett, B.S., 1991. Fenton chemistry. Amino acid oxidation. *J. Biol. Chem.* 266, 17201–17211.
- Toth, P., et al., 2011. Microalbuminuria, indicated by total versus immunoreactive urinary albumins, in acute ischemic stroke patients. *J. Stroke Cerebrovasc. Dis.* 20 (November (6)), 510–516.
- Tyml, K., 2011. Critical role for oxidative stress, platelets, and coagulation in capillary blood flow impairment in sepsis. *Microcirculation* 18, 152–162.