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Role of Tyrosine Isomers in Acute and Chronic Diseases Leading to Oxidative Stress - A Review

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Abstract: Oxidative stress plays a major role in the pathogenesis of a variety of acute and chronic diseases. Measurement of the oxidative stress-related end products may be performed, *e.g.* that of structural isomers of the physiological para-tyrosine, namely meta- and ortho-tyrosine, that are oxidized derivatives of phenylalanine. Recent data suggest that in sepsis, serum level of meta-tyrosine increases, which peaks on the 2nd and 3rd days ($p<0.05$ *vs*. controls), and the kinetics follows the intensity of the systemic inflammation correlating with serum procalcitonin levels. In a similar study subset, urinary meta-tyrosine excretion correlated with both need of daily insulin dose and the insulin-glucose product in non-diabetic septic cases ($p<0.01$ for both). Using linear regression model, meta-tyrosine excretion, urinary meta-tyrosine/para-tyrosine, urinary ortho-tyrosine/para-tyrosine and urinary (meta- + ortho-tyrosine)/para-tyrosine proved to be markers of carbohydrate homeostasis.

In a chronic rodent model, we tried to compensate the abnormal tyrosine isomers using para-tyrosine, the physiological amino acid. Rats were fed a standard high cholesterol-diet, and were given para-tyrosine or vehicle orally. High-cholesterol feeding lead to a significant increase in aortic wall meta-tyrosine content and a decreased vasorelaxation of the aorta to insulin and the glucagon-like peptide-1 analogue, liraglutide, that both could be prevented by administration of para-tyrosine.

Concluding, these data suggest that meta- and ortho-tyrosine are potential markers of oxidative stress in acute diseases related to oxidative stress, and may also interfere with insulin action in septic humans. Competition of meta- and ortho-tyrosine by supplementation of para-tyrosine may exert a protective role in oxidative stress-related diseases.

Keywords: Meta-tyrosine, ortho-tyrosine, para-tyrosine, sepsis, inflammation, oxidative stress, hormone resistance, carbohydrate metabolism.

1. INTRODUCTION

Oxidative stress means an imbalance between free radical production and the natural antioxidant defense [1]. Free radicals are highly reactive molecules with unpaired electrons. The classical scheme of free radical formation involves the partial reduction of oxygen, giving rise to reactive oxygen species (ROS). In the first step, a one-electron reduction of oxygen leads to superoxide free radical generation. The second step leads to the dismutation of superoxide to hydrogen peroxide (H_2O_2), this can be catalyzed by the superoxide dismutase enzyme. The H_2O_2 is not a free radical, as it possesses no unpaired electrons, but rather a ROS. With a further reductive step, H_2O_2 can give rise the production of hydroxyl free radical (·OH), one of the most reactive species. This can happen in terms of the Haber-Weiss or the Fenton reactions. In the last step, ·OH is reduced to H_2O, *i.e.* to water, inactivating the oxygen species [1,2]. There are numerous ways to detect free radicals or oxidative modifications of macromolecules that arise from free radical reactions.

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The present review focuses on oxidative modifications of the essential amino acid, phenylalanine, and the possible role of meta- and ortho-tyrosine produced during this modification, comparison of these pathological amino acids to the physiological isoform, para-tyrosine. Also, the review tries to sum up the evidences that support the hypothesis, that these abnormal amino acids may not merely be regarded as oxidative stress markers but that they also may play a ethiological role in the development of certain pathologies. Data were collected by thorough review of the literature, mainly using PubMed and additional medical resources.

2. OXIDATIVE STRESS AND INFLAMMATION

Oxidative stress may develop due to the overproduction of free radicals and ROS. Inflammatory processes are possible sources of ROS production. Polymorphonuclear leukocytes are major sources of ROS in macroinflammatory processes, such as host-defense or sepsis. One of the major sources of ROS in the inflammatory processes are nicotinamid adenine dinucleotide (phosphate) [NAD(P)H] oxidases (NOX), that were originally recognized to play an important role in cellular defense in phagocytic cells. They (especially NOX2) may be responsible for respiratory burst processes [3]. Similarly, dual oxidases (DUOX) that may be regarded as homologs of NOX, have similar functions, and contribute to host defense [4]. Xantin oxidase may also be a major source of superoxide radical in inflammatory processes [5]. Intracellular ROS production may arise among others in the phagosomes of inflammatory cells or in the peroxisomes [6]. Also, malfunctioning mitochrondria may yield ROS by oxidation of glucose, pyruvate or NADPH [6]. Furthermore, uncoupling of endothelial nitric oxide synthetase (eNOS) may also give rise to superoxide anion radical production. Superoxide may be dismutated to hydrogen peroxide that is able to react with chloride anions in the presence of myeloperoxidase, thus turning to reactive hypochloric acid, further promoting tissue damage [5].

Tissue damage may lead to a release of transition metals such as iron from the cells, these may promote ROS formation *e.g. via* autoxidation, or in Fenton's reaction [7].

Moreover, in the inflammatory processes, the expression of the inducible form of nitric oxide synthetase (iNOS) may be triggered, thus giving rise to nitric oxide (NO) – a free radical itself – and also to reactive nitrogen species (RNS) [8].

On the other hand, ROS may further trigger inflammation, *e.g.* they may lead to phosphorylation of the inhibitor of kappa-B kinase (IκK) and may this promote nuclear factor kappa-B (NF-κB) signaling, thus leading to further transcription of inflammatory proteins such as iNOS, tumor necrosis factor alpha (TNF-α) or interleukin-1β [6,9]. This may initiate a vicious cycle. Furthermore, ROS are even able to induce multi-protein platforms, the so-called inflammasomes, that may lead to caspase activation and thus to programmed cell death *via* the mitogen-activated protein kinase (MAPK) system [10].

2.1. Oxidative Stress Markers

The extent of oxidative stress could be determined in different ways, using different methodologies. The most feasible way would be direct detection of the accumulating free radicals. However, these molecules are highly reactive due to their unpaired electron, and therefore have a very short half-life time. This makes them virtually impossible to measure, unless a so-called spin trap and an electron-spin resonance device are applied. This is however a cumbersome methodology and not for routine purposes [11].

Instead of measuring the radicals directly, research tends to focus on measurement of free radical-derived stable oxidation products. Among them, advanced glycation end-products (AGE) would offer the advantage of measurement *via* autofluorescence at characteristic wavelengths (*e.g.* 355/460 or 370/440 nm), however their production largely depends on glycation processes and is somewhat less directly influenced by oxidative stress [12]. Detection of advanced protein oxidation products (AOPPs) is also problematic, since this is also a heterogeneous class of proteins that become oxidized [13]. Specific, free radical-related products may be nucleic acid-related derivatives such as 8-oxo-guanosine [14] or single/double strand DNA breaks [15], lipid peroxidation products, such a F2-isoprostanes, malondialdehyde, 4-hydroxy-nonenal or thiobarbituric acid-reactive substances (TBARS) [15,16]. Nitrosative stress is a consequence of accumulation of peroxynitrite, a product of the reaction of NO and superoxide free radicals. Since, peroxynitrite is an unstable molecule, a RNS, it is able to attack and modify structure of proteins, leading to nitrosylation of tyrosine residues (nitrotyrosine). That is why; nitrotyrosine levels are not only dependent on the extent of oxidative stress, but also on NO formation, mainly *via* the iNOS [17].

A further possible approach would be measurement of antioxidant status or antioxidant capacities, such as reduced glutathione (GSH), total antioxidant status (TAS), plasma levels of antioxidant vitamins. This is

however a rather indirect measure and is also dependent on regulatory mechanisms of antioxidant enzymes, not only on oxidative stress [11].

2.1.1. Phenylalanine-Derivatives

L-Phenylalanine (Phe) is an essential amino acid, that is, the human body is unable to synthetize it, therefore its origin is the exogenous supply *via* food [18]. In the nature, three different structural isomers of L-tyrosine (L-Tyr) exist, namely para-, meta- and ortho-tyrosine (p-Tyr, m-Tyr and o-Tyr, respectively).

In human cells, p-Tyr is the only isoform produced enzymatically, *via* the iron(II)-containing phenylalanine-hydroxylase [18]. On the other hand, hydroxyl radical is able to attack the aromatic ring of Phe, giving rise to all three isomers, *i.e.* p-, m- and o-Tyr, as well. (Fig. **1A**). This process has already been described in the '50s, where Dalgliesh has described that in a non-cellular system consisting of Phe, EDTA and iron, p-Tyr and o-Tyr were produced [19]. Some years later, o-Tyr has been verified in a biological system: the cuticle of an insect larvae has been shown to contain o-Tyr at a certain stage of development [20].

The amount of p-Tyr produced in enzymatic processes by far exceeds that of p-Tyr produced *via* oxidative stress. [21,22] Summing up, p-Tyr is the main, physiological isoform, while m- and o-Tyr are regarded as specific hydroxyl free radical markers [21,22].

p-Tyr can be further oxidized to yield 3,4-dihydroxy-phenylalanine (DOPA) in both enzymatic and free radical-derived reactions (Fig. **1B**) [21]. Hydroxylation of Phe (Fig. **1A**) or hydroxylation of m- and o-Tyr may also yield 2,3-DOPA, a less studied substance [23,24] (Fig. **1C** and **D**).

m-Tyr has also been verified to be present in soil and to be produced by some types of grasses, and to function as a natural herbicide, inhibiting the growth of neighboring grasses and plants [25,26]. The exact source of formation of m-Tyr, however is unknown, in some species it is produced through transamination from meta-hydroxyphenylpyruvate (*e.g.* in *Euphorbia myrsinites,* Fig. **2A**), or probably *via* the cytochrome p450 system (*e.g.* in *Festuca rubra,* Fig. **2B**) [27].

Recent data suggest that some bacteria-derived peptide antibiotics contain m-Tyr, and the current evidence suggest that it is produced by a specific phenylalanine-3-hydroxylase instead of the normal variant, the phenylalanine-4-hydroxylase. The former enzyme is according to data of the authors a mutated form of the normally occurring phenylalanine-4-hydroxylase [28]. In humans, there is up to now no evidence of such an enzymatic production of m-Tyr, thus m- and o-Tyr should still be regarded as markers of hydroxyl radicals.

Among amino acid-derived oxidative stress markers, phenylalanine-derivatives offer a specific advantage in regard of detection: the aromatic ring bears an autofluorescence, thus both phenylalanine, as well as the tyrosine isoforms exert a specific fluorescence: Phe at wavelengths of 258 nm (excitation) and 288 nm (emission), while p-, m- and o-Tyr at 275 nm (excitation) and 305 nm (emission). Thus, they can be detected using fluorescent methods, such as high-performance liquid chromatography (HPLC) with a fluorescent detector, without derivatization, not only using derivatization or a mass-spectrometric mthod [29,30,21,22]. Also 3,4-DOPA could be detected and was shown to accumulate in non-water-soluble component of cataract lenses using a fluorescent HPLC system [21].

3. ACUTE DISEASES ASSOCIATED WITH OXIDATIVE STRESS

3.1. Clinical Inflammation and Resulting Oxidative Stress in Sepsis

Oxidative stress may play a major role in the development of complications of sepsis. Data support among others that, due to re-distribution of blood circulation, a slow-down in splanchnic circulation is followed by an increase in levels of ROS and RNS within just a few hours [31]. Moreover, microbes and inflammatory cells contribute in sepsis to an increase in levels of so-called damage-associated molecular patterns or pathogen-associated molecular patterns (DAMPs and PAMPs), which are able in turn to activate toll-like receptors, that could lead to a further amplification of oxidative stress and inflammation [31]. These processes may play an important role in acute kidney damage developing in sepsis [31].

Another type of substances released by inflammatory cells, but also by platelets and endothelial cells during sepsis, are microparticles. They are small vesicles that detach from their host cells, and are released into the circulation; they may carry molecules from cell membranes, but also from cell cytoplasm. They can be released either *via* bleb formation form activated cells, such as inflammatory cells; or from fragments of apoptotic cells, the latter may also contain fragments of cell nuclei [32,33]. Furthermore, microparticles and DAMPs may share common properties, also because content of microparticles may include DAMPs [32]. These microparticles further contribute to inflamma-

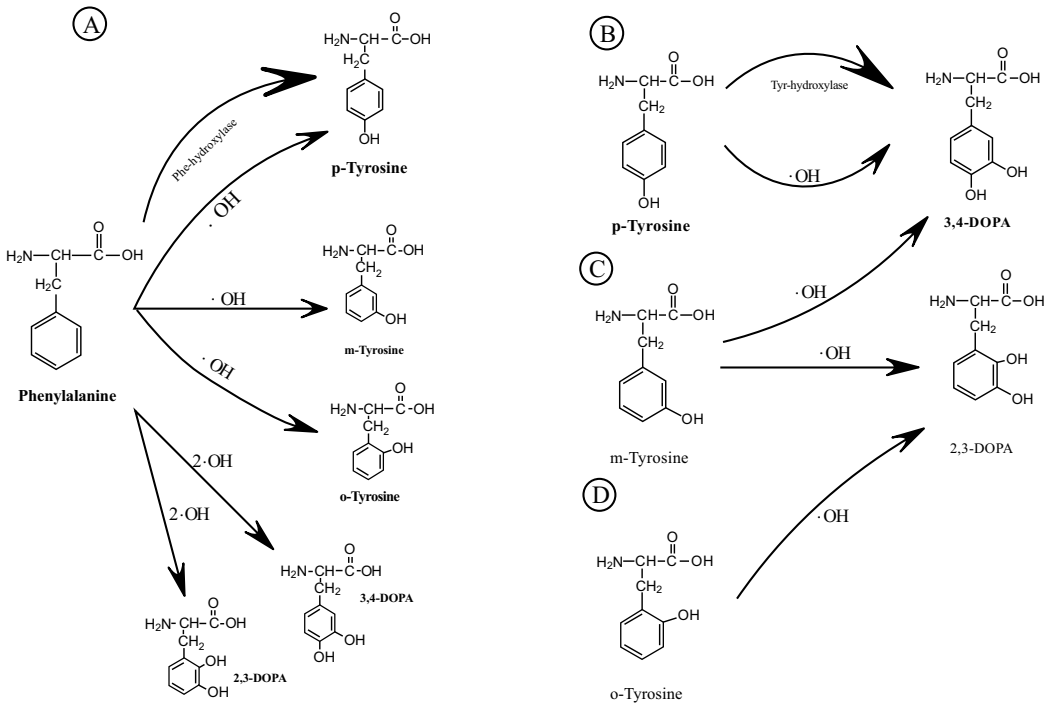

Fig. (1). Conversion reactions of phenylalanine yielding para-, meta- and ortho-tyrosine, and formation of isoforms of dihydroxy-phenylalanine (DOPA) from the different tyrosine isoforms.

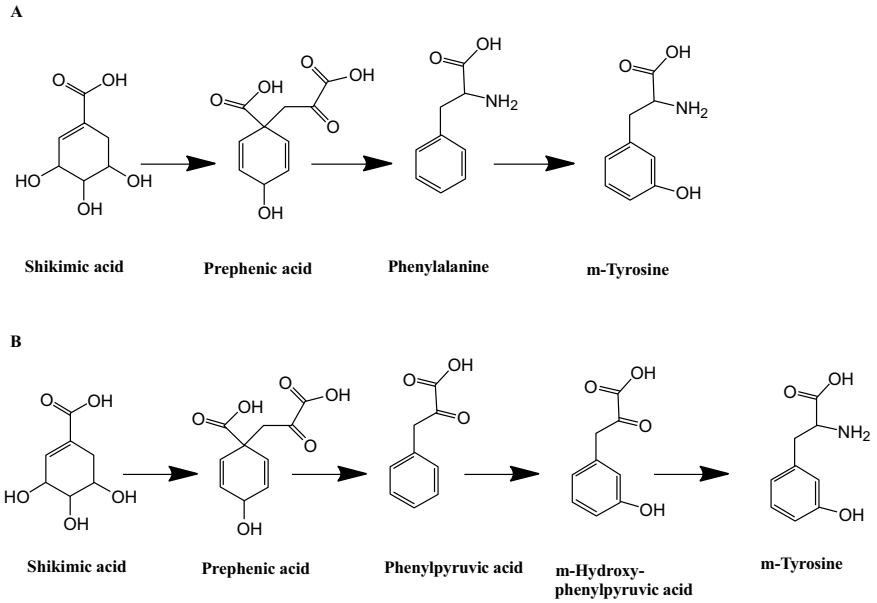

Fig. (2). Proposed synthetic routes of meta-tyrosine in plants as suggested by Huang *et al.* [36].

tion, but also lead to endothelial damage, platelet aggregation and thus to thrombosis within the small vessels. The resulting hypoxia and possible reperfusion injury will further contribute to oxidative stress and related damage [34].

Hypoxia may lead to a further increase in generation of ROS, but on the longer term also to adaptive changes in renal microcirculation and blood flow, namely, renal vasoconstriction will develop, that decreases filtration and thereby tubular flow and transport, and thus oxygen demand. This way, a new balance between oxygen need and oxygen supply could develop, however the price for that is a decline in glomerular filtration rate. ROS may in turn interrupt this (mal)adaptive processes, and lead to further hypoxic damage [35].

3.2. Oxidative Stress Markers and Antioxidants in Critically Ill Patients

Patients with septic shock have been shown to have a higher level of plasma TBARS and lower levels of antioxidants, such as Vitamin A, Vitamin E, carotene or lycopene. Moreover, levels of TBARS in plasma correlated inversely with plasma levels of tocopherol and retinol [36]. Also, reactive nitrogen species can be produced, mainly through nitric oxide synthesized *via* the iNOS, the resulting peroxynitrite and other ROS may also play a major role in protein damage and depletion of antioxidants such as reduced glutathione [37]. In sepsis, there is also a mitochondrial damage that develops in various organs, which could lead to a vicious cycle *via* increase in ROS formation and resulting further mitochondrial injury [37]. Also, xantine oxidase has been shown to be up-regulated in septic patients, contributing to oxidative stress [38,39]. Furthermore, endotoxinaemia is able to promote an increased activity of cyclooxigenase-2 (COX-2) yielding also higher amounts of ROS [40]. In a previous study of our research group, malondialdehyde and myeloperoxidase levels were elevated in early stage of sepsis [41]. ROS may not only be involved in sepsis, but also in other pathologies, such as burn injury. ROS formed in burn injury may contribute to the development of edema, may be involved in a further acceleration of inflammation, and this cycle may be interrupted with proper fluid replacement therapy, or by administration of N-acetylcysteine [42,43]. ROS formation, and especially MPO activity may be however significantly higher in sepsis than in burn injury, probably as a consequence of differences in pathogenesis [41].

3.3. Tyrosine Isomers in Acute Inflammatory Disease

An increased oxidative stress can be detected in preterm infants, especially in the case of hypoxic or ischemic changes, reperfusion injury, or in case of preterm delivery. Among others, elevated urinary o-Tyr/Phe quotients were found in urine of resuscitated newborn piglets with increase in oxygen supply during resuscitation: 19.07 *vs*. 56.9 vs. 87.7 *vs*. 148.7 µmol/L / µmol/Lx100 (FiO2: 21% *vs*. 40% *vs*. 60% *vs*. 100%, respectively), along with a less striking rise in 8-oxo-guanosin excretions [44]. Higher m-Tyr levels and o-Tyr levels were found in cerebrospinal fluids of infants with ischemic encephalopathy as compared control neonates: 20.5 +/- 24.9 *versus* 8.0 +/-3.1 nM (m-Tyr) and 20.6 +/- 18.6 *versus* 8.7 +/- 2.6 nM (o-Tyr), $p<0.005$ each [45]. Preterm infants had higher urinary o-Tyr/Phe ratios than control infants, this could however be attenuated by feeding with human milk as compared to feeding with preterm formula: 14.90 +/- 3.75 *vs*. 12.53 +/- 3.49 *vs.* 6.43 +/- 1.82 (preterm formula *v.s* human milk *vs*. controls, $p<0.01$ for preterm *vs.* control and human milk vs. control, as well as for human milk *vs*. preterm formula). The level of o-Tyr excretion showed a negative correlation with gestational age at birth ($R^2=0.383$, $p<0.01$) [46]. Preterm delivery-related oxidative stress could be ameliorated by antenatal steroid administration, as reflected by decreases in o-Tyr levels in both male and female newborns ($p<0.01$). There was a major gender difference, with male infants having higher urinary o-Tyr/Phe ratio as compared to female infants both in steroid treated and control newborns ($p<0.05$). Similar data were found for 8-oxoguanosin excretion, however no difference was found in non-steroid treated boys *vs*. girls regarding this parameter. [47]. Using a novel methodology, namely ultra performance liquid chromatography with double mass spectrometry (UPLC-MS/MS), m- and o-Tyr, along with other oxidative stress markers could be detected (Fig. **3**) in the urine of extremely low weight newborn infants (Fig. **4**) [48].

Other causes of acute oxidative stress affecting the cardiovascular system could be also characterized using Tyr-isoforms, *e.g.* striatal o-Tyr levels increased in a model of deep hypothermic cardiac arrest, but not in low-flow cardiopulmonary bypass (2.53 +/- 0.09 *vs*. 0.48 +/- 0.09 *vs*. 0.43 +/- 0.07 nmol o-Tyr/g striatal tissue, deep hypothermic circulatory arrest vs. low-flow cardiopulmonary bypass *vs*. controls) [49].

The exogenous administration of the abnormal chiral isoform of Phe, D-Phe can also be used to study *in vivo* free radical processes. While L-Phe can be a substrate of enzymatic processes, D-Phe cannot, thus the arising D-p-Tyr, D-m-Tyr and D-o-Tyr can only be produced non-enzymatically. For example, hydroxylation of D-Phe was used to verify ischemia-reperfusion injury. Highly significant increase was observed after ischemia as compared to baseline, this further increased during reperfusion, and normalized only during several hours (Fig. **5**). The temporal changes were similar during the three isomers of D-Tyr, but D-m-Tyr was mainly generated [50]. This approach is useful, can distinguish *e.g.* between enzymatic and non-enzymatic production of p-Tyr.

These data suggest that there is a marked increase in oxidative stress in ischemia, reperfusion, cardiac arrest, and resuscitation. These processes can be monitored using m- and o-Tyr levels. Furthermore, preterm labor

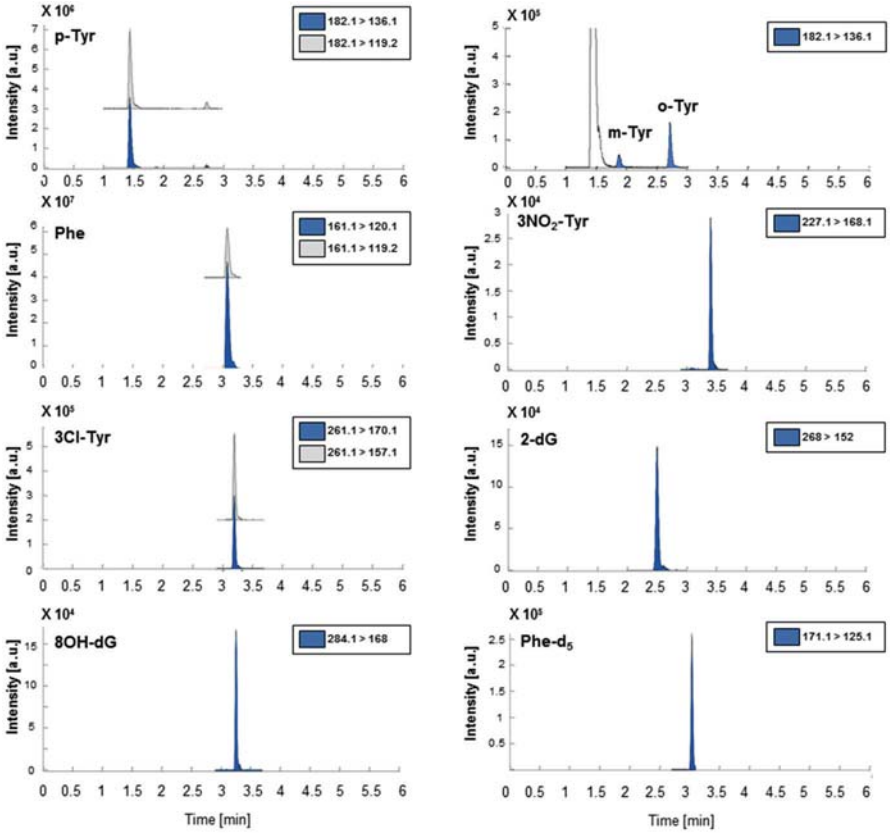

Fig. (3). Typical chromatograms of the selected biomarkers extracted from the analysis of spiked urine sample. Note: Spiking concentrations were 73 nmol/L for 8OHdG and m-Tyr, 182 nmol/L for 2-dG, o-Tyr, $3NO_2$-Tyr and 3Cl-Tyr and 23 mM for p-Tyr and Phe. Image from Kuligowski *et al. PloS One 2014,* 9: e93703 [48], reproduced under the creative commons license.

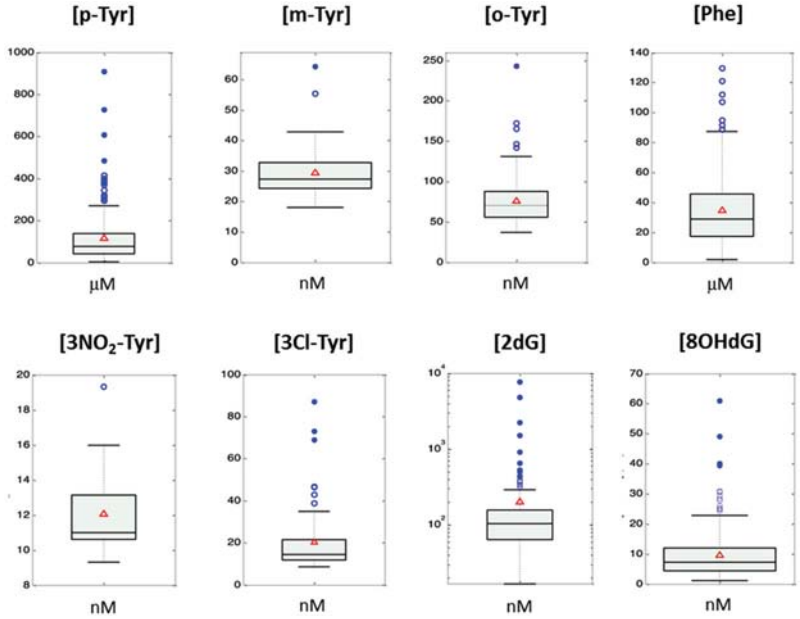

Fig. (4). Non-zero concentrations found from the analysis of 222 urine samples of extremely low birth-weight infants included in the double-blinded randomized clinical study REOX (REOX 2012-2013, EUDRACT 2088-005047-42). The percentages of concentrations, LOD in the sample set were: 15% for o-Tyr, 79% for m-Tyr, 0% for p-Tyr and Phe, 93% for NO_2-Tyr, 68% for 3Cl-Tyr, 0.4% for 8OH-dG and 0.4% for 2dG. Boxes indicate the 1st and the 3rd quartiles, the median is shown as a black line, whiskers mark the 9th and 91st percentiles, red triangles represent mean concentrations and blue circles are outliers. Image from Kuligowski *et al. PloS One* 2014, 9: e93703 [48], reproduced under the creative commons license.

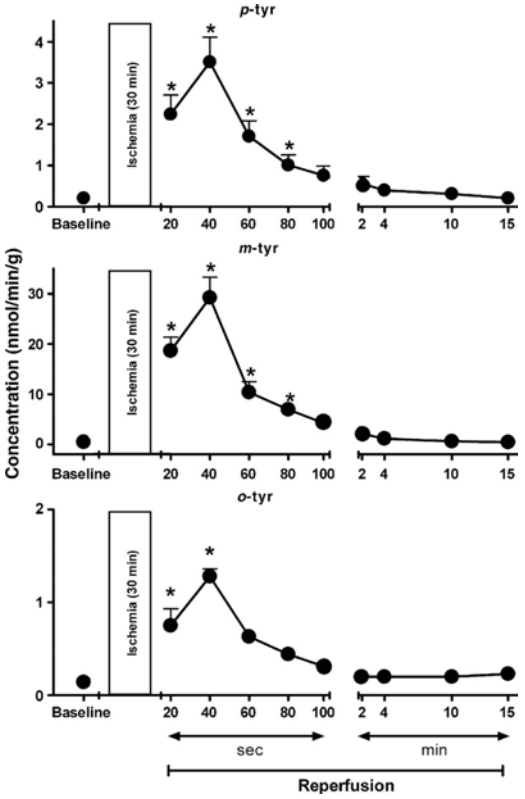

Fig. (5). Time course of myocardial release of D stereoisomer of p-, m- and o-Tyr in the cardiac effluent at baseline and at various time points during reperfusion after 30-min ischemia. Hearts were perfused with D-Phe. Data are mean + S.E.; n =8; **p* <0.05 *vs*. pre-ischemic value. Fig. reproduced from Biondi *et al. Cardiovasc. Res.* 2006, 15;71(2):322-330 [50] with permission of Oxford University Press.

is a condition associated with an increased oxidative stress that may be further triggered by ischemia, while human milk seems to protect more effectively against oxidative stress than preterm formulas. This may be due to a difference in composition in antioxidants, which may be more pronounced and more active in human milk [46].

Serum m- and o-Tyr levels were used to follow ROS-generation by inflammatory cells in different models: *e.g.* triiodothyronine administration to healthy volunteers resulted in an increase in ROS-generation by leukocytes that was accompanied by a marked rise in serum m-Tyr (4.28+/-0.73 µg/L *vs*. 3.40+/-0.47 µg/L, day 7 *vs*. baseline, $p<0.05$) and o-Tyr levels (4.18 +/-0.74 *vs*. 3.36 +/-0.46 µg/L, day 7 *vs*. baseline, $p<0.05$) [51].

In another set of experiments, levels of m- and o-Tyr were found to decrease in sera of healthy volunteers after 48 hours fast (m-Tyr, 4.00+/-0.69 *vs*. 4.21+/-0.68 µg/L and o-Tyr, 3.59+/-0.66 *vs*. 3.79+/-0.62 µg/L, 48 hours *vs*. baseline), in parallel to a decrease in ROS production and a decrease in NOX expression of polymorphonuclear leukocytes [52]. These data suggest that ROS-generation by leukocytes during inflammation results in marked changes in serum m- and o-Tyr levels.

Supporting these data, a study in patients with sepsis found that changes in inflammatory markers (CRP and procalcitonin - PCT) were paralleled by changes in serum levels of m-Tyr and urinary excretion of both m- and o-Tyr [53].

In this study, serum m-Tyr levels were significantly higher on days 2 and 3 as compared to control subjects, then the difference disappeared. In case of serum o-Tyr levels the observed differences were not significant. The elevated serum m-Tyr levels may reflect a systemic oxidative stress induced by inflammation, this notion is supported by the parallel changes of CRP and PCT levels. Serum p-Tyr levels were initially low in the septic patients as compared to controls, and then during the 5 days of follow-up, this difference has disappeared (Fig. **6**). Changes in serum p-Tyr levels may arise from a lower p-Tyr synthesis in the kidney *via* phenylalanine hydroxylase enzyme or an increased renal loss of the amino acid.

Urinary excretion of m- and o-Tyr peaked at day 1, and decreased day-by-day to the level of controls in the septic patients (Fig. **7**). These data suggest an increase in oxidative stress related to the kidneys in sepsis, the source of this may again be inflammation, as suggested by changes in CRP and PCT levels. An opposite trend could be observed for urinary p-Tyr levels, where urinary excretion of p-Tyr increased during the follow-up period (Fig. **8**).

To obtain more specific data on renal handling of the different amino acid isoforms, fractional excretion (Fe) values were calculated for p-, m- and o-Tyr, respectively. $Fe_{m\text{-}Tyr}$ and $Fe_{o\text{-}Tyr}$ were higher nearly through the whole study period than $Fe_{p\text{-}Tyr}$. $Fe_{m\text{-}Tyr}$ and $Fe_{o\text{-}Tyr}$ both peaked at study days 1-2, and showed a decrease afterwards. Especially in the case of o-Tyr, the Fe value exceeded 100% by several times, probably indicating active secretion or *in loco* release of the pathologic amino acids in sepsis. On the contrary, $Fe_{p\text{-}Tyr}$ levels stayed in the range <10% during the whole study period, indicating that a large proportion (>90%) of the filtered p-Tyr molecules can be reabsorbed in the proximal tubuli. We could observe the same tendency of initial lower, than raising values for urinary p-Tyr excretion as well as for $Fe_{p\text{-}Tyr}$ this may suggest that an

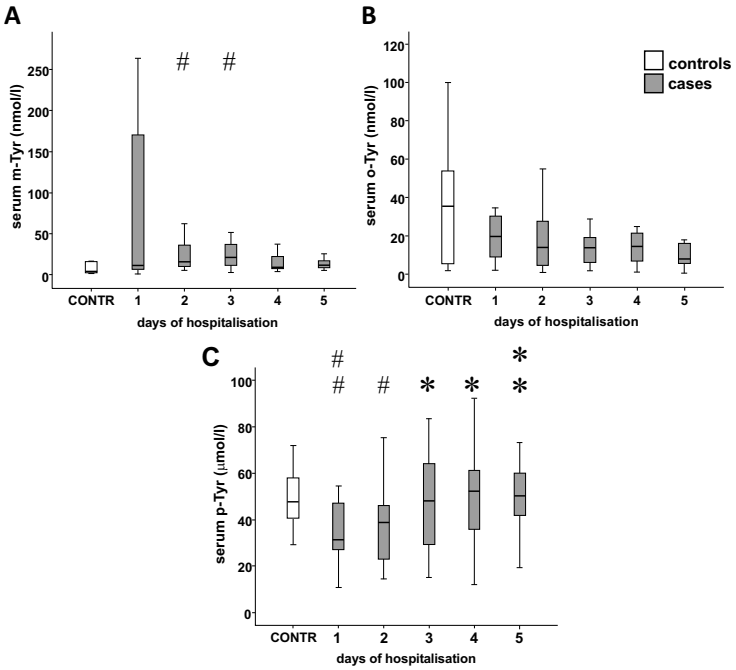

Fig. (6). Serum levels of (**A**) meta-tyrosine, (**B**) ortho-tyrosine and (**C**) para-tyrosine in septic patients. Data are expressed as median and inter-quartile range (IQR; standard 25th-75th percentile) and 5th and 95th confidence interval). Asterisks indicate statistical differences within the septic group compared to day 1 (*: p<0.05; **: p<0.01). The "#" symbols show significant differences between patients and controls (#: p<0.05; ##: p<0.01). Serum para-tyrosine levels showed a significant day-by-day elevation with trend analysis. (p=0.002) Reproduced with permission of Maney Publishing Ltd. from Szélig *et al. Redox Report* 2015. Jul. 20 [53].

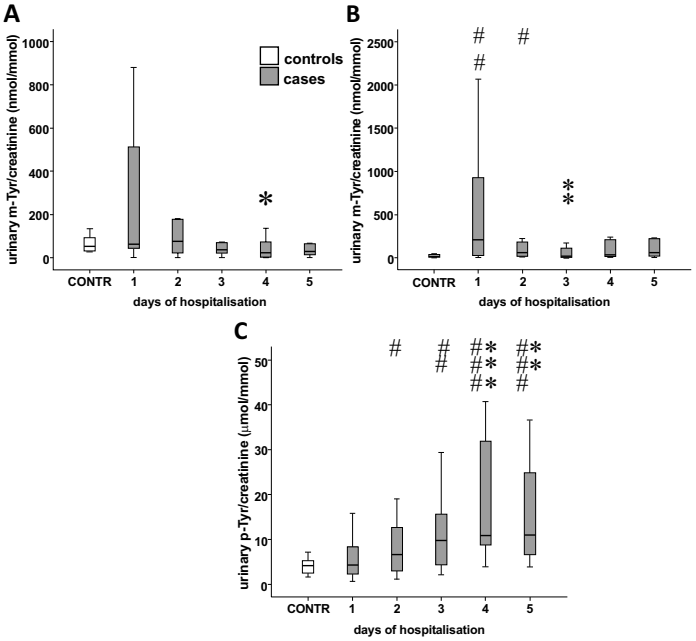

Fig. (7). Urinary (**A**) meta-tyrosine/creatinine, (**B**) ortho-tyrosine/creatinine and (**C**) para-tyrosine/creatinine ratio in septic patients. Data are expressed as median, inter-quartile range (IQR; standard 25th-75th percentile) and 5th and 95th confidence interval. Asterisks indicate statistical differences within the septic group compared to day 1. (*: p<0.05; **: p<0.01; ***: p<0.001). The "#" symbols show significant differences between patients and controls (#: p<0.05; ##: p<0.01; ###: p<0.001). Urinary meta-tyrosine/creatinine ratios had a decreasing tendency (p=0.018), while urinary para-tyrosine/creatinine ratios showed a marked increase (p=0.001). Reproduced with permission of Maney Publishing Ltd. from Szélig *et al. Redox Report* 2015. Jul. 20 [53].

altered reabsorption of p-Tyr may in the background of changes in urinary p-Tyr excretion. (Fig. **8**).

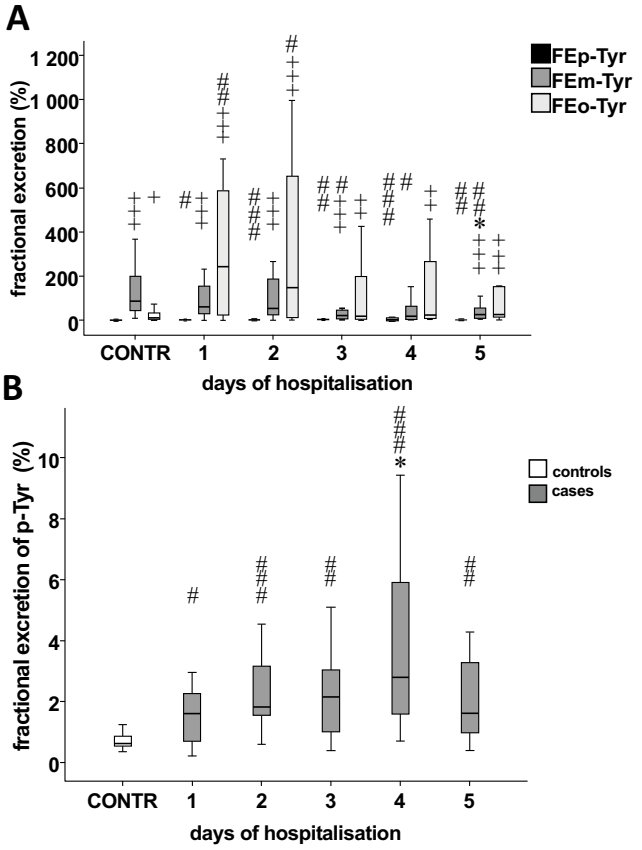

Fig. (8). Fe of para-, meta- and ortho-tyrosine (median; standard 25th-75th percentile and 5th and 95th confidence interval). Fe of m-Tyr showed a decreasing tendency (p=0.009). (**A**) Fe of para-tyrosine (median; standard 25th-75th percentile and 5th and 95th confidence interval). (**B**) Asterisk indicates a statistically relevant difference within the septic group compared to day 1 (*: p<0.05). The "#" symbols show significant differences between cases and controls (#: p<0.05; ##: p<0.01; ###: p<0.001). The "+" symbols show significant differences between Fe of meta- or ortho-tyrosine and that of para-tyrosine (+: p<0.05; ++: p<0.01; +++: p<0.001) Reproduced with permission of Maney Publishing Ltd. from Szélig *et al. Redox Report* 2015. Jul. 20 [53].

The data suggest that systemic inflammation may be in direct connection with production of m- and o-Tyr, *i.e.* it accounts for development of systemic oxidative stress. Furthermore, urinary excretion of m- and o-Tyr showed even more pronounced changes during the course of the disease. The larger concentrations of urinary m- and o-Tyr are in part due to the filtered amino acids, but results also suggest that – at least in a certain portion of the patients – the pathologic amino acids are actively secreted or produced in the kidney. A similar situation has been described in chronic conditions, where patients with type 2 diabetes also had a fractional excretion of o-Tyr exceeding 100%.

During the course of sepsis, initial lower levels of serum p-Tyr are corrected. This is probably in part related to the effective retention of p-Tyr in the tubuli and to a resolution of kidney function and probably an amelioration of phenylalanine hydroxylase activity.

In a further study, connection of the pathological amino acids m- and o-Tyr with carbohydrate metabolism of non-diabetic septic patients was evaluated [54]. It was found that urinary m-Tyr/p-Tyr ratio was significantly higher in patients with a total daily insulin dose (DID) above the median. Similarly, patients with an insulin-glucose product (IGP) over the median had higher urinary m-Tyr/p-Tyr ratio (Fig. **9**).

Glucose homeostasis in the critically ill patients is an important issue, and clinical data suggested by 2001 that glycemic control *via* insulin administration ameliorated clinical outcome [55]. Nowadays, the question in everyday work at an Intensive Care Unit is rather the question of tight *vs*. moderate glycemic control, where data of the NICE-sugar studies suggested that more intensive glycemic control has a rather adverse effect on mortality than moderate glycemic control [56]. A follow-up of a subgroup of these patients with traumatic brain injury did not verify these concerns [57]. As critically ill patients represent a heterogeneous population regarding underlying cause, age and comorbidities, personalized treatment may need to be carried out [58,59].

The connection between oxidative stress/inflammation and glycemia may be in this context a problem of "chicken and egg", there are numerous data that support that hyperglycemia is able to induce oxidative stress, however also oxidative stress and inflammation may lead to a worsening of hyperglycemia *via* increase in gluconeogenesis, change in levels and activity of glucose transporters, inhibition of cellular glucose metabolism *etc.* Stress hormones (*e.g.* cortisol, glucagone, catecholamines) released due to systemic stress may further contribute to dysglycemia, but may also trigger oxidative stress [60].

Moreover, a significant correlation was found between urinary m-Tyr excretion of urinary m-Tyr/p-Tyr ratio and DID or IGP, suggesting that oxidative stress, either systemically or rather more specifically affecting the kidneys, may have an impact on carbohydrate metabolism and may contribute to insulin resistance in this state (Fig. **10**). Connection between acute inflamma-

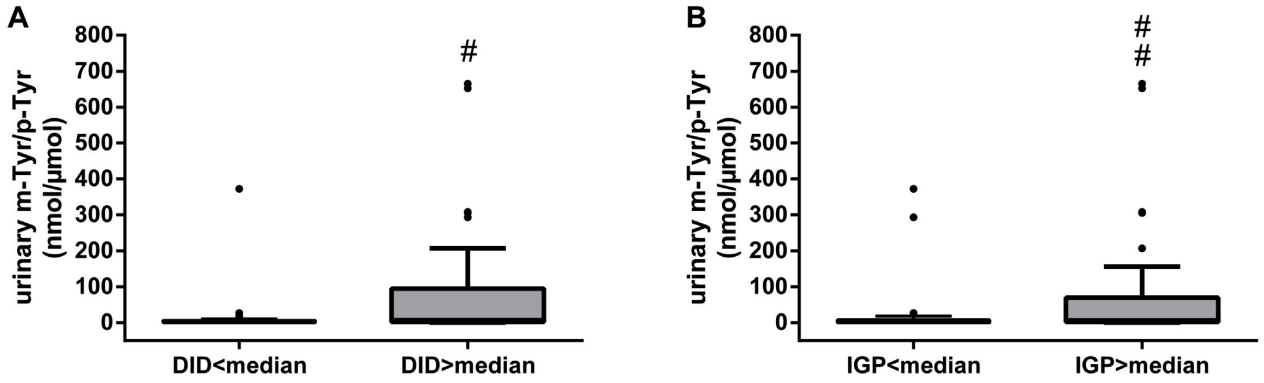

Fig. (9). Urinary m-Tyr/p-Tyr ratio in septic patients requiring insulin administration, according to (**A**) daily insulin dose or (**B**) insulin-glucose product. #: p=0.005 *vs*. DID < median; ##: p=0.01 *vs*. IGP < median. Abbreviations: DID, daily insulin dose; IGP, insulin-glucose product. Reproduced with permission of Hindawi Publishing Corporation from Kun *et al. Oxidative Medicine and Cellular Longevity,* Article ID 839748 [54].

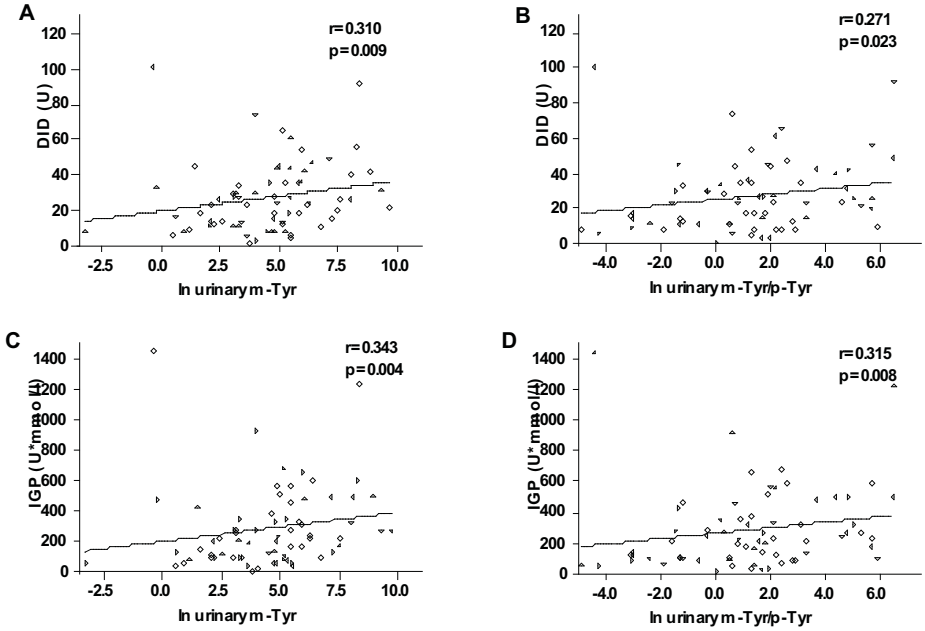

Fig. (10). Correlation of urinary m-Tyr concentration with (**A**) DID and (**C**) IGP. Correlation of urinary m-Tyr/p-Tyr ratio with (**B**) DID and (**D**) IGP in septic patients requiring insulin administration. Abbreviations: DID, daily insulin dose; IGP, insulin-glucose product. Reproduced with permission of Hindawi Publishing Corporation from Kun *et al. Oxidative Medicine and Cellular Longevity*, Article ID 839748 [54].

tion, mediators and insulin resistance is a known entity in critically ill patients, however underlying mechanisms are quite complex [63].

The observations were further supported by regression analyzes, in which serum Phe levels, the serum p-Tyr/Phe ratio and serum o-Tyr/Phe ratio showed a connection to IGP (or DID) independent of body mass, CRP, PCT levels and potential interfering medications. Also, urinary indices related to m- and o-Tyr were in connection with carbohydrate metabolism (Table **1**).

Due to the specific role of tyrosine in insulin signaling (*e.g.* insulin receptor autophosphorylation, phosphorylation of the insulin receptor substrates), the possibility also arises, that m- and o-Tyr may interfere with intracellular signaling of insulin, and that this may contribute to the development of hyperglycemia in the state with high levels of m- and o-Tyr. Longitudinal studies or interventions using antioxidants and/or anti-inflammatory agents and subsequent analysis of change of glycemia may help in future to decide, which of the two possible explanations play a stronger role.

Table 1. Predictors of carbohydrate metabolism parameters among septic patients, across the whole study period.

	DID		IGP*	
	β	*p*	β	*p*
serum Phe	-0.450	0.001	-0.460	0.001
serum p-Tyr/Phe	0.507	<0.001	0.554	<0.001
serum o-Tyr/Phe	-	-	0.280	0.049
urinary m-Tyr	0.381	0.007	0.382	0.007
urinary m-Tyr/p-Tyr	0.359	0.011	0.351	0.013
urinary o-Tyr/p-Tyr	0.322	0.023	0.308	0.030
urinary (m-Tyr + o-Tyr)/p-Tyr	0.389	0.006	0.376	0.008

Model: body weight, hsCRP, PCT, daily hydrocortisone dose, daily dobutamine dose and the actual amino acid parameter. Abbreviations: DID, daily insulin dose; IGP, insulin-glucose product. *: calculated by average daily glucose level (mmol/l) multiplied by daily insulin dose (U) Method: stepwise enter. Reproduced with permission of Hindawi Publishing Corporation from Kun *et al. Oxidative Medicine and Cellular Longevity,* Article ID 839748 [54].

Whether m- and o-Tyr could play an additional role in the diagnosis or follow-up of these pathologies, would also require interventional studies with sufficient number to analyze mortality.

4. CHRONIC DISEASES ASSOCIATED WITH OXIDATIVE STRESS

4.1. Subclinical Inflammation and Oxidative Stress in Chronic Diseases

In diabetes mellitus, there are different sources of oxidative stress, among others the mitochondrial respiratory chain, the NOX and xanthine oxidase systems, autoxidation of glucose, the pseudohypoxic state, formation of advanced glycation end products (AGEs). The resulting AGEs can bind to their receptors (receptor of AGE, RAGE), inducing activation of nuclear factor kappa B (NFκB) will promote further inflammatory processes [61]. Due to the oxidation of proteins, advanced oxidation protein products (AOPPs) will also be formed that are not only potentially functionally impaired proteins, but also share some characteristics with AGEs, among others they are also able to bind to RAGEs [62,63,13].

Chronic kidney disease per se may lead to an increase in oxidative stress, accumulation of advanced protein oxidation products. Latter may provoke further inflammation, similar to microparticles that are not only described in sepsis, but also in chronic kidney disease. Furthermore, water-soluble, low molecular weight AGEs are not cleared *via* the kidney, instead they can accumulate with worsening of the glomerular filtration rate (GFR), they may in turn bind to their receptors (RAGE, see above) and may induce further inflammation and oxidative stress [13].

Glomeruli and tubulointerstitium of patients with diabetic nephropathy exhibit accumulation of inflammatory cells that may produce pro-inflammatory cytokines, chemokines and may play a role in progression of the disease [64]. In advanced stage of diabetic nephropathy, *i.e.* stage 4-5 chronic kidney disease, typical signs of inflammation were detected in the absence of manifest (macro)inflammation, among others, elevated in both dialyzed patients as well in non-dialyzed patients [mean C-reactive protein (CRP) values: 22.4 and 23.4 mg/dl] as compared to controls (mean: 4,125 mg/dl). Also, serum albumin levels were lower in the groups as compared to controls. CRP levels showed a negative correlation with GFR in this cohort [65]. Even such a low grade of inflammation may have an impact on pathophysiological processes, such as atherosclerosis [64,66].

4.2. Oxidative Stress Markers and Antioxidants in Chronic Subclinical Inflammatory Diseases

Oxidative stress is markedly elevated in experimentally induced and in clinically overt diabetes mellitus as well. For example, elevated levels of TBARS have been found in plasma and in aortic tissue of rats with streptozotocin-induced diabetes [67], in red blood cells of patients with type 2 diabetes mellitus – especially when concomitted by liver cirrhosis [68], or in plasma of patients with type 2 diabetes [69]. Similarly, isoprostanes have been shown to accumulate in urine of patients with type 1 and type 2 diabetes mellitus [70]. Isoprostanes may play an important role in develop-

ment of atherosclerotic lesions as well, among others *via* LDL-oxidation, platelet and abnormal vacular tone [70]. Moreover, ROS have been directly implicated in the development of pancreatic β-cell dysfunction in early diabetes, especially as a consequence of decrease intracellular synthesis of reduced glutathione (GSH) [71]. Also, polycystic ovary syndrome has been characterized by high levels of malondialdehyde and low levels of GSH [72].

Advanced glycation endproducts, which can partially be regarded as oxidative stress markers, accumulate in patients with end-stage renal failure, and among these, carboxy-methyllysine levels were associated with mortality in dialyzed patients [73]. The level of TBARS and lipid hydroperoxides and protein carbonyls increased with stage of chronic kidney disease in another study, and there was an inverse change in activity of superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase and levels of vitamin E [74].

4.3. Tyrosine Isomers in Chronic Subclinical Inflammatory Diseases

Fabry's disease, a disease alpha galactosidase deficiency leads to severe vascular damage at relative early age. In a mouse model, genetic knockout of alpha galactosidase has led to abnormal caveolin structure and to lower endothelial NOS activity. This was accompanied by an increase in o-Tyr and nitrotyrosine levels, suggesting an uncoupling of eNOS as a consequence of the mutation. These findings may shed a light on the development of vascular complications of Fabry's disease, and highlight the role of free radical processes [75].

The abovementioned findings between m- and o-Tyr levels and insulin resistance in septic patients are in line with our previous results from chronic models of dysglycemia. Namely, increased urinary o-Tyr excretion was found in patients with diabetes and/or chronic kidney disease. In patients with diabetes, also a markedly elevated $Fe_{o\text{-}Tyr}$ could be observed. This suggests a connection of diabetes and the oxidized amino acid markers [22]. In a further study, the higher urinary excretion of o-Tyr in diabetic patients (change in urinary o-Tyr/Phe ratio: 0.02+/-0.046 μmol/mmol) could be attenuated by the administration of resveratrol (-0.015+/-0.014 μmol/mmol, p=0.043). This was mirrored by an improvement in the insulin resistance (IR) as seen by a decrease in the marker of IR, namely the homeostasis model assessment IR index ($HOMA_{IR}$: 0.04+/-1.4 *vs*. -1.52+/-1.18, p=0.044). These data suggest that oxidative stress (characterized by o-Tyr levels) may contribute to insulin resistance, and this may be interrupted by administration of an antioxidant [76].

We also found a connection between o-Tyr and resistance to erythropoietin (EPO) in patients with chronic kidney disease, namely plasma o-Tyr levels and o-Tyr/p-Tyr ratios were elevated in patients on renal replacement therapy as compared to controls: 162.97(191.24) nM *vs*. 489.92(726.85) nM *vs*. 54.19(262.91) nM *vs*. 9.74(9.93) nM, non-EPO-treated hemodialyzed patients *vs*. EPO-treated hemodialyzed patients *vs*. patients on continuous peritoneal dialysis *vs*. controls, all $p<0.001$, and higher levels (p=0.014) were measured in EPO-requiring dialyzed patients compared to non-EPO-requiring hemodialyzed patients. There was also direct relationship of plasma o-Tyr/p-Tyr ratio and indices of EPO-resistance, as verified using correlation (with R values of approx. 0.4 and p values < 0.005) and regression analyzes [77].

In the background, incorporation of o-Tyr into erythroid precursors and inhibition of proliferative signaling may by present, this has indeed been verified by a further study of our group, where supplementation of m- and o-Tyr to the media of erhythroblasts lead to the incorporation of these abnormal amino acids into cellular proteins and to a nearly 50% inhibition of cell proliferation [78].

More recent data of our group have verified an increase of vascular insulin resistance in the arterious system towards the central blood vessels, and that this was mirrored by an increase in vascular o-Tyr content. [79] Furthermore, long-term supplementation of rats with o-Tyr led to development of vascular insulin resistance with an accompanying increase in vascular o-Tyr levels [80].

As mentioned earlier, oxidized Phe-derivates have also been detected in humans lens protein. In an earlier study, no connection between o-Tyr levels of total lens proteins of healthy lenses and age was found, however, dityrosine, another oxidized amino acid was slightly accumulating with age [81]. In another study, their levels of o-Tyr and DOPA were significantly higher in the non-watersoluble components of the cataractous lens, as compared to total lens homogenates. This suggests that o-Tyr and DOPA especially accumulate in non-watersoluble lens proteins in cataract due to ageing or diabetes, both pathologies with well-known oxidative stress background [21]. The seeming slight discrepancy of the two studies may be explained by the fact that lenses in the study by Wells-Knecht *et al.* were originating from eye banks, *i.e.* they were non-cataractous

lenses, thus containing mainly water-soluble lens proteins [81]. Both oxidized Phe derivates are more water-soluble than Phe itself, which means that oxidation of Phe side-chains to m-, o-Tyr or DOPA does not change water-solubility directly, rather the secondary and tertiary structure of lens protein, such as crystallins may be altered and this may lead to precipitation and hence lower transparency of the lens [21].

Oxidative stress processes have been long implied in the background of important pathologies, such as cellular senescence and ageing of the organism, as well. The pathogenesis may include among others cumulative DNA-damage, mitochondrial dysfunction [82]. These damages may be ameliorated by caloric restriction, chelating agents, antioxidant foods, leading to increase in functional life span [83].

During ageing, oxidative protein modifications may occur that may lead to malfunctioning proteins. Diabetes mellitus is in some ways regarded as an accelerated ageing process, thus it should not be surprising that ageing and diabetic cataracts showed similar damage, *i.e.* accumulation of oxidized amino acids [81,21].

Summing up, the abovementioned data suggest that the oxidized amino acids m- and o-Tyr are associated with hormone resistances, such as insulin- and EPO-resistance, and might have a bad impact on the homeostasis. On the contrary, the physiological p-Tyr seems to be in connection with favorable processes. It is feasible to view m- and o-Tyr as "innocent bystanders" in this situation, which would mean that they are only markers of oxidative stress and oxidative stress itself or the underlying subclinical inflammation is the cause of hormone resistance.

The data on erythroblasts however suggest that there is direct effect of m- and o-Tyr on cell function; they are namely able to inhibit cell growth. p-Tyr, on the other hand, when administered in an 2.6x or 1.8 abundance to media containing m- or o-Tyr, respectively, could restore proliferation [78].This suggests a subacute and reversible effect of m- and o-Tyr, that seems to be competitively inhibitable using p-Tyr.

4.4. Tyrosine Isomers in an Animal Model Of Oxidative Stress and Vascular Hormone Resistance

As a proof-of-concept, a further set of experiments were carried out [84]. Rats were fed either a normal chow, or a high cholesterol diet; while latter animal were divided into two groups and received either the physiologic amino acid p-Tyr or vehicle orally. In the high cholesterol group, a lower insulin secretion could be found, that was restored by p-Tyr supplementation (Fig. **11**).

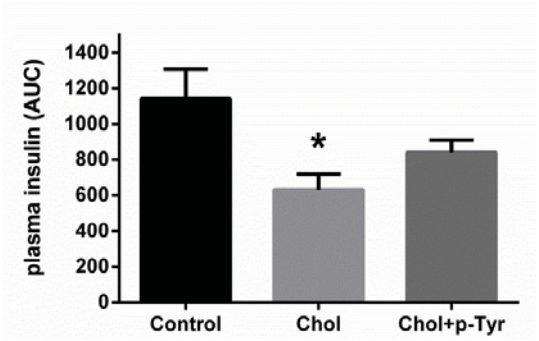

Fig. (11). Area-under-the-curve (AUC) of plasma insulin during OGTT in control rats (Control), cholesterol-fed rats without p-Tyr supplementation (Chol) and cholesterol-fed rats with p-Tyr supplementation (Chol+p-Tyr). *: $p<0.05$ *vs*. Contr. Reproduced with permission of Bentham Science Publishers from Selley *et al. Protein and Peptide Letters* 2015; 22(8): 736-742 [84].

In the cholesterol-supplemented rats, an increase in the vascular levels of m-Tyr could be observed, that could be partially reversed in high-cholesterol-fed, but p-Tyr-supplemented rats (Fig. **12**).

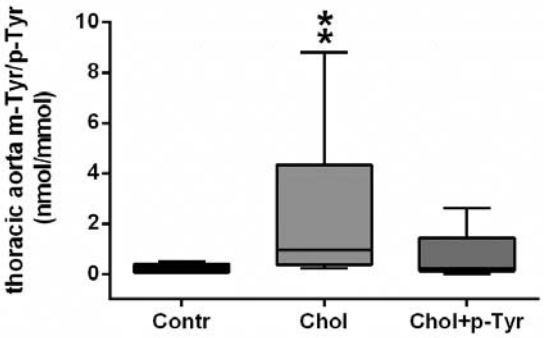

Fig. (12). Ratios of protein-bound m-Tyr and p-Tyr in thoracic aorta of control rats (Contr), cholesterol-fed rats without p-Tyr supplementation (Chol) and cholesterol-fed rats with p-Tyr supplementation (Chol+p-Tyr). **: $p<0.01$ *vs*. Contr. Reproduced with permission of Bentham Science Publishers from Selley *et al. Protein and Peptide Letters* 2015; 22(8): 736-742 [84].

Furthermore, high cholesterol feeding led to the development of vascular resistance to liraglutide (an antidiabetic medication, and analogue of the endogenous peptide hormone glucagon-like peptide-1, GLP-1) and resistance to insulin. These pathologies could be completely of nearly completely prevented by the supplementation with p-Tyr (Fig. **13**). This suggests that high cholesterol-induced vascular hormonal resistance could be attenuated by the administration of the physiologic Tyr isomer, *i.e.* p-Tyr.

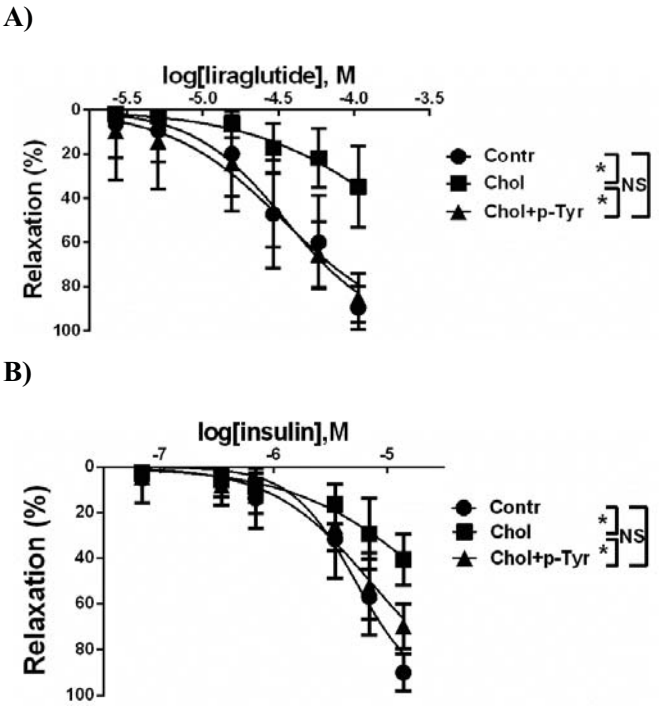

Fig. (13). Liraglutide-induced (panel A) and insulin-induced (Panel B) relaxation relaxation of the thoracic aorta of control rats (Contr), cholesterol-fed rats without p-Tyr supplementation (Chol) and cholesterol-fed rats with p-Tyr supplementation (Chol+p-Tyr). *: $p<0.05$. Reproduced with permission of Bentham Science Publishers from Selley *et al. Protein and Peptide Letters* 2015; 22(8): 736-742 [84].

4.5. Tyrosine Isomers and Concomitant Tumor Growth

The presence of a primary malignancy is able to inhibit growth of a second, concomitant malignant tumor. This phenomenon is the so-called concomitant tumor resistance. There are two major approaches to explain this effect, either *via* anti-tumor effects of the immune system or *via* an immune-independent effect. The workgroup of Ruggiero *et al.* has been looking for tumor-inhibiting factors in sera of animals with malignancies and to their surprise they have found that m-Tyr and – to a less extent – o-Tyr can be found in the background. Also, exogenous administration of m- and o-Tyr to tumor cells or macroscopic tumors exerted anti-mitogenic effects. In fact, m-Tyr inhibited both MAPK/Erk- as well as STAT-signaling, and decreased the expression of Ki-67 protein, indicating that these tumor cells moved into the G0 cell cycle phase [85]. Further data of the workgroup suggest that other signaling proteins such c-myc, BCL-XL or Cyclin-D may be involved [86]. Also, exogenous, periodic iv. administration of m-Tyr to mice lead to a significant decrease in the number of lung metastases, without causing histopathologically evident damage to major organs. However, in this model, no test regarding physiologic and pathophysiologic processes was tested yet [87]. The source of the circulating m- and o-Tyr in these models is not completely clear; however the use of anti-inflammatory approaches and antioxidants decreased the concomitant tumor resistance. This suggests that activation of inflammatory cells and resulting oxidative stress may be the underlying mechanism of m- and o-Tyr formation [85].

Previous data suggest that m- and o-Tyr could be taken up by the cells and also incorporated into cellular proteins during protein translation. Results may indicate that L-Phe-tRNA synthetase may bind m-Tyr and incorporate it into proteins, leading to cell death *e.g.* in Chinese hamster ovary cells [88]. This may resemble our data on erythroid progenitor cells, the proliferation of which was inhibited when m- or o-Tyr were added to the cell culture media [78]. However, the mechanism of concomitant tumor resistance may be somewhat different, as addition of specific amino acids, such as phenylalanine, glutamate, aspartate, glutamine or histidine could lead to a reversal of tumor-inhibition. This seems to explain also, why m- and o-Tyr only inhibit growth of the second cancer or metastases not the primary tumor, as these amino acids were proven to accumulate in the primary tumor but not the metastases [85,86].

5. RELATIVE ABUNDANCE OF TYROSINE ISOMERS AND THEIR SIGNIFICANCE

The abovementioned data suggest that levels of m- and o-Tyr are not equal to each other in different experimental settings, conditions or tissues. Also, some factor correlate rather with m-Tyr, others with o-Tyr parameters. For example, in the study on the effect of thyroxin administration on leukocytes, both m- and o-Tyr were produced nearly equimolarly. In the study on urinary excretion of tyrosine isomers in healthy rats, m-Tyr and o-Tyr were excreted also in nearly the same amounts [29]. Also in the cerebrospinal fluid of newborns, concentrations were identical [45].

In the study on cardiopulmonary bypass vs. circulatory arrest, just like in the study on resuscitation of newborn piglets, that on the effect of human milk on preterm infants, or that on the use of antenatal steroid, only o-Tyr levels were determined [49,44,46,47].

In the study on urine of low birth-weight infants, o-Tyr excretion was at least twice as high as that of m-Tyr [48]. In a paper on the effect of iron and ascorbic acid in PC12 cells, o-Tyr levels were approx. 30-50%

higher than m-Tyr levels [89]. When examining the use of D-Phe hydroxylation in ischemia-reperfusion injury, approx. 20-times higher concentrations of D-m-Tyr were reached as compared to D-o-Tyr, and D-p-Tyr levels were approx. twice as high as o-Tyr levels [50]. When analyzing hydroxyl radical production by different strains of *Enterococcus faecalis*, approx. 50-100% higher amounts of m-Tyr than o-Tyr were produced *in vitro*; while in *vivo*, after colonizing rat intestine, approx. 50-100% higher urinary excretion of o-Tyr than that of m-Tyr could be observed. That is, proportions were inversely changing *in vitro* and *in vivo*. The amount of D-p-Tyr was similar to that of o-Tyr both *in vitro* and *in vivo* [90]. In a study on hydroxyl radical-like species and diabetes, in an *in vitro* system, hydroxyl radical lead to generation of approx. twice as much o-tyr than m-Tyr, and also in the aorta of diabetic cynomolgus monkeys, the proportion of o-Tyr to m-Tyr was approx. 2:1, similarly to that of non-diabetic control animals [91]. Furthermore, both amino acid levels (m-Tyr as well as o-Tyr) correlated with glycated hemoglobin in the diabetic animals [91]. In a further paper, production of m- and o-Tyr was measured in myocardium of rats after acute exercise, and levels of o-Tyr were approx. twice as high both in mitochrondia and in the cytosol than levels of m-Tyr [92]. In the study on cataract lens proteins, m-Tyr levels were below detection limit in majority of water-soluble lens supernatants, and to a much less extent in total homogenates [21]. In the study on erythroblast proliferation, at baseline, levels of protein-bound m-Tyr were markedly lower than that of o-Tyr [78]. Also, at the same concentration, o-Tyr tended to have a higher inhibitory capacity on cell proliferation than m-Tyr did. However, p-Tyr could more effectively overcome the inhibition by o-Tyr than by m-Tyr [78]. In the study on septic patients, serum levels of m-Tyr seemed at least as high as that of o-Tyr, however, urinary concentration and fractional excretion of o-Tyr was markedly higher than that of m-Tyr in the patients [53]. In the study on glycemia in septic patients, serum m- and o-Tyr levels were identical, however urinary concentration, daily excretion, clearance and fractional excretion of o-Tyr were approx. twice as high as for m-Tyr [54]. However, parameters calculated using urinary m-Tyr levels were somewhat more tightly correlated with glycemic parameters than parameters of o-Tyr [54]. Also, in *in vitro* experiments, iron or iron complexes + hydrogen peroxide led to formation of somewhat more o-Tyr than m-Tyr [93]. In an aqueous solution, hydroxyl radical formed tended to attack the ring of Phe in the following positions: ortho > para > meta (50% *vs*. 30% *vs*. 14%) [94].

The exact background of the abovementioned differences and discrepancies is not entirely known. If the hydroxylation occurred completely in a random manner at the five hydroxylation sites of the aromatic ring, one could expect that p-, m- and o-Tyr were produced in a 1:2:2 ratio. However, in some *in vitro* systems, the proportion is 1:1:1, in others, the proportion was only slightly different: 0.32 : 0.28 : 0.40 [95]. In reactions involving radiolysis or Fenton reaction, p-, m- and o-Tyr were produced in a 33:31:38 ratio (radiolysis on air), and a 32:31:28 ratio (Fenton reaction) [24]. These data suggest that probably electrondensity is not equal at different parts of the aromatic ring.

Furthermore, in the biological probes, the direct surroundings of the amino acid (secondary and tertiary structure) may also influence binding of the radical to the aromatic ring. Also, in the living organism, both serum levels and urinary levels of tyrosine isomers are not only influenced by production, also by excretion of the products. The data suggest that o-Tyr is retained less efficiently by the kidney, in fact, some data support active secretion or in loco production of o-Tyr, *e.g.* in the kidney of patients with sepsis or diabetes [53,22]. Also, the biologic value and efficacy of m- and o-Tyr may not be equal, as suggested by data on glucose metabolism or inhibition of cell growth [54,78,85,86].

CONCLUSION

Concluding, m- and o-Tyr are reliable markers of oxidative stress in various acute and chronic diseases. Among others, they have been shown to be produced by inflammatory cells. In the study on septic patients, serum m-Tyr levels were initially markedly elevated, then – parallel with amelioration of systemic inflammation – the levels normalized. Urinary levels of m-Tyr and o-Tyr behaved similarly. We have also found a marked difference in fractional excretion between physiological and pathological tyrosine isoforms (*i.e.* p-Tyr *vs*. m- and o-Tyr). Despite the minor structural difference and maybe also due to different hydrophilic/hydrophobic properties, the physiological (p-Tyr) and pathological isomers are handled by the kidneys in a striking different way, *i.e.* p-Tyr is retained, even in patients with sepsis; while m-Tyr and o-Tyr are highly excreted or possibly produced in the kidneys. The possibility that, m- and o-Tyr could be used as diagnostic tools or to follow-up changes due to different therapies requires additional research.

In the chronic models, m- and/or o-Tyr levels or concentrations correlated with metabolic and vascular insulin resistance, with EPO-resistance, vascular liraglutide-resistance. Data suggest that this connection may even be causal, *i.e.* the pathologic isoforms may contribute to the development of hormone resistances. Furthermore, one might speculate that, the physiologic isoform, p-Tyr may have an impact on the development of hormone resistance, thus providing a possible therapeutic tool.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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Declared none.

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