World Journal of Diabetes

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4239/wjd.v6.i3.500

World J Diabetes 2015 April 15; 6(3): 500-507
ISSN 1948-9358 (online)

MINIREVIEWS

Tyrosine isomers and hormonal signaling: A possible role for the hydroxyl free radical in insulin resistance

Gergő A Molnár, Esztella Zsóka Mikolás, István András Szijártó, Szilárd Kun, Eszter Sélley, István Wittmann

Gergő A Molnár, Esztella Zsóka Mikolás, István András Szijártó, Szilárd Kun, Eszter Sélley, István Wittmann, 2nd Department of Medicine and Nephrological Center, University of Pécs, H-7624 Pécs, Hungary

Author contributions: Molnár GA and Wittmann I designed the research; Mikolás EZ, Szijártó IA, Kun S and Sélley E performed the research; Molnár GA and Wittmann I wrote the paper.

Supported by The European Union and the State of Hungary and co-financed by the European Social Fund in the framework of TÁMOP 4.2.4. A/2-11-1-2012-0001 National Excellence Program.

Conflict-of-interest: All authors have nothing to declare.

Correspondence to: István Wittmann, MD, PhD, Professor of Medicine, Head of the Department, 2nd Department of Medicine and Nephrological Center, University of Pécs, Pacsirta Str. 1, H-7624 Pécs, Hungary. istvan.wittmann@aok.pte.hu
Telephone: +36-72-536050
Fax: +36-72-536051

Received: September 3, 2014
Peer-review started: September 4, 2014
First decision: November 19, 2014
Revised: December 5, 2014
Accepted: January 9, 2015
Article in press: January 12, 2015
Published online: April 15, 2015

Abstract

Oxidative stress processes play a major role in the development of the complications associated with diabetes and other diseases *via* non-enzymatic glycation, the hexosamine pathway, the polyol pathway and diacylglycerol-protein kinase C. Oxidative stress may lead to the production of hydroxyl free radicals, which can attack macromolecules, such as lipids, nucleic acids or amino acids. Phenylalanine (Phe) can be enzymatically converted to the physiological para-tyrosine (p-Tyr); however, a hydroxyl free radical attack on Phe may yield meta- and ortho-tyrosine (m- and o-Tyr, respectively) in addition to p-Tyr. Hence, m- and o-Tyr may be regarded as markers of hydroxyl free radical-induced damage. Their accumulation has been described; *e.g.*, this accumulation has been found in the urine of patients with diabetes mellitus (DM) and/or chronic kidney disease, in cataract lenses, in vessel walls, in irradiated food and in amniotic fluid, and it may serve as an indicator of oxidative stress. The use of resveratrol to treat patients with type 2 DM led to a decrease in the urinary excretion of o-Tyr and concomitantly led to an improvement in insulin signaling and insulin sensitivity. Literature data also suggest that m- and o-Tyr may interfere with intracellular signaling. Our group has shown that erythropoietin (EPO) has insulin-like metabolic effects on fat cells in addition to its ability to promote the proliferation of erythroid precursor cells. We have shown that the supplementation of cell culture medium with m- and o-Tyr inhibits erythroblast cell proliferation, which could be ameliorated by p-Tyr. Additionally, *in vivo*, the o-Tyr/p-Tyr ratio is higher in patients with renal replacement therapy and a greater need for EPO. However, the o-Tyr/p-Tyr ratio was an independent determinant of EPO-resistance indices in our human study. The o-Tyr content of blood vessel walls inversely correlates with insulin- and acetylcholine-induced vasodilation, which could be further impaired by artificial oxidative stress and improved by the use of antioxidants. In rats that receive o-Tyr supplements, decreased vasorelaxation is detected in response to insulin. Additionally, o-Tyr supplementation led to the incorporation of the unnatural amino acid into cellular proteins and caused a decrease in the insulin-induced phosphorylation of endothelial nitric oxide synthase. Our data suggest that m- and o-Tyr may not only be markers of oxidative stress; instead, they may also be incorporated into cellular proteins, leading to resistance to insulin, EPO and acetylcholine.

Key words: Acetylcholine; Insulin resistance; Hormone resistance; Oxidative stress; Para-tyrosine; Ortho-tyrosine; Meta-tyrosine; Erythropoietin

Core tip: Hydroxyl-free radical-derived amino acids, such as meta- and ortho-tyrosine (m- and o-Tyr, respectively) are regarded as free radical markers, but they may also be taken up and incorporated into blood vessel walls, erythroid precursors and endothelial cells. These pathological amino acids can induce vascular insulin and acetylcholine, as well as cellular erythropoietin resistance.

Molnár GA, Mikolás EZ, Szijártó IA, Kun S, Sélley E, Wittmann I. Tyrosine isomers and hormonal signaling: A possible role for the hydroxyl free radical in insulin resistance. *World J Diabetes* 2015; 6(3): 500-507 Available from: URL: http://www.wjgnet.com/1948-9358/full/v6/i3/500.htm DOI: http://dx.doi.org/10.4239/wjd.v6.i3.500

OXIDATIVE STRESS IN THE OLD PERSPECTIVES

Oxidative stress plays a role in the pathogenesis of many diseases, such as diabetes mellitus (DM), chronic kidney disease (CKD) and inflammatory diseases, as well as in the development of the complications associated with these diseases. Oxidative stress, free radicals and reactive oxygen species (ROS) are tightly connected to DM in several ways. Hyperglycemia may lead to a shift in cellular metabolism toward the polyol pathway, which leads to an oxidative shift in the NADPH/NADP ratio. NADPH is in turn required for antioxidant defense, *e.g.*, for the reduction of oxidized glutathione by glutathione reductase. Furthermore, hyperglycemia activates the diacylglycerol-protein kinase C intracellular signaling pathway, which can activate NADPH oxidases; this oxidation leads to the translocation of nuclear factor κB into the nucleus and then to the transcription of proinflammatory cytokines, which results in increased oxidative stress. Additionally, hyperglycemia increases the rate of non-enzymatic glycation, which produces advanced glycation end-products (AGEs) that can bind to their receptors of AGE (RAGE), and the AGE-RAGE interaction also leads to inflammation and oxidative stress. DM involves the enhancement of not only non-enzymatic glycation but also enzymatic glycation *via* the hexosamine pathway; this enhanced glycation may also result in a proinflammatory response and oxidative stress. However, this interplay is complex; the ROS arising from oxidative stress may also contribute to the activation of the abovementioned pathways and reactions and can thus generate a vicious circle[1,2].

DETECTION OF OXIDATIVE STRESS

The study of oxidative stress processes is therefore important, albeit somewhat cumbersome. Per their definition, free radicals are highly reactive molecules with a very short lifetime; therefore, detecting these molecules requires spin traps and an electron spin resonance device[3,4], but the sensitivity of this method is usually too low for many diseases. Because of the high reactivity of free radicals, they can react with macromolecules and yield oxidation products, some of which are more chemically stable molecules. These products may include lipid peroxidation products (such as malonyldialdehyde derivatives), nucleobase products (such as 8-hydroxydeoxyguanosine), so-called advanced oxidation products or amino acid derivatives[5,6].

DETECTION OF HYDROXYL FREE RADICALS BY STABILE L-PHENYLALANINE DERIVATIVES

L-Phenylalanine (Phe) is a highly abundant essential amino acid in proteins of the human body, and Phe plays a role in forming peptides and proteins; Phe also gives rise to the semi-essential amino acid L-para-tyrosine (p-Tyr) and its derivatives, such as 3,4 dihydroxy-phenylalanine (DOPA), and derivatives thereof[7]. p-Tyr is mainly produced *via* the enzymatic reactions catalyzed by the Phe hydroxylase enzyme, especially in the kidney and liver[8]. p-Tyr synthesis becomes impaired in patients with renal failure (*e.g.*, CKD); therefore, the serum levels of p-Tyr in these patients are lower than those in patients/controls with normal renal function [CKD: 28 (26-34), DM + CKD: 32 (29-39) μmol/L *vs* controls: 56 (36-57) μmol/L][9]. However, other isomers of tyrosine, namely meta- and ortho-tyrosine (m-Tyr and o-Tyr, respectively) also exist in humans. These amino acids cannot be formed enzymatically in humans; instead, they are stable products of the reaction between the hydroxyl free radical and Phe. Additionally, p-Tyr may be formed non-enzymatically, *via* the hydroxyl radical, but the enzymatically produced p-Tyr is much more abundant than the free radical-derived p-Tyr. Therefore, p-Tyr is regarded as the physiologic isoform, whereas m- and o-Tyr are considered to be free radical markers[10-14].

All four aromatic amino acids (p-Tyr, m-Tyr, o-Tyr and Phe) can be readily detected in the nanomolar range *via* their autofluorescence and reverse-phase high performance liquid chromatography[9,15-22], as well as by gas chromatography combined with mass spectrometric detection[23] or by ultra-performance liquid chromatography combined with mass spectrometry[24].

ABUNDANCE OF THE HYDROXYL FREE RADICAL MARKERS M- AND O-TYR

In vitro measurements[25] and *in silico* calculations[26] have shown that in free radical reactions, the three isoforms (p-,

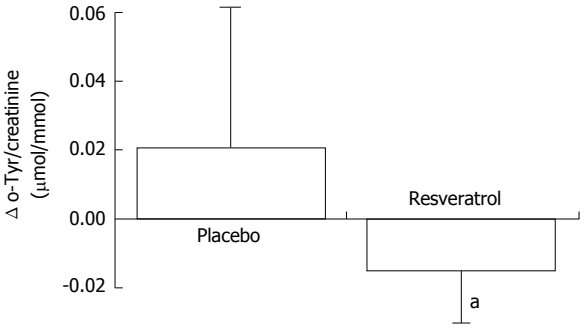

Figure 1 Changes in urinary ortho-tyrosine:creatinine excretion after resveratrol treatment. For each participant, the value measured at baseline was subtracted from that measured at week 4 (*i.e.*, Δ o-Tyr:creatinine ratio), and then the resulting values were averaged within the respective groups. The values are the means, with the standard deviations represented by vertical bars. [a]Mean values were significantly different (P = 0.043). Comparisons were performed using ANOVA and Bonferroni post hoc tests; P < 0.05 was regarded as statistically significant. Republished with permission of Cambridge University Press from Brasnyó *et al*[18]. o-Try: Ortho-tyrosine.

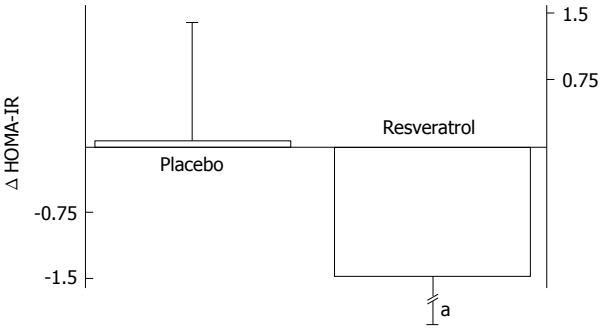

Figure 3 Decrease in homeostasis model of assessment for insulin resistance upon resveratrol treatment. For each participant, the value measured at baseline was subtracted from that measured at week 4 (*i.e.*, Δ HOMA-IR), and then the resulting values were averaged within the respective groups. Values are the means with the standard deviations represented by vertical bars. [a]Mean values were significantly different for the resveratrol group *vs* the placebo group (P = 0.044), comparisons were performed using ANOVA and Bonferroni post hoc tests; P < 0.05 was regarded as statistically significant. Republished with permission of Cambridge University Press from Brasnyó *et al*[18]. HOMA-IR: Homeostasis model of assessment for insulin resistance.

m- and o-Tyr) are not produced stoichiometrically (*i.e.*, 1:1:1), and their concentrations and ratios may vary from tissue to tissue *in vivo*. These isoforms, along with others, have been detected in cataractous lenses by our group and others[16,27], in the brain after acute oxidative stress[28], in the serum/plasma of diabetic patients with/without CKD by our group[9], after the administration of thyroid hormone[29], after ischemia-reperfusion injury[14], in the hair of the Homo tirolensis and mummies[30], in irradiated food[31], in urine specimens[9,24] and in amniotic fluid[32]. In earlier publications, we have shown that m-Tyr, o-Tyr and DOPA accumulate in the non-water soluble proteins of cataractous lenses during aging and in patients with DM[16], and we also showed that the urinary excretion of o-Tyr increases in patients with type 2 DM and/or CKD [CKD: 122 (94-183), DM: 641 (272-499), DM+CKD: 403 (258-651) nmol/d *vs* controls: 24 (0-35) nmol/d][9]. Using the so-called fractional excretion values,

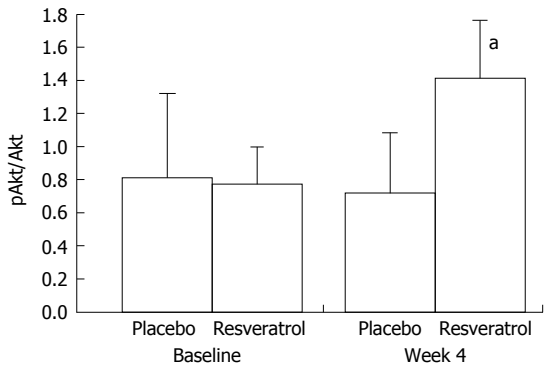

Figure 2 Increase in protein kinase B phosphorylation in platelets upon resveratrol treatment. Values are the means, with the standard deviations represented by vertical bars. [a]Mean values were significantly different for baseline *vs* week 4 within the resveratrol group (P = 0.032). Comparisons were performed using ANOVA and post-hoc tests; P < 0.05 was regarded as statistically significant. Republished with permission of Cambridge University Press from Brasnyó *et al*[18]. pAkt: Protein kinase B phosphorylation.

which show the renal handling of o-Tyr, we have found active urinary secretion or *in loco* renal synthesis of o-Tyr in the kidney of diabetic patients [DM: 125 (69-140), DM + CKD: 112 (69-187)% *vs* controls: 8 (4-12)%][9].

In patients with stroke, the urinary excretion of o-Tyr is associated with the total urinary albumin excretion; immunoreactive albumin excretion; and most significantly, urinary non-immunoreactive albumin excretion[17].

DECREASED URINARY EXCRETION OF O-TYR IS ASSOCIATED WITH AN IMPROVEMENT IN INSULIN RESISTANCE IN TYPE 2 DIABETES

In a human study, we demonstrated the protective effect of the polyphenolic compound resveratrol in patients with type 2 DM; a short-term administration of resveratrol led to a decrease in urinary o-Tyr excretion (Figure 1), an increase in the phosphorylation of the insulin signaling molecule protein kinase B (or Akt), (Figure 2) and an amelioration of the calculated marker of insulin resistance (homeostasis model assessment-insulin resistance) (Figure 3)[18].

ARE M- AND O-TYR JUST MARKERS OR ALSO MAKERS?

All of the abovementioned papers focused on m- and o-Tyr as markers of hydroxyl free radical damage. However, the last of our abovementioned studies (on the *in vivo* effects of resveratrol) raised the possibility that these molecules may not only be markers; instead, they may also play a role in the development of pathological states[18]. Independent of our results, Ruggiero *et al*[33,34] showed that m- and o-Tyr can inhibit tumor cell growth *via* influencing the mitogen-activated protein kinase/extracellular signal regulated kinase (ERK) and

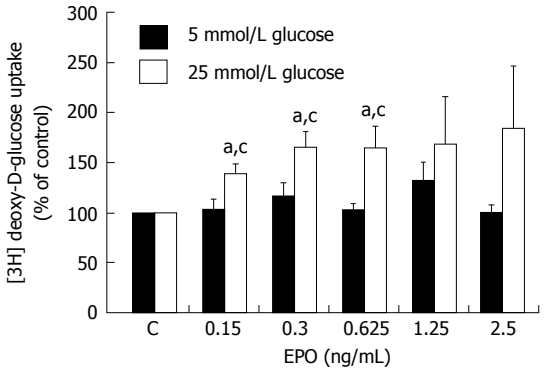

Figure 4 Effects of treatment with r-mo-erythropoietin on the rate of [3H]-deoxy-D-glucose uptake in 3T3-L1 adipocytes cultured in either normal (5 mmol/L) or high (25 mmol/L) glucose medium (insulin: *n* = 8 and *n* = 6; r-mo-erythropoietin: *n* = 6 and *n* = 5, respectively). $^{a}P < 0.05$ *vs* control in the same medium; $^{c}P < 0.05$ between the two media; Comparisons with control were performed using a one-sample *t*-test; pairwise comparisons between 5 and 25 mmol/L glucose-cultured cells were performed using a paired samples *t*-test. Republished with permission of Georg Thieme Verlag KG Stuttgart, New York from Mikolás *et al*[37]. EPO: Erythropoietin.

the signal transducer and activator of transcription (STAT) pathway. In plants, m-Tyr inhibits cell growth and plant root growth and may be considered a natural herbicide[35,36]. This finding raises the possibility that the unnatural isoforms, m- and o-Tyr, might affect cellular function in mammals and plants and may interfere with hormonal signaling.

INSULIN-LIKE EFFECT OF ERYTHROPOIETIN ON GLUCOSE METABOLISM

In a subsequent study, we have shown that under hyperglycemic circumstances, erythropoietin (EPO) exerts insulin-like effects on the uptake of isotope-labeled glucose by 3T3-L1-type fat cells (Figure 4) and can lead to the translocation of glucose transporters transporters (GLUTs) from their intracellular pools to the membrane (Figure 5). EPO also improves glucose metabolism in streptozotocin-induced diabetic rats[37].

M- AND O-TYR ARE INCORPORATED INTO CELLULAR PROTEINS AND LEAD TO ERYTHROPOIETIN RESISTANCE

In further studies, we showed that the administration of m- and o-Tyr to erythroblasts inhibited erythroblast proliferation in a time- and concentration-dependent manner. Increasing doses of p-Tyr could overcome the inhibition by m- and o-Tyr, suggesting potential competition among the structural isoforms (Figure 6).

Erythroblasts grown in cell culture medium supplemented with m- or o-Tyr incorporated the Tyr isoforms into their cellular proteins (Figure 7).

Supplementing erythroblast cells with o- or m-Tyr inhibited the EPO-dependent increase in the rate of phosphorylation of STAT5 and ERK1/2 (Figure 8). This finding indicates that the unnatural isoforms, o- and m-Tyr, can be incorporated into cellular proteins and interfere with the hormonal signaling of EPO[21].

This finding is consistent with a clinical observation of our group that the ratio of the pathological o-Tyr to the physiological p-Tyr (o-Tyr/p-Tyr ratio) is higher in patients who receive renal replacement therapy compared with controls. Additionally, the o-Tyr/p-Tyr ratio in the blood was higher in the individuals receiving hemodialysis and EPO replacement than in those patients receiving hemodialysis who did not require EPO replacement or in patients receiving peritoneal dialysis and requiring markedly lower EPO doses. Furthermore, the plasma o-Tyr/p-Tyr ratio was an independent predictor of the EPO dose and EPO-resistance indices in these patients. This finding is another indirect demonstration that o-Tyr may interfere with EPO signaling and lead to EPO resistance[22].

VASCULAR INSULIN RESISTANCE ACCORDING TO THE O-TYR CONTENT OF THE VESSEL WALL

In a further set of experiments, we analyzed the o-Tyr levels in the blood vessel walls of rodents and found that the o-Tyr content of blood vessels decreases toward the peripheral vasculature (*i.e.*, thoracic aorta > abdominal aorta > femoral artery) (Figure 9A). The o-Tyr content could be increased by treatment with hydrogen peroxide and aminotriazole or by aortic banding, and it could be inhibited using superoxide dismutase and catalase (Figure 9B).

We have shown that vascular segments with higher o-Tyr content show lower vasorelaxation in response to insulin; *i.e.*, the insulin-induced vasorelaxation is lowest in the thoracic aorta, higher in the abdominal aorta and the highest in the femoral artery (Figure 10).

Furthermore, the insulin-induced relaxation could be increased by an antioxidant (superoxide dismutase and catalase) treatment in the thoracic aorta. By contrast, pro-oxidant therapy further diminished the vasorelaxation in an ERK1/2-dependent manner[19].

VASCULAR ACETYLCHOLINE RESISTANCE ACCORDING TO THE O-TYR CONTENT OF THE VESSEL WALL

In the same set of experiments, the vasorelaxation in response to acetylcholine was also tested, and we also found an inverse relationship between the vessel wall o-Tyr content and the vasorelaxation in response to acetylcholine; *i.e.*, the blood vessels with high o-Tyr content (see Figure 9A) show less vasorelaxation in response to Ach (Figure 11, previously unpublished data).

Based on these experiments, we subjected rats

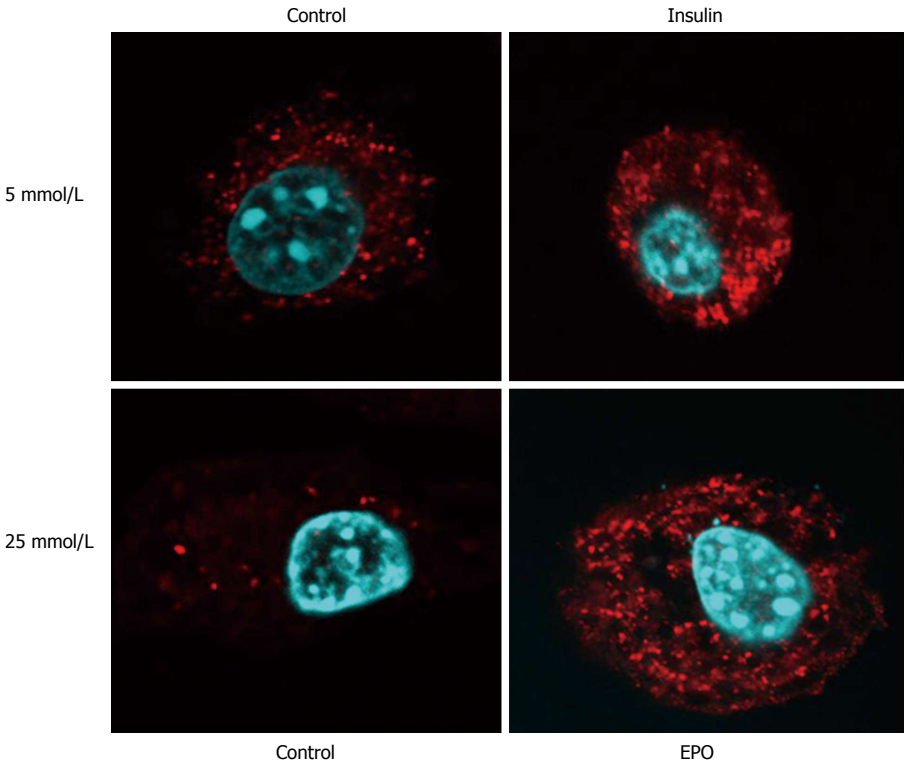

Figure 5 GLUT4 translocation (red fluorescence) after a 30 min treatment with insulin (400 nmol/L) or r-mo-erythropoietin (40 ng/mL) in 3T3-L1 adipocytes cultured in high glucose (25 mmol/L) or normal glucose (5 mmol/L) medium compared with that in untreated cells (Control). Nuclei are shown with blue fluorescence. The GLUT4 translocation was apparent after both the erythropoietin (EPO) and insulin treatments, whereas it was not present in untreated cells. Representative images are shown from *n* = 3 independent experiments. Republished with permission of Georg Thieme Verlag KG Stuttgart, New York from Mikolás *et al*[37].

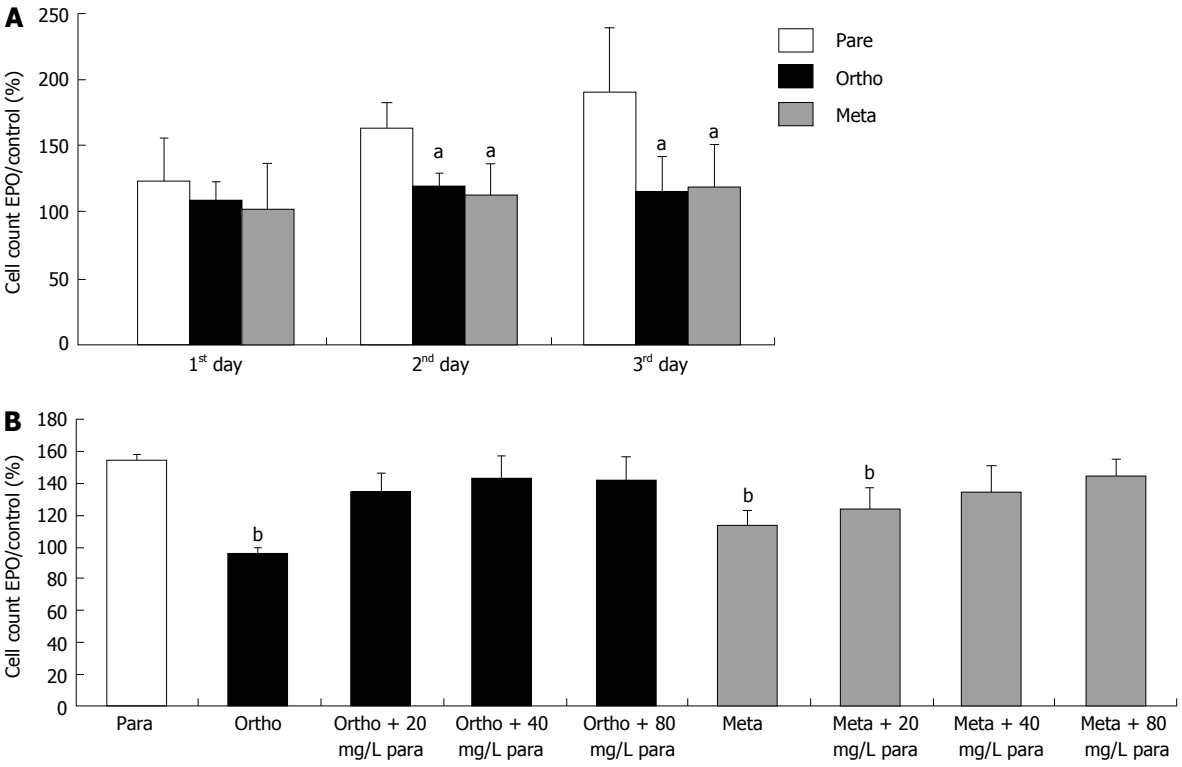

Figure 6 Effect of para-, ortho-, or meta-tyrosine supplementation on the proliferation of cells cultured in medium with or without erythropoietin. A: The time-dependent increase in cell count in medium supplemented with p-Tyr (open bars), o-Tyr (black bars) or m-Tyr (grey bars) ($^{a}P < 0.05$ *vs* p-Tyr cultured cells on the same day; *n* = 10); B: The alterations in the cell counts after incubation for 3 d in medium containing o- or m-Tyr and the indicated additional amount of para-tyrosine ($^{b}P < 0.001$ *vs* p-Tyr-containing medium; *n* = 5). The results are expressed as the ratio of the protein content of erythropoietin (EPO) and non-EPO (control) cells ($^{a}P < 0.05$ *vs* p-Tyr cultured cells; *n* = 10). Comparisons were performed using ANOVA and Bonferroni's post-hoc test. Republished with permission of S Karger AG, Basel from Mikolás *et al*[21]. p-Tyr: Para-tyrosine; o-Tyr: Ortho-tyrosine; m-Tyr: Meta-tyrosine.

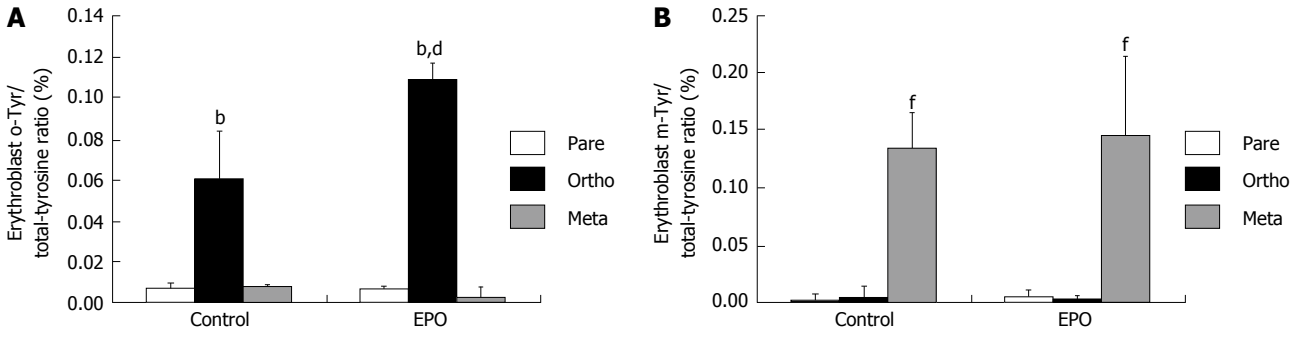

Figure 7 Relative meta- and ortho-tyrosine content (*i.e.*, ratios of meta- and ortho-tyrosine/total tyrosine) of the cellular proteins of TF-1 cells (A and B, respectively). $^{b}P < 0.001$ *vs* p- and m-Tyr cultured cells; $^{d}P < 0.001$ *vs* o-Tyr cultured control cells; $^{f}P < 0.001$ *vs* p- and o-Tyr cultured erythroblasts; $n = 10$. Comparisons were performed using ANOVA and Bonferroni's post-hoc test. Republished with permission of S Karger AG, basel from Mikolás *et al*[21]. p-Tyr: Para-tyrosine; o-Tyr: Ortho-tyrosine; m-Tyr: Meta-tyrosine.

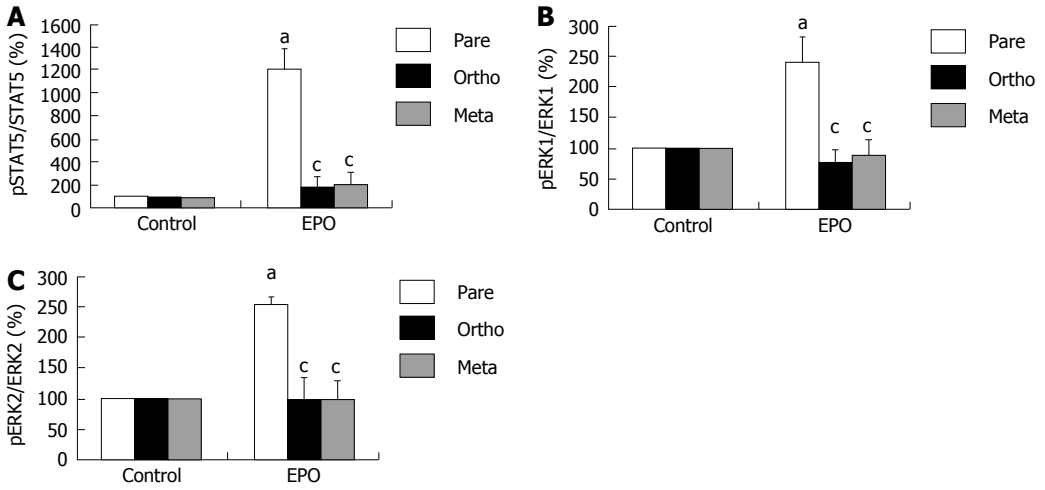

Figure 8 Densitometric analyses of the phosphorylation of signal transducer and activator of transcription 5 (A: $n = 3$); densitometric analysis of extracellular signal regulated kinase 1 (B: $n = 4$) and extracellular signal regulated kinase 2 (C: $n = 4$). The data are expressed as the percent of untreated (control) cells. $^{a}P < 0.05$; erythropoietin (EPO) *vs* control (one-sample *t*-test); $^{c}P < 0.05$ *vs* para EPO (one-way ANOVA). Republished with permission of S Karger AG, basel from Mikolás *et al*[21].

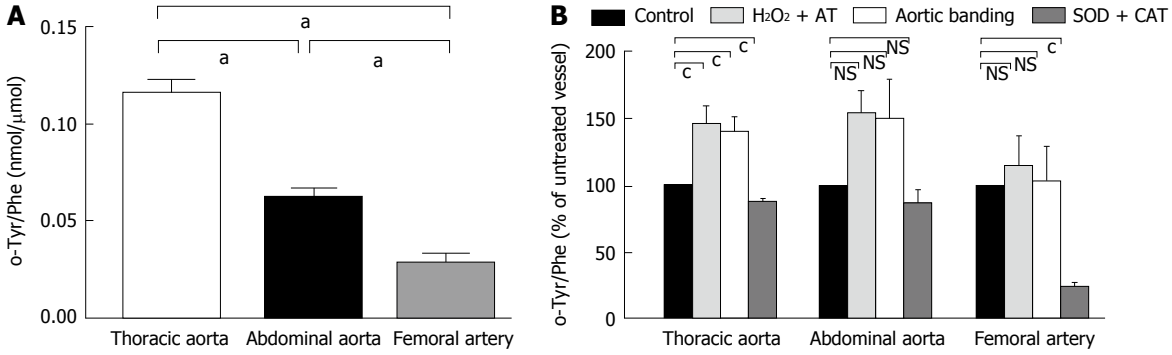

Figure 9 The ortho-tyrosine levels of consecutive arterial segments isolated from rats (A). There were significant differences ($^{a}P < 0.05$) in the o-Tyr levels among the thoracic aorta ($n = 7$), abdominal aorta ($n = 7$), and the femoral artery ($n = 9$) of rats. The changes in the o-Tyr levels in the consecutive arterial segments of rats after H_2O_2 + AT treatment (*i.e.*, high oxidative state), SOD + CAT treatment (*i.e.*, low oxidative state), and aortic banding were compared with those of the control vessels (B). Control: untreated, control vessels (thoracic: $n = 7$; abdominal: $n = 7$; femoral: $n = 9$); H_2O_2 + AT: hydrogen peroxide- and aminotriazole-incubated vessels (aminotriazole is an inhibitor of catalase) (thoracic: $n = 5$; abdominal: $n = 5$; femoral: $n = 4$); SOD + CAT: superoxide dismutase- and catalase-incubated vessels (thoracic: $n = 5$; abdominal: $n = 5$; femoral: $n = 5$); aortic banding (thoracic: $n = 5$; abdominal: $n = 4$; femoral: $n = 4$). Data are the mean ± SEM. The o-Tyr levels are relative to the phenylalanine (Phe) levels (Panels A and B) and are expressed as the percentage of the control vessels (Pane B). $^{c}P < 0.05$; NS, $P > 0.05$ (ANOVA). Republished with permission of Informa Healthcare from Szijártó *et al*[19]. o-Tyr: Ortho-tyrosine; NS: Non-significant; SEM: Standard error of the mean.

to vehicle or o-Tyr supplementation for 4 wk. By the end of the 4 wk, we showed decreased vasorelaxation in response to insulin in the o-Tyr-supplemented rats compared with the controls. Additionally, the endothelial cells that received o-Tyr supplementation incorporated o-Tyr into their cellular proteins. In these cells, the

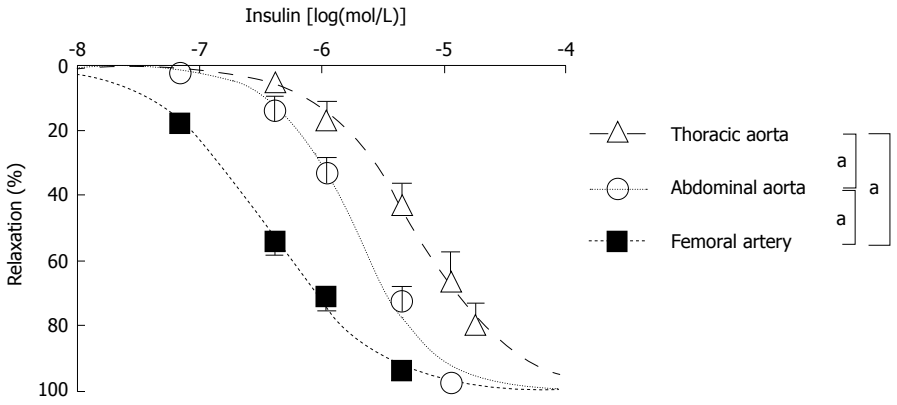

Figure 10 The dose–response curve of the insulin-induced relaxation of consecutive arterial segments isolated from rats. The relaxation in response to insulin was the greatest in the femoral artery (n = 7) and was less pronounced in the abdominal aorta (n = 7) and even less in the thoracic aorta (n = 7). The relaxation is depicted as a function of the logarithm of the insulin dose. Data are the mean ± SEM. $^{a}P < 0.05$; NS, $P > 0.05$ (Extra sum-of-squares F test). Republished with permission of Informa Healthcare from Szijártó *et al*[19]. NS: Non-significant; SEM: Standard error of the mean.

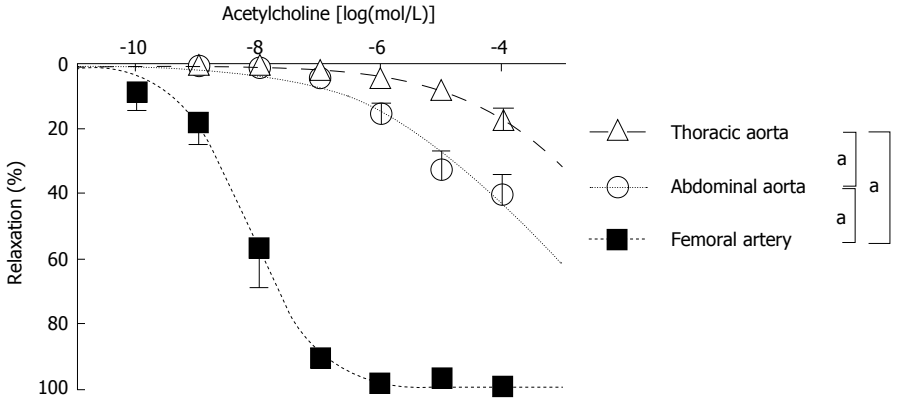

Figure 11 Acetylcholine-induced vasorelaxation in the abdominal aorta and femoral artery. Data are the means ± SEM. $^{a}P < 0.05$; NS, $P > 0.05$ (Extra sum-of-squares F test). NS: Non-significant.

insulin-induced increase in endothelial nitric oxide synthase phosphorylation was diminished compared with that in the control cells[20] (data not shown).

CONCLUSION

Our results, which are consistent with previous findings, indicate that m- and o-Tyr are valuable markers of oxidative stress and other types of stress in patients or experimental animals with DM. Furthermore, the results suggest that these unnatural amino acids may also perform a pathogenic role, *i.e.*, interfere with the signaling of three distinct hormones: insulin (vascular and perhaps metabolic signaling), acetylcholine and erythropoietin (also has metabolic effects). This inhibition may be even more pronounced in patients who have high levels of the pathological amino acids m- or o-Tyr (*e.g.*, in DM) and simultaneously have lower levels of physiological p-Tyr (*e.g.*, patients with impaired kidney function). This finding, together with the effect in which p-Tyr overcomes the inhibitory effect of m- and o-Tyr, raises the possibility that the physiological amino acid p-Tyr could be a therapeutic tool in hormone resistance in states with increased oxidative stress (*e.g.*, in DM).

REFERENCES

1 **Brownlee M**. The pathobiology of diabetic complications: a unifying mechanism. *Diabetes* 2005; **54**: 1615-1625 [PMID: 15919781]
2 **Giacco F**, Brownlee M. Oxidative stress and diabetic complications. *Circ Res* 2010; **107**: 1058-1070 [PMID: 21030723]
3 **Rashba-Step J**, Turro NJ, Cederbaum AI. ESR studies on the production of reactive oxygen intermediates by rat liver microsomes in the presence of NADPH or NADH. *Arch Biochem Biophys* 1993; **300**: 391-400 [PMID: 8380968]
4 **Berliner LJ**, Khramtsov V, Fujii H, Clanton TL. Unique in vivo applications of spin traps. *Free Radic Biol Med* 2001; **30**: 489-499 [PMID: 11182519]
5 **Maritim AC**, Sanders RA, Watkins JB. Diabetes, oxidative stress, and antioxidants: a review. *J Biochem Mol Toxicol* 2003; **17**: 24-38 [PMID: 12616644]
6 **Ghiselli A**, Serafini M, Natella F, Scaccini C. Total antioxidant capacity as a tool to assess redox status: critical view and experimental data. *Free Radic Biol Med* 2000; **29**: 1106-1114 [PMID: 11121717]
7 **Stadtman ER**, Levine RL. Free radical-mediated oxidation of free amino acids and amino acid residues in proteins. *Amino Acids* 2003; **25**: 207-218 [PMID: 14661084]
8 **Bergeron M**, Scriver CR. Pathophysiology of renal hyperaminoacidurias and glucosuria. In Seldin DW, Giebisch G, editors. The kidney. Physiology and pathophysiology, New York: Raven Press,

1985: 1725-1745
9 **Molnár GA**, Wagner Z, Markó L, Kó Szegi T, Mohás M, Kocsis B, Matus Z, Wagner L, Tamaskó M, Mazák I, Laczy B, Nagy J, Wittmann I. Urinary ortho-tyrosine excretion in diabetes mellitus and renal failure: evidence for hydroxyl radical production. *Kidney Int* 2005; **68**: 2281-2287 [PMID: 16221230]
10 **Themann C**, Teismann P, Kuschinsky K, Ferger B. Comparison of two independent aromatic hydroxylation assays in combination with intracerebral microdialysis to determine hydroxyl free radicals. *J Neurosci Methods* 2001; **108**: 57-64 [PMID: 11459618]
11 **Stadtman ER**, Berlett BS. Fenton chemistry. Amino acid oxidation. *J Biol Chem* 1991; **266**: 17201-17211 [PMID: 1894614]
12 **Davies MJ**, Fu S, Wang H, Dean RT. Stable markers of oxidant damage to proteins and their application in the study of human disease. *Free Radic Biol Med* 1999; **27**: 1151-1163 [PMID: 10641706]
13 **Brennan ML**, Hazen SL. Amino acid and protein oxidation in cardiovascular disease. *Amino Acids* 2003; **25**: 365-374 [PMID: 14661097]
14 **Sun JZ**, Kaur H, Halliwell B, Li XY, Bolli R. Use of aromatic hydroxylation of phenylalanine to measure production of hydroxyl radicals after myocardial ischemia in vivo. Direct evidence for a pathogenetic role of the hydroxyl radical in myocardial stunning. *Circ Res* 1993; **73**: 534-549 [PMID: 8394226]
15 **Ishimitsu S**, Fujimoto S, Ohara A. Determination of m-tyrosine and o-tyrosine in human serum by high-performance liquid chromatography with fluorimetric detection. *J Chromatogr* 1986; **378**: 222-225 [PMID: 3733974]
16 **Molnár GA**, Nemes V, Biró Z, Ludány A, Wagner Z, Wittmann I. Accumulation of the hydroxyl free radical markers meta-, ortho-tyrosine and DOPA in cataractous lenses is accompanied by a lower protein and phenylalanine content of the water-soluble phase. *Free Radic Res* 2005; **39**: 1359-1366 [PMID: 16298866]
17 **Toth P**, Koller A, Pusch G, Bosnyak E, Szapary L, Komoly S, Marko L, Nagy J, Wittmann I. Microalbuminuria, indicated by total versus immunoreactive urinary albumins, in acute ischemic stroke patients. *J Stroke Cerebrovasc Dis* 2011; **20**: 510-516 [PMID: 20813547]
18 **Brasnyó P**, Molnár GA, Mohás M, Markó L, Laczy B, Cseh J, Mikolás E, Szijártó IA, Mérei A, Halmai R, Mészáros LG, Sümegi B, Wittmann I. Resveratrol improves insulin sensitivity, reduces oxidative stress and activates the Akt pathway in type 2 diabetic patients. *Br J Nutr* 2011; **106**: 383-389 [PMID: 21385509]
19 **Szijártó IA**, Molnár GA, Mikolás E, Fisi V, Laczy B, Gollasch M, Koller A, Wittmann I. Increase in insulin-induced relaxation of consecutive arterial segments toward the periphery: Role of vascular oxidative state. *Free Radic Res* 2014; **48**: 749-757 [PMID: 24628420]
20 **Szijártó IA**, Molnár GA, Mikolás E, Fisi V, Cseh J, Laczy B, Kovács T, Böddi K, Takátsy A, Gollasch M, Koller A, Wittmann I. Elevated vascular level of ortho-tyrosine contributes to the impairment of insulin-induced arterial relaxation. *Horm Metab Res* 2014; **46**: 749-752 [PMID: 25208272]
21 **Mikolás E**, Kun S, Laczy B, Molnár GA, Sélley E, Kőszegi T, Wittmann I. Incorporation of ortho- and meta-tyrosine into cellular proteins leads to erythropoietin-resistance in an erythroid cell line. *Kidney Blood Press Res* 2013; **38**: 217-225 [PMID: 24751667]
22 **Kun S**, Mikolás E, Molnár GA, Sélley E, Laczy B, Csiky B, Kovács T, Wittmann I. Association of plasma ortho-tyrosine/para-tyrosine ratio with responsiveness of erythropoiesis-stimulating agent in dialyzed patients. *Redox Rep* 2014; **19**: 190-198 [PMID: 24693974]
23 **Leeuwenburgh C**, Rasmussen JE, Hsu FF, Mueller DM, Pennathur S, Heinecke JW. Mass spectrometric quantification of markers for protein oxidation by tyrosyl radical, copper, and hydroxyl radical in low density lipoprotein isolated from human atherosclerotic plaques. *J Biol Chem* 1997; **272**: 3520-3526 [PMID: 9013599]
24 **Kuligowski J**, Torres-Cuevas I, Quintás G, Rook D, van Goudoever JB, Cubells E, Asensi M, Lliso I, Nuñez A, Vento M, Escobar J. Assessment of oxidative damage to proteins and DNA in urine of newborn infants by a validated UPLC-MS/MS approach. *PLoS One* 2014; **9**: e93703 [PMID: 24695409]
25 **Molnár GA**. Pathogenesis of the chronic complications of type 2 diabetes mellitus, chronic kidney disease and ageing. The role of oxidative stress, endothelial dysfunction and the renin-angiotensin system (PhD Theses). University of Pécs, 2007. Available from: URL: http: //aok.pte.hu/en/egyseg/phd_dolgozatok/1670/docs/phd/file/dolgozatok/2007/Molnar_Gergo_Attila_PhD_dolgozat.pdf
26 **Galano A**, Cruz-Torres A. OH radical reactions with phenylalanine in free and peptide forms. *Org Biomol Chem* 2008; **6**: 732-738 [PMID: 18264574]
27 **Fu S**, Dean R, Southan M, Truscott R. The hydroxyl radical in lens nuclear cataractogenesis. *J Biol Chem* 1998; **273**: 28603-28609 [PMID: 9786852]
28 **Lubec B**, Hayn M, Denk W, Bauer G. Brain lipid peroxidation and hydroxy radical attack following the intravenous infusion of hydrogen peroxide in an infant. *Free Radic Biol Med* 1996; **21**: 219-223 [PMID: 8818637]
29 **Magsino CH**, Hamouda W, Ghanim H, Browne R, Aljada A, Dandona P. Effect of triiodothyronine on reactive oxygen species generation by leukocytes, indices of oxidative damage, and antioxidant reserve. *Metabolism* 2000; **49**: 799-803 [PMID: 10877210]
30 **Lubec G**, Weninger M, Anderson SR. Racemization and oxidation studies of hair protein in the Homo tirolensis. *FASEB J* 1994; **8**: 1166-1169 [PMID: 7958623]
31 **Hein WG**, Simat TJ, Steinhart H: Detection of irradiated food. Determination of non-protein bound o-tyrosine as a marker for the detection of irradiated shrimps. *Eur Food Res Technol* 2000; **210**: 299-304
32 **Escobar J**, Teramo K, Stefanovic V, Andersson S, Asensi MA, Arduini A, Cubells E, Sastre J, Vento M. Amniotic fluid oxidative and nitrosative stress biomarkers correlate with fetal chronic hypoxia in diabetic pregnancies. *Neonatology* 2013; **103**: 193-198 [PMID: 23295371]
33 **Ruggiero RA**, Bruzzo J, Chiarella P, di Gianni P, Isturiz MA, Linskens S, Speziale N, Meiss RP, Bustuoabad OD, Pasqualini CD. Tyrosine isomers mediate the classical phenomenon of concomitant tumor resistance. *Cancer Res* 2011; **71**: 7113-7124 [PMID: 22084446]
34 **Ruggiero RA**, Bruzzo J, Chiarella P, Bustuoabad OD, Meiss RP, Pasqualini CD. Concomitant tumor resistance: the role of tyrosine isomers in the mechanisms of metastases control. *Cancer Res* 2012; **72**: 1043-1050 [PMID: 22315349]
35 **Bertin C**, Weston LA, Huang T, Jander G, Owens T, Meinwald J, Schroeder FC. Grass roots chemistry: meta-tyrosine, an herbicidal nonprotein amino acid. *Proc Natl Acad Sci USA* 2007; **104**: 16964-16969 [PMID: 17940026]
36 **Bertin C**, Harmon R, Akaogi M, Weidenhamer JD, Weston LA. Assessment of the phytotoxic potential of m-tyrosine in laboratory soil bioassays. *J Chem Ecol* 2009; **35**: 1288-1294 [PMID: 19924485]
37 **Mikolás E**, Cseh J, Pap M, Szijárto IA, Balogh A, Laczy B, Bekő V, Fisi V, Molnár GA, Mérei A, Szeberényi J, Wittmann I. Effects of erythropoietin on glucose metabolism. *Horm Metab Res* 2012; **44**: 279-285 [PMID: 22351476]

P- Reviewer: Tsuruya K **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Liu SQ

Published by **Baishideng Publishing Group Inc**
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com