Journal of Plastic, Reconstructive & Aesthetic Surgery (2012) 65, 1706–1711

JPRAS
An International Journal of Surgical Reconstruction
www.JPRASurg.com

The role of antioxidants in ischaemia-reperfusion in a human DIEP flap model☆

M.G.W. van den Heuvel [a,*], A. Bast [b], G.R.M.M. Haenen [b], A.W. Ambergen [c], J.F. Mermans [a], R.R.W.J. van der Hulst [a]

[a] *Department of Plastic, Reconstructive and Hand Surgery, Maastricht University Medical Centre+, Maastricht, The Netherlands*
[b] *Department of Pharmacology and Toxicology, Maastricht University Medical Centre+, Maastricht, The Netherlands*
[c] *Department of Methodology and Statistics, Maastricht University Medical Centre+, Maastricht, The Netherlands*

Received 16 June 2010; accepted 11 June 2012

KEYWORDS
Antioxidant;
TEAC;
Ischaemia;
Reperfusion;
(DIEP) Flap

Summary *Background:* A better understanding of the pathophysiology of ischaemia-reperfusion injury and a possible treatment for it, is of great importance. The deep inferior epigastric perforator (DIEP) flap is an innovative, clinical model of ischaemia-reperfusion. There has been an ongoing interest in the health benefits and medical applications of antioxidants. We hypothesised that during ischaemia-reperfusion, specific antioxidants are depleted.
Methods: Seventeen DIEP flaps were performed in 15 patients undergoing breast reconstruction. In each free flap, 3-mm skin biopsies were taken from the DIEP flap at four different time points during and after surgery. In those tissue biopsies, concentrations of the antioxidants vitamin E, glutathione (GSH) and uric acid and total antioxidant capacity (TEAC) were measured.
Results: Unexpectedly, no immediate change was observed in GSH concentrations during ischaemia-reperfusion. Uric acid concentrations were significantly increased at all time points following reperfusion. Vitamin E concentrations also showed a significant incline 30 min and 1 h after reperfusion. However, 1 h after reperfusion, a significant decrease in total hydrophilic antioxidant capacity (TEAC) was seen. In the next hour, this capacity recovered.
Conclusions: During ischaemia-reperfusion, a deficiency in hydrophilic antioxidant capacity develops. This is a potential cause for the development of ischaemia-reperfusion injury by reactive oxygen species.

☆ This paper was presented at the Spring Congress of the Dutch Society of Plastic Surgery of 30 May–1 June, 2008.

* Corresponding author. Department of Plastic, Reconstructive and Hand Surgery, Maastricht University Medical Centre+, P.O. Box 5800, 6202 AZ Maastricht, The Netherlands. Tel.: +31 0433877481.
E-mail address: m.vandenheuvel@AH.unimaas.nl (M.G.W. van den Heuvel).

1748-6815/$ - see front matter
http://dx.doi.org/10.1016/j.bjps.2012.06.010

This clinical trial is registered on Clinical Trials: http://www.clinicaltrials.gov/.
Trial registry name: The DIEP-flap as a model of ischaemia-reperfusion.
Registration identification number (NCT): 00482469.

Introduction

Ischaemia-reperfusion injury plays an important role in the dysfunction and failure of tissue and organ transplants.[1,2] A better understanding of the pathophysiology of ischaemia-reperfusion injury and a possible treatment for it is of great importance. In this study, the deep inferior epigastric perforator free flap (FF-DIEP) is used as a human, clinical model of ischaemia-reperfusion. This tissue flap is dissected from the abdomen and transplanted to the patient's thorax for breast reconstruction. This new model of ischaemia-reperfusion has several advantages over other models of this condition. The free flap is visible and within reach after surgery, and interference of donor incompatibility is avoided. It provides the opportunity to study ischaemia-reperfusion over time.

In this study, we focussed on the role of antioxidants in ischaemia-reperfusion injury. There has been an ongoing interest in the health benefits and medical applications of antioxidants. They are compounds that own the capacity to neutralise reactive oxygen species (ROS) and thereby prevent the development of tissue damage when ROS arise during reperfusion.[3] Up-regulation of endogenous antioxidants is observed during oxidative stress.[4] However, as many ROS evolve in a short period of time, the system of antioxidants is flooded.[5] As a result, the antioxidants are consumed quickly and concentrations in the body decline.[3,6] We hypothesised that during ischaemia-reperfusion, specific antioxidants are depleted.

Materials and methods

Population

Seventeen DIEP flaps were performed in 15 patients undergoing secondary breast reconstruction at the department of Plastic and Reconstructive Surgery in the Maastricht University Medical Centre^{+} in The Netherlands. Two patients underwent bilateral surgery. The population consisted of women who had undergone breast amputation or mastectomy due to breast cancer or to prevent breast cancer. All patients aged 18 years or older who were scheduled for DIEP surgery, were asked to participate in the research. An informed consent procedure was followed. No patients met the exclusion criteria that were a medical history of diabetes mellitus, diseases of the kidneys or liver or the use of immunosuppressants. The study was conducted according to the principles of the Declaration of Helsinki (5th version, 24/07/2001) and in accordance with the Medical Research Involving Human Subjects Act (WMO) approved by the Ethical Committee of Maastricht University Medical Centre^{+}.

Structure of the experiment

During and after the DIEP operation, four 3-mm skin biopsies were taken from the DIEP flap at four different time points (Table 1). The first biopsy was performed at the start of the operation to determine the patient's antioxidant condition (baseline measurement). This dynamic condition is dependent on a combination of internal and external factors, including nutritional status. The next three biopsies were taken after reperfusion to evaluate the effect of ischaemia-reperfusion on the patient's antioxidant condition.

Baseline characteristics that might intervene with the main study parameters (confounders) were registered (Table 2). Amongst them, is duration of ischaemia. The length of ischaemia time is relatively constant with this surgical procedure and is around 90 min.

Biopsy procedure

Skin biopsies were taken from zone 1 of the free flap (which is the central zone near the vascular pedicle) to ensure adequate reperfusion. The biopsy needle (3 mm dermal biopsy punch, Miltex, York, PA, USA) was put onto the flap and turned around 360° once. The biopsies were taken from the lower border of the free flap. Biopsies were obtained during surgery, except for the last biopsy that was usually taken after surgery ended. No local anaesthesia was given for the taking the biopsies, because patients were under general anaesthesia and the free flap was not innervated neurologically after surgery. Skin samples were rapidly frozen in liquid nitrogen, stored at −80 °C and analysed at the laboratory at a later point of time.

Tissue preparation

Prior to analyses, the skin biopsies were homogenised in 5% trichloro acetic acid (5—15 mg in 220 μl TCA) and centrifuged. The supernatant was used to measure GSH, uric acid and TEAC. The pellet was used for the measurement of vitamin E.

Table 1 Biopsy schedule.

Biopsy number	Time point
1	At start of surgery
2	0.5 h after reperfusion
3	1 h after reperfusion
4	2 h after reperfusion

Time points at which biopsies were taken. In total four biopsies were taken from each patient.

Table 2 Baseline values.

Time point	Baseline values
Pre-operative	Age, BMI, smoking, previous chemotherapy or radiation therapy.
Per-operative	Weight of flap, duration of ischaemia, operative time.
Post-operative	Bleeding, infection, necrosis, flap loss.

Baseline values that were included integrated as covariates in statistical analysis. In the end, only those values that showed significant interaction ($P \leq 0.05$) with antioxidant concentrations were included in the linear mixed models analysis (see Results section).

Laboratory analyses

Chemicals

2,2′-Azinobis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), uric acid and butylated hydroxytoluene (BHT) were obtained from Sigma Chemical Co., St. Louis, USA. 2,2′-Azinobis(2-amidinopropane) dihydrochloride (ABAP) was obtained from Brunschwig Chemicals, Amsterdam, The Netherlands. All chemicals were of analytical purity.

Uric acid assay

Uric acid, the reaction product of xanthine oxidase activity, was determined using a high-performance liquid chromatography (HPLC) method described by Lux et al.[7]

Total antioxidant capacity assay

Total antioxidant capacity assay was performed as described by Fischer et al.[8] Briefly, 950 μl of ABTS•+ radical solution was incubated for 1 min at 37 °C; thereafter 50 μl of supernatant was added. After 5 min of incubation, the absorption at 734 nm was measured. The decrease in absorption after 5 min relative to blank (buffer) was related to that of Trolox calibrators. The TEAC value gives the concentration of trolox that has a similar antioxidant capacity as the sample.

Skin GSH content

Glutathione (GSH) concentrations were measured in the supernatant of homogenised skin biopsies, as described extensively by Anderson.[9] Total protein concentrations in the homogenates were measured using the bicinchoninic acid method, as described by Smith et al.[10] Final concentrations of GSH were expressed per mg protein.

Skin levels of α-tocopherol

The analysis of the concentrations of the α-tocopherols in the pellet of homogenised skin biopsies was performed as described by Broekmans et al.[11] Final concentrations of α-tocopherol (vitamin E) were expressed per mg protein.

Calculations and statistics

Statistical analysis by Statistical Package for Social Sciences (SPSS) linear mixed models has been performed.[12] This analysis corrects for dependency in repeated measurements. Antioxidant concentrations were compared to that person's concentrations at the start of surgery. Baseline characteristics (Table 2) that significantly influenced antioxidant concentrations were integrated included in the final model as covariates. Statistical correction had taken place for patients who underwent bilateral DIEP surgery. Significance was considered present at $P \leq 0.05$.

Results

Out of 17 free flaps, one flap showed partial necrosis (around 33%). All the other free flaps displayed no complications postoperatively. Concentrations of the antioxidants are shown in Figure 1. *P*-values for antioxidant concentrations compared to baseline measurement are shown in Table 3.

GSH

Unexpected, no immediate change was observed in GSH concentrations compared to the concentrations at the start of surgery. However, concentrations 2 h after reperfusion displayed an increasing trend ($P = 0.088$).

Uric acid

The concentrations of uric acid showed obvious changes. In comparison to the baseline measurements, concentrations were significantly increased at all time points following reperfusion.

Vitamin E

Vitamin E concentrations were significantly increased 30 min and 1 h following reperfusion. Two hours after reperfusion, vitamin E concentration had normalised again to baseline level.

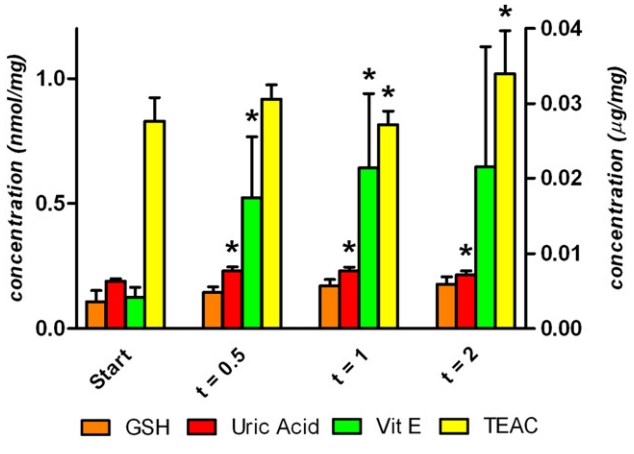

Figure 1 Antioxidant concentrations. Antioxidant concentrations at the start of surgery and 30 min, 1 h and 2 h after reperfusion. GSH, uric acid, and TEAC are expressed in nmol/mg, Vit E in μg/mg. Error bars represent SEMs. Significance compared to baseline is marked by an asterisk.

Table 3 *P*-values for antioxidant concentrations compared to baseline measurement.

	0.5 h	1 h	2 h
GSH	0.328	0.132	0.088↑
Uric acid	0.002↑	0.000↑	0.040↑
Vitamin E	0.000↑	0.046↑	0.847
TEAC	0.373	0.046↓	0.000

P-values for antioxidant concentration measurements 30 minutes, 1 hour and 2 hours after reperfusion compared to baseline concentrations. Values marked red show significance, values marked yellow show a trend. Significance was considered present at $p \leq 0.05$. Arrows indicate an increase or a decrease in concentration.

TEAC

TEAC is a measure for the cumulative capacity of the present hydrophilic antioxidants in neutralising ROS. Thirty minutes after reperfusion, TEAC concentrations did not significantly differ from those at the start of surgery. However, 1 h after reperfusion, a significant decrease in TEAC concentration was seen. In the next hour TEAC concentrations recovered, followed by a significant increase in concentration 2 h after reperfusion.

Discussion

Through the process of ischaemia-reperfusion, ROS are produced. We hypothesised that during ischaemia-reperfusion, a depletion of certain antioxidants would occur. Depletion of antioxidants does not necessarily mean oxidative tissue damage has occurred: it might simply indicate that the antioxidant system has removed the ROS and thereby consumed the present antioxidants to protect the tissues.[13] However, if the amount of produced ROS exceeds the capacity of the antioxidant system, tissue damage may develop. Except for one, the free flaps in our experiment recovered from the ischaemia-reperfusion without tissue damage. The damaged free flap remained included in the study and its antioxidant concentrations did not differ from concentrations of undamaged free flaps. A possible explanation might be that necrosis occurred in the lateral part of the free flap, while biopsies were performed in the central part of the flap.

Vitamin E

The antioxidants that were focussed on in our research are GSH, uric acid and vitamin E. Vitamin E (α-tocopherol) is one of the most important fat-soluble antioxidants and represents in this study the lipophilic antioxidant compartment, while GSH and uric acid account for the hydrophilic compartment. Vitamin E is localised in cellular membranes. It plays an important role in stabilisation of these membranes and in protecting them by capturing peroxyl radicals.[4] It is also important in protecting low-density lipoprotein (LDL) from oxidation and in processing glucose in the body. Through acting as a chain-breaking antioxidant, α-tocopherol is transformed into the tocopheroxyl radical.[13] After antioxidants have donated an electron to neutralise a free radical, they become free radicals themselves. Subsequently, they can be regenerated by other antioxidants.[4] Ubiquinol, GSH, vitamin C (ascorbic acid) and some flavonoids are able to recycle the tocopheroxyl radical.[3,4,13] However, despite evidence, it is generally believed that recycling by ascorbic acid is the most important recycling system *in vivo*.[13]

The results of our experiment show that directly after reperfusion a compensatory increase in the tissue concentration of vitamin E occurs. An increase in vitamin E concentrations is against expectations, as other studies have shown that serum concentrations of α-tocopherol decrease after reperfusion.[14,15] Zinchuk et al. as well as Kadkhodaee et al. demonstrated that not only α-tocopherol levels in venous blood, but also in tissue decreased significantly at the end of reperfusion.[16,17] Others confirmed this decrease in tissue vitamin E concentration, while some experiments showed no change in tissue α-tocopherol concentration.[18,19] A possible explanation for these differences is that the concentration of an antioxidant is dependent on the types of free radicals that are formed. If lipophilic free radicals are formed and cell membranes are damaged, lipophilic antioxidants (e.g., vitamin E) will be consumed. If hydrophilic antioxidants are formed, only hydrophilic antioxidants can scavenge the free radicals and will therefore decrease in concentration. In our study, the latter is the case.

Uric acid

The second antioxidant studied is uric acid. Uric acid is the final product of purine metabolism and is thought to display antioxidant capacities.[20] It is produced from hypoxanthine and xanthine by the enzymes xanthine oxidase and xanthine dehydrogenase. Ames et al. demonstrated in 1981 that uric acid is a potent scavenger of ROS *in vitro*.[13,20] By being favourably oxidised as a substrate for oxidation by haem protein or H_2O_2 systems, uric acid can protect tissues from oxidative damage.[13] It has been demonstrated that by infusion of uric acid, neuroprotective effects can be exerted in animal models.[13] Uric acid is a potent antioxidant,[20] whose concentrations have shown to increase strongly after ischaemia and after reperfusion.[21–23] It is known that in patients who are subjected to oxidative stress, concentrations of uric acid degradation products are increased.[13] The expected increase in uric acid concentration following ischaemia-reperfusion was obviously visible in our experiment. At every time point following ischaemia-reperfusion, uric acid concentrations were significantly elevated compared to baseline levels. This confirms that the antioxidant uric acid plays an important role in attenuating oxidative stress caused by ischaemia-reperfusion.

GSH

GSH is a tri-peptide that plays an important role in regulating the cellular redox potential in general.[4] It is synthesised in the cytoplasm of all cells, the liver being most active.[13] It is produced from cysteine, glycine and glutamic

acid by the enzymes glutamate—cysteine ligase and glutathione synthetase.[13] Important functions of GSH are protecting the cell against oxidative damage or damage due to radiation, and regenerating antioxidant vitamins.[4] During oxidative stress, GSH donates an electron to neutralise hydrogen peroxides and lipoperoxides.[24] Through this, the GSH changes into oxidised GSH, glutathione disulphide (GSSG).[24] GSSG is subsequently reduced to GSH by GSSG-reductase.[4]

Reduced GSH plays an important role in protecting cells against ROS. Research has proven this by showing that inhibition of the endogenous production of GSH during ischaemia-reperfusion of the heart causes an increase in tissue damage.[5] Furthermore, it has recently been demonstrated that suppletion of GSH before the onset of ischaemia-reperfusion of the heart decreases myocardial damage.[5] On the contrary to what literature describes,[4,25] ischaemia-reperfusion did not significantly alter GSH concentrations in our study. This is against expectations, as GSH is one of the most important antioxidants of the body.[24,25] It is present in high concentrations in every body cell and one of the first to come into action during ischaemia-reperfusion. Most animal studies have shown that ischaemia-reperfusion is characterised by a decrease in GSH tissue concentration.[18,24,25] However, others did not observe changes in total GSH concentration during ischaemia and reperfusion in myocardial and liver tissue.[19,26] A study in rats only showed a significant decrease in myocardial GSH level after 40 min of ischaemia and 40 min of reperfusion; shorter periods of ischaemia and reperfusion induced no significant changes in GSH concentration.[27]

Possibly, the unchanged GSH concentrations in our study can be explained by the fact that skin is less sensitive to ischaemia-reperfusion than for example muscle, fat tissue or liver.[25,28] This can prevent major antioxidant defence reactions (and thus changes in antioxidant concentration) from occurring. Furthermore, the human metabolism is slower than the rat metabolism and for that reason a longer ischaemia duration might be necessary in humans to display the same effects as seen in animals. Another possible explanation for differences with other research can be that our model is a model of true ischaemia-reperfusion, while related studies are possibly looking at ischaemia while there is no adequate reperfusion.

TEAC

TEAC is a measure for the cumulative capacity of the present hydrophilic antioxidants in neutralising ROS. Major components of TEAC are uric acid (35—65%), plasma proteins (10—50%) and ascorbic acid (up to 24%).[13] In some analyses, TEAC can also include some vitamin E activity. In this study, however, TEAC was measured in the supernatant of the tissue homogenates. Because no cell membranes are present, no vitamin E can be measured. Our TEAC analysis represents activity of *hydrophilic* antioxidants only. TEAC does not only represent the known antioxidants, it also displays activity of an unidentified antioxidant component. This can either be an antioxidant not yet identified or be caused by synergy between antioxidants.[13]

One hour after reperfusion, a significant decrease in TEAC was seen. This is remarkable because the tissue concentrations of its major components uric acid and GSH did not display a decrease. This indicates that the decrease in hydrophilic antioxidant capacity is caused by hydrophilic antioxidants other than those measured in our experiment. Which antioxidant component(s) is (are) responsible for the decrease in TEAC, is not clear. An antioxidant not yet identified or synergy between antioxidants as described in the literature,[13] or an antioxidant that is known but is not measured in our study could be responsible for this phenomenon. The latter could for instance be ascorbic acid.

It is probably the same antioxidant(s) that, 2 h after reperfusion, causes the TEAC to recover and even to increase concentrations above baseline levels. Indeed, the increase in hydrophilic antioxidant capacity can only be partially explained by the changes in concentrations of the hydrophilic antioxidants measured. A slight increase in GSH concentration is shown (trend, $P = 0{\cdot}088$) and uric acid concentration is significantly increased. However, this cannot explain the overall change in TEAC concentration, as the increase in GSH is only mild and an increased uric acid concentration was also seen 1 h after reperfusion, when TEAC dropped.

Our results correspond with the literature. The rat study of Sivonova et al. also demonstrated an increase in total antioxidant capacity after 3 h of reperfusion, which was followed by a decrease during late reperfusion (after 72 h).[22] The patient study of Monge et al. confirmed a significant decrease in total antioxidant values 1 day after ischaemia-reperfusion.[29] Remarkably, some rat studies showed an increase in serum total antioxidant capacity at the end of ischaemia and during reperfusion.[23,30] However, the absence of a drop in TEAC concentration after ischaemia could be explained by the fact that rats are able to produce ascorbate (vitamin C) *de novo*, which enables these animals to increase their TEAC.[23]

Conclusion

From these data, it can be concluded that during ischaemia-reperfusion a deficiency in hydrophilic antioxidant capacity develops. An unknown hydrophilic antioxidant is probably responsible for it. This is a potential cause for the development of ischaemia-reperfusion injury by ROS. Suppletion with hydrophilic antioxidants may intercept the initial decline in hydrophilic antioxidant capacity and thereby prevent the development of tissue damage.

Conflict of interest and source of funding statement

There are no funds supporting our work and no financial benefits. No products have been used in this article.

Acknowledgement

The authors would like to thank M.J.H.J. Drittij-Reijnders for her assistance with the laboratory analyses.

References

1. Kupiec-Weglinski JW, Busuttil RW. Ischemia and reperfusion injury in liver transplantation. *Transplant Proc* 2005;**37**(4): 1653—6.
2. Burns AT, Davies DR, McLaren AJ, Cerundolo L, Morris PJ, Fuggle SV. Apoptosis in ischemia/reperfusion injury of human renal allografts. *Transplantation* 1998;**66**(7):872—6.
3. Tangney CC, Hankins JS, Murtaugh MA, Piccione Jr W. Plasma vitamins E and C concentrations of adult patients during cardiopulmonary bypass. *J Am Coll Nutr* 1998;**17**(2):162—70.
4. Chan AC, Chow CK, Chiu D. Interaction of antioxidants and their implication in genetic anemia. *Proc Soc Exp Biol Med* 1999;**222**(3):274—82.
5. Ramires PR, Ji LL. Glutathione supplementation and training increases myocardial resistance to ischemia-reperfusion in vivo. *Am J Physiol Heart Circ Physiol* 2001;**281**(2):H679—88.
6. Ozmen D, Boydak B, Mutaf I, et al. The state of lipid peroxidation and antioxidants following thrombolytic therapy with rt-PA and streptokinase in acute myocardial infarction. *Jpn Heart J* 1999;**40**(3):267—73.
7. Lux O, Naidoo D, Salonikas C. Improved HPLC method for the simultaneous measurement of allantoin and uric acid in plasma. *Ann Clin Biochem* 1992;**29**(Pt 6):674—5.
8. Fischer MA, Gransier TJ, Beckers LM, Bekers O, Bast A, Haenen GR. Determination of the antioxidant capacity in blood. *Clin Chem Lab Med* 2005;**43**(7):735—40.
9. Anderson ME. Determination of glutathione and glutathione disulfide in biological samples. *Methods Enzymol* 1995;**113**: 548—55.
10. Smith PK, Krohn RI, Hermanson GT, et al. Measurement of protein using bicinchoninic acid. *Anal Biochem* 1985;**150**(1):76—85.
11. Broekmans WM, Berendschot TT, Klopping-Ketelaars IA, et al. Macular pigment density in relation to serum and adipose tissue concentrations of lutein and serum concentrations of zeaxanthin. *Am J Clin Nutr* 2002;**76**(3):595—603.
12. Verbeke GMG. *Linear mixed models for longitudinal data*. New York: Springer; 2000.
13. Halliwell BGJ. In: *Free radicals in biology and medicine*. 4th ed. New York: Oxford University Press Inc.; 2007.
14. Muzakova V, Kandar R, Vojtisek P, et al. Antioxidant vitamin levels and glutathione peroxidase activity during ischemia/reperfusion in myocardial infarction. *Physiol Res* 2001;**50**(4): 389—96.
15. Bartels M, Biesalski HK, Engelhart K, Sendlhofer G, Rehak P, Nagel E. Pilot study on the effect of parenteral vitamin E on ischemia and reperfusion induced liver injury: a double blind, randomized, placebo-controlled trial. *Clin Nutr* 2004;**23**(6): 1360—70.
16. Kadkhodaee M, Aryamanesh S, Faghihi M, Zahmatkesh M. Protection of rat renal vitamin E levels by ischemic-preconditioning. *BMC Nephrol* 2004;**5**:6.
17. Zinchuk VV, Khodosovsky MN, Maslakov DA. Influence of different oxygen modes on the blood oxygen transport and prooxidant-antioxidant status during hepatic ischemia/reperfusion. *Physiol Res* 2003;**52**(5):533—44.
18. Aydemir O, Naziroglu M, Celebi S, Yilmaz T, Kukner AS. Antioxidant effects of alpha-, gamma- and succinate-tocopherols in guinea pig retina during ischemia-reperfusion injury. *Pathophysiology* 2004;**11**(3):167—71.
19. Hack B, Witting PK, Rayner BS, Stocker R, Headrick JP. Oxidant stress and damage in post-ischemic mouse hearts: effects of adenosine. *Mol Cell Biochem* 2006;**287**(1, 2):165—75.
20. Ames BN, Cathcart R, Schwiers E, Hochstein P. Uric acid provides an antioxidant defense in humans against oxidant- and radical-caused aging and cancer: a hypothesis. *Proc Natl Acad Sci USA* 1981;**78**(11):6858—62.
21. Strazzullo P, Puig JG. Uric acid and oxidative stress: relative impact on cardiovascular risk? *Nutr Metab Cardiovasc Dis* 2007; **17**(6):409—14.
22. Sivonova M, Kaplan P, Durackova Z, et al. Time course of peripheral oxidative stress as consequence of global ischaemic brain injury in rats. *Cell Mol Neurobiol* 2008;**28**(3):431—41.
23. Cizova H, Lojek A, Kubala L, Ciz M. The effect of intestinal ischemia duration on changes in plasma antioxidant defense status in rats. *Physiol Res* 2004;**53**(5):523—31.
24. Serviddio G, Di Venosa N, Federici A, et al. Brief hypoxia before normoxic reperfusion (postconditioning) protects the heart against ischemia-reperfusion injury by preventing mitochondria peroxyde production and glutathione depletion. *FASEB J* 2005;**19**(3):354—61.
25. Coban YK, Kurutas EB, Ciralik H. Ischemia-reperfusion injury of adipofascial tissue: an experimental study evaluating early histologic and biochemical alterations in rats. *Mediators Inflamm* 2005;**2005**(5):304—8.
26. Codoner-Franch P, Muniz P, Gasco E, Domingo JV, Valls-Belles V. Effect of a diet supplemented with alpha-tocopherol and beta-carotene on atp and antioxidant levels after hepatic ischemia-reperfusion. *J Clin Biochem Nutr* 2008; **43**(1):13—8.
27. Ko KM, Mak DH, Poon MK, Yiu HY. Altered susceptibility to ischemia-reperfusion injury in isolated-perfused hearts of short-term diabetic rats associated with changes in non-enzymatic antioxidants. *Jpn J Pharmacol* 2001;**85**(4): 435—42.
28. Kupinski AM, Shah DM, Bell DR. Transvascular albumin flux in rabbit hindlimb after tourniquet ischemia. *Am J Physiol* 1993; **264**(3 Pt 2):H901—8.
29. Monge M, Ledeme N, Mazouz H, et al. Insulin maintains plasma antioxidant capacity at an early phase of kidney transplantation. *Nephrol Dial Transplant* 2007;**22**(7):1979—85.
30. Slavikova H, Lojek A, Hamar J, et al. Total antioxidant capacity of serum increased in early but not late period after intestinal ischemia in rats. *Free Radic Biol Med* 1998;**25**(1):9—18.