Effects of melatonin on low dose lipopolysaccharide-induced oxidative stress in the mouse liver, muscle and kidney

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<tr>
<td>Keyword:</td>
<td>melatonin, LPS, oxidative stress, lipids oxygenation, oxidatively modified proteins, antioxidant enzymes</td>
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Effects of melatonin on low dose lipopolysaccharide-induced oxidative stress in the mouse liver, muscle and kidney

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Short title: Melatonin ameliorates low grade endotoxaemia
Abstract

Lipopolysaccharide (LPS) administration in an in vivo experimental mice model causes oxidative damage in the liver, muscle and kidney. We aimed to determine specific mechanisms underlying melatonin’s antioxidant protective role.

Assays were carried out in quadruplicate in the control, melatonin (10 mg/kg, 10 days), acute LPS administration (once 150 µg) and LPS plus melatonin groups.

LPS stimulated lipid peroxidation processes (dienes and malondialdehyde) and antioxidant enzyme concentrations (superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase) were assessed in all investigated tissues. Protein oxidation processes (measured as aldehyde and kenotic carbonyl protein derivatives) were enhanced by LPS in the kidney and liver but not in the muscle. Melatonin, reversed LPS-induced changes, with the exception of muscle protein oxidation.

LPS-induced oxidative stress resulted in augmented early stage diene conjugated and end stage malondialdehyde lipid peroxidation processes and affected antioxidant activity in liver, kidney and muscle tissues. LPS activated protein oxidation processes in the kidney and liver. Melatonin ameliorated oxidative damage in the liver, kidney and partially in the muscle.

Melatonin modulates the oxidative stress-induced states. Potential synergism between melatonin and systemic inflammation in terms of oxidative modification of muscle proteins needs to be clarified in further studies.

Keywords: melatonin, LPS, oxidative stress, lipids oxygenation, oxidatively modified proteins, antioxidant enzymes
Most of the pathological processes in the human organism are associated with disturbances in the prooxidant-antioxidant balance (Halliwell 2007; Valko et al. 2007). Oxidation processes are accompanied by the formation of very active, toxic forms of oxygen such as reactive oxygen species (ROS) or organic and inorganic peroxy compounds. ROS and the antioxidant mechanisms inactivating them constitute an integral part of the functioning of a living organism (Shinde at al. 2012). The system of antioxidant protection is an autoregulated, multicomponent metabolic complex, in which the components function with and complement each other. During pronounced and prolonged activation of lipid peroxidation (LPO) processes, endogenous bio-antioxidants are depleted. Additional intake of them is slowed down due to impaired circulation and reduced permeability of cell membranes (Valko et al. 2007).

Lipopolysaccharide (LPS), the main component of the Gram-negative bacterial membrane, administration results in the oxidative stress (Wang and Quinn 2010). LPS exposure enhances the expression of cytokines, adhesion molecules, oxygenases and the inducible isoform of nitric oxide (NO)-synthase in effector cells (Snowden and Kirkman 2002; Pancher et al. 2007). Consequently, LPS-induced oxidative stress is, at least partially, NO-dependent (Cobb and Marsha 2015). The human body is constantly exposed to pro-inflammatory cytokines and ROS (Stigger et al. 2013). Oxidative stress is an important defense mechanism against invading bacteria (e.g. the oxidative burst used by immune cells to kill bacteria) and decreasing oxidative stress could actually be detrimental to host defenses during a bacterial invasion. Low doses of LPS ensure the maintenance of homeostasis and adaptation of the organism to stressful effects (Snowden and Kirkman 2002). High doses of LPS lead to oxidative damage, impaired microcirculation and tissues oxygenation, and result in hypoxia at the tissue level (Cadenas and Cadenas 2002).

An example of low grade systemic inflammation is, associated with obesity, so called “metabolic endotoxaemia”, a condition of chronically elevated plasma LPS at levels 10–50 times lower than during septic conditions (Cani et al. 2007; Boutagy et al. 2016). Under physiological conditions, the gut microbiota promotes gut barrier function. However, a high fat diet unfavorably alters the gut microbial composition, leading to increased intestinal permeability and subsequently elevated circulating plasma endotoxin (Cani et al. 2009). Over 35% of US adults and >100 billion people worldwide are overweight or obese (Flegal et al. 2016) what makes the “metabolic endotoxaemia” very relevant from clinical perspective.
Quite interestingly, injection of 300 µg/kg/day of LPS in mice produces similar derangements of diet induced obesity in terms of inflammation and oxidative stress (Cani et al. 2007).

It is necessary to find new pharmacological agents with a pronounced antioxidant and membrane stabilizing action (Saravanan et al. 2007; Shinde et al., 2012). The results of experimental and clinical studies in recent years indicate that the hormone melatonin (N-acetyl-5-methoxytryptamine) is an adaptation factor involved in the coordination and synchronization of neuro and immuno-physiological processes (Shin et al. 2015). Melatonin is a powerful antioxidant secreted from the pineal gland and is capable of reducing ROS activity (Sewerynek et al. 1995; Bonnefont-Rousselot and Collin 2010; Kurhaluk et al. 2017). The effect of melatonin is manifested by the maintenance of normal bioelectrical activity of the brain, circadian rhythm, sleep, regulation of hypothalamic-pituitary activity and the immune system, and in general anti-stress properties (Escames et al. 1997). The disruption of melatonin production and action may represent one of the pathogenic links between neuro-immunological disorders and oxidative stress. Melatonin has been used in various areas of preventive and clinical medicine (Kim et al. 2005; De Filippis et al. 2008). Importantly, melatonin, as an indole, possesses amphiphilic properties (Petrosillo et al. 2008). As a consequence, it easily overcomes tissue barriers and passes through the cell membrane to affect intracellular processes (Reiter et al. 2003). Melatonin interacts with G-proteins via membrane receptors, and thereby modulates the state of intracellular messengers and/or membrane ion channels (Sewerynek et al. 1996).

LPS administration in an in vivo experimental mouse model causes oxidative damage in the liver, muscle and kidney. We aimed to determine specific mechanisms underlying melatonin’s protective role on the intensity of lipid and protein peroxidation processes, and antioxidant enzyme activities in these organs.

Materials and methods

Animals and experimental design.

All authors confirm that the experiments were performed in accordance with the Guidelines of the European Union Council and the current laws in Ukraine, and approved by the Ethical Commission of National Pedagogical University in Chernihiv (2612/2016) where the experiments were carried out. The animals were cared for in accordance with Guide to the Care and Use of Experimental Animals (Vol. 1, 2nd ed., 1993, and Vol. 2, 1984, available...
from the Canadian Council on Animal Care (CCAC), 190 O’Connor St, Suite 800, Ottawa, ON K2P 2R3, Canada).

All experiments were performed between 10:00 am and 12:00 pm to compensate for the circadian rhythm. Healthy male white mice (Mus musculus) Balb/c weighing about 20-30 grams and aged about 2-3 months were used in the experiments. The data were collected from 24 adult animals divided into four groups. The mice were housed at a constant temperature of 20 ±2°C. The animals had free access to food and water throughout the experiments.

**Drugs and chemicals.**

EDTA, HEPES, KCl, K$_2$CO$_3$, KH$_2$PO$_4$, EDTA, 2-tiobarbituric acid were purchased from Sigma-Aldrich (Sigma-Aldrich Sp. z.o.o, Poznan, Poland). All drugs were freshly prepared. All other used reagents were of analytical reagent grade. Melatonin (cat. no. M5250, Sigma-Aldrich, Deisenhofen, Germany) and LPS (E. coli serotype 026:B6, Sigma-Aldrich Sp. z.o.o, Poznan, Poland) were purchased from these companies.

**Tissue isolation.**

The tissues were obtained after decapitation of the animals. Briefly, the tissues was excised, weighed, washed in ice-cold buffer and minced. The minced tissue was rinsed with cold isolation buffer to remove blood and homogenized in a glass Potter-Elvehjem homogenizer with a motor-driven Teflon pestle on ice. The isolation buffer consisted of 120 mM KCl, 2 mM K$_2$CO$_3$, 10 mM HEPES and 1 mM EDTA; pH of 7.2 was adjusted with KOH.

**Experimental groups.**

The mice were randomly assigned into four groups: untreated controls (6 animals), melatonin group (6 animals), LPS group (6 animals) and LPS + melatonin group (6 animals). Melatonin was given as daily intraperitoneal injections with 10 mg/kg of melatonin during 10 days. Melatonin was dissolved in a minimum volume of ethanol and diluted in 0.9% NaCl to yield a dose of 10 mg/kg body weight, as described in previous studies (Bonnefont-Rousselot and Collin, 2010) and Shin et al. (2015). LPS (E. coli serotype 026:B6, Sigma-Aldrich) was injected once intraperitoneally in 150 µg doses per mouse, as described by Yang et al., (2013) and Blanque et al. (1999). Control mice were given 0.9% NaCl intraperitoneally.

**Biochemical assays**

**Thiobarbituric acid reactive substances (TBARS) assay.**

TBARS were estimated using the method of Kamysnykov (2004). The TBARS level was analyzed according to the following principle: distilled water, 20% trichloracetic acid and
0.8% 2-tiobarbituric acid (TBA) reagent were added to liver homogenate and boiled in a water bath at 100°C for 10 min. TBARS products were then cooled in ice-cold water, centrifuged at 3,000g for 10 min.; and measured by spectrophotometry at 540 nm. TBARS levels were expressed in µmol of malondialdehyde (MDA) per L of plasma.

**Protein carbonyl derivatives assay.**

The oxidatively modified proteins (OMP) rate was estimated using the reaction detecting carbonyl derivatives of amino acids with 2,4-dinitrophenyl hydrazine (DNFH) as described by Levine et al. (1990) and modified by Dubinina et al. (1995). The method is based on the reaction of carbonyl derivatives of amino acids with 2, 4-dinitrophenylhydrazine after adding tissue homogenate. The final solution was centrifuged to remove any insoluble material. The carbonyl content was calculated from the absorbance measurement at 370 nm and 430 nm and an absorption coefficient 22,000 M⁻¹·cm⁻¹. Carbonyl groups were determined spectrophotometrically at 370 nm (aldehyde derivatives (AD), OMP₃₇₀) and 430 nm (ketone derivatives (KD), OMP₄₃₀), and expressed in nmol per mL of blood.

**Superoxide dismutase activity assay.**

Superoxide dismutase (SOD, E.C. 1.15.1.1) activity in the supernatant was determined according to Kostiuk et al. (1990). SOD activity was assessed by a colorimetric method based on the principle of measuring absorbance of the coloured quercetin autoxidation complex in an alkaline medium (pH 10.0) against water blank. Absorbance at 406 nm was measured immediately and after 20 min. Activity is expressed in units of SOD per mL of blood.

**Catalase activity assay.**

Catalase (CAT, E.C. 1.11.1.6) activity was determined by measuring the decrease of H₂O₂ in the reaction mixture using the method of Koroliuk et al. (1988). Activity of the enzyme was estimated by the decrease in absorbance of hydrogen peroxide at 410 nm. The reaction mixture contained tissue homogenate diluted in incubation medium (1:10), H₂O₂ and ammonium molybdate. One unit of CAT activity is defined as the amount of enzyme required for decomposition of 1 µmol H₂O₂ per min per L of plasma.

**Glutathione reductase activity assay.**

Glutathione reductase (GR, E.C. 1.6.4.2) activity in the blood and tissues was measured according to the method of Glatzle et al. (1974). The enzymatic activity was assayed spectrophotometrically by measuring nicotinamide adenine dinucleotide phosphate (NADPH) consumption in sodium phosphate buffer (pH 6.6). The method is based on the measurement of changes in absorbance at a wavelength of 340 nm, caused by the NADPH oxidation. A
blank without NADPH was used and the GR activity was expressed as nmol NADPH per min per mL of blood or per mg protein.

**Glutathione peroxidase activity assay.**

Glutathione peroxidase (GPx, EC 1.11.1.9) activity was determined by the detection of non-enzymatic utilization of reduced glutathione (GSH) as the reacting substrate at 412 nm after incubation with 5,5-dithiobis-2-nitrobenzoic acid (DTNB) according to the method of Moin (1986). The assay mixture contained 0.1 M Tris–HCl buffer, 6 mM EDTA, 12 mM sodium azide (pH 8.9), 4.8 mM GSH, tissue homogenate sample, 20 mM t-butylhydroperoxide, and 0.1 mL of 0.01 M 5, 5-dithiobis-2-nitrobenzoic acid. GPx activity is expressed as nmol GSH per min per mL of blood.

**Total antioxidant capacity (TAC) assay.**

The TAC level in the plasma and liver tissue was estimated spectrophotometrically with Tween 80 oxidation and measuring the TBARS level following the method described by Galaktionova et al. (1998). The level of TAC in the sample (%) was calculated according to the absorbance of the blank.

For the quantification of proteins, the Bradford method (1976) was used with bovine serum albumin as the standard. Absorbance was recorded at 595 nm. All enzymatic assays were carried out at 22 ± 0.5°C using a Specol 11 spectrophotometer (Carl Zeiss Jena, Germany) in duplicate.

**Statistical analysis.**

Results are expressed as mean ± S.E.M. All variables were tested for a normal distribution using the Kolmogorov-Smirnov and Lilliefors tests (p>0.05) and the homogeneity of variance was checked by using Levene’s test. The significance of differences in the level of lipid peroxidation, amino acid carbonyl derivatives and antioxidant enzyme activities between the control and test groups were examined using one-way analysis of variance (ANOVA). We used Bonferonni’s post-hoc test. Differences were considered significant at p<0.05. In addition, the associations between data of all individuals were evaluated using Pearson’s correlation analysis. All statistical calculations were performed using data from each individual with STATISTICA 8.0 software (StatSoft Inc., Poland).

**Results**

LPS administration was associated with the free radical oxidation of lipids. As the process occurs in several stages, we decided to assess the degree of change at the beginning and the end of the lipoperoxidation process (LPO). Diene conjugation is considered to be a
primary product following the formation of diene conjugates and ketodienes (Fig. 1). Consequently, we estimated the initial substrate accumulation in this stage of free radical oxidation in different tissues. After LPS treatment, the concentration of conjugated dienes was significantly higher in the liver (F=11.34, p=0.000), muscle (F=4.22, p=0.001) and kidney (F=14.12, p=0.000) compared to those observed in control mice. Melatonin statistically decreased the concentration of conjugated dienes in all tissues compared to the LPS-treated mice.

The MDA concentration, as an end product of the terminal stages of free radical oxidation of lipids, and an oxidative stress marker, namely TBARS, may be used to estimate the level of oxidative stress; this was the next step in our investigation (Fig. 2). The MDA value changed significantly in all analyzed tissues. In the liver (F=10.94, p=0.000), muscle (F=4.64, p=0.002) and kidney (F=3.64, p=0.001), the intensity of the lipid peroxidation process was significantly higher after LPS administration compared to the control group. We found a statistically significant decrease in MDA in those tissues after melatonin treatment in LPS-treated mice.

ROS are implicated as important pathologic agents in many disorders. In the present study, we determined the level of OMP (Table 1); OMP-AD and KD derivatives were higher in the liver (F=18.78, p=0.000 for AD and F=22.23, p=0.000 for KD respectively), muscle (F=13.25, p=0.000 for AD) and kidney (F=19.03, p=0.000 for KD) following LPS treatment. Our results show a consistent trend toward an increase in the concentration of OMP such as AD and KD in all investigated tissues in the LPS-treated group. In muscle tissue, we observed an increased OMP KD concentration after melatonin administration in LPS-treated mice. Otherwise, melatonin either decreased OMP levels (AD in the liver, KD in the kidney) or levels did not change.

Our results show that the TAC level (Fig. 3) was statistically lower after LPS treatment in the liver (F=10.34, p=0.000), muscle (F=10.17, p=0.000) and kidney (F=13.45, p=0.000) compared to the control. In liver tissue, melatonin treatment increased the TAC level compared to the control group. Melatonin administration to LPS-treated mice either restored or even increased the TAC concentration in all investigated tissues compared to the control group.

Antioxidant enzyme activity in the selected tissues, i.e. the liver, muscle and kidney in response to inflammation processes induced by LPS and melatonin, was found to be highly tissue-specific (Table 2). In the liver, LPS treatment increased SOD, GR and GPx, while it diminished the CAT concentration. Melatonin treatment reversed the effects of LPS on SOD,
CAT and GR activity, and further increased GPx activity. In the kidney, LPS administration augmented SOD and GR, while it decreased CAT and GPx activity. Melatonin reversed the effects of LPS in kidney. In the muscle, LPS injection elevated CAT, GR and GPx activity, and did not influence the SOD concentration. Melatonin administration further increased GPx, augmented SOD and did not change CAT and GR. In the present study, we observed correlative dependences between the analyzed oxidative stress data and the activity of antioxidant enzymes upon LPS and melatonin treatment. After LPS, the following interdependences were observed: in the liver, a significant relationship between the SOD and MDA (r=0.97, p=0.020), conjugated dienes and TAC (r=0.89, p=0.001), and OMP AD and TAC (r=0.89, p=0.002) was found; in the muscle, interrelations were observed between the OMP AD and CAT (r=0.98, p=0.000), TAC and GPx (r=0.88, p=0.003), and MDA and GR (r=0.92, p=0.002). Melatonin treatment in LPS-treated mice resulted in the following interdependencies in the liver: MDA and TAC (r= -0.90, p=0.000), as well as MDA and CAT (r= -0.87, p=0.002).

Discussion

We assessed the effects of a single low dose intraperitoneal LPS injection on oxidative stress in selected organs, namely the liver, kidney and muscle. We measured biomarkers of lipid peroxidation processes at the initial (diene conjugation concentration) and end stages (MDA concentration), OMP contents (aldehyde and ketone derivatives) and TAC as a measure of the pro/antioxidant balance of the main antioxidant enzymes such as SOD, CAT, GR and GPx. Finally, based on out in vivo experimental mouse model, we assessed the effect of melatonin treatment in control and LPS-treated mice. There are several new findings of this study: 1) LPS administration results in augmented LPO in skeletal muscle, 2) melatonin ameliorates LPO in skeletal muscle, 3) melatonin enhances oxidative modification of muscle proteins, 4) melatonin diminishes kidney and liver protein oxidation induced by low grade endotoxaemia.

Our results indicate that dienes (early products of the free radical oxidation of lipids) and MDA (end product of the terminal stages of free radical lipid oxidation) were significantly higher in the kidney, liver and muscle after LPS administration compared to the control group. High concentrations of dienes and MDA in the kidney and liver of LPS-treated animals have been already reported by several authors (Requintina and Oxenkrug 2007; Yazar et al. 2010). LPS-induced lipid peroxidation has been shown in smooth muscle (Maziere et al.
1999), the heart (Lipton et al. 2001) and the diaphragm (Taillé et al. 2001). To the best of our knowledge, we are the first to demonstrate a similar effect in skeletal muscle.

Melatonin has been recently been shown to ameliorate muscular oxidative stress and inflammation induced by strenuous exercise (Borges et al. 2015). Quite interestingly, as exercise increases ROS production, the intracellular half-life of melatonin is shortened as it is rapidly used as a free radical scavenger (Troiani et al. 1987; Ueck et al. 1988). Actually, a similar mechanism may play a role in chronic inflammation induced by LPS (Alamili et al. 2013). Our results indicate that melatonin administration effectively decreased LPS-induced LPO in muscle. Melatonin is known to decrease LPO evoked by LPS administration in the kidney and liver (Requintina and Oxenkrug 2003, 2007). LPS is often used as a model of low intensity chronic inflammation (Cadenas and Cadenas 2002). It has been shown recently by our team that melatonin also ameliorates alcohol-induced LPO in the kidney, liver and muscle (Kurhaluk et al. 2017). Thus, it is likely that the protective effect of melatonin can be generalized to most factors resulting in elevated diene and MDA concentrations.

The available data referring to the influence of LPS on the generation of OMP are scarce. Prolonged exercise results in elevated OMP concentrations in skeletal muscle (Radak et al. 1999). The oxidative modification of proteins is believed to play a role in muscle ageing (Choksi et al. 2008) and diaphragm dysfunction in subjects with chronic obstructive pulmonary disease (Marin-Corral et al. 2009). Our results indicate that LPS itself had little influence on the OMP concentration; however, in combination with melatonin, LPS tended to increase the oxidative modification of proteins. In resistance-trained athletes, melatonin prevents increases in advanced oxidation protein product concentrations (Leonardo-Mendonça et al. 2017). However, in breast cancer subjects receiving melatonin as a supporting therapy, fatigue was mentioned as a relatively frequent adverse reaction (Schernhammer et al. 2012). Therefore, our data may suggest an unknown synergism between melatonin and systemic inflammation in terms of oxidative modification of muscle proteins.

Melatonin diminishes kidney protein oxidation related to ischemia-reperfusion injury, thermal injury and chronic renal failure in animals (Sener et al. 2002a,b, 2004). Our study demonstrates the protective action of melatonin against LPS-induced oxidative nephrotoxicity. This is an important finding from the clinical perspective, as endotoxaemia remains one of the main factors leading to acute kidney injury (reviewed recently by Herring and Winklewski, 2017). The beneficial action of melatonin has been reported in several clinical conditions leading to liver protein oxidation, such as exposure to bile acids (for example due to cholestasis) or alcohol toxicity (Kurcer et al. 2007; Fuentes-Broto et al. 2009;
Antioxidant enzymes (SOD, CAT, GPx, and GR) play an important role in protecting cells from the harmful effects of ROS. Free radicals can be defined as molecules or molecular fragments containing one or more unpaired electrons in atomic or molecular orbitals. The addition of one electron to dioxygen forms the superoxide anion radical (O$_2^{-}$). The superoxide anion, arising either through metabolic processes or following oxygen “activation” by physical irradiation, is considered the “primary” ROS and can further interact with other molecules to generate “secondary” ROS, either directly or prevalently through enzyme- or metal-catalyzed processes (Valko et al. 2007). It has been shown that antioxidants of various origins can be useful for the prevention of oxidative damage caused by endotoxins and therefore are of great importance for the correction of such conditions in vivo and in vitro (Sakaguchi and Furusawa 2006, Sudnikovich et al. 2007). It is known that melatonin is a powerful antioxidant (Sönmez et al. 2012). The ability of this hormone to penetrate through biomembranes provides the possibility of protection against oxidative damage to both membrane structures and nuclear DNA (Reiter et al. 2003). It has been noted that melatonin, on the one hand, exhibits direct antioxidant properties by acting as a “scavenger” of free radicals and suppresses the activity of LPO processes, while on the other hand it can enhance the activity of antioxidant enzymes and can maintain their level (SOD, CAT, GPx) (Kurhaluk et al. 2017).

The interrelationships between antioxidant enzymes and lipoperoxidation products became visible after LPS exposure. We can thus expect an important role of the significant relationship between SOD and MDA, conjugated dienes and OMP AD, and conjugated dienes and TAC in maintaining the level of redox reactions in cells of the selected tissues, i.e. the liver, for example. Initiated by LPS, fenton-like reactions are commonly associated with most membrane fractions and include ROS production by mitochondria, microsomes and peroxysomes. The inflammatory state induced by LPS activates phagocytic cells and may be another important source of ROS production. Furthermore, in the muscle, the ability of the tissue to generate ROS by redox cycling caused by endotoxin involved interrelations between the production of oxidized modified proteins and CAT, TAC and GPx, and MDA and GR. On the basis of our results, we have provided evidence for the mutual induction of antioxidant enzymatic activity and lipoperoxidation processes in different biochemical redox reactions, demonstrated by the effect of melatonin in LPS-treated mice. Correlation and regression
analysis demonstrated strong independencies in the liver between MDA and TAC, as well as MDA and CAT.

It is well known that inflammation evoke oxidative stress and oxidative stress leads to inflammatory response. Melatonin ameliorates low-grade inflammation in metabolic syndrome and diabetes mellitus (Negi et al. 2011; Agil et al. 2013). Based on our experiments we are not able to precisely differentiate melatonin anti-inflammatory from anti-oxidative actions. Moreover, melatonin exerts its anti-oxidative function also directly through its radical scavenging ability and diminishes ROS production through protection of the mitochondrial electron transport chain (Hardeland 2017; Mortezaee and Khanlarkhani 2018). We did not assess these effects. Nevertheless, in this study, we specified several novel mechanisms of LPS and melatonin actions. In particular we demonstrated that LPS administration results in augmented LPO in skeletal muscle, while melatonin plays an ameliorative role. Furthermore melatonin diminishes kidney and liver protein oxidation induced by low grade endotoxaemia. The effect of melatonin on skeletal muscle seems ambiguous as it enhances oxidative modification of muscle proteins.

Metabolic syndrome, non-alcoholic fatty liver disease and insulin resistance associated with atherogenic dyslipidaemia lead to subclinical chronic proinflammatory state, activate prothrombotic factors and profibrogenic molecules promoting vascular and renal damage. Accumulating evidence indicates a strong association between non-alcoholic fatty liver disease and chronic kidney disease (Targher and Byrne 2017). Melatonin can be potentially used as the supportive therapy of subclinical endotoxaemia.

Conclusions

LPS-induced oxidative stress increases diene conjugates at an early stage and MDA lipid peroxidation processes at a later stage in the liver, kidney and muscle. Moreover, LPS activates protein oxidation processes and affects the total antioxidant activity. Melatonin ameliorates LPS endotoxemia-induced oxidative damage in the liver and kidney, and partially in the muscle. Taken together, these findings suggest that melatonin modulates the LPS-induced oxidative stress state. Potential synergism between melatonin and low grade systemic inflammation in terms of the oxidative modification of muscle proteins needs to be clarified in further studies.

Conflict of interest
Author contributions

The experiments were performed in Department of Ecology and Nature Protection, National Pedagogical University of Chernihiv, Chernihiv, Ukraine. The authors contributed to the following aspects of the study:

1. conception or design of the work: NK, AS, PJW
2. acquisition, analysis, or interpretation of data for the work: NK, OZ, AS, SK
3. drafting the work or revising it critically for important intellectual content: NK, AS, OZ, AS, SK, PJW

All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

Founding

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Table 1. Effects of melatonin on protein carbonyl derivative contents in selected tissues during LPS-induced stress in mice

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<td>6.59±0.51</td>
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Control – control animals
Mel – melatonin administration
LPS – LPS-induced stress model
LPS+Mel – LPS-induced stress model and melatonin administration
AD – aldehyde derivatives, KD – ketone derivatives
Significant differences between groups are designated as follows:
<sup>a</sup> – LPS or Melatonin group vs. Control group
<sup>aa</sup> – LPS+Mel group vs. LPS group
<sup>ab</sup> – LPS+Mel group vs. Mel group
Table 2. Antioxidant enzyme activity in the liver, muscle, kidney and plasma following the induction of LPS-induced toxicity and melatonin (Mel) administration in mice

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Mel</th>
<th>LPS</th>
<th>LPS+ Mel</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>a</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
<td>aa</td>
</tr>
<tr>
<td>SOD, U·mg⁻¹ protein</td>
<td>360.84±16.19</td>
<td>435.65±43.37</td>
<td>598.23±36.14</td>
<td>478.41±23.16</td>
</tr>
<tr>
<td>CAT, µmol·min⁻¹·mg⁻¹ protein</td>
<td>10.43±0.40</td>
<td>9.42±0.39</td>
<td>6.98±0.93</td>
<td>14.81±1.06</td>
</tr>
<tr>
<td>GR, nmol NADPH₂·min⁻¹·mg⁻¹ protein</td>
<td>128.20±6.80</td>
<td>143.61±7.06</td>
<td>188.31±11.32</td>
<td>155.94±9.26</td>
</tr>
<tr>
<td>GPx, nmol GSH·min⁻¹·mg⁻¹ protein</td>
<td>43.17±1.31</td>
<td>58.94±1.83</td>
<td>78.33±9.25</td>
<td>96.81±5.04</td>
</tr>
<tr>
<td>Muscle</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOD, U·mg⁻¹ protein</td>
<td>443.87±37.76</td>
<td>339.87±16.16</td>
<td>433.12±21.61</td>
<td>732.09±22.80</td>
</tr>
<tr>
<td>CAT, µmol·min⁻¹·mg⁻¹ protein</td>
<td>11.19±0.75</td>
<td>9.28±0.77</td>
<td>14.08±0.93</td>
<td>13.74±0.95</td>
</tr>
<tr>
<td>GR, nmol NADPH₂·min⁻¹·mg⁻¹ protein</td>
<td>76.70±6.85</td>
<td>99.92±2.26</td>
<td>143.77±8.01</td>
<td>136.44±11.54</td>
</tr>
<tr>
<td>GPx, nmol GSH·min⁻¹·mg⁻¹ protein</td>
<td>27.85±2.19</td>
<td>51.22±2.95</td>
<td>49.86±8.33</td>
<td>77.93±8.13</td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOD, U·mg⁻¹ protein</td>
<td>479.14±31.66</td>
<td>533.64±59.08</td>
<td>703.64±30.12</td>
<td>618.22±31.95</td>
</tr>
<tr>
<td>CAT, µmol·min⁻¹·mg⁻¹ protein</td>
<td>11.63±0.74</td>
<td>11.25±0.76</td>
<td>6.12±0.83</td>
<td>14.17±1.01</td>
</tr>
<tr>
<td>GR, nmol NADPH₂·min⁻¹·mg⁻¹ protein</td>
<td>128.20±6.80</td>
<td>143.61±7.06</td>
<td>188.46±9.11</td>
<td>159.16±11.36</td>
</tr>
<tr>
<td>GPx, nmol GSH·min⁻¹·mg⁻¹ protein</td>
<td>50.65±3.42</td>
<td>59.09±4.22</td>
<td>34.21±4.26</td>
<td>99.86±8.19</td>
</tr>
</tbody>
</table>

Significant differences between groups are designated as follows:
- a – LPS or Melatonin group vs. Control group
- aa – LPS+Mel group vs. LPS group
- ab – LPS+Mel group vs. Mel group
Fig. 1. Effect of melatonin (Mel) on conjugated diene contents during LPS-induced toxicity (E233·mg⁻¹ protein or E233·ml) in the liver (A), muscle (B) and kidney (C) of mice.

Fig. 2. Effect of melatonin on LPS-induced lipid peroxide levels (µmol MDA·mg⁻¹ protein or µmol MDA·ml) in the liver (A), muscle (B) and kidney (C) of mice.

Fig. 3. Effect of melatonin (Mel) on the total antioxidant capacity (%) in the liver (A), muscle (B), kidney (C) and plasma (D) during LPS-induced stress in mice.
Figure 1

A

- LPS+Mel
- LPS
- Melatonin
- Control

E233 mg protein

B

- LPS+Mel
- LPS
- Melatonin
- Control

E233 mg protein
Fig. 1. Effect of melatonin (Mel) on conjugated diene contents during LPS-induced toxicity (E233·mg⁻¹ protein or E233·ml) in the liver (A), muscle (B) and kidney (C) of mice. Significant differences between groups are designated as follows:

- a – LPS or Melatonin group vs. Control group
- aa – LPS+Mel group vs. LPS group
- ab – LPS+Mel group vs. Mel group
Figure 2

A

![Graph A showing levels of MDA•mg-1 protein for Control, Melatonin, LPS, and LPS+Mel. The graph indicates differences with letters a, aa, and ab.]

B

![Graph B showing levels of MDA•mg-1 protein for Control, Melatonin, LPS, and LPS+Mel. The graph indicates differences with letters a and aa.]

Fig. 2. Effect of melatonin on LPS-induced lipid peroxide levels (µmol MDA·mg⁻¹ protein or µmol MDA·ml) in the liver (A), muscle (B) and kidney (C) of mice. Significant differences between groups are designated as follows:

- a – LPS or Melatonin group vs. Control group
- aa – LPS+Mel group vs. LPS group
- ab – LPS+Mel group vs. Mel group
Figure 3

A

B
**Fig. 3.** Effect of melatonin (Mel) on the total antioxidant capacity (%) in the liver (A), muscle (B), kidney (C) and plasma (D) during LPS-induced stress in mice.

Control – control animals
Mel – melatonin administration
LPS – LPS-induced stress model
LPS+Mel – LPS-induced stress model and melatonin administration

Significant differences between groups are designated as follows:
- a – LPS or Melatonin group vs. Control group
- aa – LPS+Mel group vs. LPS group
- ab – LPS+Mel group vs. Mel group