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Antioxidant activity of melatonin in diabetes in relation to the regulation and levels of plasma Cu, Zn, Fe, Mn, and Se in Zucker diabetic fatty rats

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ABSTRACT

Objective: To study the antioxidant activity of melatonin in diabetes in relation to the regulation and levels of plasma copper (Cu), zinc (Zn), iron (Fe), manganese (Mn), and selenium (Se) in Zucker diabetic fatty (ZDF) and lean (ZL) rats.
Methods: At 6 wk of age, both ZDF (n = 30) and ZL (n = 30) animals were subdivided into three groups: control (C) (n = 10), vehicle (V) (n = 10), and melatonin-treated (M) (10 mg/kg/d; n = 10) rats for a 6-wk period. At the end of treatment period, plasma mineral levels were measured by flame (Cu, Zn, and Fe), electrothermal (Mn), and hydride generation (Se) atomic absorption spectrometry.
Results: ZDF rats had significantly higher Cu, Fe, and Mn plasma levels than did ZL rats ($P < 0.05$). No significant differences were found between control and vehicle groups ($P > 0.05$). Melatonin treatment did not influence plasma levels of these antioxidant minerals (Cu, Zn, Fe, and Mn) in ZDF groups (M-ZDF versus C-ZDF group) and ZL (M-ZL versus C-ZL group) rats with the exception of Zn, whose mean plasma level was lower in the M-ZL versus C-ZL group. However, plasma Se levels increased significantly ($P < 0.05$) after melatonin supplementation in both groups (M-ZDF and M-ZL).
Conclusion: The higher mean plasma Cu, Fe, and Mn levels in the ZDF group are related to the enhanced oxidative stress in diabetes and obesity. Melatonin administration significantly enhanced plasma Se levels in both groups (M-ZDF and M-ZL). This is the first study to report that melatonin treatment increases plasma Se levels.

Miguel Navarro-Alarcon was responsible for study design, mineral analysis, data acquisition, and interpretation, drafting, critical revision, and approval of manuscript; Francisco J. Ruiz-Ojeda was responsible for data acquisition, determinations, and critical revision and approval of manuscript; Rosa M. Blanca-Herrera was responsible for study design, second drafting of manuscript, and critical revision and approval of manuscript; Ahmad Agil was the principal investigator of the research group and was responsible for study design, sample acquisition, data acquisition of antioxidant minerals, drafting, critical revision of the manuscript, and approval of the article.

Introduction

Human diet has progressively diverged from that of our ancestors. This discrepancy between our ancient, genetically determined biology and the present nutritional pattern of Western populations has contributed to a major rise in the incidence of cardiovascular diseases (CVDs), metabolic syndrome, cancer, and diabetes [1]. Diseases related to overweight and obesity also are reaching epidemic proportions worldwide. Obesity during childhood and adolescence increases the risk for metabolic syndrome, which is associated with an increase in oxidative stress and inflammatory processes [2,3]. Recent strategies developed to prevent and treat these diseases include the use of functional foods fortified with biologically active compounds from plants. One of these compounds is melatonin, which is present in food plants (fruit, vegetables, cereals, edible seeds, and nuts), medicinal herbs, and processed foods (e.g., wine and beer) [4–7].

An excess of reactive oxygen species (ROS) in mitochondria can overcome antioxidant defenses, causing bioenergetic failure and metabolic complications. Melatonin, an amphipatic antioxidant, enters the mitochondria in a dose- and time-dependent manner [8] and exerts its antioxidant effect via multiple

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mechanisms [6], as scavenging several radical species (e.g., hydroxyl and peroxil radicals), regulating the activity of antioxidant enzymes (as indirect antioxidant properties), reducing oxygen consumption, maintaining membrane potential, or abating superoxide radical production [9]. It is widely documented that this antioxidant indolamine, and its endogenous metabolites can detoxify harmful reactants and reduce molecular damage [10–14]. Furthermore, various animal and human studies on different diseases have reported that melatonin significantly increases the activity of several antioxidant enzymes, including cytoplasmic and mitochondrial superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) [11,15–21]. All of these enzymes are known to have different antioxidant elements as cofactors, namely copper (Cu), zinc (Zn), and manganese (Mn), iron (Fe), and selenium (Se), respectively. We have lately studied the mechanism whereby melatonin treatment, abrogates increased plasma lipid peroxidation in basal (endogenous) condition and after challenge with hydoxil radical ex vivo [22]. Notwithstanding, melatonin can exert its antioxidant properties in these Zucker diabetic fatty (ZDF) animals through its capacity to reduce serum levels of the prooxidant adipokine leptin [23], and by increasing adiponectin [23] and high-density lipoprotein (HDL) cholesterol concentrations [24], both possessing antioxidant properties [25,26]. The administration of melatonin also was found to normalize elevated erythrocyte SOD activity in patients with Alzheimer's disease [27]. However, no research data are available on the effect of melatonin supplementation on the body's homeostasis of these antioxidant elements (Cu, Zn, Fe, Mn, and Fe) in young diabetic Zucker rats and their lean littermates.

To date, few data have been published correlating the protective effects of melatonin with the metabolic complications derived from obesity and diabetes in relation to its potential regulation of the homeostasis of transition metals that act as cofactors of antioxidant enzymes. The aim of the present study was to determine the antioxidant activity of melatonin in diabetes in relation to the regulation and levels of plasma Cu, Zn, Fe, Mn, and Se in ZDF and lean (ZL) rats. The ZDF (fa/fa) is a rodent model with genetic obesity that develops type 2 diabetes mellitus (T2DM) similar to that in humans and is associated with cardiovascular complications at 9 to 10 wk. However, their lean ZL littermates (fa/−) develop insulin resistance but not diabetes.

Our group previously reported that melatonin supplementation significantly diminished the overweight condition and low-density lipoprotein and free fatty-acid levels in young ZDF rats and significantly increased HDL levels in ZDF and ZL rats [23,24]. These previous studies demonstrated that oral melatonin administration ameliorates glucose homeostasis in young ZDF rats by improving both insulin action and β-cell function. In the present study, we investigated the effects of melatonin on the regulation of antioxidant mineral homeostasis in young ZDF rats and their lean littermates.

Materials and methods

Animals and experimental protocol

Male ZDF (fa/fa; 180–200 g body weight [BW]; n = 30) and male ZL littermates (fa/−; 120–140 g BW; n = 30) were obtained at an age of 5 wk from Charles River (Barcelona, Spain). Animals were maintained on Purina 5008 rat chow (23% protein, 6.5% fat, 58.5% carbohydrates, 4% fiber, and 6.8% ash [13, 73, 230, 71 and 0.23 ppm for Cu, Zn, Fe, Mn, and Se, respectively]; Charles River) and tap water ad libitum [23,24]. The food and water intake in both ZL and ZDF groups (control and melatonin-treated) were published elsewhere [24]; however, the ratio of ingested food for ZDF/ZL rat groups is about 2.5 times (during 6 wk of treatment). Animals were housed in clear plastic cages (three to four animals per cage) in a controlled room under a 12-h dark/light cycle (lights on at 07:00 h). The study complied with European Community Council Directives and was approved by the ethics committee of our university. At 6 wk of age, ZDF and ZL rats were subdivided into three groups of 10 animals each, giving a total of six groups: control ZDF and ZL (C-ZDF and C-ZL) groups; vehicle-treated ZDF and ZL (V-ZDF and V-ZL) groups; and melatonin-treated (10 mg/kg/d) ZDF and ZL (M-ZDF and M-ZL) groups. Vehicle (0.066% ethanol) and melatonin were administered in the drinking water. The water intake in both ZL and ZDF groups (control and melatonin-treated) also was shown previously [24]; while the ratio of water ingested for ZDF/ZL rat groups is about 2.9 times (during 6 wk of treatment), the melatonin treatment significantly reduced the weight gain in ZDF rats without food and water intake differences [24]. At the end of the 6-wk treatment period (12 wk old rats), the animals were fasted overnight and were then anesthetized with sodium thiobarbital (thiopental) and sacrificed between 09:00 and 11:00 h the next day. Blood was collected by heart puncture into ethylenediaminetetraacetic acid (EDTA) vacutainer tubes and centrifuged, and the plasma was aliquoted and frozen at −80°C for subsequent determinations of multiple biochemical parameters [22–24] and antioxidant minerals.

Apparatus

A Perkin-Elmer 1110B double-beam atomic absorption spectrophotometer equipped with a deuterium background corrector and Cu, Fe, Zn, Mn, and Se hollow cathode lamps were used together with an HGA-700 furnace spectrophotometer or a MHS-10 hydride generator (Perkin-Elmer, Norwalk CT, USA). A thermostatic multiplace digestion block (Selecta, S.A., Barcelona, Spain) was also used.

Reagents

Melatonin was obtained from Sigma Chemicals Madrid, Spain. Commercially available standard solutions of Cu, Zn, Fe, Mn, and Se (1000 mg/L; Tritisol, Merck, Darmstadt, Germany) were used to prepare calibration graphs. All solutions were prepared from analytical grade reagents (Suprapur, Merck): 65% HNO_3, 65% $HClO_4$, 37% HCl, Triton-X 100, $NaBH_4$, and NaOH. Double- distilled deionized water with a specific resistivity of 18 mΩ/cm was used to prepare standards for calibration and dilutions and was obtained immediately before use by filtering distilled water through a Milli-Q purifier (Millipore, Waters, Mildford, MA, USA).

Determination of plasma Cu, Zn, Fe, Mn, and Se levels by atomic absorption spectrometry

Plasma samples from ZDF and ZL rats were thawed and homogenized for the Cu, Zn, and Fe determinations. A 0.150-mL aliquot was diluted with double-distilled deionized water (1:5) following procedures reported elsewhere [28,29]. Cu, Zn, and Fe levels were determined by flame (direct aspiration) atomic absorption spectrophotometry. In an accuracy test, Cu and Zn concentrations obtained by this method in certified reference material (Contox Trace Serum Metal Control A Level I, Kaulson Laboratories Inc., NJ, USA [91.6 ± 1.6 and 78.9 ± 4.2 μg/dL, respectively]) did not significantly differ ($P > 0.05$) from certified levels (90.0 ± 7.5 and 80.0 ± 6.0 μg/dL, respectively). Likewise, the mean Fe concentration obtained by this method in the reference material (Seronorm™ CRM M10181 Trace Elements in Serum from Sero AS Nycomed Pharma AS, Billingstad, Norway [157 ± 10 μg/dL]) did not significantly differ ($P > 0.05$) from the certified level (154 ± 8 μg/dL).

A previously optimized hydride generation atomic absorption spectrometry procedure was used for Se determinations [30]. In an accuracy test, the mean Se concentration obtained by this method in the reference material (0148 Contox trace metal serum control Panel C from the Kaulson Laboratories Inc., NY, USA [15.64 ± 1.10 μg/dL]) did not significantly differ ($P > 0.05$) from the certified level (15.05 ± 0.49 μg/dL).

Mn was directly measured in plasma samples diluted with 0.1% Trition X 100 in water (1:1) after optimizing the volume of the diluted samples. The linear calibration method was applied for the determination, using a micropipette to manually inject 20 μL of diluted sample through a graphite tube without L'Vov platform [31]. Furnace conditions for electrothermal atomic absorption spectrometry at 279.5 nm were previously reported [31]. The mean Mn concentration obtained by this method in the reference material (CRM M10181) (1.28 ± 0.01 μg/dL) did not significantly differ ($P > 0.05$) from the certified level (1.30 ± 0.015 μg/dL).

Statistical analysis

The Statistical Package for the Social Sciences (version 15.0; SPSS, Chicago, IL) was used for data analyses. Results were expressed as arithmetic means and SD, and the normal distribution of data and the homogeneity of variances were checked by Kolmogorov-Smirnov's and Levene's tests respectively. ANOVA and Duncan multiple range tests were used for comparisons for parametric variables, the Kruskall-Wallis test for non-parametric variables. $P < 0.05$ was considered significant.

Table 1
Comparison of plasma Cu, Zn, Fe, Mn, and Se levels between ZDF and ZL rats

Mineral	*P*-value	ZDF rats		ZL rats	
		n	Mean ± SD	n	Mean ± SD
Cu (μg/dL)	0.007	30	141.1 ± 3.3	30	123.5 ± 5.1
Zn (μg/dL)	0.149	30	158.7 ± 6.1	30	150.5 ± 9.6
Fe (μg/dL)	0.002	30	844.0 ± 77.3	30	425.0 ± 146.0
Mn (ng/dL)	0.023	30	123.6 ± 12.5	30	102.3 ± 20.1
Se (ng/dL)	0.101	30	1257 ± 35	30	1137 ± 61.5

Cu, copper; Fe, iron; Mn, manganese; Se, selenium; Zn, zinc; ZDF, Zucker diabetic fatty; ZL, Zucker lean

Results and discussion

No statistically significant differences in plasma concentrations of Cu, Zn, Fe, Mn, and Se were found between the naïve (C-ZL, N-ZDF) and vehicle (V-ZL, V-ZDF) groups ($P > 0.05$). Hence, the vehicle used for the melatonin administration had no effect on the plasma levels of these antioxidant minerals.

As shown in Table 1, plasma Cu, Fe, and Mn levels were significantly higher in the ZDF groups (C-ZDF, M-ZDF) than in the ZL groups (C-ZL plus M-ZL group) ($P < 0.05$), which may be related to the enhanced oxidative stress that is characteristic of diabetes and obesity [32,33]. In the ZDF rats, however, melatonin administration (M-ZDF group) did not significantly reduce these elevated plasma Cu, Zn, Fe, or and Mn levels in comparison with the untreated animals (C-ZDF group) (Fig. 1–4, respectively). This may be related to the need for ZDF rats to maintain high plasma Cu, Zn, Fe, and Mn levels in response to the increase in antioxidant enzymes (SOD and CAT) induced by melatonin treatment [11,15–21]. However, little is known about the precise nature of oxidative stress in diabetes and the mechanism by which it causes tissue damage [34]. Cooper implicated excessive transition metal-catalyzed oxidative stress in this process [33]. The role of Cu and Fe ions as catalyzers of oxidative stress in diabetes is supported by our finding of elevated plasma Cu and Fe levels in the ZDF rats. Elevated serum Cu values were previously described in rats [35]. Nevertheless, no significant difference in serum Cu levels has been observed between diabetics and healthy controls [36,37].

Among the control rats, the plasma Fe level was significantly higher in the C-ZDF group than in the C-ZL group ($P = 0.003$). The C-ZDF group also showed a borderline significant tendency to higher plasma Cu ($P = 0.051$), Zn ($P = 0.065$), and Mn ($P = 0.051$) concentrations.

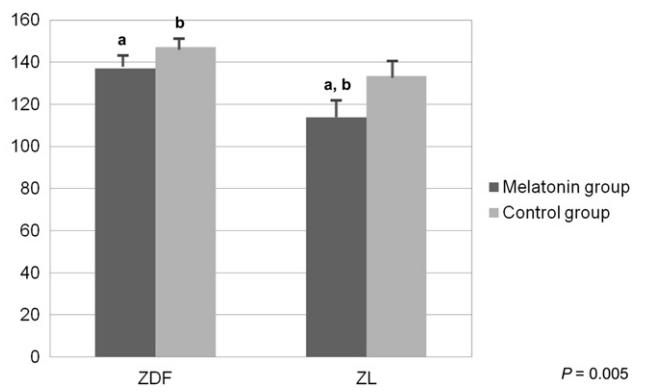

Fig. 1. Plasma Cu levels in control (C-ZL and C-ZDF) and melatonin-treated (M-ZL and M-ZDF) animals. Values are means ± SEM (n = 10, each group). [a,b]Rat groups with the same superscript were significantly different ($P < 0.01$). ZDF, Zucker diabetic fatty; ZL, Zucker lean.

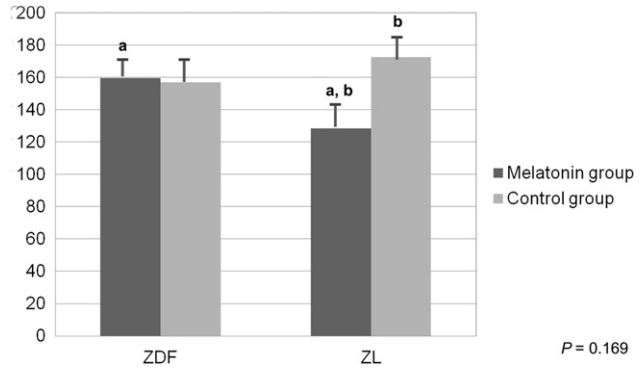

Fig. 2. Plasma Zn levels in control (C-ZL and C-ZDF) and melatonin-treated (M-ZL and M-ZDF) animals. Values are means ± SEM (n = 10, each group). [a,b]Duncan multiple range test showed significant differences between rat groups with the same superscript. ZDF, Zucker diabetic fatty; ZL, Zucker lean.

The effects of melatonin administration on plasma Cu, Zn, Fe, Mn, and Se levels are depicted in Figures 1 to 5. Over the 6-wk treatment period, plasma Cu (Fig. 1), Zn (Fig. 2), and Fe (Fig. 3) levels were significantly lower ($P < 0.05$) in the M-ZL group than in the M-ZDF group (113.8 ± 6.9, 128.6 ± 12.9 and 382.5 ± 167.5 versus 137.1 ± 4.0, 159.6 ± 7.3, and 961.8 ± 96.7 μg/dL; respectively). The mean plasma Cu concentration was significantly lower ($P < 0.01$) in the M-ZL group (113.8 ± 6.9 μg/dL) than in the C-ZDF group (147.1 ± 4.9 μg/dL) (Fig. 1). The Duncan multiple range test results showed that melatonin supplementation significantly reduced plasma Zn levels in the ZL group (C-ZL, 172.4 ± 12.9 μg/dL; M-ZL, 128.6 ± 12.9 μg/dL) (Fig. 2). No significant differences in plasma Mn concentrations were found among M-ZDF (133.7 ± 18.1 ng/dL), C-ZDF (113.6 ± 18.1 ng/dL), M-ZL (85.3 ± 31.3 ng/dL), and C-ZL (115.0 ± 27.1 ng/dL) groups (Fig. 4), although a tendency to significance was observed (Kruskal-Wallis test, $P = 0.086$).

A previous report that melatonin increases the absorption of Zn in the digestive system [38] is not supported by the present results. Other animal studies found that plasma Zn levels were increased by melatonin supplementation [39,40] even in pinealectomized rats [41]. In one of these studies, plasma Zn levels [39] were significantly higher after melatonin treatment versus controls in middle-aged rats but not in old rats.

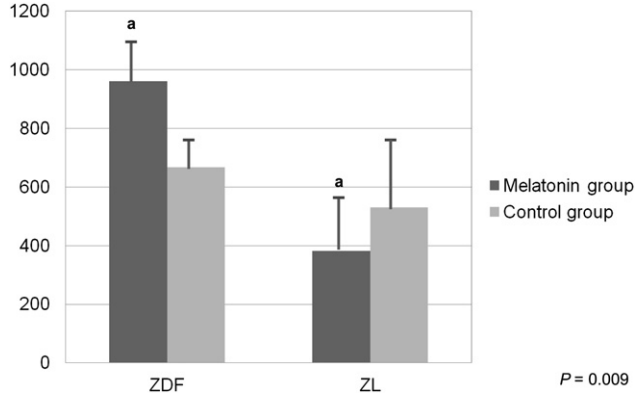

Fig. 3. Plasma Fe levels in control (C-ZL and C-ZDF) and melatonin-treated (M-ZL and M-ZDF) animals. Values are means ± SEM (n = 10, each group). [a]Rat groups with the same superscript were significantly different ($P < 0.01$). ZDF, Zucker diabetic fatty; ZL, Zucker lean.

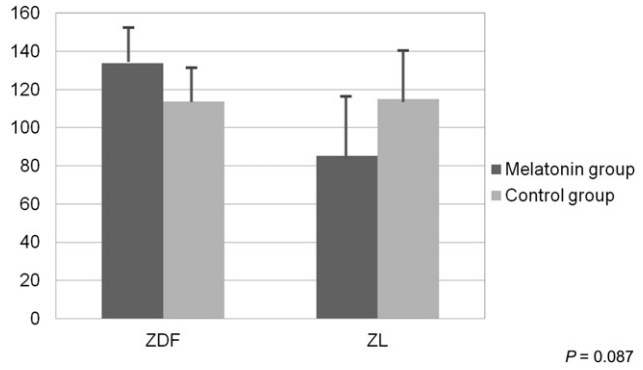

Fig. 4. Plasma Mn levels in control (C-ZL and C-ZDF) and melatonin-treated (M-ZL and M-ZDF) animals. Values are means ± SEM (n = 10, each group). ZDF, Zucker diabetic fatty; ZL, Zucker lean.

Some authors reported that melatonin ameliorated oxidative stress by controlling Fe, after finding that adriamycin-treated rats that received melatonin normalized plasma Fe levels to concentrations found in controls [11]. However, melatonin did not affect plasma Zn or Se levels. The researchers concluded that the damaging action of ROS, lipids, and proteins requires a catalyst such as Fe [11]. This finding may be related to other reports that melatonin and its derivatives possess Fe-binding properties [42] and contribute to Fe homeostasis by maintaining Fe pools at appropriate levels. Other researchers also found that the elevated total Fe levels induced by CCl_4 injections were significantly reduced by melatonin [40]. However, this effect was not observed in the present study, given that melatonin treatment only significantly diminished plasma Zn levels in ZL rats, as confirmed by applying the Duncan multiple range tests. This result also may be related to the Zn-binding properties of melatonin and its derivatives, as previously reported for Fe [42]. A similar tendency to statistical significance was found for lower plasma Mn concentrations in ZL rats ($P = 0.087$). These findings reveal a differential effect of melatonin in ZDF and ZL rats on regulation of the plasma homeostasis of Zn and probably Mn.

It has been reported that melatonin supplementation significantly enhanced the activity of SOD and CAT enzymes in non–insulin-dependent diabetes mellitus [15]. Other researchers also found that melatonin treatment improves antioxidant status by increasing the activity of antioxidant enzymes such as SOD, CAT, and GPx [11,15–19]. It also has been reported that Zn deficiency reduces Cu-Zn SOD activity [19]. The melatonin-induced increase in Cu-Zn SOD activity counteracts the elevated increase in oxidative stress in diabetes [15]. In the situation of lower oxidative stress and inflammation characteristic of ZL rats, the lower plasma levels of Cu, Zn, and Fe measured in melatonin-treated rats (M-ZL versus M-ZDF group) may be related to the reduced activities of cytoplasmic SOD and CAT.

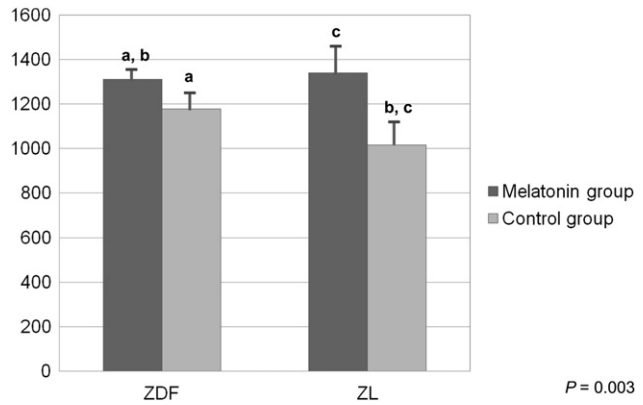

Fig. 5. Plasma Se (B) levels in control (C-ZL and C-ZDF) and melatonin-treated (M-ZL and M-ZDF) animals. Values are means ± SEM (n = 10, each group). [a,b,c]Rat groups with the same superscript were significantly different ($P < 0.01$). ZDF, Zucker diabetic fatty; ZL, Zucker lean.

Although melatonin administration did not significantly influence plasma Cu, Zn, Fe, and Mn levels in the ZDF rats, other researchers found that this indolamine interacts with metals, neutralizing their toxic effects in some cases [43–45].

The regulation of Se plasma levels by melatonin showed a distinct behavior. Thus, plasma Se levels were significantly higher ($P < 0.01$) in the M-ZDF (1320 ± 38.5) versus C-ZDF (1177 ± 47.1 ng/dL) groups (Fig. 5) and in the M-ZL (1340 ± 85.5) versus C-ZL (1016 ± 66.5 ng/dL) (Fig. 5). The enhancement in Se levels after melatonin administration (10 mg/kg/d) may be related to the release of Se from body stores for use in the synthesis of the GPx antioxidant enzyme. Previous studies have reported an increased activity of this enzyme after melatonin treatment [20,21,46]. Probably this result is related with a previous finding of our research group on that melatonin improved basal lipid peroxidation (LPO) in ZDF rats as well as the total antioxidant capacity (measured as Fe^{2+}/H_2O_2–induced LPO), in both ZL and ZDF rats [22].

Conclusions

In conclusion, the elevated mean plasma Cu, Fe, and Mn levels measured in the ZDF rats are related to the enhanced oxidative stress characteristic of diabetes and obesity. Melatonin administration enhances plasma Se levels in M-ZDF and M-ZL rats.

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