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**Melatonin increases magnesium concentrations in white adipose tissue and pancreas of diabetic obese rats**

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A B S T R A C T

Melatonin is a natural bioactive compound, whose intake by obese diabetic Zucker (ZDF) rats improves this pathology. Hypomagnesaemia has also been observed in diabetes, and magnesium (Mg) is known to play an essential role in carbohydrate metabolism. In this study we have determined the effect of melatonin intake on Mg concentrations in white adipose tissues and organs in ZDF rats. This study reveals for the first time that melatonin intake increases Mg concentrations in subcutaneous lumbar, visceral, omentum, and gonadal adipose tissue and in the pancreas. These findings may be related to an improvement in the homeostatic regulation of adipocytokines produced by white adipose tissues, and to a reduction in plasmatic oxidative stress, which would lead to a decrease in insulin resistance and improvement in glucose homeostasis. These results open up the beneficial use of melatonin for the development of functional foods to ameliorate glucose homeostasis in obesity-associated diabetes.

*Keywords:*

Melatonin

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White adipose tissue and organs

**1. Introduction**

Melatonin (*N*-acetyl-5-methoxytryptamine) is produced in the pineal gland and in peripheral organs and tissues. The amount of melatonin in tissues (extrapineal) is far in excess of that in the circulation, but only circulating melatonin possesses chronobiotic properties (Hardeland et al., 2011; Acuña-Castroviejo et al*.*, 2014; Ribas-Latre et al., 2015). Although melatonin was initially characterized as a hormone primarily involved in the circadian regulation of physiological and neuroendocrine functions, subsequent studies found that it can counteract free radical damage by various mechanisms of action (Tan et al., 2002; Othman, El-Missiry, Amer & Arafa, 2008; Reiter et al., 2016; Zhang & Zhang, 2014), supporting a role for this indoleamine against oxidative stress and inflammation. There is also considerable evidence on the beneficial effects of melatonin supplementation against obesity and related comorbidities (Wolden-Hanson, 2000; Prunet-Marcassus, 2003; Hussein, Ahmed, Hassan & Ahmed, 2007; Tan, Manchester, Fuentes-Broto, Paredes & Reiter, 2011; Navarro-Alarcon et al., 2014a; Favero et al., 2015). In previous studies, we investigated the anti-obesity and antidysmetabolic effect of chronic melatonin administration in ZDF rats, demonstrating that melatonin limits obesity and improves metabolic syndrome (Agil et al., 2011, 2012, 2013) in part through via browning of subcutaneous white adipose tissue (WAT; Jiménez et al., 2013). Moreover, recently we reported that melatonin increased their interscapular brown adipose tissue (BAT) amounts and their mitochondrial mass and function probably by inducing proliferation and at the same time promotes differentiation of pre-adipocytes into thermogenic adipocytes (Fernández-Vázquez et al., 2018). In this line, our group previously reported that chronic oral melatonin administration improved glucose homeostasis in young Zucker Diabetic Fatty (ZDF) rats, by intensifying β-cell function and insulin action (Agil, et al., 2012) and had a positive effect on dyslipidemia, the pro-inflammatory state, and oxidative stress, which underlie development of the metabolic syndrome (Agil et al., 2011, 2013; Fernández-Vázquez, Reiter & Agil, 2018). Furthermore, pinealectomy has been related to a deficiency in insulin signaling pathways and a reduction in GLUT4 gene expression, inducing insulin resistance and glucose intolerance (Zanquetta, Seraphim, Sumida, Cipolla-Nieto & Machado, 2003).

 Agil et al. (2012) previously verified that melatonin supplementation improved glucose homeostasis in ZDF rats, reducing fasting insulinemia, the HOMA-IR insulin resistance index, and blood leptin levels, and increasing adiponectin blood levels*.* Given that leptin and adiponectin are synthetized by WATs, melatonin administration would significantly affect their homeostasis and functioning, reducing leptin resistance and thereby producing a decrease in blood leptin levels and an increase in satiety. This would facilitate weight reduction in melatonin-treated ZDF rats in comparison to controls (Agil et al., 2011). Melatonin supplementation increased blood adiponectin concentrations, reducing the insulin resistance of ZDF rats and improving their glucose homeostasis (Agil et al., 2012).

Type 2 diabetes mellitus has frequently been associated with hypomagnesemia (Sales & Pedrosa, 2006; Guerrero-Romero, Rascón-Pacheco, Rodríguez-Morán, Escobedo de la Peña & Wache, 2008; Simmons, Joshi & Shaw, 2010; Rodríguez-Morán & Guerrero-Romero, 2011; Gommers, Hoenderop, Bindels & De Baaij, 2016) which has been reported in 14-48 % of diabetic patients *versus* 2.5-15 % of healthy controls (Pham, Pham, Pham, Miller & Pham, 2007). In a recent cross-sectional study, hypomagnesemia was associated with an increased risk of complications, including cardiovascular disease (Kunutsor, Khan & Laukkanen, 2016). In animal studies, plasma magnesium (Mg2+) concentrations were found to be lower in ZDF rats than in their lean littermates (ZL rats; Navarro-Alarcon et al., 2014b). Importantly, Mg2+ supplementation has been reported to have beneficial effects on insulin sensitivity and metabolic control, suggesting that Mg2+ may be a key factor in the etiology and management of diabetes (Rodriguez-Moran & Guerrero-Romero, 2003; Dong, Xun, He & Qin, 2011; Guerrero-Romero & Rodriguez-Morán, 2014; Guerrero-Romero, Simental-Mendía, Hernández-Ronquillo & Rodriguez-Morán, 2015; Veronese et al., 2016).

 With this background, we hypothesized that melatonin supplementation would regulate Mg2+ concentrations at cellular level in different tissues and thereby contribute to improving Mg2+ homeostasis in diabetes. Therefore, the objective of this study was to evaluate the effects of melatonin administration on Mg2+ homeostasis in muscle, brain, liver, pancreas, and gonadal, visceral, renal, omental, and subcutaneous lumbar adipose tissue in young male ZDF and ZL rats.

**2. Material and methods**

***2.1. Animals and experimental protocols***

Five-week-old male ZDF rats (fa/fa; 180–200 g body weight [BW]; *n* = 20) and male lean littermates (ZL, fa/-; 120–140 g BW; *n* = 20) from Charles River (Barcelona, Spain) were kept on Purina 5008 rat chow (23 % protein, 6.5 % fat, 58.5 % carbohydrates, 4 % fiber, 6.8 % ash) and tap water *ad libitum*. Animals were housed 3/4 per clear plastic cage in a controlled room with a 12 h dark–light cycle (lights on at 07:00 h). The study complied with European Union guidelines for animal care and protection and was approved by the Ethics Committee of the University of Granada (Granada, Spain). At the age of 6 weeks, ZDF and ZL rat groups were each subdivided into two groups (*n* = 10): control groups (ZDF-C and ZL-C) that received no treatment; and melatonin-treated groups (ZDF-M and ZL-M). A vehicle group was not included, as previous research found no significant differences between untreated and vehicle-treated groups (Agil, et al., 2014). Melatonin was dissolved in a minimum volume of absolute ethanol and diluted to a final solution of 0.066 % (w/v) in the drinking water, providing treated animals with a daily dose of 10 mg/kg BW. Fresh melatonin and vehicle solutions were prepared twice a week, adjusting the melatonin dose to the body weight. Water bottles were protected from light by an aluminum foil cover. After 6 weeks of treatment and overnight fasting, animals were anesthetized with sodium thiobarbital (thiopental) and killed between 09:00 and 11:00 h.

 Subcutaneous lumbar and visceral WAT samples (peri-renal, gonadal, and omentum adipose tissues) and samples from other tissues (liver, muscle, pancreas, brain) were obtained by surgical extirpation, washed in saline solution, and kept at -80º C until analysis (Agil, et al., 2014).

***2.2. Equipment and reagents***

Mg2+ concentrations were measured using a 1100 B Atomic Absorption Spectrometer equipped with a Ca2+ and Mg2+ multi-element hollow cathode lamp (Perkin-Elmer, Germany). Reagent grade water was obtained using the R015 Milli-Q system (Waters, Medford, MA), and tissue samples were mineralized in a Multiplaces Selecta mineralization block (Barcelona, Spain; Agil et al., 2014).

 Melatonin was purchased from Sigma Chemicals (Madrid, Spain). A standard solution of Mg2+ (1000 mg/L; Tritisol, Merck, Darmstadt, Germany) was used to prepare calibration graphs. Analytical grade reagents were used to prepare all solutions: HNO3 (65 %) and Triton X-100 (Suprapur, Merck). Standards for calibration and dilutions were prepared immediately before use using deionized water with a specific resistivity of 18 mΩ cm (Millipore, Waters, Mildford, MA).

***2.3. Magnesium determination by flame atomic absorption spectrometry (AAS)***

Tissue samples (from subcutaneous lumbar, peri-renal, gonadal, or omentum adipose tissue, liver, muscle, pancreas, or brain) of 0.100 - 0.200 g were weighed in a Pyrex glass tube and 0.8 mL HNO3 was added, followed by heating for 15 min at 80 °C, transfer to a multi-stage mineralization block (Multiplazas Selecta; Barcelona, Spain), and subsequent heating for 45 min at 180 °C. Next, 0.8 mL HNO3:HClO4 (4:1) was added, and the mixture was then heated at 200 °C for a further 90 min. The solution obtained was diluted to 2.5 mL with milli-Q water, and Mg2+ concentrations were determined by direct aspiration in the flame of the atomic absorption spectrophotometer using a previously optimized linear calibration method (Gámez et al., 1997). The mean Mg2+ concentration of 2.11 ± 0.07 mg/dL obtained for the reference material (CRM Human Serum Chengdu Shuyang Medition Factory, Chengdu, China; National Research Center for CRM′1, Beijing, China, United Analysis and Measurement Center of Sichnan, Chengdu, China) did not significantly differ (*p* > 0.05) from the certified value of 2.04 ± 0.08 mg/dL.

***2.4. Statistical analysis***

SPSS 15.0 for Windows (IBM, Chicago, IL) was used for statistical analyses. Results are expressed as the arithmetic mean and the standard error of the mean (SEM). Normal distribution was checked with the Kolmogorov–Smirnov test and the homogeneity of variance with Levene's test. Parametric variables were compared using two-way ANOVA and Bonferroni’s multiple comparisons test, considering *p* < 0.05 as statistically significant. Non-parametric variables were compared using the Kruskall-Wallis test, considering *p* < 0.05 as statistically significant.

**3. Results and discussion**

Figure 1A depicts the Mg2+ concentrations measured in white adipose tissue (WAT) samples of subcutaneous lumbar and visceral adipose tissue (peri-renal, gonadal, and omentum adipose tissues) from young male ZDF rats and their lean littermates (ZL); figure 1B exhibits the concentrations found in liver, muscle, pancreas, and brain samples. Mg2+ concentrations did not significantly differ between ZDF-C and ZL-C rats, indicating that concentrations in these tissues are not significantly influenced by the presence of diabetes. In ZDF rats, melatonin treatment significantly increased Mg2+ concentrations in subcutaneous lumbar, peri-renal, gonadal, and omentum adipose tissue (Figure 1A) and in the pancreas (Figure 1B; *p* < 0.05). Mean omemtum Mg2+ concentrations were significantly higher in ZDF-M rats than in ZL-M and ZL-C animals (*p* < 0.05), but no significant differences were found between ZDF-M and ZDF-C rats in liver, muscle, or brain Mg2+ concentrations (Figure 1B; *p* > 0.05). According to these findings, neither obesity nor diabetes influences the tissue distribution of Mg2+.

 Mg2+ is an essential cofactor involved in key metabolic pathways with a major role in glucose and lipid metabolism (Paolisso, Scheen, Donofrio & Lefèbvre, 1990; Paolisso & M. Barbagallo, 1997). Thus, hypomagnesemia and low dietary Mg2+ intake have been strongly associated with an increased risk of glucose metabolism disorders (Guerrero-Romero, Rascón-Pacheco, Rodríguez-Morán, Rscobedo de la Peña & Wacher, 2008; Simmons, Joshi & Shaw, 2010; Rodríguez-Morán & Guerrero-Romero, 2011; Hruby, Meigs, O'Donnell, Jacques & McKeown, 2014; Fang et al., 2016; Sarrafzadegan, Khosravi-Boroujeni, Lotfizadeh, Pourmogaddas &. Salehi-Abargouei, 2016). The relationship between Mg2+ deficiency and diabetes mellitus has not been fully elucidated, but some authors have described a vicious circle in which low serum Mg2+ concentrations cause insulin resistance, and serum Mg2+ concentrations are reduced by insulin resistance (Gommers, Hoenderop, Bindels & De Baaij, 2016), which may be related to micro- and macro-vascular complications observed in diabetes, including cardiovascular disease (De LeeuwI, Vansant & Van Gaal, 1992; Sales & Pedrosa, 2006; Bertinato et al., 2015; Kunutsor, Khan & Laukkanen, 2016). In addition, because obesity is usually associated with hypomagnesemia, it has been suggested that magnesium status may be worsened by obesity, thereby contributing to impaired insulin action (Bertinato et al., 2015). However, the relationship between obesity and magnesium status remains poorly understood. It was proposed in a recent study that hypomagnesemia is an epiphenomenon related to obesity but the relationship is not causal; accordingly, hyperglycemia can be the cause of hypomagnesemia in non-diabetic individuals, regardless of their weight (Guerrero-Romero, Flores-García, Saldaña-Guerrero, Simental-Mendía & Rodríguez-Morán, 2016).

 The present finding of no relationship between diabetes and tissue Mg2+ concentrations contrasts with our previously published observation of an association between diabetic conditions and low plasma concentrations in the same rat model (Othman, El-Missiry, Amer & Arafa, 2008). This discrepancy is likely explained by the more transient change in Mg2+ homeostasis in plasma than in tissues.

Metabolic alterations in cellular Mg2+, which may play the role of a second messenger for insulin action, are known to contribute to insulin resistance (Takaya, Higashino & Kobayashi, 2004).Moreover, insulin itself is a major factor regulating intracellular Mg2+ accumulation, suggesting that insulin resistance may cause a reduction in intracellular Mg2+ levels (Paolisso & Barbagallo, 1997).

 Staniek et al. (2013) compared metal concentrations among ZL, ZF, and ZDF rats and observed lower liver Mg concentrations in ZF rats than in ZDF or ZL rats, attributed to their increased liver fat content. In the present study, liver Mg concentrations were non-significantly higher in ZDF rats than in ZL controls (Fig. 1B).

 Zimmermann et al. (2000) fed Sprague-Dawley rats with diets containing different Mg concentrations (70, 1000, and 9000 ppm) and found no significant differences among the three groups in liver Mg2+ concentrations, in line with the present finding that melatonin supplementation did not modify liver Mg2+ concentrations. Contrarily, others reported that melatonin supplementation in diabetes and forced exercise enhanced the Mg2+ levels in the liver tissue of rats (Bicer et al., 2015).

 Our research group previously reported that melatonin administration significantly improved the lipid profile of young ZDF rats, reducing triglyceride and LDL cholesterol levels and increasing HDL levels; it was also found to reduced their weight gain (Agil et al., 2011), which is closely related to metabolic disorders (Rasmussen, Boldt, Wilkinson, Yellow & Matsumoto, 1999; Wolden-Hanson et al., 2000). Findings in rodents suggest that melatonin may increase energy expenditure by activating adipose tissue thermogenesis (Jimenéz-Aranda et al., 2013). According to these data, melatonin increases both the mass and activity of these WATs. It has been proposed that melatonin may act *via* M1/M2 membrane receptors, but the mechanisms involved are not well understood. Jiménez-Aranda et al. (2013) found that thermogenesis activation in inguinal WAT results from its melatonin-induced transformation into beige adipose tissue, which expresses larger amounts of uncoupling proteins UCP-1 and PGC-1α, which regulate mitochondrial energetic metabolism.

 A study of mitochondrial isolates from subcutaneous white and beige adipose tissue of ZDF rats(Jimenéz-Aranda, Fernández-Vázquez, Serrano, Reiter & Agil, 2014) found that melatonin supplementation improves mitochondrial function by facilitating adipocyte differentiation and energy balance, with a reduction in oxidative stress. Mitochondrial adipocyte dysfunction may be the cause or consequence of diabetes and obesity, because it can produce oxidative stress, apoptosis, and inflammation, thereby contributing to the development of insulin resistance (Agil et al., 2013). Others (Mogulkoc et al., 2006) also reported that melatonin prevented oxidant damage in cerebral, liver and cardiac tissues in hyperthyroid rats.

Melatonin is a key molecule for the environmental integration and circadian cycle distribution of the physiological processes required for healthy metabolism, alongside energy balance optimization and body weight regulation (Agil et al., 2011). Melatonin acts by enhancing the action of insulin through the regulation of GLUT4 expression or activation of its signaling pathway. It therefore induces insulin receptor phosphorylation *via* its G-protein coupled membrane receptors. Melatonin is responsible for establishing an appropriate energy balance, mainly by regulating the flow of energy to and from depots, simultaneously and directly controlling energy expenditure by activating beige adipose tissue. In this sense, Kaya, Kilic, Celik, Baltaci & Mogulkoc (2010) found that increased plasma glucose levels in Sprague-Dawley rats in a 30-mimutes acute swimming exercise were diminished after melatonin supplementation, which was related with its protective effect on liver glycogen reserves in rats subjected to acute swimming exercise. In addition, this indolamine is responsible for transforming white into beige tissue and thereby assisting in body weight regulation (Cipolla-Neto, Amaral, Afeche, Tan & Reiter, 2014) as reported by other researchers (Jimenéz-Aranda et al., 2014).

The absence of melatonin or its reduced production related to aging, nightshift work, or night-time exposure to light can induce insulin resistance, glucose intolerance, and metabolic disorders, with adverse health consequences and an increased risk of obesity (Cipolla-Neto, Amaral, Afeche, Tan & Reiter, 2014; Navarro-Alarcon et al., 2014a).

 Adipose tissue is an endocrine organ that participates in metabolic homeostasis, producing adipocytokines such as leptin and adiponectin (Ahima, 2006). There is abnormal adipocytokine action and production in metabolic syndrome-related obesity, with elevated leptin concentrations and reduced adiponectin concentrations (Matsuzawa, Funahashi, Kihara & Shimomura, 2004; Satoh et al., 2004; Ryo et al., 2004; Cote et al., 2005; Beltowski, 2006; Nrata et al., 2007; Antuna-Puente, Feve, Fellahi & Bastard, 2008; Gnacinska, Malgorzewicz, Stojek, Lysiak-Szydlowska & Sworczak, 2009). ZDF rats have hyperleptinemia due to a genetic resistance that leads to reduced energy expenditure, giving rise to obesity (Kasiske, O**\_**donnell & Keane, 1992).

 Adipocytes are the body cells that store energy and also play an essential role in metabolic syndrome development. The deregulated adipocytokine production and secretion that takes place in obesity is related to metabolic disease processes. A study by Favero et al. (2015) in obese mice hypothesized that dietary melatonin would support an anti-inflammatory response and play an important role in the energy metabolism of subcutaneous visceral adipose tissues that may counteract some of the detrimental effects of obesity. In the vehicle-treated obese mice fat depots, inflammation, and adipocytokine deregulation increased, adiponectin decreased, and tumor necrosis factor α, resistin, and visfatin increased in adipose tissue depots. However, these effects were partially reverted when melatonin was pre-administered (Favero et al. 2015).7

 In the present study, we verified that melatonin supplementation (ZDF-M groups) increased Mg concentrations in all four types of WAT studied in ZDF rats (Figure 1A), which may be attributable, as proposed above, to the improved function of these producers of leptin, adiponectin, tumor necrosis factor α, IL-6, and CRP. This would result in improved glucose homeostasis, given that hypomagnesemia has been found to be characteristic of different tissues in diabetes mellitus animal models (Guerrero-Romero, Rascón-Pacheco, Rodríguez-Morán, Escobedo de la Peña & Wacher, 2008; Rodríguez-Morán & Guerrero-Romero, 2011; Simmons, Joshi & Shaw, 2010; Del Gobbo, 2012) and of plasma in the present animal model (ZDF-C *vs.* ZL-C; Navarro-Alarcon et al, 2014b).

 Agil et al. (2013) found higher plasma concentrations of IL-6, TNF−α, and CRP in ZDF obese rats than in non-diabetic lean control ZL rats and observed that these inflammatory biomarkers, associated with diabetes and obesity, were partially reduced in melatonin-treated ZDF rats. It has also been reported that Mg2+ acts as an anti-inflammatory factor in adipose tissue, reducing IL-1 and TNF-α secretion (Gommers, Hoenderop, Bindels & De Baaij, 2016). In the present study, melatonin supplementation increased Mg2+ concentrations in all adipose tissues studied (subcutaneous lumbar, peri-renal, gonadal, and omentum). This increase may underlie the anti-inflammatory effect of melatonin observed in the ZDF rats Agil et al. (2013) by producing a reduction in TNF-α, IL-6, and RCP levels, and oxidative stress, shown by a decrease in basal plasma lipid peroxidation (LPO) and Fe2+/H2O2-induced plasma lipid peroxidation (Agil et al., 2013). The lower pro-inflammatory status and oxidative stress produced by the melatonin-induced increase in WAT Mg2+ concentrations would favor an environment that would reduce the insulin resistance characteristic of obesity-related type 2 diabetes mellitus (Rodríguez-Morán & Guerrero-Romero, 2011; Günther, 2010).

 Pancreatic β-cell activity was reported to be impaired in type 2 diabetic patients with hypomagnesemia, and dietary Mg2+ supplementation improved their glucose metabolism and insulin sensitivity (Gommers, Hoenderop, Bindels & De Baaij, 2016). Hypomagnesemia was previously reported in ZDF rats (Navarro-Alarcon et al., 2014b) and in rats with reduced levels of insulin receptor phosphorylation, mimicking insulin resistance (Suárez, et al., 1995; Paxton & Ye, 2005). It has also been reported (Gommers, Hoenderop, Bindels & De Baaij, 2016) that intracellular Mg2+in pancreatic β-cells regulates glucokinase, KATP channels, and L-type Ca2+ channels, preceding insulin secretion. In the present study, melatonin administration significantly increased pancreatic Mg2+ concentrations in ZDF rats, which may be related to their enhanced insulin sensitivity and glucose metabolism, previously reported in this animal model of diabesity (Agil et al., 2012). Mg2+ concentrations in pancreatic β-cells are known to antagonize the role of Ca2+ and regulate insulin secretion according to the Ca2+-to-Mg2+ ratio (Atwater, Frankel, Rojas & Grodsky, 1983). Mg2+ concentrations were found to increase in perfused rat pancreas and mouse islets due to an alteration in the Ca2+-to-Mg2+ ratio, reducing insulin vesicle release (Gommers, Hoenderop, Bindels & De Baaij, 2016). Melatonin administration increases pancreatic concentrations of Mg2+, which may be associated with Ca2+ influx and L-type Ca2+ channel closing and with a reduction in insulin release (Atwater, Frankel, Rojas & Grodsky, 1983). This would eventually reduce the insulinemia and HOMA-IR previously observed in these ZDF rats after melatonin supplementation (Agil et al., 2012). The reduction in insulin resistance by melatonin administration and consequent improvement in insulin function would reduce the need for insulin release from pancreatic β-cells. It has also been reported that melatonin supplementation increases pancreatic Ca2+ concentrations in ZDF rats (Agil et al., 2014), which is opposite to the present finding. However, when Ca2+:Mg2+ ratios were based on Ca2+ data from the aforementioned study (Agil et al., 2014), and the present Mg2+ data,a reduction from 1.48 to 1.34 was observed after melatonin administration at pharmacological doses.

 Although further in-depth research is required on the specific mechanisms by which pharmacological doses of melatonin reduce insulin resistance in ZDF rats, the present findings point to an increase in WAT Mg2+ concentrations as a possible pathway. This increase would improve the modulation of WAT production of leptin, adiponectin, and proinflammatory adipocytokines (Agil et al., 2012) and decrease plasmatic oxidative stress (Agil et al., 2013), leading to an improvement in insulin sensitivity (Agil et al., 2012). Consequently, plasma glucose levels would be lowered and the lipid profile would improve, reducing insulin resistance (Agil et al., 2011, 2012). This lower glycemia would reduce the entry of glucose into pancreatic cells, resulting in lower ATP production by pancreatic β-cells and, alongside the increased Mg2+ concentrations in these cells (present finding of increased Ca2+-to-Mg2+ ratio), would increase sensitivity to insulin and therefore reduce its secretion.

**4. Conclusion**

We report for the first time that melatonin intake to obese and diabetic Zucker rats increases Mg concentrations in subcutaneous lumbar, peri-renal, gonadal, and omentum adipose tissue, and pancreas. This finding may be related to an improved homeostatic regulation of other hormones produced in WAT, such as leptin and adiponectin, with a decrease in proinflammatory factor synthesis (TNF-α, IL-6, and CRP) and oxidative stress, leading to lowering of insulin resistance and improvement in glucose homeostasis, as previously reported. The increase in pancreatic Mg concentrations may also be related to a reduction in insulin secretion by pancreatic β-cells as a result of lower insulin resistance. This study provides a novel mechanistic insight into the anti-diabetic effects of melatonin and supports its possible use for the development of functional foods.

**Conflict of interest**

None.

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**Figure caption**

**Fig. 1A**: Subcutaneous lumbar and visceral adipose tissue Mg2+ concentrations (peri-renal, gonadal, and omentum) in ZL and ZDF rats (control *vs.* melatonin-treated). **1B**: Liver, muscle, pancreas, and brain Mg2+ concentrations in ZL and ZDF rats (control *vs.* melatonin-treated). Values are given as means ± SEM (*n*= 10 per group). *P* < 0.05 is considered statistically significant.

