Melatonin administration in diabetes: regulation of plasma Cr, V, and Mg in young male Zucker diabetic fatty rats

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The use of melatonin, a neurohormone present in plants, represents an exciting approach for the maintenance of optimum health conditions. Melatonin administration ameliorates glucose homeostasis in Zucker diabetic fatty (ZDF) rats. The objective of this study was to investigate the effects of melatonin in diabetes in relation to the levels and regulation of plasma chromium (Cr), vanadium (V), and magnesium (Mg) in Zucker diabetic fatty (ZDF) and Zucker lean (ZL) rats. At the age of 6 weeks, ZDF ($n = 30$) and ZL ($n = 30$) groups were each subdivided into three groups: control (C) ($n = 10$), vehicle-treated (V′) ($n = 10$) and melatonin-treated (M) (10 mg kg^{-1} per day; $n = 10$) groups for a 6 week period. After treatment, plasma mineral concentrations were measured by flame (Mg) and electrothermal (Cr and V) atomic absorption spectrometry. No significant differences were found between the C and V′ groups ($p > 0.05$). Plasma Mg levels were significantly lower in C-ZDF vs. C-ZL rats, demonstrating the presence of hypomagnesemia in this diabetes mellitus model. Plasma V and Cr levels were significantly higher in M-ZDF vs. C-ZDF rats. Plasma Mg levels in ZDF rats were not affected by melatonin treatment ($p > 0.05$). Melatonin administration ameliorates the diabetic status of ZDF rats by enhancing plasma Cr and V concentrations. This appears to be the first report of a beneficial effect of melatonin treatment on plasma Cr and V regulation in ZDF rats.

Introduction

Being overweight and obesity are immediate precursors of type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD).[1] Obesity-associated cases of T2DM are expected to increase two-fold by 2030.[2] Although the etiology of diabetes and its complications are not fully understood, other factors besides obesity, such as age and oxidative damage, have also been implicated.[3]

Chromium (Cr), vanadium (V), and magnesium (Mg) are known to play a role in diabetes. Cr is widely used as a supplement by diabetics and dieters, usually in the form of propionate, picolinate, or chromium(III) chloride.[4] Chromium(III) is known to be essential for carbohydrate and lipid metabolism, but the effects of Cr on these processes are controversial. Cr administration was found to enhance glucose tolerance and insulin action and to lower lipids in diabetic rats,[5] and the majority of clinical trials in diabetic patients have reported a positive effect on glycemic control.[6,7] However, other studies have found that Cr has no beneficial effects on glycemic control or insulin sensitivity.[8] Conversely, some researchers concluded that Cr metabolism is disturbed by glucose intolerance.[9]

Various reports show that V and its complexes mimic the action of insulin by enhancing the uptake of glucose,[10] stimulating lipogenesis and the synthesis of glycogen, improving oxidation and glucose transport and augmenting the activity of insulin-receptor tyrosine kinase.[11] In another study, blood glucose levels were normalized and HbA1c levels were reduced by the administration of vanadyl sulfate supplements in T2DM patients.[11] In diabetic rats, V supplementation significantly decreased serum levels of antioxidant enzymes which were significantly elevated in muscle tissue by diabetes, indicating that V may be of potential value in preventing the complications of diabetes.[12]

Hypomagnesemia is frequently present in diabetic patients,[13,14] although the mechanism underlying this relationship has not been definitively established. It has been suggested that low plasma Mg levels are directly related to some of the micro- and macrovascular complications observed in diabetes, including cardiovascular disease.[15]

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Melatonin present in plants represents an exciting alternative for the maintenance of optimum health conditions.[16] The neurohormone melatonin is produced nocturnally, principally by the pineal gland, but also in numerous tissues.[17] Pineal melatonin mediates the photoperiodic control of endogenous circadian rhythms and participates in energy balance regulation, among many other functions.[16,18] Indirect evidence suggesting that the intake of grape juice results in a rise in circulating melatonin levels has been reported.[19] Few data are available regarding the potential protective action of melatonin on the metabolic complications of obesity and DM through regulation of the homeostasis of Cr, V, and Mg, which are related to altered lipid and carbohydrate metabolism in these diseases. We hypothesized that enhancement of the plasma status and homeostatic regulation of these transition metals by melatonin supplementation might improve DM complications and even prevent T2DM development. The specific objective of the present study was to examine the effect of melatonin on the plasma levels and regulation of Cr, V, and Mg in young male Zucker diabetic fatty (ZDF) rats, an experimental model of T2DM and the metabolic syndrome, and in their lean littermates (ZL).

Results and discussion

Our group previously reported that the administration of melatonin improves glucose homeostasis in young ZDF rats by enhancing insulin action and β-cell function, with improvements in fasting glycemia, fasting insulinemia, leptinemia, HOMA-IR, adiponectinemia, free fatty acid levels, and HbA1c % ($p < 0.05$).[20] In another study we found that melatonin ameliorates the low-grade inflammation and oxidative stress underlying the development of insulin resistance and its metabolic consequences.[21] In a third study, melatonin was found to augment plasma Se levels,[22] which may be attributable to Se release from body stores for synthesis of the antioxidant enzyme glutathione peroxidase. In a continuation of this line of research, the present investigation explored the effects of melatonin on the homeostasis of minerals related to glucose homeostasis and insulin metabolism (Cr, V and Mg) in ZDF rats and their lean littermates, and on their status in DM. The vehicle used for melatonin administration did not affect plasma levels of these minerals, given that no differences were observed between the control and vehicle groups (C-ZDF and C-ZL vs. V′-ZDF and V′-ZL, respectively; $p > 0.05$).

Cr was previously found to be beneficial in glycemic control,[5,7] and the present study presents the first set of evidence that the administration of melatonin increases its circulating levels in young ZDF rats (Fig. 1). Thus, plasma Cr levels were significantly higher in melatonin-treated vs. vehicle-treated and control ZDF rats after applying multiple range tests [3.39 (SEM = 0.914) vs. 0.66 (SEM = 0.125) and 0.67 (SEM = 0.117) μg dL^{-1}, respectively]. However, no significant differences were found between melatonin-treated and control ZL groups (Fig. 1). Hence, the significant reduction in circulating insulin and glucose found by other authors after melatonin administration to high fat diet-fed rats[23] and by our group in
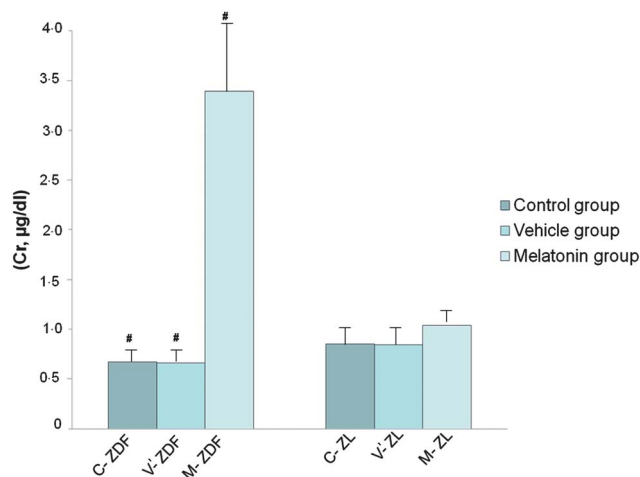

Fig. 1 Plasma Cr levels in control (C-ZDF (Zucker diabetic fatty) and C-ZL (Zucker lean)), vehicle-treated (V′-ZDF and V′-ZL) and melatonin-treated (M-ZDF and M-ZL) animals. Values are given as means (±SEM) ($n = 10$ per group). $^{\#}p < 0.05$ (significant differences determined by the multiple range test).

ZDF rats[21] would also be related to a significant enhancement in plasma Cr concentration.

Various organic and inorganic Cr compounds have been associated with anti-diabetic (insulin-sensitizing) and anti-hyperlipidemic effects.[24] In the present study, we report for the first time that melatonin treatment significantly enhances plasma Cr levels in ZDF rats, indicating an additional mechanism by which melatonin diminishes insulin resistance.[20] The mechanism underlying the action of Cr on DM has not been fully elucidated. An in vivo study reported that Cr may directly influence the insulin receptor and enhance its tyrosine kinase activity.[25] Cr may also exert its insulin-sensitizing effect by stimulating the translocation of glucose transporter-4 (GLUT4) to the plasma membrane, which is associated with a decrease in plasma and membrane cholesterol.[26] Animals fed with a fat-free diet containing Cr showed reduced plasma insulin levels,[27] and Cr was found to exert a hypoinsulinemic effect in other animal[28] and human studies.[6] The present study demonstrates that in the situation of high oxidative stress and inflammation that is a characteristic of ZDF rats, plasma Cr levels are increased by melatonin supplementation, as previously reported for Se in ZDF and ZL rats.[22] These results may also be related to the potential anti-diabetic capacity of melatonin observed in the same animal model.[18,20] Hence, Cr appears to have significant anti-diabetic (insulin-sensitizing) and antihyperlipidemic potential and may be a promising candidate as a therapeutic agent in DM.[23,27]

Insulin-like effects of inorganic and organic V compounds have been described in vitro and in vivo,[11,29,30] but V supplementation trials have reported severe toxicological effects, including pro-neoplastic actions,[31] enhanced spontaneous lipid peroxidation, and a lower total antioxidant status in hepatic tissue[32] and erythrocyte lysates.[33] Those groups found that the separate or combined 12-week administration of sodium metavanadate (0.100 mg mL^{-1}) and chromium chloride (0.004 mg mL^{-1}) in the

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drinking water of rats had no effect on lipid oxidation or on Cu/Zn-SOD, CAT, or cGSH-Px activity; *i.e.*, neither metal showed pro-oxidant potential for red blood cells at these doses.[34] Other groups[35] have stated that further research is required on the role of V as a micronutrient and on its hypoglycemic and toxicological effects.

Several animal and human studies have reported that melatonin supplementation ameliorates glucose homeostasis, mainly by enhancing insulin sensitivity,[20,21,36] although the mechanism underlying this relationship remains controversial. Melatonin may exert its antihyperglycemic effect by enhancing the action (as an insulin-sensitizer) or secretion of insulin, or both.[20] However, the specific effect of melatonin on V plasma levels and regulation has not previously been addressed. We found plasma V levels to be significantly higher in melatonin-treated ZDF rats compared to ZDF vehicle-treated and control rats [1.02 (SEM = 0.311) *vs.* 0.182 (SEM = 0.032) and 0.181 (SEM = 0.030) μg dL^{-1}, respectively] after applying multiple range tests (Fig. 2). Accordingly, melatonin may exert a direct and/or indirect beneficial effect in T2DM. Firstly, melatonin directly acts as an antioxidant and anti-inflammatory molecule against the diabetogenic factors associated with obesity.[20,21] Secondly, melatonin may improve the complications of diabetes in an indirect manner, given that adequate plasma V levels appear to have positive effects on this pathology. This is the first time that the anti-diabetic effect of melatonin has been related to an enhancement in plasma V levels in ZDF rats. Importantly, the improvement in these plasma levels was achieved without the need for elevated, and therefore potentially toxic, V supplementation.

On the other hand, the reported ability of melatonin to promote weight loss[18] may be attributable to a decrease in the storage of white adipose tissue (WAT) and/or an increase in energy expenditure by brown adipose tissue (BAT).[18] There is increasing evidence, largely from rodent studies, that melatonin might increase energy expenditure by activating non-shivering thermogenesis in BAT.[18] Various studies in rodents have reported that melatonin increases BAT mass and activity. The precise mechanisms underlying the influence of melatonin on BAT physiology remain unknown, but several possible explanations are suggested by indirect evidence.[18] We have recently demonstrated that chronic oral administration of melatonin drives WAT to function like BAT in ZDF rats,[37] which could be related to a redistribution and change in tissue as well as plasma levels of some minerals like Cr and V. Therefore, future studies focused on measuring Cr and V levels in different body tissues (WAT and BAT from different body locations, brain, kidney, pancreas, liver, and muscle) after the oral administration of melatonin in this animal model would help to understand exactly how it modifies Cr and V homeostasis to ultimately increase their plasma levels.

Results for plasma Mg levels differed from those observed with the other two transition metals in our study. Thus, plasma Mg levels were shown to be significantly lower in ZDF control rats than in ZL vehicle-treated and control rats after applying multiple range tests [29.6 (SEM = 3.19) *vs.* 42.5 (SEM = 3.30) and 41.5 (SEM = 3.49) mg dL^{-1}, respectively] (Fig. 3). Other authors have described an association between obesity and/or T2DM and hypomagnesemia.[13,14,38,39] Guerrero-Romero and Rodríguez-Morán[40] reported that a three-month course of $MgCl_2$ supplementation in non-diabetic individuals with significant hypomagnesemia improved the ability of β-cells to compensate for insulin sensitivity variations. They also found[13] a reduction in the first and second phases of insulin secretion in non-diabetic subjects with hypomagnesemia. In this study, the reduced plasma Mg levels of ZDF rats were not improved by melatonin supplementation (M-ZDF *vs.* C-ZDF group) ($p > 0.05$).
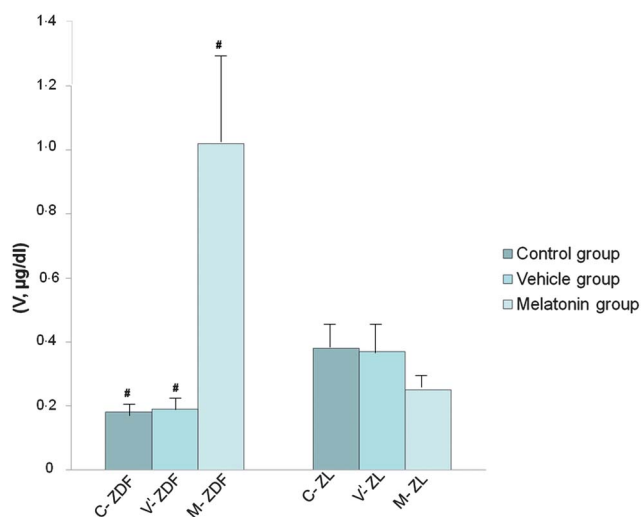

Fig. 2 Plasma V levels in control (C-ZDF (Zucker diabetic fatty) and C-ZL (Zucker lean)), vehicle-treated (V′-ZDF and V′-ZL) and melatonin-treated (M-ZDF and M-ZL) animals. Values are given as means (±SEM) (*n* = 10 per group). #*p* < 0.05 (significant differences determined by the multiple range test).
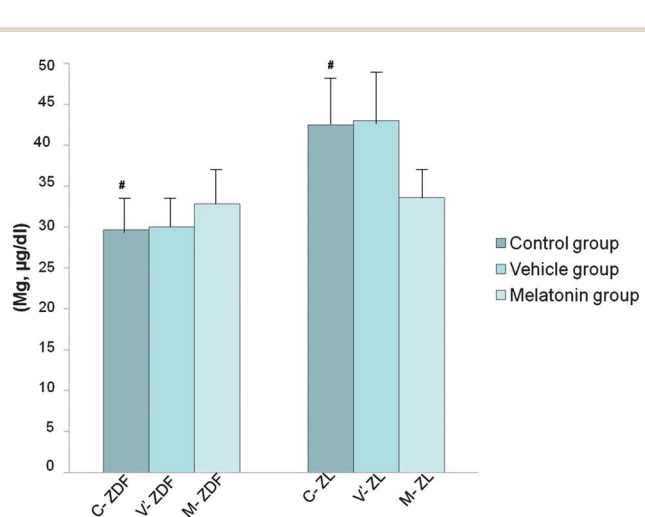

Fig. 3 Plasma Mg levels in control (C-ZDF (Zucker diabetic fatty) and C-ZL (Zucker lean)), vehicle-treated (V′-ZDF and V′-ZL) and melatonin-treated (M-ZDF and M-ZL) animals. Values are given as means (±SEM) (*n* = 10 per group). #*p* < 0.05 (significant differences determined by the multiple range test).

Experimental

Reagents

Melatonin was purchased from Sigma Chemicals (Madrid, Spain). Standard solutions of Cr, V, and Mg (1000 mg L^{-1}; Tritisol, Merck, Darmstadt, Germany) were used to prepare calibration graphs. Analytical grade reagents were used to prepare all solutions: HNO$_3$ (65%) and Triton X-100 (Suprapur, Merck). Standards for calibration and dilutions were prepared immediately before use by employing deionized water with a specific resistivity of 18 mΩ cm (Millipore, Waters, Mildford, MA, USA).

In vivo experimentation

Five-week-old male ZDF rats (fa/fa; 180–200 g body weight [BW]; $n = 30$) and male lean littermates (ZL, fa/-; 120–140 g BW; $n = 30$) from Charles River (Barcelona, Spain) were maintained on Purina 5008 rat chow (23% protein, 6.5% fat, 58.5% carbohydrates, 4% fiber, 6.8% ash, 1.4 ppm Cr, and 2000 ppm Mg; the label provided no information on V content) and tap water *ad libitum*. The food and water intake of control and melatonin-treated ZL and ZDF groups was previously reported, with a 2.5-fold higher food intake and a 2.9-fold higher water intake observed in ZDF rats compared to ZL rats over the 6-week treatment period. The same study found a lower weight gain in melatonin-treated *versus* non-treated ZDF rats, with no differences between them with respect to water or food intake.[41] 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 Ethical Committee of the University of Granada (Granada, Spain). At the age of 6 weeks, the ZDF and ZL groups were each subdivided into three groups ($n = 10$): control groups (C-ZDF and C-ZL) receiving no treatment; vehicle-treated groups (V'-ZDF and V'-ZL), and melatonin-treated groups (M-ZDF and M-ZL). Melatonin was dissolved in a minimum volume of absolute ethanol and then diluted to a final solution of 0.066% (w/v) in the drinking water, providing a daily dose of 10 mg kg^{-1} body weight for treated animals. Fresh melatonin and vehicle solutions were prepared twice a week, when the melatonin dose was adjusted according to the body weight. Water bottles were protected from light by an aluminum foil cover and the drinking fluid was changed twice weekly.[40] At the end of the treatment period and after overnight fasting, the (12 week old) animals were anesthetized with sodium thiobarbital (thiopental) and killed between 09:00 and 11:00 h. Blood was drawn by cardiac puncture into EDTA Vacutainer tubes. Samples were then centrifuged, and the plasma was aliquoted and frozen at −80 °C for later analysis of multiple biochemical parameters[20,21,41] and minerals.[22]

Mineral analysis

The Perkin-Elmer 5100 Zeeman AAS atomic absorption spectrometer, equipped with a HGA-5100 graphite furnace (Perkin-Elmer, Germany) and AS-90 autosampler (Perkin-Elmer, Germany), was used.

Cr was directly measured by a linear calibration method using a 10 μL matrix modifier solution [0.2% (v/v) HNO$_3$], by manually injecting plasma samples (10 μL) by micropipette using a graphite tube without a L'Vov platform. Furnace conditions for Cr determination by electrothermal atomic absorption spectrometry at 279.5 nm were based on previous assays.[42] The mean Cr concentration [0.533 (SD = 0.045) μg dL^{-1}] obtained for the reference material (SERO203105 trace element in human serum, level 2, European Reference Materials, LGC Standards, Middlesex, UK) did not significantly differ ($p > 0.05$) from the certified value (0.520 μg dL^{-1}).

V was directly measured by a linear calibration method after optimization of conditions, using a micropipette to inject plasma samples (10 μL) diluted with 0.05% Triton X-100 using a graphite tube without a L'Vov platform. Furnace conditions were optimized for V determination by electrothermal atomic absorption spectrometry at 318.5 nm. The mean V concentration [0.114 (SD = 0.009 μg dL^{-1})] obtained for the reference material (SERO203105) did not significantly differ ($p > 0.05$) from the certified value (0.112 μg dL^{-1}).

For Mg determinations, plasma samples were thawed and homogenized, and an aliquot of 0.150 mL was diluted to 100 ml with bi-distilled ultrapure water. Mg levels were measured by direct aspiration of this solution into the flame of the atomic absorption spectrophotometer.[43] The mean Mg concentration [2.11 (SD = 0.07) mg dL^{-1}] 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 [2.04 (SD = 0.08) μg dL^{-1}].

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 using the Kolmogorov–Smirnov test and the homogeneity of variance with Levene's test. Parametric variables were compared using ANOVA and Duncan's multiple range test with $p < 0.05$ considered statistically significant.

Conclusions

In conclusion, melatonin treatment increases plasma Cr and V levels in young male ZDF rats but not in young male ZL rats, and it does not affect plasma Mg levels in either ZDF or ZL rats. These findings support the potential therapeutic value of melatonin with respect to insulin resistance, suggesting this effect may be mediated by enhancing plasma levels of Cr and V, which are both directly involved in regulating glucose and insulin homeostasis.

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