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Beneficial Effects of Magnesium Treatment on Heart Rate Variability and Cardiac Ventricular Function in Diabetic Rats

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Abstract

Background: Diabetes mellitus induces life-threatening cardiovascular complications such as cardiac autonomic neuropathy and ventricular dysfunction, and is associated with hypomagnesemia. In this study, we investigated the short-term effects of magnesium (Mg^{2+}) treatment on streptozotocin (STZ)-induced diabetic cardiac complications. **Methods:** Adult Wistar rats were treated once with STZ (50 mg/kg, ip) or vehicle (citrate), and then daily for seven days with $MgSO_4$ (270 mg/kg, ip) or saline. On the eighth day, *in vivo* tail-pulse plethysmography was recorded for heart rate variability (HRV) analysis and *ex vivo* Langendorff-based left ventricular (LV) pressure-volume parameters were measured using an intraventricular balloon. Measurements of plasma lipid- and Mg^{2+} levels as well as blood glucose and cardiac tissue Mg^{2+} levels were also performed. **Results:** Mg^{2+} treatment prevented diabetes-induced alterations of the standard deviation of the averages of normal-to-normal (NN) intervals (SDANN), root mean square differences of successive NN intervals (RMSSD), heart rate, and low-frequency (LF) power/high-frequency (HF) power ratio. In addition, Mg^{2+} restored orthostatic stress-induced changes in SDANN, RMSSD, and LF/HF ratio in diabetic rats. In isolated hearts, Mg^{2+} reversed the diabetes-induced decrease in LV end-diastolic elastance and the right shift of end-diastolic equilibrium volume intercept, without altering LV developed pressure or end-systolic elastance. However, Mg^{2+} did not prevent the elevation of blood glucose, total cholesterol, and triglycerides, or the decrease in high-density lipoprotein cholesterol in diabetes. Plasma- or tissue Mg^{2+} was not different among the treatment groups. **Conclusions:** These results suggest that Mg^{2+} treatment may attenuate diabetes-induced reduction in HRV and improve LV diastolic distensibility, without preventing hyperglycemia and dyslipidemia. Thus, Mg^{2+} may have a modulatory role in the early stages of diabetic cardiovascular complications.

Keywords cardiac, diabetes, heart rate variability, magnesium, pressure-volume relationship

Introduction

Diabetes mellitus is a major health-economic burden worldwide,[1] and has a high mortality rate mainly due to its cardiovascular complications.[2] Diabetic cardiovascular complications account for almost half of diabetic complications, and include cardiomyopathy, arrhythmias, and vasculopathies like coronary artery diseases.[2] Such complications occur through pathological structural- and electrical remodeling of the heart as well as through pro-atherosclerotic changes in blood vessels.[3] In addition, diabetes induces cardiac autonomic neuropathy, which manifests as impaired hemodynamic responses to stress and reduced heart rate variability (HRV), an independent risk factor for fatal myocardial infarction.[4] Although the onset of such complications may occur insidiously in asymptomatic patients,[5] the overall diabetic prognosis worsens once the complications become established, hence the importance of early intervention. Unfortunately, there is still a lack of effective treatment options to prevent or modulate the onset of cardiovascular complications.

Diabetes is associated with several electrolyte disturbances such as those involving sodium, magnesium (Mg^{2+}), and potassium.[6] Among these disturbances, hypomagnesemia is common in diabetes,[7,8] and is an independent cardiovascular risk factor.[9] In addition, hypomagnesemia in diabetes has been linked to atherosclerosis[10] and arrhythmogenesis.[11] Conversely, insulin deficiency in diabetes disrupts cardiac Mg^{2+} homeostasis.[12] Mg^{2+} is an essential cofactor for several enzymes involved in cellular metabolic processes, and has beneficial effects in diabetes by improving insulin sensitivity and metabolic control.[13] Extracellular Mg^{2+} also minimizes cromakalim-induced arrhythmias during ischemia/reperfusion injury in diabetic hearts,[14] but the effects of Mg^{2+} on the onset and nature of diabetic cardiovascular complications are not fully understood. In this study, we therefore investigated the short-term effects of Mg^{2+} treatment on cardiac autonomic neuropathy and ventricular dysfunction in a streptozotocin (STZ)-induced diabetic rat model.

Furthermore, in order to infer the possible underlying mechanisms, we evaluated changes in

plasma Mg^{2+} and metabolic markers such as glucose and lipids.

Materials and Methods

Animals

The study was approved by the Faculty of Health Sciences Animal Research Ethics

Committee of the University of Cape Town and was performed in compliance with the *Guide*

for the Care and Use of Laboratory Animals (National Research Council, National Academy

Press, 2011). Adult male Wistar rats (250–300 g) were obtained from the University of

Stellenbosch Animal Unit and housed in an animal facility at the University of Cape Town

under standardized conditions (12-h light/dark cycle and temperature of ~ 23°C) and had free

access to food and water.

Treatment Protocols

STZ (dissolved in 0.1 mol/L citrate buffer, pH 4.5) was used to induce diabetes mellitus,

whereas $MgSO_4$ (dissolved in physiological saline) was used for Mg^{2+} treatment. STZ has

cytotoxic effects on insulin-secreting pancreatic β-cells, and, at moderate doses (such as

50 mg/kg), it induces non-ketotic diabetes in rats.[15] Seventy–one rats were randomly divided

into four treatment groups (Figure 1A), and each rat was identified by a code. One subset of

rats (n = 9–10) was used to study HRV and cardiac ventricular function, whereas the other

subset (n = 7–12) was used for biochemical assays. An additional 33 rats (n = 7–10) were used

for cardiac tissue Mg^{2+} assays. On the first day of treatment, rats were fasted for 6 h prior to

intraperitoneal (ip) drug/vehicle injections. The STZ group was injected with a single dose of

STZ 50 mg/kg on the first day and with saline daily for 7 consecutive days. The STZ solution

was prepared on the day of treatment. The $STZ + Mg^{2+}$ group was injected with a single dose

of STZ 50 mg/kg on the first day and with $MgSO_4$ 270 mg/kg daily for 7 days.[16,17] The Mg^{2+}

group was injected with a single dose of citrate buffer on the first day and with $MgSO_4$

270 mg/kg daily for 7 days. The Control group was injected with a single dose of citrate buffer

on the first day and with saline daily for 7 days. Tail-prick blood samples were taken for measurements of blood glucose using a glucometer (Accu-Chek, Roche, SA), which was validated by comparison with blood glucose measurements performed on a Cholestech LDX system (Alere, SA). A random blood glucose level (measured in non-fasted rats) ≥ 15 mmol/L was taken to indicate diabetes mellitus.

In Vivo Heart Rate Variability Measurements

On day eight, HRV measurements were made using pulse plethysmography.[18] The rats were initially familiarized with the HRV recording process for 5 days. HRV parameters were recorded on awake rats placed in a rodent restrainer in a quiet, temperature-regulated environment (~ 23°C). Pulse plethysmography was recorded with a piezo-electric pulse transducer (TN1012) placed over the ventral artery at the base of the tail. Measurements were first done in the horizontal position for 5 min, before orthostatic stress was induced by tilting the restrainer (70°-angle head-up)[19] and recording for another 5 min. HRV parameters were recorded using the PowerLab data-acquisition system via LabChart 7 software (ADInstruments, Australia), and analyzed using the LabChart Pro HRV Analysis Module (ADInstruments, Australia). Ectopic beats and artefacts were manually excluded in the LabChart 'Beat Classifier' view. The indices of HRV that were quantified included time domain parameters such as the standard deviation of the averages of normal-to-normal (NN) intervals (SDANN) and root mean square differences of successive NN intervals (RMSSD), as well as frequency domain parameters such as low frequency (LF) power (0.2–0.8 Hz), high frequency (HF) power (0.8–2.8 Hz), and LF/HF ratio.

Heart Isolation and Langendorff Perfusion

For *ex vivo* experiments, hearts were harvested and perfused as previously described.[16] Briefly, rats were anticoagulated with heparin (500 I.U./kg, ip) and anesthetized with sodium pentobarbital (60 mg/kg, ip). Hearts were rapidly extracted and placed in cold, filtered, modified Krebs-Henseleit (K-H) perfusate containing (in mmol/L): 118.5 NaCl, 4.7 KCl, 25 NaHCO$_3$, 1.2 MgSO$_4$, 1.8 CaCl$_2$, 1.2 KH$_2$PO$_4$ and 11 glucose (pH 7.4). The aorta was immediately cannulated, and hearts were retrogradely perfused with the K-H solution on a constant-pressure (74 mm Hg) Langendorff apparatus, while suspended in a temperature-regulated organ chamber. Hearts were excluded if there were technical problems or delays (> 3 min) during cannulation. The perfusate was gassed with carbogen (95% O$_2$ and 5% CO$_2$) and maintained at 37°C. Blood collected during heart excision was centrifuged to obtain plasma, which was stored at −80°C for assays performed later.

Ventricular Pressure-Volume Measurements

Left ventricular (LV) pressure was measured using an intraventricular balloon inserted through the left atrial appendage and mitral valve. The balloon was mounted at the tip of a catheter connected to a pressure transducer (MLT1199) and amplifier (Bridge Amp ML221, ADInstruments, Australia). After 20 min of stabilization, pressure-volume responses were assessed as previously described by Kameyama et al.[20] Briefly, incremental volumes (10 μL at a time) of bubble-free, distilled water were injected into the intraventricular balloon (starting from an empty balloon) using a Hamilton syringe (Sigma-Aldrich, SA). The heart was left to equilibrate at a given balloon volume for 3 min before the volume was changed. The maximum LV end-diastolic pressure (LVEDP) was limited to ~ 30 mm Hg in order to avoid over-distension of the ventricle, as was determined in preliminary experiments.

Hemodynamic parameters were recorded using the PowerLab data-acquisition system via the LabChart 7 software (ADInstruments, Australia) and analyzed using the

LabChart 7 Pro BP module (ADInstruments, Australia). The end-diastolic equilibrium volume intercept was read off as the balloon volume at a LVEDP of zero mm Hg. The LV developed pressure (LVDP) was calculated as the difference between LV end-systolic pressure (ESP) and LVEDP. The end-diastolic elastance was derived as the gradient of EDP-volume relationship, whereas end-systolic elastance was the gradient of ESP-volume relationship.

Lipid Profile Assays

The plasma concentrations of total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C) were measured using colorimetric assays (Wako Pure Chemicals, USA). TC and TG were measured directly from plasma, whereas HDL-C was first separated from the other TC components (low- and very low-density lipoprotein cholesterol) by precipitation of apoB-associated lipoproteins using the sodium heparin sulfate-manganese chloride precipitation assay.[21] In each case, the final lipid concentration was measured by spectrophotometry (SpectraMax, Labotec, SA) as proportional to the absorbance of the pigment formed by the reaction between the chromogen dimethoxyaniline sodium salt (DAOS) and the hydrogen peroxide produced in the reactions describe below.

TC and HDL-C were determined by a cholesterol oxidase-DAOS enzymatic reaction colorimetric assay (Wako Pure Chemicals, USA) as described by Allain et al.[22] In this assay, free cholesterol, formed from the decomposition of cholesterol esters by cholesterol esterase, was oxidized by cholesterol oxidase to produce hydrogen peroxide. TG were determined by glycerol-3-phosphate oxidase (GPO)-DAOS enzymatic reaction colorimetric assay (Wako Pure Chemicals, USA). In this assay, glycerol, formed from the hydrolysis of TG by lipoprotein lipase, was converted to glycerol-3-phosphate (G3P) by glycerol-kinase. G3P was then oxidized by GPO to produce hydrogen peroxide.

Cardiac Tissue and Plasma Mg^{2+} Measurements

Ventricular tissue (0.1 g) was minced on ice in distilled water (1 ml) and sonicated (Soniprep, MSE, UK) twice for 15 sec, and cooled for 5 min between each round. Samples were then centrifuged at 10 000 g for 10 min and the supernatant was collected for Mg^{2+} assay. Plasma Mg^{2+} was measured from samples obtained on the eighth day, 24 h after the final MgSO$_4$ or equivalent saline injection. Ionized Mg^{2+} concentration was measured using automated photometric assays (Beckman AU, PathCare, SA).

Chemicals and Reagents

Sodium pentobarbitone was purchased from Kyron Laboratories (Johannesburg, SA). All other drugs and chemicals were obtained from Sigma (Sigma-Aldrich, SA).

Data Analysis

Data are expressed as mean and standard error of the mean (SEM), with *n* indicating the number of rats studied under each condition. Statistical analysis was conducted using Prism 5 (GraphPad, USA). Differences among multiple groups for data with normal distribution (Kolmogorov-Smirnov and Shapiro-Wilk normality tests) were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's *post-hoc* test. For data without normal distribution, a Kruskal-Wallis test was conducted followed by Dunn's *post-hoc* test. Serial measurements were compared using repeated measures ANOVA. Comparison of HRV data between the horizontal- and head-up tilt positions was carried out using paired *t*-test. A two-tailed $P \leq .05$ was considered statistically significant.

Results

General Parameters

STZ induced a significant elevation of blood glucose concentration (Figure 1B) as well as decreased body weight (Figure 1C), starting from 2–3 days post-treatment ($P < .05$, STZ vs Control). Treatment with Mg^{2+} did not prevent STZ-induced hyperglycemia ($P > .05$, STZ + Mg^{2+} vs STZ), but significantly minimized the STZ-induced loss of body weight ($P < .05$, STZ + Mg^{2+} vs STZ; Figure 1C). Mg^{2+} alone had no significant effect on blood glucose or body weight compared to Control rats ($P > .05$, Mg^{2+} vs Control; Figure 1B and C). The plasma Mg^{2+} concentrations measured on the eighth day were not significantly different among the treatment groups (plasma Mg^{2+} in mmol/L: 091 ± 0.05 for Control, 1.04 ± 0.03 for STZ, 1.08 ± 0.05 for STZ + Mg^{2+}, and 0.95 ± 0.04 for Mg^{2+}, $P > .05$, n = 5–6). There were also no statistically-significant differences in the ventricular tissue Mg^{2+} levels (tissue Mg^{2+} in mmol/g: 062 ± 0.04 for Control, 0.57 ± 0.01 for STZ, 0.62 ± 0.02 for STZ + Mg^{2+}, and 0.65 ± 0.01 for Mg^{2+}, $P = .07$, n = 7–10).

Effects of Treatments on HRV Parameters

STZ significantly decreased the time domain HRV parameters RMSSD and SDANN compared to Control ($P < .01$), and these STZ-induced effects were prevented by treatment with Mg^{2+} ($P < .05$, STZ + Mg^{2+} vs STZ; Figure 2A and B). Mg^{2+} treatment alone had no significant effect on RMSSD or SDANN compared to Control.

Representative screen-shot images of the frequency power spectra (Figure 2C) highlight a decrease in power (especially HF power) in STZ-treated rats compared to the others. STZ significantly increased the LF/HF ratio compared to Control ($P < .001$; Figure 2D). Treatment with Mg^{2+} prevented the STZ-induced increase in LF/HF ratio ($P < .001$, STZ + Mg^{2+} vs STZ). Mg^{2+} treatment alone had no significant effect on LF/HF ratio

compared to Control ($P > .05$). STZ also significantly decreased heart rate compared to Control, and this effect of STZ was reversed by co-treatment with Mg^{2+} (Figure 2E). Mg^{2+} treatment alone had no significant effect on heart rate compared to Control.

Effects of Treatments on HRV Response to Orthostatic Stress

The responses of HRV parameters to orthostatic stress (Figure 3) showed that, in Control rats, a change in body position from horizontal to head-up tilt resulted in a significant decrease in RMSSD and SDANN ($P < .001$) as well as a significant increase in LF/HF ratio ($P < .01$). In contrast, STZ-treated rats did not show a significant change in RMSSD, SDANN or LF/HF ratio in response to head-up tilt position ($P > .05$, head-up vs horizontal position). Mg^{2+} co-treatment with STZ restored the head-up tilt-induced decrease in RMSSD and SDANN as well as the increase in LF/HF ratio ($P < .001$, head-up vs horizontal position in STZ + Mg^{2+} rats). Rats treated with Mg^{2+} alone showed responses to orthostatic stress that were similar to those in Control rats ($P < .05$, head-up vs horizontal position in Mg^{2+} rats).

Ventricular Pressure-Volume Response

Representative LV pressure tracings recorded at the same ventricular filling volume (Figure 4A) show that the STZ-treated heart had a lower LVEDP compared to the others. STZ treatment induced a right shift of LVEDP-volume relationship compared to Control hearts (Figure 4B). Mg^{2+} treatment reversed the shift of LVEDP-volume relationship in STZ-treated rats by decreasing the end-diastolic equilibrium volume intercept from 49 ± 6 μL to 25 ± 5 μL ($P = .014$; Figure 4B). A similar pattern of results was obtained when the equilibrium volume intercept was normalized to heart weight (normalized equilibrium volume in μL/g: 16.9 ± 2.5 for Control, 30.4 ± 3.6 for STZ, 16.1 ± 3.1 for STZ + Mg^{2+}, and 13.8 ± 3.7 for Mg^{2+}; n = 6–7). Mg^{2+} also reversed the STZ-induced decrease in end-diastolic

elastance (P < .05, STZ + Mg^{2+} vs STZ; Figure 4C), with similar results obtained when the end-diastolic elastance was normalized to heart weight. Mg^{2+} treatment alone had no significant effect on equilibrium volume intercept or end-diastolic elastance compared to Control (Figure 4B and C). The LVDP-volume relationship and end-systolic elastance were not significantly different among the treatment groups (P > .05; Figure 4D and E).

Lipid Profile Parameters

The plasma concentrations of TC and TG in STZ-treated rats were significantly elevated compared to those in Control rats, whereas that of HDL-C was significantly decreased (P < .05, STZ vs Control; Figure 5A–C). However, Mg^{2+} treatment had no significant effect on these STZ-induced changes in plasma lipids (P > .05, STZ + Mg^{2+} vs STZ). Mg^{2+} treatment alone had no significant effect on the baseline lipid profile (P > .05, Mg^{2+} vs Control).

Discussion

In this study, we demonstrated that, even in the short term, diabetes induces abnormal HRV and ventricular diastolic dysfunction. These cardiovascular complications of diabetes were attenuated by Mg^{2+} treatment. However, Mg^{2+} did not prevent hyperglycemia and dyslipidemia in diabetes.

The STZ-induced diabetic rat model is known to mimic diabetic complications in humans.[15] The impaired orthostatic stress response in diabetic rats in our study indicated the early onset (at one week) of cardiac autonomic neuropathy. Further evidence for this neuropathy was the decreased RMSSD and SDANN, which suggest decreased vagal tone or enhanced sympathetic modulation of the sino-atrial node, as well as the increased LF/HF ratio, which indicates sympathetic dominance.[23,24] Therefore, the type of cardiac autonomic neuropathy observed in this study was that of a shift of the sympatho-vagal balance towards sympathetic predominance. Our results are consistent with those of studies showing the relatively early onset (at 3 weeks) of parasympathetic failure in STZ-induced diabetic rats.[25,26] In those studies, the mechanisms proposed to underlie autonomic neuropathy were depletion of cholinergic neurotransmitter vesicles in cardiac parasympathetic nerve endings[26] and autonomic nerve remodeling via the modulation of enzymes involved in neurotransmitter synthesis.[25] In our study, although there was no evaluation of the neuronal ultrastructural- or biochemical changes, similar mechanisms are possibly taking place in the same type of diabetes model.

The reversal of the effects of STZ on HRV and orthostatic stress response in our study suggest that Mg^{2+} prevents diabetic cardiac autonomic neuropathy, possibly by attenuating the above-mentioned cardiac parasympathetic synaptic degeneration. A related effect (outside the context of diabetes) has been reported in rat brain tissue, where magnesium-L-threonate enhanced hippocampal presynaptic functional sites and synaptic plasticity.[27] In addition,

Mg^{2+} also alleviated signs of neuropathic hyperalgesia in STZ-induced diabetic rats, via the modulation of the phosphorylation of N-methyl-D-aspartate receptor subunit in the spinal cord synapses.[28] In other cardiac conditions unrelated to diabetes, Mg^{2+} partially improved HRV in patients with systolic heart failure[29] and in a pre-eclamptic rat disease model,[30] but had no effect on HRV in patients with atrial fibrillation.[31]

The STZ-induced decrease in heart rate observed in our study is not consistent with the above-mentioned evidence of parasympathetic failure, where tachycardia would be expected. The basis of this decrease in heart rate is uncertain, but a similar finding was also reported in STZ-induced diabetic rats at day 6 post-induction.[26] Nonetheless, our present results provided further knowledge regarding this condition by demonstrating its attenuation by Mg^{2+} treatment.

Our results also showed diastolic dysfunction in diabetes, as was evidenced by an increase in end-diastolic equilibrium volume intercept and a decrease in end-diastolic elastance, both of which are indicative of increased ventricular distensibility. In contrast, systolic parameters (end-systolic elastance and developed pressure) were unaltered in this diabetes model. Diabetic diastolic dysfunction is known to precede systolic impairment in type 1 diabetic patients[5] and STZ-induced diabetic rats,[32] and occurs even in short-term conditions like gestational diabetes.[33] However, the nature of the diastolic pathology reported in most chronic diabetes studies is ventricular stiffness,[34] which has been attributed to hyperglycemia-induced deposition of advanced glycation end-products (AGEs) and myocardial fibrosis.[35] The increased ventricular distensibility observed in our study is therefore a unique finding in short-term diabetes, and may represent an early feature of cardiac remodeling. Thus, the reversal of this increased ventricular distensibility by Mg^{2+} in our study demonstrated a novel effect of Mg^{2+} in diabetes. The increased distensiblity is also consistent with the dilated cardiomyopathy (reported at 4 weeks in diabetic rats) that has been

attributed to diabetes-induced oxidative stress,[36] but the effects of Mg^{2+} in that condition are not known.

The mechanisms underlying the cardioprotective effects of Mg^{2+} in our study seem to be unrelated to the prevention of key metabolic cardiovascular risk factors in diabetes i.e., dyslipidemia and hyperglycemia,[3] despite Mg^{2+} being able to minimize the degree of loss of body weight. Similarly, in a neurological study, Mg^{2+} did not alter hyperglycemia in STZ-induced diabetic rats, despite modulating diabetic neuropathic pain.[28] In contrast, in chronic STZ-induced diabetic rats, magnesium valproate was found to prevent hyperglycemia and dyslipidemia as well as to decrease LV hypertrophy and fibrosis.[37] These anti-diabetic effects of magnesium valproate were proposed to be mediated by a reduction in oxidative stress, but it remains unclear whether the active component in that case was the Mg^{2+} ion or the valproate moiety. In chronic spontaneously-diabetic rats, Mg^{2+} also reduced the degree of hyperglycemia and the incidence of diabetes,[38] but the underlying mechanisms are unknown.

Furthermore, the effects of Mg^{2+} in our study were unrelated to a correction of plasma hypomagnesemia in diabetes, as there were no differences in plasma Mg^{2+} levels among the treatment groups at the time of measurement. The absence of hypomagnesemia was probably in keeping with the short-term diabetes model used in our study, because hypomagnesemia is reported to be more common in chronic, poorly-controlled diabetes.[7,8] There was also no statistically-significant difference in cardiac tissue Mg^{2+} concentrations, but diabetic hearts had the lowest Mg^{2+} levels ($P = .07$), suggesting the presence of a certain degree of Mg^{2+} deficiency in diabetic hearts that was probably correctable by Mg^{2+} treatment. However, this possibility still requires to be clarified in further studies.

A limitation of this study is that, although the STZ-induced rat diabetic model is known to mimic human complications,[15] it is an artificial model of diabetes and therefore the beneficial effects observed in this study may not be present in the natural disease model and

may therefore not be corroborated by clinical trials in humans. Moreover, the sustainability of cardiovascular effects of Mg^{2+} in chronic diabetes or after stoppage of Mg^{2+} supplementation was not explored since only short-term benefits of Mg^{2+} were studied. There is also uncertainty regarding the transferability of HRV results to clinical situations, because of species-related differences in HRV responses and reference points.[24,39] Furthermore, although rodents have more rapid heart rates compared to humans, the short (5 min) HRV recording still makes the analysis of time domain parameters less definitive.[23] Finally, the exact mechanism underlying the beneficial actions of Mg^{2+} remains unestablished, albeit the indirect evidence in this study showing that it was unrelated to alterations of diabetic metabolic derangements or hypomagnesemia.

The results of this study suggest that Mg^{2+} treatment may attenuate diabetic cardiac autonomic neuropathy and improve left ventricular pressure-volume response, without preventing diabetes-induced dyslipidemia or hyperglycemia. However, the exact mechanisms underlying these cardiovascular preservation effects of Mg^{2+} in diabetes require further investigations. Clinically, the results imply that Mg^{2+} supplementation therapy in pre-diabetic states may attenuate the onset of cardiovascular complications in the early stages of diabetes.

Declaration of Conflicting Interests

The authors declare no conflict of interest.

References

1.   Guariguata L, Whiting DR, Hambleton I, Beagley J, Linnenkamp U, Shaw JE. Global estimates of diabetes prevalence for 2013 and projections for 2035. *Diabetes Res Clin Pract.* 2014;103(2):137-149.

2.   Peterson LR, McKenzie CR, Schaffer JE. Diabetic cardiovascular disease: getting to the heart of the matter. *J Cardiovasc Transl Res.* 2012;5(4):436-445.

3.   Eguchi M, Xu G, Li RK, Sweeney G. Diabetes influences cardiac extracellular matrix remodelling after myocardial infarction and subsequent development of cardiac dysfunction. *J Cell Mol Med.* 2012;16(12):2925-2934.

4.   Kleiger RE, Miller JP, Bigger JT, Jr., Moss AJ. Decreased heart rate variability and its association with increased mortality after acute myocardial infarction. *Am J Cardiol.* 1987;59(4):256-262.

5.   Raev DC. Which left ventricular function is impaired earlier in the evolution of diabetic cardiomyopathy? An echocardiographic study of young type I diabetic patients. *Diabetes Care.* 1994;17(7):633-639.

6.   Liamis G, Liberopoulos E, Barkas F, Elisaf M. Diabetes mellitus and electrolyte disorders. *World J Clin Cases.* 2014;2(10):488-496.

7.   Sjogren A, Floren CH, Nilsson A. Magnesium deficiency in IDDM related to level of glycosylated hemoglobin. *Diabetes.* 1986;35(4):459-463.

8.   Nagase N. Hypertension and serum Mg in the patients with diabetes and coronary heart disease. *Hypertens Res.* 1996;19 Suppl 1:S65-68.

9.   Peters KE, Chubb SA, Davis WA, Davis TM. The relationship between hypomagnesemia, metformin therapy and cardiovascular disease complicating type 2 diabetes: the Fremantle Diabetes Study. *PLoS One.* 2013;8(9):e74355.

10.   Atabek ME, Kurtoglu S, Pirgon O, Baykara M. Serum magnesium concentrations in type 1 diabetic patients: relation to early atherosclerosis. *Diabetes Res Clin Pract.* 2006;72(1):42-47.

11.   Del Gobbo LC, Song Y, Poirier P, Dewailly E, Elin RJ, Egeland GM. Low serum magnesium concentrations are associated with a high prevalence of premature ventricular complexes in obese adults with type 2 diabetes. *Cardiovasc Diabetol.* 2012;11:23.

12.   Reed G, Cefaratti C, Berti-Mattera LN, Romani A. Lack of insulin impairs Mg^{2+} homeostasis and transport in cardiac cells of streptozotocin-injected diabetic rats. *J Cell Biochem.* 2008;104(3):1034-1053.

13.   Sales CH, Pedrosa Lde F. Magnesium and diabetes mellitus: their relation. *Clin Nutr.* 2006;25(4):554-562.

14.   Tosaki A, Das DK. Extracellular Mg^{2+} manipulation prevents the proarrhythmic activity of cromakalim in ischemic/reperfused diabetic hearts. *J Pharmacol Exp Ther.* 1997;282(1):309-317.

15.   Wei M, Ong L, Smith MT, et al. The streptozotocin-diabetic rat as a model of the chronic complications of human diabetes. *Heart Lung Circ.* 2003;12(1):44-50.

16.   Amoni M, Kelly-Laubscher R, Petersen M, Gwanyanya A. Cardioprotective and Anti-arrhythmic Effects of Magnesium Pretreatment Against Ischaemia/Reperfusion Injury in Isoprenaline-Induced Hypertrophic Rat Heart. *Cardiovasc Toxicol.* 2015. doi: 10.1007/s12012-015-9355-6.

17.   Sameshima H, Ota A, Ikenoue T. Pretreatment with magnesium sulfate protects against hypoxic-ischemic brain injury but postasphyxial treatment worsens brain damage in seven-day-old rats. *Am J Obstet Gynecol.* 1999;180(3 Pt 1):725-730.

18. Linder JR, Stauss HM, Gindes H, et al. Finger volume pulse waveforms facilitate reliable assessment of heart rate variability, but not blood pressure variability or baroreflex function. *BMC Cardiovasc Disord.* 2014;14:180.

19. Bedette D, Santos RA, Fontes MA. Cardiovascular reactivity after blockade of angiotensin AT1 receptors in the experimental model of tilting test in conscious rats. *Br J Pharmacol.* 2008;153(5):966-971.

20. Kameyama T, Chen Z, Bell SP, Fabian J, LeWinter MM. Mechanoenergetic studies in isolated mouse hearts. *Am J Physiol.* 1998;274(1 Pt 2):H366-374.

21. Gidez LI, Miller GJ, Burstein M, Slagle S, Eder HA. Separation and quantitation of subclasses of human plasma high density lipoproteins by a simple precipitation procedure. *J Lipid Res.* 1982;23(8):1206-1223.

22. Allain CC, Poon LS, Chan CS, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. *Clin Chem.* 1974;20(4):470-475.

23. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Heart rate variability: standards of measurement, physiological interpretation and clinical use. *Circulation.* 1996;93(5):1043-1065.

24. von Borell E, Langbein J, Despres G, et al. Heart rate variability as a measure of autonomic regulation of cardiac activity for assessing stress and welfare in farm animals -- a review. *Physiol Behav.* 2007;92(3):293-316.

25. Li X, Jiang YH, Jiang P, et al. Analysis of Heart Rate Variability and Cardiac Autonomic Nerve Remodeling in Streptozotocin-induced Diabetic Rats. *Exp Clin Endocrinol Diabetes.* 2015;123(5):272-281.

26. Sanyal SN, Wada T, Yamabe M, et al. Synaptic degradation of cardiac autonomic nerves in streptozotocin-induced diabetic rats. *Pathophysiology.* 2012;19(4):299-307.

27. Slutsky I, Abumaria N, Wu LJ, et al. Enhancement of learning and memory by elevating brain magnesium. *Neuron.* 2010;65(2):165-177.

28. Rondon LJ, Privat AM, Daulhac L, et al. Magnesium attenuates chronic hypersensitivity and spinal cord NMDA receptor phosphorylation in a rat model of diabetic neuropathic pain. *J Physiol.* 2010;588(Pt 21):4205-4215.

29. Almoznino-Sarafian D, Sarafian G, Berman S, et al. Magnesium administration may improve heart rate variability in patients with heart failure. *Nutr Metab Cardiovasc Dis.* 2009;19(9):641-645.

30. Zhou Q, Shen J, Zhou G, Shen L, Zhou S, Li X. Effects of magnesium sulfate on heart rate, blood pressure variability and baroreflex sensitivity in preeclamptic rats treated with L-NAME. *Hypertens Pregnancy.* 2013;32(4):422-431.

31. Frick M, Ostergren J, Rosenqvist M. Effect of intravenous magnesium on heart rate and heart rate variability in patients with chronic atrial fibrillation. *Am J Cardiol.* 1999;84(1):104-108, A109.

32. Mourouzis I, Mantzouratou P, Galanopoulos G, et al. The beneficial effects of ranolazine on cardiac function after myocardial infarction are greater in diabetic than in nondiabetic rats. *J Cardiovasc Pharmacol Ther.* 2014;19(5):457-469.

33. Freire CM, Nunes Mdo C, Barbosa MM, et al. Gestational diabetes: a condition of early diastolic abnormalities in young women. *J Am Soc Echocardiogr.* 2006;19(10):1251-1256.

34. Litwin SE, Raya TE, Anderson PG, Daugherty S, Goldman S. Abnormal cardiac function in the streptozotocin-diabetic rat. Changes in active and passive properties of the left ventricle. *J Clin Invest.* 1990;86(2):481-488.

35.   van Heerebeek L, Hamdani N, Handoko ML, et al. Diastolic stiffness of the failing

diabetic heart: importance of fibrosis, advanced glycation end products, and myocyte

resting tension. *Circulation.* 2008;117(1):43-51.

36.   Crespo MJ, Zalacain J, Dunbar DC, Cruz N, Arocho L. Cardiac oxidative stress is

elevated at the onset of dilated cardiomyopathy in streptozotocin-diabetic rats. *J

Cardiovasc Pharmacol Ther.* 2008;13(1):64-71.

37.   Patel BM, Raghunathan S, Porwal U. Cardioprotective effects of magnesium

valproate in type 2 diabetes mellitus. *Eur J Pharmacol.* 2014;728:128-134.

38.   Balon TW, Gu JL, Tokuyama Y, Jasman AP, Nadler JL. Magnesium supplementation

reduces development of diabetes in a rat model of spontaneous NIDDM. *Am J

Physiol.* 1995;269(4 Pt 1):E745-752.

39.   Manzo A, Ootaki Y, Ootaki C, Kamohara K, Fukamachi K. Comparative study of

heart rate variability between healthy human subjects and healthy dogs, rabbits and

calves. *Lab Anim.* 2009;43(1):41-45.

Legends

Figure 1. Treatment protocols and general parameters**.** A, *In vivo* treatment protocols for different groups of rats [Control, streptozotocin (STZ), STZ + Mg^{2+} (STZ + Mg), and Mg^{2+} (Mg)]. B, Daily random blood sugar concentrations. C, Body weight. Values are mean ± SEM; n = 16–22; *P < .05 versus Control; [#]P < .05 versus STZ.

Figure 2. Effects of STZ and Mg^{2+} on heart rate variability (HRV) parameters. A and B, Summary data of root mean square differences of successive normal-to-normal (NN) intervals (RMSSD) and standard deviation of the averages of NN intervals (SDANN). C, Screen-shot images of frequency power spectra. D, Ratios of low frequency (LF) power to high frequency (HF) power. E, Heart rate measurements. Values are mean ± SEM; n = 9–10; **P < .01, ***P < .001 versus Control; [#]P < .05, [##]P < .01, [###]P < .001 versus STZ.

Figure 3. Effects of orthostatic stress on HRV parameters. A–C, Data of RMSSD, SDANN, and LF/HF ratios, measured from rats in the horizontal position (grey columns), followed by head-up tilt position (black columns). Values are mean ± SEM; n = 9–10; *P < .05, **P < .01, ***P < .001.
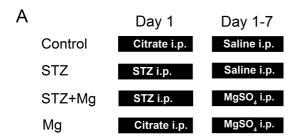
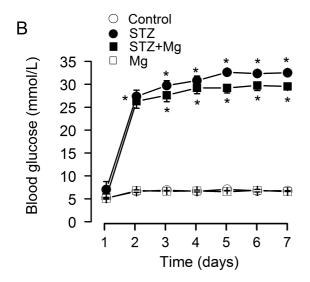**Figure 4.** Effects of treatments on left ventricular pressure-volume characteristics. A, Representative ventricular pressure tracings recorded at an intraventricular balloon volume of 70 μL. Note the relatively lower diastolic pressure in the STZ-treated heart compared to the others. B and D, Left ventricular (LV) end-diastolic pressure (LVEDP)-volume relationship and LV developed pressure (LVDP)-volume relationship in different treatment groups. Inset in B, End-diastolic equilibrium volume intercept. C and E, End-diastolic

elastance and end-systolic elastance. Values are mean ± SEM; n = 6–7; *$P < .05$ versus Control; [#]$P < .05$ versus STZ.

Figure 5. Effects of various treatments on the plasma lipid profile. A–C, Plasma concentrations of total cholesterol, triglycerides, and high-density lipoprotein (HDL) cholesterol, measured on day 8 in the different treatment groups. Values are mean ± SEM; n = 7–12; *$P < .05$ versus Control.
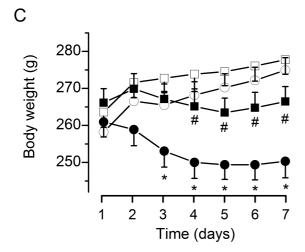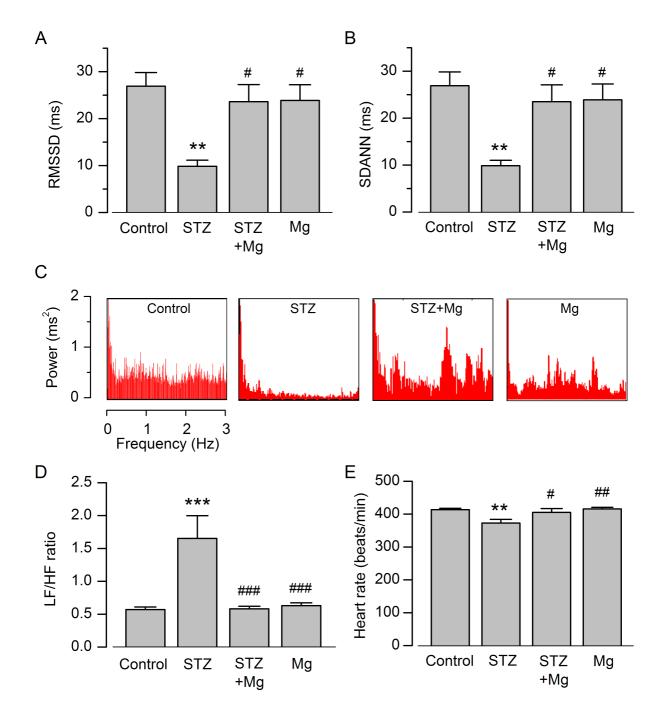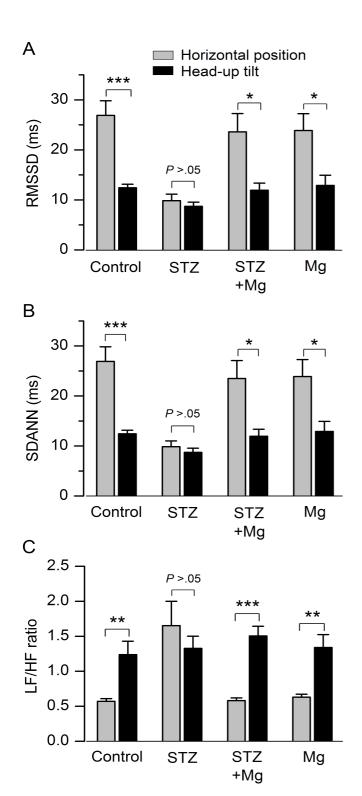
Figure 1.

A

	Day 1	Day 1-7
Control	Citrate i.p.	Saline i.p.
STZ	STZ i.p.	Saline i.p.
STZ+Mg	STZ i.p.	MgSO$_4$ i.p.
Mg	Citrate i.p.	MgSO$_4$ i.p.

Figure 2.

Figure 3.

A

B

C

Figure 4.

Figure 5.