Improvement of cardiac ventricular function by magnesium treatment in chronic streptozotocin-induced diabetic rat heart

Hamida Aboalgasm, Morea Petersen, Asfree Gwanyanya

Abstract

Objective: Chronic diabetes mellitus is associated with detrimental cardiovascular complications and electrolyte imbalances such as hypomagnesaemia. We investigated the effect of magnesium (Mg²⁺) on cardiac function and the possible role of histological and electrical alterations in chronic, streptozotocin-induced diabetic rats.

Methods: Wistar rats were treated once intraperitoneally with streptozotocin or citrate, and then daily with MgSO₄ or saline for four weeks. Cardiac contractile and electrocardiographic parameters were measured on Langendorff-perfused hearts. Other hearts were histologically stained or immunoblotted for the mitochondrial ATP synthase (ATP5A).

Results: In diabetic hearts, Mg²⁺ prevented a diabetes-induced decrease in left ventricular developed pressure and improved contractility indices, as well as attenuated the reduction in heart rate and prolongation of QT interval, but not the QT interval corrected for heart rate (QTc). Histologically, there were neither differences in cardiomyocyte width nor interstitial collagen. The expression of ATP5A was not different among the treatment groups.

Conclusion: Mg²⁺ supplementation improved cardiac contractile activity in chronic diabetic hearts via mechanisms unrelated to electrocardiographic or histologically detectable myocardial alterations.

Keywords: magnesium, cardiac, diabetes, ventricular function, streptozotocin

Cardiovascular complications are a major cause of mortality in diabetes mellitus. These complications are a result of the pathological remodelling processes in the heart and blood vessels that are induced by metabolic derangements in diabetes, such as hyperglycaemia, dyslipidaemia, acid–base imbalances and electrolyte disturbances. The resultant diabetic cardiomyopathy and coronary artery disease predispose the heart to cardiac contractile dysfunction, ischaemic heart disease and dysrhythmias. In addition, the macrovascular and microvascular angiopathies in diabetes induce target-organ damage in other tissues, such as the brain, kidneys and eyes. Therefore, diabetes mellitus has been proposed to be a cardiovascular disease, and the modulation of pathological cardiovascular remodelling could represent one aspect of diabetic treatment. However, the mechanisms of remodelling are not fully understood.

Hypomagnesaemia is a common and detrimental type of electrolyte disturbance in diabetes, especially in chronic, poorly controlled diabetes. In diabetic patients, hypomagnesaemia is associated with cardiovascular conditions such as atherosclerosis, coronary artery disease, and arrhythmias. However, although magnesium (Mg²⁺) has been shown to modulate insulin receptors and to improve metabolic control in diabetic rats, the role of Mg²⁺ in cardiovascular pathological remodelling remains unclear.

An area of difficulty in determining the role of Mg²⁺ at tissue level is that Mg²⁺ tissue deficits are not readily detectable, given that Mg²⁺ is largely an intracellular ion, binds to cellular components, and has relatively slow shifts across the cell membrane. Furthermore, clinical hypomagnesaemia is indicative of decreased ionised Mg²⁺ in serum and may not necessarily reflect cellular deficits or the degree of imbalance between extracellular and intracellular concentrations. These issues suggest that a possible way to offset the occurrence of subtle, but detrimental Mg²⁺ tissue deficits and imbalances that may be induced by pathological stress conditions such as diabetes would be to prevent subclinical intracellular Mg²⁺ deficiency through Mg²⁺ supplementation.

We previously showed that Mg²⁺ supplementation improved cardiac ventricular compliance and cardiac autonomic function in the early stages of diabetes in rats, but the long-term efficacy of Mg²⁺ in chronic diabetes and the underlying mechanisms remain unknown. In this study, we investigated the long-term effect of Mg²⁺ treatment on cardiac ventricular dysfunction in chronic diabetes and explored the possible role of electrical and myocardial histological alterations.
Methods

The study was approved by the Faculty of Health Sciences Animal Research Ethics Committee of the University of Cape Town (AEC Protocol 014-014). All procedures on animals were 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 (n = 275 g) were used in this study. Rats were housed under standardised conditions (12-hour light/dark cycle and temperature of ~ 23°C) and had free access to rat chow and drinking water.

Unless stated otherwise, drugs and chemicals were obtained from Sigma-Aldrich (SA). Streptozotocin (STZ) was used to induce a moderate form of diabetes mellitus, as previously described.14 Rats were fasted of food (but not water) for six hours to improve the uptake of STZ before being injected intraperitoneally (i.p.) with STZ (50 mg/kg). The STZ was freshly dissolved in 0.1 M citrate buffer (pH 4.5) before administration.

Blood glucose was measured from tail vein blood samples obtained at similar times of the day using a glucometer (Accu-Chek, Roche, SA).14 Rats with a random blood glucose concentration ≥ 15 mmol/l were considered diabetic.

Magnesium was administered as MgSO4 (270 mg/kg, i.p.) dissolved in normal saline.15,16 The i.p. route was chosen for Mg2+ to achieve more reliable uptake compared to oral administration in water or food where the uptake may vary in diabetes due to polydipsia and polyphagia.

The rats were randomly divided into four treatment groups, and each rat was identified by a unique label on the tail. The control group was injected i.p. with a single dose of citrate buffer on the first day, and with saline i.p. once daily for 28 consecutive days. The STZ group was injected i.p. with a single dose of STZ 50 mg/kg on the first day, and with saline i.p. once daily for 28 days. The STZ + Mg2+ group was injected i.p. with a single dose of STZ 50 mg/kg on the first day, and with MgSO4 270 mg/kg i.p. once daily for 28 days. The Mg2+ group was injected i.p. with a single dose of citrate buffer on the first day, and with MgSO4 270 mg/kg i.p. once daily for 28 days.

Rat hearts were surgically removed under anaesthesia to euthanise the rats, as previously described.14 Briefly, rats were anaesthetised with sodium pentobarbital (70 mg/kg, i.p., Vetserv, SA). Upon loss of the pedal withdrawal reflexes, the hearts were excised via a thoracotomy incision and placed in cold (4°C), filtered (7-μm pore Whatman filter paper, Sigma-Aldrich, SA), modified Krebs-Henseleit (KH) solution containing (in mmol/l): 118.5 NaCl, 4.7 KCl, 25 NaHCO3, 1.2 MgSO4, 1.8 CaCl2, 1.2 KH2PO4 and 11 glucose (pH 7.4). CaCl2 was added after the optimisation of pH to prevent precipitation of calcium with phosphate. Some hearts were used for cardiac perfusion studies, whereas the others were either histologically analysed or snap-frozen in liquid nitrogen and stored at ~80°C for Western blot analysis.

For perfusion studies, the hearts were retrogradely perfused with K-H solution through an aortic cannula on a constant-pressure (74 mmHg) Langendorff apparatus. To ensure optimal cardiac tissue viability, the time lapse between excision of the heart and commencement of perfusion was limited to three minutes. The K-H solution was gassed with carbogen (95% O2 and 5% CO2) and was maintained at 37°C. The coronary flow rate was measured by collecting coronary effluent over time and was normalised to heart weight. Blood samples used for Mg2+ assays were collected at the time of removal of the heart and centrifuged at 15 000 g (Beckman microfuge, USA) to obtain plasma, which was frozen until further analysis.

Electrocardiographic (ECG) and haemodynamic parameters were measured using the PowerLab data-acquisition system and LabChart Pro 7 software (ADInstruments, Australia), as previously described.15 ECG was recorded using apex-to-base electrodes via a transducer (ML136) and was analysed using the LabChart Pro ECG module (ADInstruments, Australia). The QT interval, corrected for heart rate (QTc) was calculated using Bazett’s formula. Left ventricular (LV) pressure was measured using a water-filled, intraventricular balloon connected to a pressure transducer (MLT1199) and amplifier (ML221, ADInstruments, Australia).

The hearts were stabilised for 20 minutes and the LV end-diastolic pressure (LVEDP) was set at 5–10 mmHg. The LabChart 7 Pro blood pressure module (ADInstruments, Australia) was used to analyse haemodynamic data and to derive the maximal rate of pressure increase (+dP/dtmax), the maximal rate of pressure decline (−dP/dtmin), contractility index and the time constant of ventricular relaxation (tau). The LV developed pressure (LVPD) was calculated as the difference between LV peak systolic pressure and LVEDP.

Transverse sections of cardiac ventricular tissue were stained with either haematoxylin and eosin (H&E) or Masson’s trichrome, as previously described.14 Histological images were taken using a charge-coupled device camera (Zeiss AxioCam, Germany) attached to an optical microscope (Zeiss AxioSkop, Germany). The cardiomyocyte width on H&E images was analysed using ImageJ software (NIH, USA). The average width of five cells on each of four sections of the heart was calculated for each heart. The degree of interstitial and perivascular fibrosis on Masson’s trichrome images was semi-quantitatively scored, as done previously,14 based on a scoring system described by Buwa et al.17 as follows: none (−), mild (+), moderate (++) and severe (+++).

Frozen LV tissues were homogenised on ice by sonication in a modified radioimmunoprecipitation assay buffer (50 mM Tris-HCl, 150 mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulphate, pH 7.4) containing a protease/phosphatase inhibitor cocktail (Thermo Scientific, USA). Protein concentrations were quantified (Piezec protein assay kit, Thermo Scientific, USA) and protein samples (40 µg) were loaded and electrophoresed on 12% sodium dodecyl sulphate-polyacrylamide gels (Mini-Protein Tetra Cell, BioRad, SA) and transferred to isopropanol-soaked polyvinylidene fluoride membranes (Trans-Blot Turbo, Bio-Rad, SA).

The membranes were blocked with 5% bovine serum albumin (BSA) in 0.1% Tween20 phosphate-buffered saline (PBS-T) for one hour at room temperature, and incubated with anti-ATP5A mouse antibody (1:5000, #136178, Santa Cruz Biotechnology, USA) in 5% BSA in PBS-T overnight at 4°C. The primary antibody was excluded in the negative control in order to rule out non-specific binding of the secondary antibody. The membranes were washed with PBST and incubated with horseradish peroxidase-conjugated secondary antibody (1:10000, #170-6516, Bio-Rad, SA) in 5% BSA in PBS-T for two hours at room temperature.

The membranes were then washed with PBS-T, incubated with enhanced chemiluminescence substrate (Bio-Rad, SA)
and exposed to X-ray film in the dark room. The membranes were stripped, blocked and re-probed with anti-β-actin rabbit antibody (1:10000, #16039, Abcam, USA) and goat anti-rabbit secondary antibody (1:10 000, #6721, Abcam, USA). The bands on films were analysed using ImageJ software (NIH, USA) and were normalised to those of the housekeeping protein β-actin.

The Mg²⁺ concentration was measured in the plasma samples prepared at exsanguination, 18–24 hours after the final dose of MgSO₄ had been administered. Ionised Mg²⁺ concentration was measured using automated spectrophotometric and potentiometric analyses (Beckman AU Chemistry Analyzer, PathCare, SA).¹⁴

**Statistical analysis**

Data are expressed as mean and standard error of the mean (SEM) or as box plots and the mean, and n indicates the number of replicates. Statistical analysis was conducted using Statistica 13. 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. A two-tailed p value ≤ 0.05 was considered statistically significant.

**Results**

*In vivo* treatment with STZ significantly increased the blood glucose concentration and decreased the rat body weight (Fig. 1, starting from the first week after treatment (*p* < 0.05, STZ vs control for each parameter). Overall, treatment with Mg⁰⁺ did not prevent STZ-induced hyperglycaemia (*p* > 0.05, STZ + Mg⁰⁺ vs STZ), except for the transient dips in blood glucose concentration observed in the first and third weeks (Fig. 1A). Mg⁰⁺ also did not prevent the STZ-induced loss of body weight (*p* > 0.05, STZ + Mg⁰⁺ vs STZ; Fig. 1B). Mg⁰⁺ treatment alone had no significant effect on blood glucose concentration or on body weight (*p* > 0.05, Mg⁰⁺ vs control for each parameter).

STZ induced a significant decrease in the LVDP (*p* < 0.05, STZ vs control), and this STZ-induced hypotensive effect was prevented by Mg⁰⁺ treatment (*p* = 0.03, STZ + Mg⁰⁺ vs STZ; Fig. 2A). Mg⁰⁺ treatment on its own had no significant effect on LVDP (*p* > 0.05, Mg⁰⁺ vs control; Fig. 2A). STZ-treated hearts also exhibited significant reductions in the indices of LV contraction (+dP/dtₑ₄₀) and relaxation (−dP/dtₑ₄₀) as well as in the overall contractility index (*p* < 0.05, STZ vs control for each parameter; Fig. 2B–D). Among these changes, Mg⁰⁺ treatment reversed the STZ-induced reduction of +dP/dtₑ₄₀ and contractility index (*p* < 0.05, STZ + Mg⁰⁺ vs STZ for each parameter; Fig. 2B, C). Mg⁰⁺ treatment alone had no detrimental effect on +dP/dtₑ₄₀, −dP/dtₑ₄₀, or the contractility index (*p* > 0.05, Mg⁰⁺ vs control; Fig. 2B–D).

In addition, there were no significant differences in coronary flow rate or in the ratio of heart weight to body weight among the different treatment groups (Fig. 2E, F). There were also no significant differences in the diastolic time constant of ventricular relaxation (τₑ₄₀) among the groups (τₑ₄₀: 0.043 ± 0.065 s for control, 0.073 ± 0.030 s for STZ, 0.064 ± 0.023 s for STZ + Mg⁰⁺, 0.080 ± 0.033 s for Mg⁰⁺; values are mean ± SEM, *p* > 0.05, *n* = 6 per group).

Representative ECG traces recorded on isolated hearts (Fig. 3) showed typical apex-to-base electrical waveforms that resembled lead II tracing on a surface ECG recording. Qualitatively, the traces highlight a reduction in the heart rate of STZ-treated hearts (Fig. 3B) compared to controls (Fig. 3A), but without significant effects on R-wave amplitude and contractility index (*p* > 0.05, STZ + Mg⁰⁺ vs STZ, *n* = 6 per group).

Fig. 1. General parameters. A: Random blood glucose concentration. B: Rat body weight. The parameters were measured weekly in different treatment groups of rats [○, control; ●, streptozotocin (STZ); ▲, STZ + Mg⁰⁺; ■, Mg⁰⁺]. Values are mean ± standard error of the mean; *n* = 12–15 per group; *p* < 0.05, **p < 0.01 versus control; ***p < 0.01 versus STZ.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>STZ</th>
<th>STZ + Mg⁰⁺</th>
<th>Mg⁰⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (bpm)</td>
<td>233 ± 8</td>
<td>178 ± 14*</td>
<td>218 ± 8</td>
<td>234 ± 13</td>
</tr>
<tr>
<td>R-wave amplitude (mV)</td>
<td>5.22 ± 0.79</td>
<td>5.67 ± 1.31</td>
<td>6.24 ± 1.17</td>
<td>6.22 ± 0.85</td>
</tr>
<tr>
<td>S-wave amplitude (mV)</td>
<td>1.75 ± 0.27</td>
<td>2.13 ± 0.63</td>
<td>2.35 ± 0.73</td>
<td>0.40 ± 1.38</td>
</tr>
<tr>
<td>T-wave amplitude (mV)</td>
<td>2.12 ± 0.53</td>
<td>2.56 ± 0.67</td>
<td>2.73 ± 0.95</td>
<td>1.76 ± 0.46</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>233 ± 8</td>
<td>178 ± 14*</td>
<td>218 ± 8</td>
<td>234 ± 13</td>
</tr>
<tr>
<td>QT interval (s)</td>
<td>0.124 ± 0.006</td>
<td>0.137 ± 0.006</td>
<td>0.137 ± 0.006</td>
<td>0.119 ± 0.007</td>
</tr>
<tr>
<td>QTc (s)</td>
<td>0.124 ± 0.006</td>
<td>0.137 ± 0.006</td>
<td>0.137 ± 0.006</td>
<td>0.119 ± 0.007</td>
</tr>
</tbody>
</table>
| QTc represents QT interval corrected for heart rate. Values are mean ± standard error of the mean; *n* = 7–11 per group; *p* < 0.05 vs control; **p < 0.01 versus control; ***p < 0.01 versus STZ.
noticeable alterations of the ECG waveform patterns. Summary data of ECG parameters (Table 1) show that STZ significantly decreased the heart rate and prolonged the QT interval ($p < 0.01$ vs control for each parameter), and both these STZ effects could be prevented by Mg$^{2+}$ treatment. Mg$^{2+}$ treatment alone had no significant effect on heart rate or QT interval. There were no significant differences in the R-, S- or T-wave amplitudes and QRS and QTc intervals among the treatment groups.

Representative images of ventricular slices stained with either H&E or Masson's trichrome are shown in Fig. 4. The H&E images showed normal cardiomyocyte structural outlines, separated by extracellular spaces that were relatively free of cellular
components or other infiltrates (Fig. 4A). There were also no apparent distortions in the arrangement of the myofibrils. There were no significant differences in cardiomyocyte width among the treatment groups (p > 0.05; Fig. 4C). The Masson’s trichrome images showed no differences in the interstitial or perivascular fibrosis score among the treatment groups (Fig. 4B, D).

To explore the role of cardiac metabolic stress, Western blot analysis was performed for the mitochondrial ATP synthase (ATP5A), a key component of the mitochondrial respiratory function. Representative images on Western blot films (Fig. 5A) showed bands of ATP5A and β-actin proteins in the ventricles of different hearts. Semi-quantitatively, there were no significant differences in the expression of ATP5A among the treatment groups (Fig. 5B).

There were no significant differences in the plasma Mg²⁺ concentration among the groups (concentration of ionised Mg²⁺: 0.89 ± 0.01 mmol/l for control, 0.94 ± 0.05 mmol/l for STZ, 0.85 ± 0.04 mmol/l for STZ+Mg²⁺, 0.83 ± 0.01 mmol/l for Mg²⁺; values are mean ± SEM, p > 0.05, n = 8 per group).

Discussion

The onset and severity of cardiovascular complications in poorly controlled diabetes mellitus are time-dependent entities. In this study, we showed that Mg²⁺ treatment induced long-term improvements in LV contractile function and stabilised heart rate in chronic diabetic rats.

Our results indicated the presence of diabetes-induced ventricular systolic dysfunction in chronic diabetes, as was evidenced by the reduction in LVDP, +dP/dt max and the contractility index in diabetic hearts. These findings are consistent with the systolic dysfunction reported in chronic type 1 diabetes patients and in STZ-induced diabetic rats. However, the results are in contrast to the lack of systolic impairment that we previously observed in the acute diabetes disease model, where only diastolic dysfunction was observed, suggesting a time-dependent progression of diabetic cardiac complications.

In the present study, except for the unaltered time constant of relaxation (τ), diastolic dysfunction was not further evaluated since the LVEDP had to be pre-set to a fixed value in order to measure LVDP. Nonetheless, in this study, the systolic dysfunction in diabetes was reversed by Mg²⁺ treatment. Recently, Mg²⁺ was also shown to improve diastolic function and mitochondrial activity in fat-fed chronic diabetic mice. Given that diabetic diastolic dysfunction is known to precede systolic impairment in type 1 diabetic patients and in STZ-induced diabetic rats, and that diastolic dysfunction is a common cause of systolic heart failure in diabetes, the improvement of systolic...
Fig. 4. Histological analyses of ventricular tissue. A: Representative images of different ventricular tissue sections stained with haematoxylin and eosin (H&E). Scale bar = 20 µm (× 40 magnification). B: Representative images of different ventricular tissue sections stained with Masson's trichrome. Insets: Images of perivascular tissue. Scale bar = 20 µm (× 40 magnification). C: Summary data of ventricular cardiomyocyte width. D: Arbitrary score of the degree of interstitial and perivascular fibrosis: –, none; +, mild. Data are shown as box plots and the mean (■); n = 6 per group.
activity by Mg²⁺ observed in our study could be secondary to the diastolic modulation observed in the acute diabetes disease model.\(^\text{14}\)

In the present study, there were no detectable cardiac morphological changes to account for the contractile dysfunction induced by diabetes. The gross heart weight was unaltered, and histologically, there was neither a change in cardiomyocyte size nor interstitial fibrosis. In addition, there was no significant coronary perivascular fibrosis or cellular infiltrates that would have been expected to impair coronary perfusion, a finding that was also consistent with the lack of change in coronary flow rate observed in this study.

These findings are in agreement with those in other studies on chronic STZ-induced diabetic rats in which the cardiac dysfunction was not accompanied by histological evidence of cardiac cellular hypertrophy or fibrosis.\(^\text{20}\) In contrast, other studies in chronic STZ-induced diabetic rats showed that there was cardiac dysfunction together with histological evidence of cardiomyocyte hypertrophy and fibrosis.\(^\text{24}\) These histological differences are likely to be related to the duration of diabetes, given that in diabetic patients, the deposition of collagen in cardiac tissue only becomes more prominent in the later stages of heart failure when there is a low ejection fraction.\(^\text{25}\)

In our study, there were no significant cardiac histological changes to account for the effect of Mg²⁺. Taken together, the lack of histological alterations in our study supports the concept that the nature of diabetic ventricular dysfunction and the effect of Mg²⁺ were functional, rather than structural.

The STZ-induced decrease in heart rate observed in the present study and its prevention by Mg²⁺ were consistent with our previous findings in the acute-diabetes model where the relative bradycardia was also observed \textit{in vivo}.\(^\text{14}\) The bradycardia in STZ-induced diabetic rats has also been reported in other studies,\(^\text{20,26}\) and has been attributed to cardiac autonomic synaptic degradation,\(^\text{26}\) but the basis of the bradycardia in our study remains unclear. In this study, the bradycardia seemed to be unrelated to the modulation of cardiac electrical activity since there were no significant changes in ECG waves. The prolongation of the QT interval in diabetes was probably related to changes in heart rate because the QT interval, corrected for the heart rate (QTc), was not significantly different among the treatment groups. Taken together, the occurrence of bradycardia both \textit{in vivo} and \textit{ex vivo} and its prevention by Mg²⁺ suggest that these effects were intrinsic to the heart.

Despite the improvements in cardiac function by Mg²⁺, there were no significant differences in the cardiac expression of ATP5A, a cardiac biomarker that could have accounted for the Mg²⁺ effects at a molecular level. Mg²⁺ is a key co-factor of several co-enzymes that may alter the cardiac metabolic status, it also contributes to cellular energetics via its coupling with ATP to form MgATP,\(^\text{27}\) and it may therefore alter mitochondrial function. However, in our study, there were no changes in the metabolic indices, as was indicated by the mitochondrial metabolic component ATP5A. Therefore, further molecular studies such as those evaluating aspects of mitochondrial fusion/fission are required to elucidate the role of Mg²⁺ at the cardiac cellular level.

Limitations of this study include the use of an artificial, STZ-induced diabetic model, in which the Mg²⁺ effects may not be readily translatable to the natural disease. However, the STZ-induced diabetic rat model is known to mimic diabetic complications in humans.\(^\text{28}\) We also previously showed the value of this disease model in that, apart from mimicking type 1 diabetes, it also exhibited features of type 2 diabetes, such as dyslipidaemia.\(^\text{14}\) Also, the clinical relevance of the Mg²⁺ dose used in this study remains unclear, given that that the dose (270 mg/kg) is higher than that used via the oral route in human supplementation, and is only comparable to the loading intravenous/intramuscular dose used in eclampsia (~230 mg/kg).\(^\text{29}\) Nonetheless, the peak increases at 3.5 hours of ~0.7 mmol/l, achievable under our experimental conditions,\(^\text{30}\) are still within the therapeutic ranges of other clinical conditions.\(^\text{31}\) Finally, since the experiments were performed at cardiac tissue level, the presence of an intracellular Mg²⁺ deficit cannot be excluded, and therefore requires further investigations at a cellular level.

**Conclusion**

The results of this study show that Mg²⁺ improved cardiac contractile function and stabilised heart rate in the STZ-induced chronic diabetes rat model, without preventing metabolic derangements such as hyperglycaemia. The mechanisms underlying the attenuation of cardiac dysfunction in chronic diabetes mellitus by Mg²⁺ were unrelated to electrocardiographi-
cally or histologically detectable changes, but the exact pathways involved require further investigation.

The study was supported by the South African Medical Research Council (MRC, Grant No 29841) and by the National Research Foundation (NRF) of South Africa (Grant No 91514).

References

2. Deluyker D, Evens L, Bito V. Advanced glycation end products (AGEs) and cardiovascular dysfunction: focus on high molecular weight AGEs. Amino Acids 2017; 49(9): 1535–1541.