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ORIGINAL ARTICLE

The Effects of Subchronic Intake of Magnesium Hydrocarbonate-Rich Mineral Water on Cardiometabolic Markers and Electrolytes in Rats With Streptozotocin-Induced Diabetes

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Abstract

Background/Aim: Hypomagnesaemia is one of the most detected electrolyte abnormalities in diabetics. Modulation of numerous cardiovascular pathophysiological processes is a potential goal for anti-diabetic therapy. Magnesium supplementation prevents subclinical tissue magnesium deficiency, thus delaying the onset of metabolic imbalance in diabetes, but long-term effects of magnesium supplementation in chronic diabetes and numerous pathophysiological processes remain unknown. Aim of this study was to determine the effects of subchronic intake of magnesium hydrocarbonate-rich mineral water on cardiometabolic markers and electrolytes in rats with streptozotocin-induced diabetes.

Methods: A total of 28 Wistar, male rats, body weight 160 g at start, were divided into four groups of 7 each: two controls, group that drank tap water and received a single ip injection of saline (0.9 % NaCl) (TW-C), group that drank mineral water rich in magnesium hydrocarbonate and received a single ip injection of saline (0.9 % NaCl) (MW-C); and two experimental groups with streptozotocin-induced diabetes, group that drank tap water and received a single ip injection of streptozotocin (100 mg/kg) in saline (0.9 % NaCl, 1 mL) (TW-DM), group that drank mineral water rich in magnesium hydrocarbonate and received a single ip injection of streptozotocin (100 mg/kg) in saline (0.9 % NaCl, 1 mL) (MW-DM).

Results: Regarding the biochemical parameters, a decrease was observed in the MW-C group for vitamin B_{12} and proteins, while triglycerides were higher compared to the TW-C group. By comparing the haemostatic biomarkers between TW-C and MW-C groups, a statistically significant decrease was found for fibrinogen, while the electrolyte analysis showed an increase in phosphates for the MW-C group. Biochemical value comparison between TW-DM and MW-DM groups showed that magnesium hydrocarbonate usage in diabetic rats did not significantly reduce glycaemia although the average glycaemic values were lower in the group treated with magnesium hydrocarbonate. Regarding the electrolyte values, a statistically significant decrease was observed for sodium, potassium and phosphate in the MW-DM group. The MW-DM group also showed a significant increase in iron value compared to TW-DM group.

Conclusion: Subchronic intake of magnesium hydrocarbonate-rich mineral water, as a form of magnesium supplementation, did not cause a significant improvement in glycaemia or normalisation of diabetes-induced dyslipidaemia. This study showed the reduction of fibrinogen value, thus indicating the possibility of usage of this form of magnesium supplementation in different pro-thrombogenic conditions.

Key words: Magnesium; Streptozotocin-induced diabetes; Cardiometabolic markers; Electrolytes; Rat.

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Introduction

Diabetes mellitus (DM) is one of the most important public health problems globally, with ever increasing incidence and prevalence.[1] Besides hyperglycaemia, which is the most frequent sign, diabetes is associated with impaired effects and secretion of insulin, metabolism of carbohydrates, lipids, as well as an increased risk for the development of various micro- and macrovascular complications.[2] Cardiovascular complications are one of the most important causes of death in patients with diabetes.[3] These complications represent the result of the pathological process of remodelling of the heart and blood vessels, which is directly induced by metabolic disorders that occur with diabetes, such as hyperglycaemia, dyslipidaemia, acid-base disturbances and the electrolyte levels.[4-6] The ensuing diabetic cardiomyopathy and coronary artery disease predisposes the myocardium for the development of the contractile dysfunction, ischaemic heart disease or various forms of the disturbance of the heart rhythm. Additionally, micro- and macrovascular angiopathy, as key pathoanatomic substrates in diabetes, induce lesions in other target tissues, such as brain, kidney or eyes.[7] Because of the consequences that it leaves on the cardiovascular system, diabetes should be viewed not just classically, as a metabolic disease, but also as a cardiovascular disease, because of which the modulation of the pathological process of remodelling of the cardiovascular system, the mechanism of which is not known yet, could be one of the main goals of the anti-diabetic therapy.[8]

Hypomagnesaemia is one of the most frequent electrolytic impairment seen in diabetic patients, especially in those ones with poorly regulated diabetes.[9-10] The decreased body concentration of magnesium (Mg^{2+}) is closely related to atherosclerosis, coronary artery disease and cardiac dysrhythmias.[11-13] However, although it is shown that use of magnesium in rats with experimentally induced diabetes results in modulation of insulin receptors and improvement of the metabolic balance, the role of magnesium in the process of cardiovascular remodelling remains unclear.[14] A problem of the revealing the concrete role that magnesium has in target tissues in the pathogenesis of diabetes is in the fact that tissue magnesium deficits are practically non-detectable, since Mg^{2+} is predominantly intracellular cation, bound to cellular components that does not readily pass into the extracellular fluid.[15] In addition to this, clinical hypomagnesaemia reflects the decrease in the concentration of ionised serum Mg^{2+} that cannot be used for estimation of the magnitude of its tissue deficit, since blood magnesium accounts for only 0.3 % of the total body magnesium.[16-21] Use of Mg^{2+} in a form of supplements can prevent the occurrence of subclinical deficits of intracellular magnesium and potentially postpone the occurrence of metabolic disbalance caused by conditions stressful for the organism, such as diabetes. It has been previously shown that the administration of magnesium as a supplement in rats in the early phase of diabetes leads to improved compliance of heart ventricles, as well as to the normalisation of the autonomous system of the myocardium, while the long-term effects of magnesium supplementation and their mechanisms remain unknown.[22]

The aim of this study was to ascertain the effect of subchronic intake of magnesium hydrocarbonate -rich mineral water, as a form of magnesium supplementation, on cardiometabolic markers and electrolyte levels in rats with streptozotocin-induced diabetes.

Methods

This research was done as a part of the project of the Ministry of Education, Science and Technological Development of the Republic of Serbia (No 200110/00402). Ethics consent was obtained from the Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia and based on the ethics approval issued by the Ethics Committee for the Protection of Welfare of Experimental Animals of the Faculty of Medicine, University of Belgrade. In this 6-week-long experimental study 25-30 days old and 160 g of weight male Wistar rats obtained from the vivarium of the Military Medical Academy in Belgrade were used. Rats were kept individually in plexiglas cages with constant ambient conditions (temperature 21 ± 2 ºC; humidity 55 ± 5 %; light-dark cycle of 12 h with the start of the light cycle at 07:30 h) and their daily food and water consumptions were registered. The animals were allowed access to food and water *ad libitum*. Diabetes mellitus was induced with a single injection of streptozotocin ($C_8H_{15}N_3O_7$, *Sigma-Aldrich, Darmstadt, Germany*) 100 mg/

kg) dissolved in physiological saline (0.9 % NaCl, 1 mL). Samples of blood from the tail vein were drawn 72 h after the streptozotocin injection and after that on a weekly basis. Rats were allowed to drink either the standard tap water or mineral water rich in magnesium hydrocarbonate (oligomineral, magnesium hydrocarbonate, natural spring mineral water "Mg Mivela" produced by *Nova Sloga d.o.o. Trstenik, Serbia*; with mineral composition (mg/L), cations: Mg^{2+} 343, Na^{+} 138, Ca^{2+} 21.670, K^{+} 9.510, anions: HCO_3^- 2109.400, Cl^- 13.400, SO_4^{2-} < 1.000, F^- 0.205, according to the manufacturer's declaration on the purchased products).

A total of 28 animals were divided into four groups of 7 each:

1. group that drank tap water and received a single ip injection of saline (0.9 % NaCl) (TW-C),
2. group that drank mineral water rich in magnesium hydrocarbonate and received a single ip injection of saline (0.9 % NaCl) (MW-C),
3. group that drank tap water and received a single ip injection of streptozotocin (100 mg/kg) in saline (0.9 % NaCl, 1 mL) (TW-DM),
4. group that that drank mineral water rich in magnesium hydrocarbonate and received a single ip injection of streptozotocin (100 mg/kg) in saline (0.9 % NaCl, 1 mL) (MW-DM).

After completion of a 6-week-long treatment, animals were sacrificed and their blood was collected for analyses of biochemical parameters in plasma and serum, including biomarkers of cardiac and neural injury: aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), creatine kinase (CK), high-sensitivity troponin T (hs-TnT), homocysteine (Hcy) and vitamin B_{12}; haemostatic biomarkers: fibrinogen, von Willebrand factor (vWF), vWF %; lipid profile: total cholesterol, low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol, triglycerides; pancreato-hepatorenal biomarkers: glucose, urea, creatinine, uric acid, proteins, albumin, alkaline phosphatase (ALP), amylase; as well as the ionogram (Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}, Fe^{2+}, HCO_3^-, Cl^-, PO_4^{3-}).

Analyses were performed by commercial kits (*Siemens Healthcare Diagnostics Ltd., Frimley, Camberley, UK*) on automated analyser (*Dimension Xpand, Siemens, Erlangen, Germany*), on atomic spectrophotometer and using spectrophotometric method. Concentration of glucose in blood drawn from tail vein was determined by use of ACCU-CHEK analyser (*Roche Diabetes Care, Inc, Indianapolis, USA*).

Statistical analysis

Statistical analysis was performed by using the GraphPad Prism 5 program. Normality of the data distribution was checked with Shapiro-Wilk test, since there were less than 50 units of observance within the groups, with values of the test $p > 0.05$ indicating the normal distribution of data. All the parameters in the present study were numeric and continual and, provided their distribution was normal, t-test for independent samples was used. In case that the data did not show normal distribution, Mann-Whitney test was used. Values of $p < 0.05$ were considered statistically significant.

Results

Comparison of the parameter values between the groups of rats subchronically treated with tap water (TW) or water rich in magnesium hydrocarbonate (MW)

Among the monitored biochemical parameters, values of the biomarkers of cardiac and neural injury (AST, ALT, LDH, CK, hs-TnT, Hcy) were not significantly decreased in the group of animals subchronically exposed to water rich in magnesium hydrocarbonate (Table 1). A significant decrease in the vitamin B_{12} levels was found (MW-C: 297.0 (288.0 – 359.0); TW-C: 400.0 (382.0 – 426.0)) ($p < 0.05$). Among the investigated haemostatic biomarkers, a significant decrease in fibrinogen levels was found in rats with subchronic intake of magnesium hydrocarbonate -rich mineral water (MW-C: 2.004 ± 0.063), in comparison with the control group who drank tap water (TW-C: 2.173 ± 0.177) (Table 1). Increase in the levels of triglycerides (MW-C: 1.110 ± 0.403; TW-C: 1.101 ±0.145) ($p < 0.05$) was detected in MW-C group compared to TW-C group (Table 1), while there was no significant difference between the lipid profiles (LDL and HDL cholesterol) of the two groups ($p > 0.05$). Decrease of protein levels (MW-C: 60.00 (57.00 – 60.00); TW-C: 61.00 (60.00 – 61.00)) ($p < 0.05$) (Table 1) was found in the MW-C group in comparison with the TW-C group of animals. After studying the ionograms

between the groups, an increase in the phosphate levels was found in the MW-C group (MW-C: 2.336 ± 0.199), as compared to the control group of rats that drank tap water (TW-C: 2.166 ± 0.056) (Table 2).

Table 1: Values of biochemical parameters in the control group of rats subchronically exposed to tap water (TW-C) or water rich in magnesium hydrocarbonate (MW-C)

Parameter	Groups		*p* value
	TW-C	MW-C	
Biomarkers of cardiac and neural injury			
AST [a]	172.286 ± 27.103	187.857 ± 46.323	0.386
ALT [a]	54.714 ± 6.184	57.429 ± 7.955	0.901
LDH [b]	4182 (3946 – 5807)	5371 (3332 – 6013)	0.482
CK [a]	6447.143 ± 1592.075	6642.333 ± 1731.076	0.932
hsTnT[a]	19.143 ± 9.547	21.500 ± 15.859	0.373
Hcy [a]	13.724 ± 3.597	14.200 ± 2.433	0.586
Vitamin B12 [b]	400.0 (382.0 – 426.0)	297.0 (288.0 – 359.0)	0.018 *
Haemostatic biomarkers			
Fibrinogen[a]	2.173 ± 0.177	2.004 ± 0.063	0.012 *
vWF[a]	0.324 ± 0.027	0.299 ± 0.031	0.600
vWF %[a]	200.086 ± 31.695	172.943 ± 34.829	0.661
Lipid profile			
Total cholesterol [a]	1.654 ± 0.237	1.479 ± 0.167	0.451
LDL cholesterol [a]	0.411 ± 0.156	0.357 ± 0.148	0.918
HDL cholesterol [b]	0.720 (0.690 – 0.750)	0.650 (0.610 – 0.720)	0.063
Triglycerides [a]	1.101 ± 0.145	1.110 ± 0.403	0.017 *
Pancreato-hepatorenal biomarkers			
Glucose [a]	6.600 ± 0.258	6.457 ± 0.597	0.057
Urea [a]	7.600 ± 0.686	7.200 ± 0.978	0.194
Creatinine [b]	47.00 (47.00 – 48.00)	47.00 (44.00 – 47.00)	0.246
Uric acid [b]	70.00 (62.50 – 70.00)	70.00 (60.00 – 100.00)	0.643
Proteins [b]	61.00 (60.00 – 61.00)	60.00 (57.00 – 60.00)	0.019 *
Albumin [b]	29.00 (28.00 – 30.00)	29.00 (29.00 – 30.00)	0.945
ALP [a]	299.000 ± 29.586	278.143 ± 43.071	0.239
Amylase [a]	1739.286 ± 219.445	1625.000 ± 275.451	0.696

a – Values are presented as mean ± SD. p < 0.05, t test;*
*b – Values are presented as median (25% – 75% percentile). * p < 0.05, Mann-Whitney test;*
AST: aspartate aminotransferase; ALT: alanine aminotransferase; LDH: lactate dehydrogenase; CK: creatine kinase; hsTnT: high-sensitivity troponin T; Hcy: homocysteine; vWF: von Willebrand factor; LDL: low density lipoprotein; HDL: high density lipoprotein (HDL); ALP: alkaline phosphatase

Table 2: Serum ion concentrations in the control groups of rats with subchronic intake of tap water (TW-C) or mineral water rich in magnesium hydrocarbonate (MW-C)

Parameter	Groups		*p* value
	TW-C	MW-C	
Na^+	140.140 ± 1.069	140.28 ± 1.380	0.351
K^+	5.350 ± 0.423	5.583 ± 0.542	0.789
Ca^{2+}	2.461 ± 0.046	2.470 ± 0.073	0.220
Mg^{2+}	0.896 ± 0.036	0.951 ± 0.055	0.246
Fe^{2+}	34.614 ± 5.189	45.314 ± 4.167	0.774 *
HCO_3^-	23.286 ± 1.604	22.714 ± 1.496	0.833
Cl^-	101.000 ± 1.633	101.000 ± 0.817	0.230
PO_4^{3-}	2.166 ± 0.056	2.336 ± 0.199	0.023

*Results are presented as mean ± SD. * p < 0.05, t test;*
Na^+: sodium; K^+: potassium; Ca^{2+}: calcium; Mg^{2+}: magnesium; Fe^{2+}: iron; HCO_3^-: bicarbonate; Cl^-: chloride; PO_4^{3-}: phosphate

Comparison of parameters between the experimental groups of rats with streptozotocin-induced diabetes mellitus subchronically exposed to tap water (TW-DM) or magnesium hydrocarbonate-rich water (MW-DM)

Comparison of the values of the biochemical parameters between groups, including biomarkers of cardiac and neural injury, haemostatic biomarkers and lipid profile, did not reveal any significant difference (p > 0.05) (Table 3).

Table 3: Values of biochemical parameters in experimental group of rats with streptozotocin-induced diabetes mellitus subchronically treated with tap water (TW-DM) of water rich in magnesium hydrocarbonate (MW-DM)

Parameter	Groups		*p* value
	TW-DM	MW-DM	
Biomarkers of cardiac and neural injury			
AST [a]	213.667 ± 29.092	223.000 ± 42.426	0.453
ALT [a]	118.333 ± 60.451	102.867 ± 47.894	0.566
LDH [b]	3484 (2589 – 4453)	4415 (2187 – 6305)	0.724
CK [a]	5985.667 ± 3266.870	3939.333 ± 1133.324	0.167
hsTnT[a]	39.000 ± 36.497	22.000 ± 20.753	0.209
Hcy [a]	5.653 ± 1.483	9.223 ± 5.735	0.075
Vitamin B12 [b]	511.0 (388.5 – 698.0)	417.0 (300.0 – 612.8)	0.386
Haemostatic biomarkers			
Fibrinogen[a]	2.780 ± 0.726	1.120 ± 1.160	0.282
vWF %[a]	278.300 ± 95.529	278.367 ± 54.658	0.355
Lipid profile			
Total cholesterol [a]	7.770 ± 7.632	7.375 ± 6.966	0.153
LDL cholesterol [a]	0.550 ± 0.673	0.760 ± 0.905	0.548
HDL cholesterol [b]	0.860 (0.570 – 0.990)	0.970 (0.370 – 1.10)	0.827
Triglycerides [a]	3.357 ± 4.016	3.260 ± 2.483	0.287
Pancreato-hepatorenal biomarkers			
Glucose[a]	30.813 ± 17.580	13.973 ± 5.337	0.102
Urea [a]	9.267 ± 1.222	11.133 ± 4.881	0.153
Creatinine [b]	62.00 (46.00 – 63.00)	54.00 (47.00 – 76.00)	0.827
Uric acid [b]	70.00 (70.00 – 70.00)	120.00 (100.00 – 140.00)	0.221
Proteins [b]	62.00 (56.00 – 66.00)	61.00 (56.00 – 134.00)	1.000
Albumin [b]	22.00 (20.00 – 27.00)	27.00 (22.00 – 30.00)	0.261
ALP [a]	1021.750 ± 503.407	1138.000 ± 680.090	0.634
Amylase [a]	1446.500 ± 332.591	1079.333 ± 355.213	0.935

a – Values are presented as mean ± SD;
b – Values are presented as median (25% – 75% percentile);
AST: aspartate aminotransferase; ALT: alanine aminotransferase; LDH: lactate dehydrogenase; CK: creatine kinase; hsTnT: high-sensitivity troponin T; Hcy: homocysteine; vWF: von Willebrand factor; LDL: low density lipoprotein; HDL: high density lipoprotein (HDL); ALP: alkaline phosphatase

In vivo treatment of rats with magnesium hydrocarbonate did not induce a significant decrease in serum glycaemia (p > 0.05), although the mean glucose concentrations in rats with diabetes tended to be lower in rats treated with magnesium hydrocarbonate (MW-DM: 13.973 ± 5.337) in comparison with the diabetic rats that drank tap water (TW-DM: 30.813 ± 17.580).

Table 4: Serum ion concentrations in the experimental group of rats with streptozotocin-induced diabetes mellitus subchronically treated with tap water (TW-DM) of water rich in magnesium hydrocarbonate (MW-DM)

Parameter	Groups		*p* value
	TW-DM	MW-DM	
Na^+	134.800 ± 4.902	125.330 ± 17.954	0.042 *
K^+	5.795 ± 0.434	5.423 ± 1.525	0.047 *
Ca^{2+}	2.620 ± 0.252	2.410 ± 0.099	0.316
Mg^{2+}	0.920 ± 0.053	1.000 ± 0.115	0.177
Fe^{2+}	31.000 ± 0.557	40.400 ± 22.345	0.000 **
HCO_3^-	29.333 ± 3.786	22.000 ± 5.657	0.349
Cl^-	92.000 ± 7.810	84.875 ± 9.852	0.774
PO_4^{3-}	2.077 ± 0.047	1.970 ± 0.516	0.042 *

*Results are presented as mean ± SD. * $p < 0.05$, ** $p < 0.001$, t test; Na^+: sodium; K^+: potassium; Ca^{2+}: calcium; Mg^{2+}: magnesium; Fe^{2+}: iron; HCO_3^-: bicarbonate; Cl^-: chloride; PO_4^{3-}: phosphate*

(Table 3). A significant decrease was found for sodium cations (MW-DM: 125.330 ± 17.954; TW-DM: 134.800 ± 4.902); and for potassium cations (MW-DM: 5.423 ± 1.525; TW-DM: 5.795 ± 0.434). The group of rats with streptozotocin-induced diabetes exposed subchronically to magnesium supplementation demonstrated an increase in the concentrations of iron ($p < 0.001$) (MW-DM: 40.400 ± 22.345) in comparison with the diabetic rats who drank tap water (TW-DM: 31.000 ±0.557). Phosphate anions were significantly decreased in MW-DM compared to TW-DM group (MW-DM: 1.970 ± 0.516; TW-DM: 2.077 ± 0.047). (Table 4).

Discussion

Experiments dealing with similar topic found that induction of diabetes with streptozotocin in rats resulted in the impaired glucose metabolism, change in the lipid profile, systemic oxidative stress as well as insulin resistance, because of which use of streptozotocin is considered a highly efficient prototype of diabetes mellitus.[23-25]

Certain results of the present study are in accordance with the literature data, since mean values of serum glycaemia, as well as of the main components of the lipid profile (total and LDL cholesterol, triglycerides) were significantly higher in groups of animals with streptozotocin-induced diabetes (MW-DM, TW-DM), in comparison with both control groups (MW-C, TW-C). Under the conditions of normal metabolism, glucose tolerance is attained by increased secretion of insulin in response to a postprandial hyperglycaemia registered by insulin receptors. Because of that, decreased secretion of insulin and/or loss of sensitivity of insulin receptors results in impaired tolerance to glucose that is found in rats with streprozotocin-induced diabetes.[26] Although the present study has shown the occurrence of hyperglycaemia in diabetic rats, subchronic intake of magnesium hydrocarbonate-rich mineral water has not reduced the glucose concentration. Contrary to that, others have demonstrated that a four-week-long magnesium supplementation leads to the reversal of insulin resistance, improvement of the insulin receptor sensitivity and normalisation of glucose metabolism in general.[27] Similar effect on glucose homeostasis was shown in healthy rats with body hypomagnesaemia and this finding led to the assumption that magnesium might be a natural sensitizer of insulin receptors, which warrants its use in chronic diabetic patients and explains the link between the daily intake of magnesium and the decrease in risk for development of type 2 diabetes mellitus in humans.[28-30]

Although the values of the components of lipid profile (total and LDL cholesterol, triglycerides) were higher in the groups of rats with streptozotocin-induced diabetes, the comparison between these values in rats with and without subchronic intake of magnesium hydrocarbonate-rich mineral water could not detect any significant difference. On the contrary, an increase in the triglyceride levels was found in the MW-C group, which is contradicted by the literature findings that use of magnesium in diabetic patients prevents or reverses the development of diabetes-induced dyslipidaemia.[27] Other studies have shown that the magnesium deficit plays a significant role in the development of lipid imbalance in the process of atherosclerosis, since endothelial cells under *in vitro* conditions and magnesium ion deprivation, express the nuclear factor-*kappa beta*, but also secrete the key factors of atherogenesis, including RANTES (Regulated on Activation, Normal T-cell Expressed and Secreted), IL-8, as well as the platelet-derived growth factor-BB (PDGF-BB), which together cause an impairment of the exchange of serum lipids between blood and the vessel wall, increase in the concentrations of serum triglycerides, accumulation of oxalate in arteries and decreased transport of cholesterol with HDL.[31-32] Other reports also suggest that the magnesium deficit directly affects the lipoprotein metabolism, which contributes to the progression

of atherosclerosis.[31] Although in some studies a decrease in the serum concentrations of HDL was found as a consequence of a decreased magnesium alimentary intake, the present study did not find a significant increase in the levels of HDL after subchronic intake of magnesium hydrocarbonate -rich mineral water between the control groups and groups with diabetes.[33]

The results of the present study have shown a decrease in the fibrinogen levels due to subchronic intake of magnesium hydrocarbonate -rich mineral water in the control group of rats without diabetes, which suggests that this form of magnesium supplementation may act protectively on the potential thrombosis. Similarly, in various experimental models it was demonstrated the correlation between the magnesium deficit and the general pro-thrombotic state.[31] Parsons and co-workers found that patients with previously diagnosed angina pectoris or previous acute myocardial infarction, had a significant decrease in mortality rate from 30 % to only 1 %, provided they received magnesium sulphate intramuscularly. The same study described the importance of use of magnesium as a therapeutic modality in cardiac patients, with the most prominent advantage being disinhibition of serum plasmin, an enzyme that plays the key role in the process of degradation of fibrin clots.[34] It was shown that use of magnesium in a form of a slow intravenous infusion slows down thrombosis after acute myocardial infarction and reduces the concentration of certain coagulation factors in pregnant women with pre-eclampsia.[35-36] It seems that the key mechanism that explains the anti-thrombogenic effect of magnesium is the inhibition of ADP-induced platelets aggregation.[37] This mechanism could explain the significant decrease in the levels of fibrinogen that results from the subchronic intake of magnesium hydrocarbonate-rich mineral water in the present study. Besides, Paolisso and co-workers also suggested that use of magnesium could reduce the hypercoagulability of platelets in patients with type 2 diabetes mellitus, which also explains the decrease of fibrinogen concentrations found in the present study.[38]

The comparison of the concentration of ions between the group of rats with diabetes showed a decrease in potassium as a consequence of the subchronic intake of magnesium hydrocarbonate -rich mineral water. Various publications showed causality of the connection among the serum concentrations of potassium and magnesium and that hypokalaemia occurs as a regular electrolyte abnormality in the preclinical phase of body hypomagnesaemia.[31] Thiazide diuretics, most frequently prescribed drugs in patients with congestive heart failure, cause an increased renal loss of both potassium and magnesium.[39-42] It was also shown that the normalisation of the intracellular concentrations of potassium in myocytes can be achieved exclusively by regulating the body hypomagnesaemia along with potassium supplementation, in spite of the fact that the supplements on their own cause the normalisation of serum potassium.[39, 43-44] Since use of water rich in magnesium hydrocarbonate brings about increase in the tissue magnesium depots and an increase in the intracellular potassium concentrations, transfer of potassium from extra- to intracellular compartment in order to replenish the tissue deficit could explain the decrease of serum potassium levels found in the present study.

One of the interesting findings in this experimental study was an increase in the serum iron due to subchronic intake of magnesium hydrocarbonate-rich mineral water in rats with diabetes. Similar studies published so far reported a reverse ratio between magnesium and iron levels in erythrocytes. It was hypothesised that magnesium supplementation acts protectively by bringing down the serum ferraemia, which in turn eliminates the additional oxidative tissue injury in diabetic patients.[45] Under physiological conditions, magnesium prevents the exposure of body to high serum iron concentrations by inhibiting the pathological process of haemolysis that would end in iron liberation from the erythrocytes into serum.[46] Some studies found that the decreased magnesium concentrations in erythrocytes were linked to the increased iron concentrations in various tissues and that the decreased alimentary intake of magnesium results in an increased intestinal absorption of iron, but also in decreased number of viable erythrocytes.[47] Under conditions of chronic body magnesium deprivation, erythrocytes with abnormal shape and function, prone to haemolysis, are formed, which explains a decrease in the haemoglobin levels and an increase in serum iron when body contains depleted depots of magnesium.[46-47]

In spite of some significant results, this experimental study has some limitations. An investigation in a larger group of rats is needed to investigate the effect of subchronic magnesium

supplementation on serum glycaemia and components of lipid profile in diabetes. Besides, a statistical comparison between the complementary control groups and groups of rats with diabetes should be performed to demonstrate the adequacy of the experimental prototype for type 2 diabetes mellitus.

Conclusion

The aim of this study was to ascertain the effects of subchronic intake of magnesium hydrocarbonate-rich mineral water on values of various cardiometabolic parameters, but also on the serum levels of ions in rats with an experimental model of diabetes. Investigation was organised with the assumption that *in vivo* supplementation with magnesium might be a potential therapeutic modality for improvement of levels of cardiometabolic markers in patients with diabetes. Although the results of this study demonstrated the existence of the increased values of serum glycaemia and the impairment of the lipid profile in diabetic rats, in comparison with the healthy controls, subchronic intake of magnesium hydrocarbonate -rich mineral water alone did not result in any significant changes. The results obtained are not completely in accordance with previous studies that reported that the use of magnesium supplementation resulted in the reversal of insulin resistance, improved sensitivity of insulin receptors and general improvement of the metabolism of glucose. The present study however showed that subchronic intake of magnesium hydrocarbonate-rich mineral water in the control group of rats, results in decreased concentrations of fibrinogen, which suggests possible modality in the treatment of various pro-thrombogenic conditions.

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Conflict of interest

None.

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