Changes on Erythrocytic and Serum Magnesium in Streptozotocin-Diabetic Rats after Maximal Exercise

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Zusammenfassung

Die vorliegende Studie befaßt sich mit der Analyse von Veränderungen in Serum und erythrozytärem Mg nach einem kurzen Schwimmtest bei diabetischen Ratten. Die Tiere wurden jeweils 1 von 4 Gruppen zugeteilt: Kontrollgruppe in Ruhe (CR), Kontrollgruppe + Schwimmtest (CE), diabetische Gruppe in Ruhe (DR) und diabetische Gruppe + Schwimmtest (DE). Der experimentelle Diabetes wurde mittels intraperitonealer Injektion mit Streptozotozin (STZ) (60 mg/kg) erzeugt. Serumglukose, Serum-Mg, Säure-Basen-Status, Hämatokrit (Ht) und erythrozytäre Mg-Konzentrationen wurden gemessen. Nach der Test-Übung stieg die Serum-Mg-Konzentration in beiden Gruppen an (CE und DE); ein höherer Anstieg wurde bei den normalen Ratten festgestellt (+49% gegen +20%). Im Gegensatz dazu nahm der erythrozytäre Mg-Spiegel nach der Übung ab, besonders bei den normalen Ratten (CE) (-15% gegen -9%). Im Ruhezustand war der erythrozytäre Mg-Spiegel bei den diabetischen Tieren (DR) ($p < 0{,}01$) niedriger als bei der Kontrollgruppe (CR). Die durch diese Studie gewonnenen Daten lassen darauf schließen, daß bei unkompensierten diabetischen Ratten die erythrozytären Mg-Konzentrationen niedriger sind im Vergleich zur Kontrollgruppe, bei ermüdender Übung leicht abnehmen und die im Serum-Mg-Spiegel verursachten Veränderungen geringer sind als bei der Kontrollgruppe, was wahrscheinlich auf die kürzere Schwimmzeit zurückzuführen ist.

Summary

The present paper was designed to analyze the changes on serum and erythrocytic Mg after a short term exercise in diabetic rats. The animals were assigned to 1 of 4 experimental groups: control at rest (CR), control plus exercise (CE), diabetic at rest (DR), and diabetic plus exercise (DE). Experimental diabetes was produced by a single intraperitoneal injection of streptozotocin (STZ) (60 mg/kg). Serum glucose, serum Mg, acid-base status, hematocrit (Ht) and erythrocytic Mg concentrations were determined. After exercise, serum Mg concentration increases in both groups (CE and DE); the higher increase is found in normal rats (+49% versus +20%). In contrast, the erythrocytic Mg level decreases after exercise, more so in normal rats (CE) (-15% versus -9%). At rest, the levels of erythrocytic Mg are lower in diabetic animals (DR) ($p < 0.01$) than those found in control rats (CR). The data found in this paper, suggest that in uncompensated diabetic rats, erythrocytic concentrations of Mg are diminished in relation to the control group, and fall slightly with exhaustive exercise, and variations induced in serum Mg levels are lower than in control rats, due probably to the shorter swimming time.

Résumé

Le présent travail a été dessiné pour analyser les changes du Mg sérique et erythrocytaire en rats diabétiques après un exercice maximum. Les rats ont été divisés dans 4 groupes: rats contrôle au répos (CR), rats contrôle après l'exercice (CE), rats diabétiques au répos (DR) et rats diabétiques après l'exercice (DE). Le diabète a été produit avec une seule injection intrapéritonéale de streptozotocine (STZ) (60 mg/kg). Nous avons déterminé le glucose sérique, le Mg sérique, le status acide-bacique, l'hématocrite et le Mg érythrocytaire. Après les exercises, la concentration de Mg sérique a été augmentée dans les rats contrôle et diabétiques (CE et DE). Par contre, le Mg érythrocytaire a été diminué après l'exercice, proportionalement plus dans les rats contrôle (CE). Au répos, le taux du Mg érythrocytaire a été plus bas dans les diabétiques (DR) que dans les rats contrôle (CR). Les données trouvées dans ce travail indiquent que dans les rats diabétiques incompensatés les concentrations de Mg érythrocytaire ont été diminuées par rapport au groupe contrôle, et dimuées légèrement avec l'exercice exhaustive. Les variations provoquées dans le Mg sérique ont été plus diminuées que dans les rats contrôle, probablement dû au temps de nage des rats plus court.

Introduction

Magnesium is involved in several metabolic processes. It is necessary for the synthesis and utilization of rich energy compounds and plays an important role in carbohydrate metabolism in general and particularly on insulin actions [1]. It has been suggested that there is an association between diabetes and magnesium [1, 2, 3]. In this sense, hypomagnesemia remains a characteristic of a significant number of human and experimental diabetic studies [1, 2]. Hypomagnesemia may play a role in several problems observed in patients with diabetes mellitus such as hypertension, retinopathy, altered platelet function and abnormal glucose disposition. Moreover, in 1992 the American Diabetes Association (ADA) released a consensous statement regarding magnesium supplementation in the treatment of diabetes mellitus [3].

Physical training has sometimes, but not always, resulted in an improved glucose homeostasis [4]. *Goodyear* et al. [5] proposed that exercise training may

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improve glucose homeostasis in animals with milder degrees of diabetes. Substrate utilization and glucose homeostasis during exercise are controlled by the effects of changes in insulin, glucagon and the catecholamines [6]. Uncompensated diabetes is also an agent provoking Mg deficiency [7].

The experiences in the last years have shown that the relation between physical acitivity and magnesium involves different aspects [8, 9]. *Haralambie* [10] and *Olha* et al. [11] have shown that a long duration physical effort in men produces a decrease of extracellular Mg due to a transient shift between extracellular and intracellular Mg compartments. Other authors [12, 13] have informed that anaerobic exercise leads to a transient decrease of plasmatic Mg accompanied with the increase of Mg in red blood cells and of urinary Mg excretion.

Taking into account these facts and as there is a lack of information concerning magnesium behaviour after exercise in diabetes, we have initiated such studies using the streptozotocin-diabetic (STZ) rats. The present paper was designed to analyze the changes on serum and erythrocytic Mg after a short term exercise in diabetic rats.

Material and Methods

40 male Wistar rats were used, weighing 240 g ± 10 g at the beginning of this experiment. Animals were housed separately in groups of 10 rats (n = 10) in plastic cages (5 rats/cage), and were allowed to acclimate to the vivarium which was maintained automatically on a 12 hour light-dark cycle at a temperature of 21 °C. The animals were assigned to 1 of 4 experimental groups: control at rest (CR), control plus exercise (CE), diabetic at rest (DR), and diabetic plus exercise (DE). Experimental diabetes was produced by a single intraperitoneal injection of streptozotocin (STZ) (60 mg/kg). Before the injection, streptozotocin was freshly prepared in a 0.10 M citrate buffer solution that was adjusted to a pH of 4.5. The rats were assigned to the diabetic group after blood glucose levels from the caudal vein (under anaesthesia light) had been measured (3 days after injection STZ); they were then pair matched by blood glucose concentrations. 1 member of each pair was assigned to the DR group, and its partner was assigned to the DE group. 30 days after injection of streptozotocin, the animals of groups CE and DE (5 at one time) were forced to acute exercise (swimming) until exhaustion (defined by no swimming for 15 seconds), in a tank (50 × 70 × 100 cm) with water maintained at a constant temperature (25°C) using a thermostat. At the end of the experiment the animals were withdrawn from the bath and immediately anaesthetized (pentobarbital sodium 5 mg/100 g). Rectal temperature (RT) was measured and arterial samples were collected in plastic tubes free from mineral elements. Following centrifugation at 1 500 × g, 10 minutes, serum was recovered.

Arterial blood samples for acid base determinations (ABL-330 Radiometer) were kept in icewater until they were analyzed. The following variables were measured: pH, pCO_2, HCO_3^-, SBE (base excess) and $Sat.O_2$ (Oxygen saturation). Aliquots were transferred into 2 different tubes: a) polypropylene tube with no anticoagulant for serum analyses, b) lithium heparin tube for Mg determination in erythrocytes. Glucose was assayed by colorimetric procedures (autoanalyzer Hitachi 705), hematocrit (Ht) value by micromethod (centrifuge) and serum and erythrocytic Mg concentrations by atomic absorption spectrophotometry (Perkin Elmer 272). The determination of intraerythrocytic Mg was performed as follows.

Whole blood was hemolyzed by dilution with deionized water, mixed Vortex and then frozen. After Mg determination in whole blood by atomic absorption spectrophotometry, erythrocyte element concentration was estimated according to the following formula:

$$Mg_{erythrocyte} = Mg_{whole\ blood} - (1 - Ht) \times Mg_{serum}/Ht$$

Statistical analysis: Data are presented as the mean ± SD. The two-way analysis of variance (significant for $p < 0.05$) and Student Newman-Kleus multiple test were used to analyze the data obtained, after checking the normality of their distribution by the Kolmogorow-Smirnov test. Statistical significance was considered $p < 0.05$.

Results

Fig. 1 shows the results of different biochemical parameters analyzed in normal and diabetic rats at rest and after exercise. After exercise, serum Mg concentration increases in both groups (CE and DE); the higher increase is found in normal rats (+49% versus + 20%). In contrast, the erythrocytic Mg level decreases after exercise, more in normal rats (CE) (-15% versus -9%). At rest, the levels of erythrocytic Mg are lower in diabetic animals (DR) ($p < 0.01$) than those found in control rats (CR).

Metabolic acidosis was observed after exercise in both groups, CE and DE (tab. 1). The bicarbonate decrease in diabetic rats with respect to control groups. The control group of diabetic rats shows a compensated metabolic acidosis at rest. The diabetic rats at rest (DR) exhibit a decrease in the pCO_2 compared with the control group at rest (CR). The same variation occurs between the groups under exercise (DE versus CE), although the decrease is not significant.

The glucose serum levels (tab. 2) confirm the diabetic condition in the group treated with STZ. The glucose concentration decreases significantly ($p < 0.001$) after exercise in normal rats (CE) but not in diabetic animals (DE versus DR). The swimming time (see tab. 2) until exhaustion is significantly less ($p < 0.001$) in diabetic rats compared to the normal rats. The hematocrit increase after maximal exercise in both groups, normal (CE) and diabetic rats (DE). Rectal temperature shows similar changes in normal and diabetic rats.

Discussion

Diabetes is a pathological condition in which secondary magnesium deficit occurs [1, 2]. Also, magnesium deficit can be induced by insufficient intake (Mg deficiency) or by a dysregulation on Mg metabolism (depletion) [3] and Mg deficit observed after exercise may

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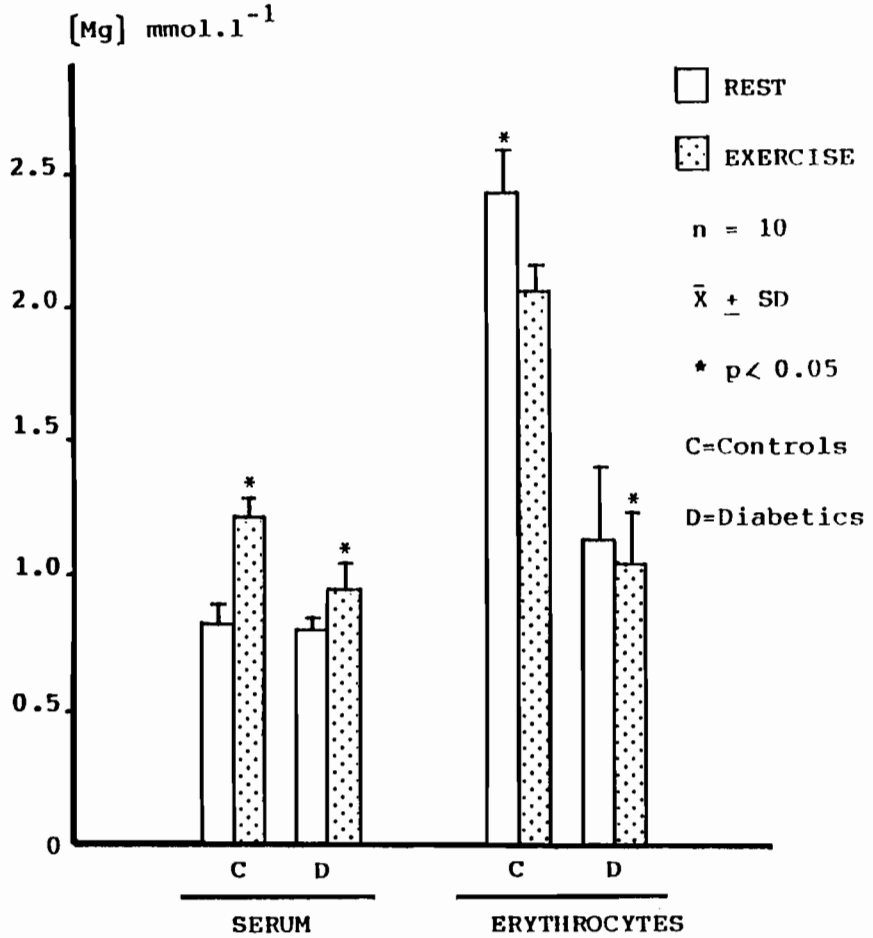

Fig. 1: Changes in serum and erythrocytic Mg from control (CR, CE) and diabetic (DR, DE) groups. Data are expresed as $\bar{x} \pm$ SD (n = 10). Significantly differences * = $p < 0.05$.

Tab. 1: Variations in acid-base parameters ($\bar{x} \pm$ SD) in normal and diabetic (STZ) rats at rest and after exercise (n = 10). Significant differences for $p < 0.05$: *CE versus CR and DE versus DR; [a]: DR versus CR; [b]: DE versus CE.

	Control		Diabetics	
	Rest (CR)	Exercise (CE)	Rest (DR)	Exercise (DE)
pH	7.39 ±0.03	7.18 ± 0.09*	7.37 ± 0.06	7.19 ± 0.07*
pCO_2 (mm Hg)	40.5 ±4.72	38.1 ± 10.1	34.3 ± 6.4	31.3 ± 14.7
HCO_3^- (mmol · l^{-1})	24.6 ±1.43	16.5 ± 4.3*	19.8 ± 2.1[a]	17.3 ± 3.04
SBE# (mmol · l^{-1})	0.36 ±0.78	-9.5 ± 5.4*	-4.0 ± 2.0[a]	-9.9 ± 13*
Sat.O_2 (%)	95.8 ±0.98	99.1 ± 0.28*	93.3 ± 4.4	98.7 ± 0.6*

SBE: Base excess

Tab. 2: Changes in serum glucose, hematocrit, rectal temperature and swimming time in normal and diabetic rats, at rest and after exercise ($\bar{x} \pm$ SD) (n = 10). Significant differences for $p < 0.05$: *CE versus CR and CE versus DR; [a]: DR versus CR; [b]: DE versus CE.

	Control		Diabetics	
	Rest (CR)	Exercise (CE)	Rest (DR)	Exercise (DE)
Glucose (mg/dl)	171 ± 22.1	131.0 ± 19.7*	403.0 ± 146[a]	392.0 ± 133[b]
Hematocrit (%)	42.55 ± 1.19	47.41 ± 1.74*	43.37 ± 1.83	47.52 ± 1.22*
Rectal T (°C)	37.0 ± 1.2	27.0 ± 1.5*	37.1 ± 1.1[a]	27.2 ± 1.6 *
Swimming time (min)		118 ± 30		42 ± 7.8[b]

depend, at least in part, of these mechanisms [14]. Since 99% of total body Mg is located in the intracellular stores or in bone, plasma Mg levels do not always reflect intracellular or total body Mg stores; however plasmatic Mg is determined systematically in all studies. Erythrocyte Mg concentrations reflect chronic Mg status [1].

The exercise-induced changes on Mg metabolism lead to modifications both on serum and erythrocyte (RBC) Mg concentrations [8–14]. Previously, it has been communicated increases in plasmatic Mg in normal rats after swimming until exhaustion [15–19]. The results presented in this paper, concerning the increases of plasmatic Mg after strenuous exercise in normal and in diabetic rats, are in agreement with these results previously reported. Other authors [16] have shown that in rats, a treadmill test produce no significant modification in RBC Mg, but significantly reduces erythrocyte Mg in animals fed with a deficient diet in this element [16]. Most of authors [11, 12, 17–19], have found increases in serum Mg after exercise, with decreases in the erythrocytic concentrations. In general, clinical data reveal that whereas short-term, high intensity exercise leads to hypermagnesemia, prolonged submaximal exercise is accompanied by hypomagnesemia [16]. However, in relation to the variations of erythrocytic Mg concentrations after exercise, some authors have reported increases [13, 20, 21] and in other studies the RBC Mg does not change [12, 22]. The decrease in RBC Mg may depend on adaptation to exercise; the better the subject is adapted, the smaller the variation in Mg [19].

In relation to RBC Mg, several clinical studies have shown reduced RBC Mg concentration of diabetic [2, 23, 24]. Moreover, experimental diabetes studies have reported significantly depressed RBC Mg concentrations in diabetic mice and in rabbits [25, 26]. Our results show a significant decrease of RBC Mg in diabetic rats, both at rest and after exercise, and are in agreement with those before mentioned. On this sense *Paolisso* et al. reports that the insulin carency impairs erythrocyte magnesium accumulation of diabetics.

However, in other studies no changes have been observed [1, 2].

These results of hypermagnesemia leads to a Mg depletion, which have been explained by several factors. Hypermagnesemia appears as the consequence of a decrease in plasma volume [27], the shift between the cellular and extracellular compartments resulting from acidosis and muscle contraction [9]. Moreover, contributing to Mg depletion, the exercise-induced acidosis also provokes the release of Mg bound to proteins [11] and the exercise rise in blood lactic acid, which causes an increase in plasma phosphorus and metabolic acidosis, which in turn, provokes magnesiuria by reducing renal tubular reabsorption of Mg [7, 28]. In this paper, hematocrit and pH variations are similar in both groups (normal and diabetic rats) after exercise, so these factors can not be considered to explain the serum and erythrocytic Mg variations found in the diabetic rats.

Another interesting factor responsible for the changes in serum Mg during exercise is work duration. The increase in serum Mg after exercise is lower in the diabetic group (DE), which could be attributed to the shorter swimming time and to the greater decline in the cellular stocks of Mg (decreased erythrocytic values) in diabetics [29]. Other factors such as sweat losses [14], and stress [1, 14] are involved in Mg depletion. Physical exercise produces an alarm reaction, a stress situation that can impair magnesium homeostasis by 2 ways: a) stress increases ADH, thyroid hormones and corticoid hormones that are reported to cause hypermagnesiuria by reducing tubular reabsorption of Mg [11, 17], b) stress replaces hypersecretion of physiological doses of adrenaline by large amounts of catecholamines that causes hypomagnesemia [1, 14]. On the other hand, rhabdomiolysis that occurs during strenuous physical effort would also contribute to Mg losses [14]. First, during exercise Mg fluxes from muscle to serum causing hypermagnesemia. After exercise, when urinary excretion increases and reaches levels higher than those measured before exercise [30], hypermagnesemia turns into hypermagnesuria.

The shortening in swimming time in diabetic rats (42 ± 7.8 min), accounted only for 35.6% of the swimming time performed by control exercise rats (118 ± 30 min). This fact could be a consequence of the morphological and biochemical alterations produced in the lungs of diabetic rats [31]. Other alterations in the values of several enzymes have been also reported [32, 33] which may affect the energetic metabolism and finally, the baseline acidosis in diabetic rats, which suggests a lower capacity to buffer the lactic acid produced during exercise.

The data found in this paper, suggest that in uncompensated diabetic rats erythrocytic concentrations of Mg are diminished in relation to the control group, and fall slightly with exhaustive exercise, and variations induced in serum Mg levels are lower than in control rats, due probably to the shorter swimming time.

References

[1] *Durlach, J.:* Magnésium in clinical practique. John Libbey, London 1988.

[2] *Garland, H. O.:* New experimental data on the relationship between diabetes mellitus and magnesium. Magnesium Res. **5** (1992) 193–202.

[3] *White, J. R.; Campbell, R. T.:* Magnesium and diabetes: A review. Ann. Pharmacother. **27** (1993) 775–780.

[4] *Horton, E. S.:* The role and management of exercise in diabetes mellitus. Diabetes Care **11** (1988) 201–211.

[5] *Goodyear, L. J.; Hirshman, M. F.; Knutson, S. M.; Horton, E. D.; Horton, E. S.:* Effect of exercise training on glucose homeostasis in normal and insulin-deficient diabetic rats. J. Appl. Physiol. **65** (1988) 844–851.

[6] *Wasserman, D. H.; Abumrad, N. N.:* Physiological bases for the treatment of the physically active individual with diabetes. Sports Med. **7** (1989) 376–392.

[7] *Durlach, J.:* Magnesium in clinical practice. Highlights of the 5th International Magnesium Symposium. Drugs Today **25** (1989) 279–283.

[8] *Niquet, G.; Milbled, G.; Guerrin, F.:* Magnésium et sport. Larc. Med. **3** (1984) 165–172.

[9] *Riche, D.:* Les déficits en magnésium et sport. Science & Sports **4** (1989) 41–52.

[10] *Haralambie, G.:* Electrolytes, trace-elements and vitamins in exercise. Med. Sport. **13** (1981) 134–152.

[11] *Olha, A. E.; Klissouras, V.; Sullivan, J. D.; Skoryna, S. C.:* Effect of exercise on concentration of elements in the serum. J. Sports Med. **22** (1982) 414–425.

[12] *Deuster, P. A.; Dolev, E.; Kyle, S. B.; Anderson, R. A.; Schoomaker, E. B.:* Magnesium homeostasis during high-intensity anaerobic exercise in men. J. Appl. Physiol. **62** (1987) 545–550.

[13] *Córdova, A.:* Changes on plasmatic and erythrocytic magnesium levels after high-intensity exercise in men. Physiol. Behav. **52** (1992) 819–821.

[14] *Rayssiguier, Y.; Guezennec, C. Y.; Durlach, J.:* New experimental and clinical data on the relationship between magnesium and sport. Magnesium Res. **3** (1990) 93–102.

[15] *Córdova, A.; Gimenez, M.; Escanero, J. F.:* Changes of plasma zinc, copper and magnesium, and acid-base balance at different times of swimming until exhaustion in the rat. J. Trace Elements Electrolytes Health Dis. **4** (1990) 189–193.

[16] *Laires, M. J.; Rayssiguier, Y.; Guezennec, C. Y.; Alves, F.; Halpern, M. J.:* Effect of magnesium deficiency on exercise capacity in rats. Magnesium Res. **2** (1989) 136.

[17] *Golf, S. W.; Happel, O.; Graef, V.:* Plasma aldosterone, cortisol and electrolyte concentration in physical exercise after magnesium supplementation. J. Clin. Chem. Clin. Biochem. **22** (1984) 717–721.

[18] *Joborn, H.; Akerstrom, G.; Ljunghall, S.:* Effects of exogenous catecholamines and exercise on plasma magnesium concentrations. Clin. Endocrinol. **23** (1985) 219–226.

[19] *Vizinova, H.; Stejskal, P.; Steidl, L.:* Serum Mg after maximal effort and its relation to some indicators of physical fitness. Cas. Lek. Cesk. **122** (1983) 18–21.

[20] *Refsum, H. E.; Treit, B.; Meen, H. D.; Stromme, S. B.:* Serum electrolyte, fluid and acid-base balance after prolonged heavy exercise at low environmental temperature. Scand. J. Clin. Lab. Invest. **32** (1973) 117–122.

[21] *Stromme, S. B.; Stensvold, I. C.; Meen, H. D.; Refsum, H. E.:* Magnesium metabolism during prolonged heavy exercise. In: *Howald, H.; Poortmans, J. R.* (eds.): Metabolic adaptation to prolonged physical exercise. Birkhäuser Verlag, Basel 1975, pp. 361–366.

[22] *Hespel, P.; Lijnen, P.; Fiocchi, R.; Denys, B.; Lissens, W.; Kabangu, J.; Amery, A.:* Cationic concentrations and transmembrane fluxes in erythrocytes of humans during exercise. J. Appl. Physiol. **61** (1986) 37–43.

[23] *Vanroelen, W. F.; Van Gaal, L. F.; Van Rooy, P. E.; De Leeuw, I. H.:* Serum and erythrocyte magnesium levels in type Y and type II diabetics. Acta Diabetol. Lat. **22** (1985) 185–190.

[24] *Paolisso, G.; Sgambato, S.; Giugliano, D.* et al.: Impaired insulin-induced erythrocyte magnesium accumulation is correlated to impaired insulin mediated glucose disposal in type 2 (non-insulin-

dependent) diabetic patients. Diabetologia **31** (1988) 910–915.

[25] *Bhimji, S.; Godin, D. V.; McNeill, J. H.:* The status of magnesium in rabbits with experimentally-induced diabetes mellitus. Horm. Metab. Res. **18** (1986) 734–738.

[26] *Nagase, N.; Saijo, Y.; Nitta, H.* et al.: Myocardial disorders caused by magnesium deficieny in diabetic KK mice. Magnesium **8** (1989) 307–315.

[27] *Costill, D. L.; Cote, R.; Fink, W.:* Muscle water and electrolytes following varied levels of dehydration in man. J. Appl. Physiol. **40** (1976) 6–11.

[28] *O'Connor, L. R.; Klein, K. L.; Bethune, J. E.:* Hyperphosphatemia in lactic acidosis. N. Engl. J. Med. **297** (1977) 707–709.

[29] *Refsum, H. E.; Treit, B.; Meen, H. D.; Stromme, S. B.:* Serum electrolyte, fluid and acid-base balance after prolonged heavy exercise at low environmental temperature. Scand. J. Clin. Lab. Invest. **32** (1973) 117–122.

[30] *Lijnen, P.; Hespel, P.; Fagard, R.; Lysens, R.; Van den Eynde, E.; Amery, A.:* Erythrocyte plasma and urinary magnesium in men before and after a marathon. Eur. J. Appl. Physiol. **58** (1988) 252–256.

[31] *Sahebjami, H.; Denholm, D.:* Effects of streptozotocin-induced diabetes on lung mechanics and biochemistry in rats. J. Appl. Physiol. **64** (1988) 147–153.

[32] *Armstrong, R. B.; Gollnick, P. D.; Inauzzo, C. D.:* Histochemical properties of skeletal muscle fibers in streptozotocin-diabetic rats. Cell Tissue Res. **162** (1976) 387–394.

[33] *Armstrong, R. B.; Ianuzzo, C. D.:* Decay of succinate dehydrogenase activity in rat skeletal muscle following streptozotocin injection. Horm. Metab. Res. **8** (1976) 392–394.

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