Protective Effects of Magnesium on Release of Proteins from Muscle Cells during a Marathon Run

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Zusammenfassung

Die Konzentrationen von Myoglobin, Gesamteiweiß und Harnstoff sowie die Aktivitäten der Kreatinkinase (CK), Aspartat-Aminotransferase (GOT) und Alanin-Aminotransferase (GPT) wurden im Plasma von Leistungssportlern direkt vor und nach einem Marathonlauf gemessen.
Während des Laufes stiegen alle Parameter im Plasma an. Im Fall der Enzyme (Myoglobin, CK, GOT und GPT) war das Ausmaß des Anstiegs im Plasma von dem Molekulargewicht des Proteins abhängig; als Ausschlußgröße wurde ein Molekulargewicht von 105 000 Daltons berechnet.
Die beschriebenen Veränderungen der Parameter im Plasma waren von der Supplementierung der Leistungssportler abhängig. Eine 3 Wochen dauernde Magnesium-Supplementierung vor dem Lauf verursachte eine signifikante Abnahme des Proteinverlustes aus den Zellen in den extrazellulären Raum. Gleichzeitig wurde die Ausschlußgröße des Molekulargewichtes auf 95 000 Daltons vermindert.
Die Harnstoffbildung stieg während des Laufes um 70 mg/l Plasma an. Dieser Wert entspricht einem Abbau von 23.3 mmol Aminosäuren/l Plasma. Eine Magnesium-Supplementierung verminderte den Aminosäure-Abbau auf 13.3 mmol/Liter Plasma.

Summary

Myoglobin, total protein and urea concentrations as well as creatine kinase (CK), aspartate aminotransferase (GOT) and alanine aminotransferase (GPT) activities were measured in plasma of competitive athletes just before and after a marathon run.
All plasma parameters increased during the run. In case of myoglobin, GOT, GPT and CK, increase in plasma concentration was dependent on molecular weight. The exclusion molecular weight was 107 000 daltons.
The changes in plasma concentrations described above were dependent on supplementation of athletes. A three week magnesium supplementation prior to the run resulted in a slowing down of protein release from the cells to the plasma.
At the same time, a regression analysis of increase of protein concentration in plasma resulted in a change of exclusion molecular weight to 95 000 daltons.
Urea formation increased during the race by 70 mg/l. This corresponds to 23,3 mmol/l amino acid degradation. Magnesium supplementation lowered amino acid degradation to 13,3 mmol/l.

Résumé

On a mesuré chez des athlètes de compétition, juste avant et aprés un marathon, les concentrations de myoglobine, de protéines et d'urée ainsi que les taux de créatine kinase (CK), d'aspartate aminotransférase (GOT) et d'alaine aminotransférase (GPT) dans le plasma.
Tous les paramètres plasmatiques ont augmenté durant la course. Les augmentations des concentrations plasmatiques de la myoglobine, de la GOT, de la GPT et de la CK ont dépendu des poids moléculaires des protéines. Le poids moléculaire d'exclusion était de 107 000 daltons.
Les modifications de concentrations plasmatiques décrites ci-dessus ont été fonction du traitement de supplémentation des athlètes. Une supplémentation en magnésium au cours des trois semaines précédant la course a permis de ralentir le passage des protéines des cellules au plasma.
Une analyse de régression de l'augmentation de la concentration des protéines dans le plasma a montré dans le même temps une modification à 95 000 daltons du poids moléculaire d'exclusion.
L'azotémie s'est élévée à 70 mg/l au cours de l'épreuve, ce qui correspond à une dégradation des acides aminés de 23,3 mmol/l. La supplémentation en magnésium a réduit cette dégradation à 13,3 mmol/l.

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Introduction

Magnesium is an essential cofactor in numerous biochemical processes, especially in those processes, which are located in energy metabolism involving transphosphorylation reactions [1]. One of the complexes, where magnesium is needed, is the maintenance of cell membrane function[2]. In physical stress situations, such as a marathon run, cell proteins are released to the extracellular fluid and to the plasma [3,4]. The reason for this leakage is still nuclear.
A magnesium deficiency might be a reason for this protein loss from muscle cells to the extracellular fluid. It is known that magnesium concentration in plasma is decreased during physical exercise [5,6,7], caused by an increased magnesium uptake during the exercise, but also caused by a loss of magnesium in sweat [8,9]. This loss in sweat might be significant [9], especially in a long time physical exercise [4]. Since magnesium is essentially necessary for maintenance of membrane permeability by supporting activity of membrane

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ATPase [2], a magnesium deficient membrane might be functionally impaired.

Methods

45 Competitive athletes were assigned in equal numbers to three groups. 21 Days prior to a marathon race, group two received a 20 mmol per day magnesium aspartate hydrochloride (Verla Pharm) supplementation; group three received 18,6 mmol per day magnesium oxide in combination with 1 500 IU dl-alpha-tocopherol (APS Starke). Group one served as control. One hour before the run, and one minute after completion of the race a sample of venous blood was obtained. The blood was allowed to clot, then centrifuged, and the serum removed and frozen until analysis.

Determination of reference values for catalytic concentration of CK in serum was carried out with 353 blood samples from female and 463 male, apparently healthy students (20–30 years old).

The weather was fair with a temperature range between 17° C and 24° C. The fluids ingested during the run consisted of electrolyte solutions (Isostar®) containing 0,925 mmol magnesium ions per liter; these were supplied ad libitum.

Samples were analyzed for CK-, GOT- and GPT-activities, protein and urea concentration using the automated photometric determination by the Hitachi 705 and commercially available test reagents. Serum and urine magnesium was determined by atomic absorption spectrometry (Evans Electroselenium, SussexUK). Myoglobin concentration was assayed using a radioimmunoassay from IDW Sprendlingen, W-Germany.

Statistical calculations were carried out using the t-test with paired and unpaired data.

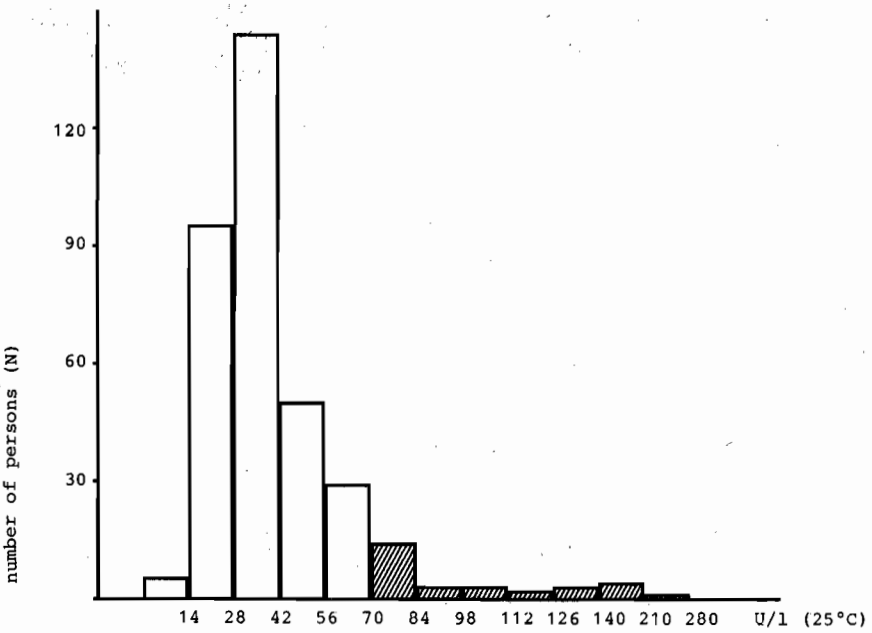

Fig. 1: Distribution of CK-activities in healthy women (N = 343), 20 to 30 years old. ▨ pathologic range

Results

Reference values for CK

Fig. 1 and 2 showed distribution of CK activities in serum of apparently healthy female and male students (20–30 years old). 24,8 % of male and 7 % of female students contained pathologic CK catalytic concentrations in serum.

Blood parameters in marathon runners

CK-, GOT- and GPT-activities as well as concentration of total protein, myoglobin and urea in serum of marathon runners just before and after the race are shown in *Tab. 1.*

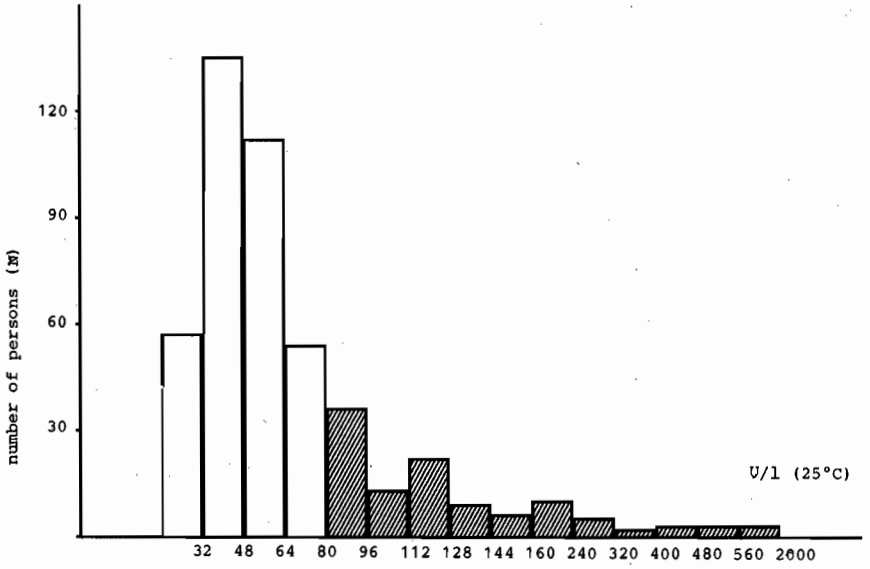

Fig. 2: Distribution of CK-activities in healthy men (N = 463), 20 to 30 years old. ▨ pathologic range

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Tab. 1: Data before and after the marathon race. Values are given as mean ± standard deviation. The P-values were obtained by comparison of corresponding values (before/after) with control values (before after)

	control		Mg-Asp-HCl				MgO-Vit E			
	before	after	before	P	after	P	before	P	after	P
Myoglobin (ng/ml)	67 ± 45	927 ± 931	51 ± 15	≤ 0.20	635 ± 341	≤ 0.20	48 ± 28	≤ 0.15	827 ± 621	≤ 0.40
Creatine kinase U/l	67 ± 31	202 ± 163	47 ± 18	≤ 0.02	112 ± 38	≤ 0.05	49 ± 18	≤ 0.05	127 ± 40	≤ 0.10
GOT (U/l)	17 ± 4	23 ± 6	14 ± 3	≤ 0.10	20 ± 3	≤ 0.15	15 ± 3	≤ 0.15	21 ± 3	≤ 0.30
GPT (U/l)	15 ± 4	17 ± 4	14 ± 3	≤ 0.25	13 ± 3	≤ 0.01	15 ± 3	≤ 0.40	14 ± 4	≤ 0.01
Total protein (g/l)	77 ± 4	78 ± 3	74 ± 4	≤ 0.25	75 ± 3	≤ 0.01	74 ± 3	≤ 0.05	74 ± 3	≤ 0.01
Urea (mg/l)	320 ± 70	420 ± 80	320 ± 80	≤ 0.50	360 ± 70	≤ 0.03	340 ± 80	≤ 0.35	370 ± 80	≤ 0.15
weight of athletes (kg)	68 ± 7	68 ± 8	68 ± 8		66 ± 8		70 ± 8		68 ± 7	
Time for the race (hour. min ± min)	3.10 ± 30		3.25 ± 25				3.06 ± 28			

Loss of weight and running time were comparable in all three groups. Magnesium had a significant effect on release of CK, GOT and GPT in plasma, and on formation of urea, which were found to be decreased after magnesium supplementation. Regression analysis showed a linear relationship between the logarithmized value of molecular weight in plasma (x), and fraction of increase (y) after the race:
control:
$y = -1096x + 5338$, $r = 0{,}997$
group II:
$y = -764x + 3813$, $r = 0{,}999$
group III:
$y = -1005x + 5013$, $r = 0{,}999$
According to these relations, proteins with a molecular weight larger than 107 000 (control), 98 000 (group II), or 97 000 (group III) are not released to the plasma.

Magnesium balance

Fig. 3 and *Tab. 2* showed magnesium excretion into urine, which was collected in four successive periods one day after the marathon. It is seen that magnesium excretion was elevated after the marathon race. In the control group magnesium excretion increased from 1,8 ± 0,6 to 4,3 ± 1,5 mmol/l one day after the run. This corresponds to an elevation of magnesium concentration of 2,5 mmol/l urine. Based on 2,5 liters of total plasma volume in man, this loss of magnesium corresponds to a decrease in blood magnesium of about 1 mmol/l.

Discussion

Myoglobin or CK-elevations in serum are the hallmark of skeletal muscle injury. Acute exertional rhabdomyolysis is a syndrome, that occurs in apparently normal, healthy individuals as a result of performing strenuous exercises. While a person may be physically fit, it may still be at risk when performing exercises, that are considered to be normal and which are carried out daily, such as bicycle riding, basketball, or jogging. The distribution patterns of CK-activities in plasma of healthy persons (Fig. 1) prove, that a considerable fraction of a normal, young population has elevated CK-values in blood without participating in strenuous exercises.
The reason for a release of cellular proteins to the extracellular fluid could be

a) a rhabdomyolysis, or
b) discrete perforations of muscle cells.

In case of rhabdomyolysis, one should expect a dependence of cell lysis on physical condition. This was indeed observed in training programs of marine recruits [10], where this syndrome occured almost exclusively in the first week of recruit training. On

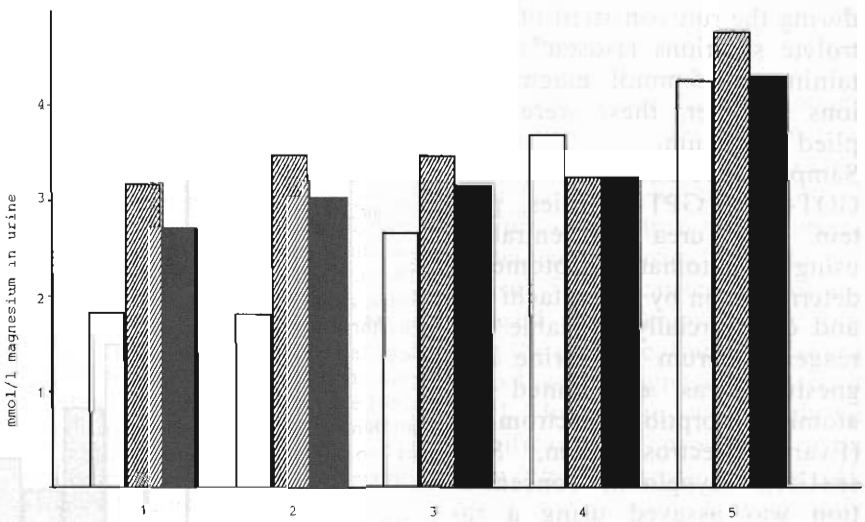

Fig. 3: Excretion of magnesium in urine before (1) and after (2 – 5) a marathon race. Urine was collected before the race (24 hours) in one fraction; after the race it was collected in four fractions. □ Control, ▨ magnesium aspartate hydrochloride supplementation, ■ magnesium oxide plus tocopherol supplementation

the other hand it was noted that highly trained Olympic ice scaters and marathon runners showed the symptom of myoglobinuria immediately after their events [11].

It must therefore be assumed that in addition to a possible rhabdomyolysis, other factors are responsible for the loss of cellular proteins to the extracellular fluid. One possible cause might be a magnesium deficiency at the cellular level. It is known, that magnesium maintains cell membrane function [2]. An impairment of membrane function must be caused by a magnesium deficiency.

On the other hand magnesium concentration in serum is decreased during a physical exercise [5,6,7]. The change in plasma concentration of magnesium is caused by an increased cellular uptake. One of the organs involved in increased uptake of magnesium during physical exercise, is the kidney, which must maintain sodium balance [12].

Physical exercises cause an increased aldosterone uptake by the kidney, an effect which is induced by magnesium [9]. This magnesium is lost in the urine (Fig. 3, Fig. 3) and cannot be reutilized again. On the average, magensium loss in urine is 2,5 mmol on the day after the race (Fig. 3). Another significant source of magnesium loss is sweet [8,9]. As a result of these events a magnesium deficiency must occur in physical exercise, and this deficiency is increased by intensity and duration of training. The magnesium deficiency causes a disfunction on cell membranes.

Discrete perforations of cell membranes are one of the reasons for translocation of cell proteins from muscle cells to the extracellular fluid. The release of cell proteins is dependent on molecular weight; the exclusion molecular weight was 105 000 daltons.

Tab. 2: Values of magnesium excretion in urine before and after a marathon run. The sampling period before the marathon was 24 hours. After the run the urine was collected in four fractions during a 24-hour period. The P-values were obtained by comparing the corresponding values (before after) with control values (before after)

Sample period	control	Mg-Asp.-HCl	P	MgO-Vit E	P
before the marathon	1,83 ± 0,63	3,17 ± 1,84	≤ 0,01	2,70 ± 1,39	≤ 0,03
after the marathon fraction 1	1,81 ± 1,55	3,48 ± 1,64	≤ 0,01	3,02 ± 1,29	≤ 0,03
fraction 2	2,66 ± 1,59	3,47 ± 1,68	≤ 0,10	3,16 ± 1,49	≤ 0,20
fraction 3	3,69 ± 1,86	3,24 ± 1,89	≤ 0,30	3,25 ± 2,15	≤ 0,30
fraction 4	4,26 ± 1,51	4,77 ± 2,32	≤ 0,25	4,31 ± 2,12	≤ 0,50

It is seen in Tab. 2, that a three week magnesium supplementation improved membrane function, based on a significant decrease in protein release from muscle cells during the run, and paralleled by a corresponding decrease in urea formation. The exclusion value for protein molecular weight is lowered to 97 000 daltons.

It must be speculated that a magnesium supplementation before a strenuous physical exercise significantly loweres loss of vital proteins necessary for energy metabolism, and accelerates regeneration of those proteins.

The coaches and officials, responsible for health and performance of competitive athletes, but also the normal individual involved in regular physical exercises, such as bicycle riding and jogging, should improve prognosis of health by a corresponding magnesium supplementation whith regular medical controls.

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