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ORIGINAL RESEARCH

Decrease in Ionized and Total Magnesium Blood Concentrations in Endurance Athletes Following an Exercise Bout Restores within Hours—Potential Consequences for Monitoring and Supplementation

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Magnesium is essential for optimal sport performance, generating an interest to monitor its status in athletes. However, before measuring magnesium status in blood could become routine, more insight into its diurnal fluctuations and effects of exercise itself is necessary. Therefore, we measured the effect of an acute bout of exercise on ionized (iMg) and total plasma magnesium (tMg) in blood obtained from 18 healthy well-trained endurance athletes (age, 31.1 ± 8.1 yr.; VO_{2max}, 50.9 ± 7.5 ml/kg/min) at multiple time points, and compared this with a resting situation. At both days, 7 blood samples were taken at set time points (8:30 fasted, 11:00, 12:30, 13:30, 15:00, 16:00, 18:30). The control day was included to correct for a putative diurnal fluctuation of magnesium. During the exercise day, athletes performed a 90 min bicycle ergometer test (70% VO_{2max}) between 11:00 and 12:30. Whole blood samples were analyzed for iMg and plasma for tMg concentrations. Both concentrations decreased significantly after exercise (0.52 ± 0.04–0.45 ± 0.03 mmol/L and 0.81 ± 0.07–0.73 ± 0.06 mmol/L, respectively, $p < .001$) while no significant decline was observed during that time-interval on control days. Both, iMg and tMg, returned to baseline, on average, 2.5 hr after exercise. These findings suggest that timing of blood sampling to analyze Mg status is important. Additional research is needed to establish the recovery time after different types of exercise to come to a general advice regarding the timing of magnesium status assessment in practice.

Keywords: micronutrients, status monitoring, blood analysis

Magnesium (Mg) is an essential micronutrient for health and performance (de Baaij et al., 2015). It is involved in numerous metabolic processes (Finstad et al., 2001). In terms of exercise capacity, magnesium is for example crucial for glycolysis (de Baaij et al., 2015; Garfinkel & Garfinkel, 1985), protein synthesis and muscular contraction (Dias et al., 2006), with ATP and calcium both depending on Mg concentrations (Aikawa, 1981; Dias et al., 2006). Research showed that magnesium deficiency can lead to muscle weakness, neuromuscular dysfunction, and muscle cramping (Finstad et al., 2001; Konishi, 1998; Laires, 2001; Lukaski, 2000, 2004; Singh et al., 2004; Welch et al., 2016). As a result, performance is highly dependent on adequate magnesium levels (Nielsen & Lukaski, 2006), generating a demand to monitor its status in athletes to prevent magnesium-related muscular dysfunction. At the same time, studies suggest that Mg supplements are frequently used by athletes (Wardenaar et al., 2016).

Currently, measurement of total serum or plasma magnesium (tMg) is most commonly used to determine magnesium status (Elin, 2010), but its reliability is subject to debate. In plasma (or serum), magnesium is present in three forms: 1) the free, ionized form, 2) complexed to anions and, 3) protein bound (mainly to albumin). Since free ionized magnesium (iMg) is the active, directly available form involved in cellular processes, it is suggested that iMg should be the preferable parameter to evaluate Mg status (Mooren et al., 2005; Rayana et al., 2005). However, iMg is not widely used because its measurement has been technically challenging so far and the availability of suitable devices limited. During the past decade, more devices for iMg measurement have become available (Dimeski et al., 2010; Rayana et al., 2005; Thode et al., 1998). Free ionized magnesium concentrations in serum/plasma account for approximately 60–70% of those for tMg, giving reference ranges according to the literature between 0.46 and 0.60 mmol/L (Ising et al., 1995).

Another problem with monitoring magnesium status is that exercise itself seems to affect both ionized and total magnesium. Previous studies showed inconclusive results for the effect of exercise on ionized and total magnesium

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status. A decrease in iMg after an incremental running exercise has been reported (Mooren et al., 2005). Contrarily, a significant increase in iMg was observed after a bicycle ergometer test (Duma et al., 1998). Furthermore, published studies have presented contradicting effects of a single bout of exercise on *total* serum/plasma magnesium (tMg) concentration as well. Decreases in tMg were found after a marathon (Buchman et al., 1998; Scherr et al., 2012) and a stepwise treadmill ergometer test (Mooren et al., 2005) while an increase was found after intensive basketball training (Zhao et al., 2015).

None of these studies measured ionized and total magnesium at different time points during an exercise day, thereby hampering evaluation of putative changes in Mg status. Moreover, previous studies did not account for a possibly underlying diurnal fluctuation. Taken together, changes in magnesium concentrations upon exercise, putative recovery to preexercise levels and the influence of diurnal fluctuations remain unclear. This information is crucial to formulate guidelines regarding reliable assessment of magnesium status.

Therefore, the aim of this study was to evaluate the effect of a single bout of exercise on ionized (whole blood) *and* total magnesium (plasma) concentrations in endurance athletes.

In addition, and hypothesizing that both parameters will decrease after exercise, we wanted to estimate the interval needed to return to baseline.

Methods

Study Population

Nine well-trained male and nine well-trained female athletes (cyclists and triathletes) participated in this study. All athletes trained regularly, for at least 5 hr per week. Participants refrained from exercise the last 24 hr before each study day. Their total plasma magnesium concentration was > 0.70 mmol/L (lower limit of normal), and subjects did not donate blood during the last six weeks before the study and did not take any calcium and/or magnesium supplements from the moment they underwent the first preliminary measurement. The study was approved by the Medical Ethical Committee of Wageningen University and all participants gave written informed consent before participation. This study was conducted in accordance with the Declaration of Helsinki.

Experimental Design

The study had a crossover design with random assignment to a control day and an exercise day, and at least two weeks between both measuring days. Before the study, preliminary measurements were performed. These included a maximal exercise test on a bicycle ergometer (Ergoline GmbH, Bitz, Germany) to establish maximal aerobic capacity (VO_{2max}). After an initial workload of 75 Watt for women and 100 Watt for men, workload was subsequently increased by 35W / 2min until the participant could not maintain the required pedaling frequency of 60 rpm. Oxygen consumption was measured with indirect calorimetry (Oxycon Carefusion, Hoechberg, Germany). In addition, body length (Seca 213 portable stadiometer, Hamburg, Germany), weight (Seca 761 scale), four-point skinfolds thickness (Holtain Tanner/Whitehouse Skinfold Caliper, Crosswell, UK) to estimate body fat percentage (Durnin & Womersley, 1974), and blood total magnesium concentration were measured. Questionnaires about supplement use, sports background, and food intake (Food Frequency Questionnaire, FFQ) (Siebelink et al., 2011; Streppel et al., 2013) were also taken.

At both experimental days, the first blood sample was taken at 08:30 a.m. (overnight fasted state) and subsequent blood samples were taken at 11:00 a.m., 12:30 p.m., 13:30 p.m., 15:00 p.m., 16:00 p.m. and 18:30 p.m. (all nonfasting). Breakfast was provided after the first blood drawing. On the exercise day a 90 min bicycle ergometer test at 70% of the athletes' individual VO_{2max} was performed between 11:00 a.m. and 12:30 p.m.. Participants were allowed to drink plain water during the test, but were not allowed to eat. During the rest of the day, participants were allowed to eat and drink their own lunch and snacks. There was no specific diet prescribed, as magnesium dietary intake has been shown not to directly affect serum magnesium concentrations (Elin, 2010; Nielsen & Johnson, 2016). However, individual food consumption was approximately the same on both test days, and consisted of typical Dutch breakfast and lunch products, like bread, cheese, yoghurt, fruits and juice. Body weight was measured directly before and after exercise.

Blood Sampling

Blood samples were taken through a cannula from the cephalic vein and collected in lithium-heparin tubes (3.5 ml LH PST II and 4.0 ml 17 I.U./ml, Becton-Dickinson, New Jersey, America). LH PST II samples were centrifuged at 3000 g for 8 min at room temperature, and plasma was analyzed the same day for total magnesium concentration (Vista 1500, Siemens HealthCare, USA). Accuracy and precision of the Vista 1500 have been validated. At 0.62 mmol/L and 1.44 mmol/L coefficients of variation (CVs) are 3.0% and 1.7%, respectively. For measurement of ionized magnesium status, whole blood collected in a LH 17 I.U./ml vacutainer was immediately analyzed using the Stat Profile pHOx Plus M analyzer (Nova Biomedical, Waltham, MA, USA) according to the manufacturers' recommendations. Precision testing of the Stat Profile pHOx Plus M Analyzer, before this study, showed CVs of 1.33% and 2.38% at 0.53 mmol/L and 0.64 mmol/L, respectively.

Statistical Analysis

Statistical analyses were performed using Statistical Package for Social Sciences 22.0 (IBM SPSS version 22.0, Armonk, New York, USA), and the level of significance was set at $p < .05$. Data are mean ± *SD* unless otherwise indicated.

The Shapiro-Wilk test was used to examine normality of the data distribution.

Linear mixed model was used to determine whether ionized and total magnesium changed over time on the exercise day. Data from the control day were used in the model to correct data from the exercise day. A top-down strategy was used to assess the model. With this approach several variables (for example: magnesium concentrations at rest day, sex, age and VO_{2max}) are added to the model. Next, these are deleted one by one when the variable does not contribute significantly to the fit of the model.

A cross-correlation between the time series of ionized and total magnesium was used to determine the cross-correlation coefficients. A Pearson correlation was used to determine the correlation between magnesium intake (estimated with FFQ) and total magnesium concentration in plasma.

Results

Characteristics of the Study Population

The participants' characteristics are shown in Table 1 as mean ± *SD* (*SD*). In spite of the weekly training load, some individual VO_{2max} values were rather modest, as reflected by the mean VO_{2max} of 54.9 ± 6.2 and 46.9 ± 6.8 ml kg^{-1} min^{-1} for men and women, respectively. Likewise, the peak power achieved was rather low for some individuals. At inclusion, (measured during preliminary measurements,) total magnesium concentration was above the lower limit of normal (0.7 mmol/L) (Hooijkaas, 2013) in all participants, range: 0.73–1.01 mmol/L. Magnesium intake (calculated with FFQ) was not associated with fasted total plasma magnesium or with fasted ionized magnesium levels at 08:30 a.m. ($p > .05$). Subsequent results will be presented for men and women together, as there were no significant differences between men and women concerning magnesium concentrations and changes upon exercise.

Table 1 Participants' Characteristics

Characteristics	Men	Women
Age (years)	33.6 ± 7.8	28.6 ± 7.9
Height (cm)	185.7 ± 5.9	173.3 ± 8.6
Weight (kg)	79.6 ± 8.6	62.1 ± 9.2
BMI (kg/m2)	23.0 ± 2.0	20.6 ± 1.6
Body fat percentage (%)	14 ± 4	23 ± 4
Plasma tMg (mmol/L)	0.88 ± 0.03	0.87 ± 0.08
Dietary Mg intake (mg)	619 ± 184	479 ± 145
Wmax (Watt)	390 ± 26	281 ± 51
VO_{2max} (ml/kg/min)	54.9 ± 6.2	46.9 ± 6.8

Note. Data are expressed as mean ± *SD*. BMI, body mass index; plasma tMg, total magnesium concentration in plasma; Dietary Mg intake was estimated from an FFQ; Wmax, maximal power output during maximal effort test; VO_{2max}, maximal oxygen consumption.

Effect of an Acute Bout of Exercise on Ionized and Total Magnesium

The ionized and total magnesium concentrations at different time points during the exercise day are shown in Figure 1. Mean ionized (whole blood) and total magnesium (plasma) concentrations early in the morning after an overnight fast (8:30 a.m.) were 0.51 ± 0.04 and 0.81 ± 0.06 mmol/L, respectively, with iMg ranging from 0.45–0.59 mmol/L and tMg ranging from 0.67–0.92 mmol/L. Two of our participants (one male, one female) had tMg values below the lower limit of normal in the fasted state: 0.67 and 0.69 mmol/L, respectively. Immediately before exercise (11:00 a.m.), ionized and total magnesium concentration did not significantly differ from early morning values (0.52 ± 0.04 and 0.81 ± 0.07 mmol/L, respectively). After exercise (12:30 p.m.), both total and ionized magnesium concentrations were significantly lower. Ionized magnesium decreased by 0.06 ± 0.03 mmol/L to 0.45 ± 0.03 mmol/L ($p < .001$). Total magnesium decreased by 0.08 ± 0.04 mmol/L to 0.73 ± 0.06 mmol/L ($p < .001$). Ionized and total magnesium concentrations at 13:30 p.m., one hour after finishing exercise, were still significantly lower compared with the concentrations before exercise ($p < .001$).

Recovery of Magnesium Concentrations After Exercise

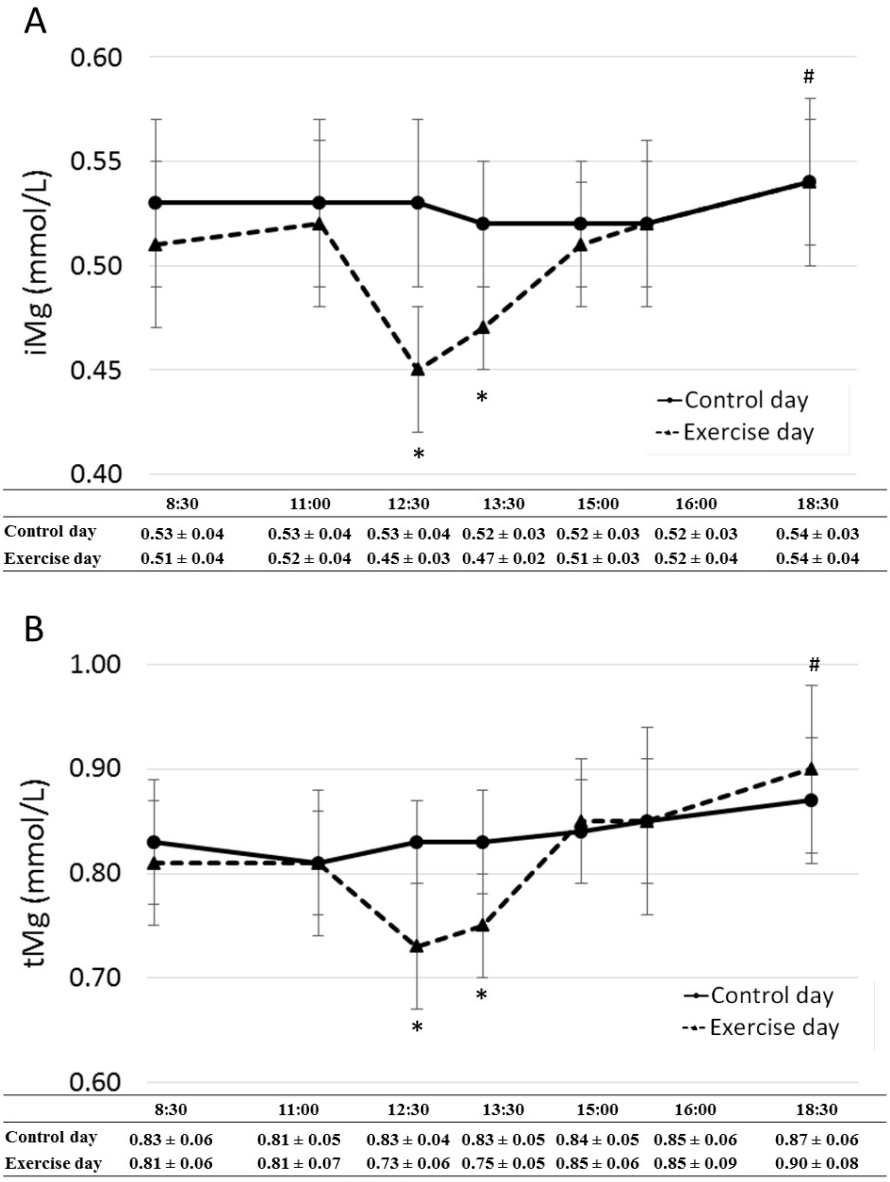
At 2.5 hr after exercise (15:00 p.m.), concentrations seemed to be recovered, as they were no longer significantly different from fasting and preexercise values. However, when individual data were analyzed, only 10 participants (out of the 18) were back at their preexercise *ionized* magnesium concentration (D Postexercise—Preexercise ³ 0 mmol/L), while 15 participants were back at their preexercise *total* magnesium concentration, see Table 2. At six hours after exercise, almost all participants were back at their preexercise *total* magnesium concentration, however, 2 participants (one man; one woman) were still below their preexercise *ionized* magnesium concentration.

Magnesium Concentrations During Control Days

Magnesium concentrations fluctuated during the control day (Figure 1). Ionized and total magnesium concentrations were both significantly higher at the end of the afternoon (18:30 p.m.). To be certain that exercise-induced changes were caused by exercise only and not due to an underlying diurnal fluctuation, these control day data were used for evaluation of the exercise day data.

Cross-Correlation Between Ionized and Total Magnesium

A cross correlation analysis revealed that iMg and tMg concentrations were significantly correlated at both the exercise day ($r = .728$, $p < .001$) and the control day ($r = .405$, $p < .001$). Both ionized and total magnesium



	8:30	11:00	12:30	13:30	15:00	16:00	18:30
Control day	0.53 ± 0.04	0.53 ± 0.04	0.53 ± 0.04	0.52 ± 0.03	0.52 ± 0.03	0.52 ± 0.03	0.54 ± 0.03
Exercise day	0.51 ± 0.04	0.52 ± 0.04	0.45 ± 0.03	0.47 ± 0.02	0.51 ± 0.03	0.52 ± 0.04	0.54 ± 0.04

	8:30	11:00	12:30	13:30	15:00	16:00	18:30
Control day	0.83 ± 0.06	0.81 ± 0.05	0.83 ± 0.04	0.83 ± 0.05	0.84 ± 0.05	0.85 ± 0.06	0.87 ± 0.06
Exercise day	0.81 ± 0.06	0.81 ± 0.07	0.73 ± 0.06	0.75 ± 0.05	0.85 ± 0.06	0.85 ± 0.09	0.90 ± 0.08

Figure 1 — Mean ionized (A) and total (B) magnesium concentrations, during exercise (dotted line) and control (black line) day. *Significantly lower concentration compared with preexercise concentrations ($p < .001$) # significantly higher concentration compared with all earlier time points. ($p < .05$).

Table 2 Recovery of Ionized and Total Magnesium After Exercise

	Ionized magnesium		Total magnesium	
	Number Recovered	Mean difference	Number Recovered	Mean difference
	(*n* / 18)	(Mean ± *SD*)	(n / 18)	(Mean ± *SD*)
directly after exercise	0 / 18	- 0.06 ± 0.03*	0 / 18	- 0.08 ± 0.04*
1 hr after exercise	1 / 18	- 0.05 ± 0.02*	1 / 18	- 0.06 ± 0.04*
2.5 hr after exercise	10 /18	- 0.007 ± 0.02	15 /18	0.04 ± 0.06
3.5 hr after exercise	13 / 18	0.00 ± 0.02	15 / 18	0.04 ± 0.06
6 hr after exercise	16 / 18	0.03 ± 0.03	18 / 18	0.09 ± 0.05

Note. Differences between preexercise and different time points after exercise. Concentrations are recovered when postexercise—preexercise difference ≥ 0 mmol/L. n represents the amount of participants recovered. * significantly lower concentration compared with preexercise concentrations ($p < .001$).

concentrations decreased immediately after exercise and were higher at the end of both the exercise and control day. There was no delay between iMg and tMg, as Lag -1 ($r = .248$) and Lag 1 ($r = .163$) in the cross correlation were lower than Lag 0.

Discussion

To our knowledge this is the first study to measure the effect of an acute bout of exercise on total *and* ionized magnesium in endurance athletes by comparing iMg and tMg 2.5 hr before, and until 6 hr after exercise in comparison with a similar situation (same time points) without exercise. Ionized magnesium has the greatest biological activity, it is readily available and diffusible. In agreement with our hypothesis, the primary finding was that both ionized and total magnesium decreased significantly after exercise. Recovery to baseline levels occurred, on average, 2.5 hr after exercise termination. This implicates that measuring Mg status the day after exercise should be sufficient to obtain reliable insight in its status.

Decreased Total and Ionized Magnesium Concentrations After Exercise

Measurement of ionized magnesium and total magnesium at consecutive time points following exercise, in comparison with a resting situation has not been performed before. With this setup we were certain that the changes found after an acute bout of exercise were caused by exercise only and not by an underlying diurnal fluctuation.

A decrease in ionized magnesium following exercise has been reported previously (Mooren et al., 2005). In that study, 20 athletes performed a progressive treadmill ergometer test until exhaustion. Blood samples were collected immediately after finishing the test and analyzed using an ion-sensitive microelectrode (AVL electrolyte analyzer 988–4). The postexercise decrease in ionized magnesium was comparable to that found in our study. Unfortunately, these authors did not measure at more time points following exercise.

Decreases in *total* magnesium were also reported before, for example, immediately after finishing a marathon (Buchman et al., 1998; Franz et al., 1985; Scherr et al., 2012). Unfortunately, not all of these studies measured total magnesium concentrations immediately before the marathon as baseline, but 1 week (Scherr et al., 2012) or 2 weeks (Buchman et al., 1998) before the marathon. By doing so one cannot be sure that the lower magnesium concentrations are caused by the exercise, or whether these concentrations were already low at the start of the marathon.

Contradicting to our results was the observation of Duma et al., who found an increase in magnesium concentrations after a bicycle ergometer test (initial power 150W increasing with 50W/min until exhaustion) (Duma et al., 1998). A possible explanation could be a decrease in plasma volume, although specific information about fluid intake and plasma volume to confirm this theory was not mentioned. The possibility that changes in plasma volume due to exercise may have influenced our results were also considered in the current study. We measured hematocrit values and noticed only a slight increase after exercise. This could indicate a decrease in plasma volume (hemoconcentration). Opposite to our findings, this would have contributed to an increase in magnesium concentration. To maintain total body water stable participants were allowed to drink water, and the lack of a change in body weight after exercise indicates that dehydration did not occur. In another study, increases in total magnesium were found 24 hr after an intensive basketball training (Zhao et al., 2015). This might be caused by the timing of blood withdrawal, as concentrations were measured 24 hr after the training and not directly after finishing the training (Zhao et al., 2015). We measured immediately after exercise and at different time points later that day. If we had measured only at 2.5 hr or later after exercise, we would not have observed the decrease in ionized and total magnesium concentration. On the other hand, we measured total and ionized magnesium up to 6 hr after exercise, so we do not know whether a putative increase may have occurred after 6 hr. In addition, individual data showed that only 10 of our participants were back at their preexercise *ionized* magnesium concentrations at 2.5 hr after exercise, while 15 participants were back at their preexercise *total* magnesium concentrations. Even 6 hr after exercise, there were still 2 participants who were not back at their preexercise ionized magnesium concentrations. These results indicate that recovery differs per individual and that individual timing of blood withdrawal is crucial. It also seems that recovery of ionized magnesium concentrations after exercise is somewhat slower in comparison with recovery of total magnesium concentrations.

It is interesting to speculate about possible causes for our observed decrease in plasma magnesium concentration following exercise. One explanation might be that this is caused by an increase of catecholamines during strenuous exercise, as catecholamines, like epinephrine and norepinephrine induce Mg2+ uptake into muscle cells and regulate the magnesium dependent Na/K ATPase pumps in skeletal muscle (Khan et al., 2013).

Another possible explanation for a decrease of plasma magnesium could be uptake of magnesium into adipocytes due to an increased rate of lipolysis (Vormann et al., 1983). Lipolysis increases during exercise, in particular when muscle glycogen levels decrease, as is the case in more prolonged exercise. As a consequence, free fatty acids are transported into the plasma. Fatty acids that remain in the adipocytes bind to magnesium, causing a decrease of the free ionized magnesium in the cells, which results in a net uptake of magnesium from the plasma into the adipocytes (Franz et al., 1985). Thus, both uptake by muscle cells and adipose tissue might explain a postexercise drop in circulating ionized and total Mg levels. Our study does not provide data to support these hypotheses. Additional studies, for example taking muscle and adipose tissue biopsies should be performed to evaluate this.

Higher Magnesium Concentrations at the End of the Day

In our study ionized and total magnesium concentrations were slightly increased at the end of the day. This might be caused by innate diurnal fluctuations, by excretion, or by dietary factors. Here again, opposite findings regarding innate diurnal fluctuations have been reported in the literature, with maximum values in the morning and minimum values later that day (Ising et al., 1995). It has also been shown that magnesium excretion fluctuates throughout the day, with maximal excretion occurring at night (Fox et al., 2001). Regarding iMg, it has been shown that concentrations are variable in healthy subjects over the course of one day (Newhouse et al., 2002). However, more research is needed to estimate the possible diurnal fluctuations of ionized and total magnesium.

Correlation Between Ionized and Total Magnesium

We found a cross-correlation between ionized magnesium and total magnesium at both the exercise day and control day. The difference in cross-correlation values between these two conditions would suggest that the equilibrium between tMg and iMg might differ after exercise and during a resting condition. However, data supporting this possibility are lacking in the current study.

A moderate correlation between ionized and total magnesium was also found by other researchers (r = .585) (Johansson & Whiss, 2007). Strong correlations were found (r = .903) in 34 critically ill and injured patients (Koch et al., 2002) as well as in patients with intestinal or liver disease and healthy controls (r = .87 in 106 patients, and r = .75 in 75 healthy controls, $p < .001$) (Saha et al., 1998). Our correlation between ionized and total magnesium at control day was lower than previously found. This might be caused by the small sample size of our study. We only included 18 athletes, whereas the other studies included more individuals. We found that ionized and total magnesium correlated better at an exercise day in comparison with a rest day.

Conclusion

In conclusion, this study showed a significant decrease in both ionized and total magnesium immediately after a single bout of exercise. Total and ionized Mg concentrations were, on average, back to preexercise levels 2.5 hr after exercise. However, we don't know whether a similar pattern is observed after another form of exercise. This implicates that the timing of blood sampling for assessment of magnesium status is important. More research is needed to estimate the recovery time after various types of exercise to facilitate a sports physician in interpreting magnesium status. Both ionized and total magnesium, showed almost similar decrease and recovery patterns, indicating that both can be used to evaluate physiological changes after exercise. Whether ionized magnesium should be the preferable parameter to evaluate Mg status in deficient participants should be studied in future.

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