Dissertation

INFLUENCE OF MENTAL OR PHYSICAL STRESS WITH OR WITHOUT SUPPLEMENTATION OF ELECTROLYTES ON SUBSEQUENT BURDEN – METABOLIC MEASUREMENTS

submitted by

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STATUTORY DECLARATION

I hereby declare that this thesis is my own original work and that I have fully acknowledged by name all of those individuals and organisations that have contributed to the research for this thesis. Due acknowledgement has been made in the text to all other material used. Throughout this thesis and in all related publications I followed the "Guidelines of the Medical University of Graz on Good Scientific Practice".

Graz, 13th December 2019 eh. Stefan Opresnik

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TABLE OF CONTENT

ABSTRACT – DEUTSCH

Die objektive Quantifizierung von mentalen (Vor-)Belastungen und nachfolgendem Stress bleibt eine Herausforderung. Ionisierte Magnesium (Mg)-Spiegel im Blut und insbesondere deren Dynamik scheinen eine entscheidende Rolle bei der sensitiven Quantifizierung solcher Kompensationsreaktionen zu spielen. Darüber hinaus ist Mg einer der wichtigsten Co-Faktoren für eine Vielzahl von enzymatischen Reaktionen, insbesondere bei metabolischen Vorgängen. Deshalb könnte eine Mg-Supplementation vorteilhaft sein, um die Leistung im Sport zu verbessern. Um diese Zusammenhänge in Situationen mit geistiger und/oder körperlicher Erregung besser zu verstehen, wurden 100 µl Kapillarblut aus der Fingerbeere vor und nach entsprechenden Situationen (2400 m Lauf, Akkordeonunterricht, Coaching-Wochenende, 2400 m Lauf nach doppelblinder Mg-Supplementation) gesammelt, um Elektrolyte und diverse Stoffwechsel- und Respirationsparameter zu bestimmen (pHOx-M-Gerät, Nova Biomedical, Waltham, USA). Herzfrequenz, systolischer und diastolischer Blutdruck wurden mit einem Beurer BM40 Blutdruckmessgerät (Beurer, Ulm, Deutschland) gemessen.

Die Analyse ergab Zusammenhänge zwischen metabolischen, respiratorischen, kardiovaskulären Parametern und Elektrolyten, abhängig von der jeweiligen Situation. Ionisierte Mg-Spiegel und die entsprechende Dynamik während einer Belastung ermöglichen die Quantifizierung der Stressintensität und zeigen mögliche Vorbelastungen auf. Positive Empfindungen und Emotionen sowie angenehme Aufgaben scheinen einen großen Einfluss auf die tatsächliche Wahrnehmung von Stress zu haben. Eine akute Mg-Supplementation, bereits eine Stunde vor der Übung, hat einen positiven Einfluss auf die Verbesserung der Leistung und die Verminderung der Anstrengung. Die Daten deuten darauf hin, dass Mg ein möglicher limitierender Faktor für die Leistungsfähigkeit zu sein scheint. Da der ionisierte Mg Spiegel im Blut mit dem Erregungszustand schwankt, sollte dieser immer zusammen mit anderen Stressparametern interpretiert werden, um den Mg-Status zu beurteilen.

ABSTRACT – ENGLISCH

The objective quantification of mental (pre)loads and subsequent stress remains challenging. The ionized magnesium (Mg) level and especially Mg dynamics seem to play a decisive role to sensitively quantify such compensation reactions. Furthermore, Mg is one of the most important co-factors for a multitude of enzymatic reactions, especially in metabolic processes. Therefore, Mg supplementation could be beneficial to improve performance in sports. To better understand those interrelationships in situations with mental and/or physical excitement, 100 µl of capillary blood from fingertip before and after particular situations (2400 m run, accordion lesson, coaching weekend, 2400 m run after double-blinded Mg supplementation) were collected to determine electrolytes and various metabolic and respiratory parameters (pHOx-M device, Nova Biomedical, Waltham, USA). Heart rate, systolic and diastolic blood pressure were measured using a Beurer BM40 sphygmomanometer (Beurer, Ulm, Germany)

Analysis revealed correlations between metabolic, respiratory, cardiovascular parameters and electrolytes, depending on the particular situation under investigation. Ionized Mg levels and respective dynamics during exercise allow the quantification of stress intensity and indicate potential preloads. Positive mental feelings and enjoyable tasks seem to have a huge impact on the way stress is perceived. Acute Mg supplementation, just one hour before the exercise has a positive impact on improvement of performance and the reduction of effort. The data suggest that Mg appears to be a potential limiting factor for performance. Since the ionized Mg level in the blood varies with the state of excitation, it should always be interpreted together with other stress parameters to reliable assess the Mg status.

1 INTRODUCTION

1.1 ACID BASE HOMEOSTASIS AND BUFFERING

The term homeostasis was first introduced by Cannon and generally summarizes the physiological processes which maintain the steady states in the organism (1).

Acid base homeostasis in blood is tightly regulated to maintain physiological processes (2). This is facilitated by different receptors, which sense fluctuations in certain variables in the organism. Via messengers, actions like protein excretion, cell recruitment or short term regulation mechanisms are initiated (2). Therefore, several buffer and regulatory systems work together to maintain a pH-value in arterial blood between 7.37 and 7.43 (3,4).

The main organs for controlling the buffer-systems are the kidneys and the lungs (5). By increasing or decreasing the respiratory minute volume or excretion or reabsorption by the kidneys, the bicarbonate-, phosphate- and proteinate-buffer systems can additionally be controlled in a fast and effective way. Those regulation mechanisms become important when an organism is dealing with internal or external stressors to maintain physiological homeostasis (3).

The bicarbonate buffer system is the most important system and consists of carbonic acid (H_2CO_3) as a weak acid and its dissociated components bicarbonate (HCO_3^-) and one H^+ ion (4). The Henderson-Hasselbalch-equation describes the relationship between pH- and pK-value (pK = negative decadic logarithm of the dissociation constant) and HCO_3^- and CO_2 concentration (3,4).

$$\text{pH} = \text{pK} + \log\left(\frac{HCO_3^-}{CO_2}\right)$$

It becomes clear that an increase in HCO_3^- concentration goes along with an increase in pH-value. Conversely, the pH value decreases as the concentration of the buffer acid CO_2 increases. For a permanent regulation of the pH value, the excretion of acids via the lungs and kidneys is necessary (3). The lungs eliminate approximately 15 mol CO_2 daily. Chemoreceptors recognize increased CO_2 and H^+ ion levels and increase respiration via the respiratory center. With the excretion of CO_2, more H^+ ions bind to HCO_3^- which increases the blood pH (3,4).

Via active transport mechanisms, the kidneys eliminate approximately 50 mmol H^+ ions daily. This proton elimination is linked to a reabsorption of bicarbonate (3).

Phosphate as an acid ($H_2PO_4^-$) and secondary phosphate as the correspondent base (HPO_4^{2-}) are the components of the phosphate buffer system. Ionizable side groups of proteins, particularly of hemoglobin and albumin, can also form buffer systems with the corresponding non-dissociated proteins. Deoxygenated hemoglobin for example has a stronger affinity for protons. Because of their low concentrations, phosphate and proteinate-buffer-systems play a minor role (3).

Alkalosis and acidosis occur if pH value increases above 7.43 or drops below 7.37, respectively (3). Table 1.1.1 describes different states and possible causes.

Table 1.1.1 Relationship between changes in blood pH, change in buffer and possible causes (3,4,6)

	BLOOD PH	CHANGE IN BUFFER	POSSIBLE CAUSE
RESPIRATORY ACIDOSIS	DECREASE	INCREASE IN PCO_2	ALVEOLAR HYPOVENTILATION
RESPIRATORY ALKALOSIS	INCREASE	DECREASE IN PCO_2	HYPERVENTILATION
METABOLIC ACIDOSIS	DECREASE	INCREASE IN PROTON CONCENTRATION, NORMAL PCO_2, BUFFER BASES DECREASED	POORLY ADJUSTED DIABETES MELLITUS
METABOLIC ALKALOSIS	INCREASE	INCREASE IN BUFFER BASES, NORMAL PCO_2	LOSS OF ACID GASTRIC JUICE

Bicarbonate, phosphate and protein buffer systems can also buffer each other. Overall, the concentration of buffer bases (proteinate and HCO_3^-) in arterial blood is 48 mmol/L (the low phosphate concentration is irrelevant in practice) and is relatively independent of changes in CO_2 concentration in the blood (3). Therefore, it is a good indicator for changes in the acid-base balance that occur independently of respiratory processes (3).

This is different for metabolic disorders: The total buffer base concentration is reduced with an increase in acidic valences and vice versa. The increase of the total buffer bases above 48 mmol/l is called base excess, a decrease is called base-deficit (3).

Instead of the base excess, also the so-called standard bicarbonate in the plasma can be determined. It is determined at a pCO_2 of 40 mmHg, a temperature of 37° Celsius and complete oxygen saturation of the hemoglobin (3). The standard bicarbonate concentration is 24 mmol/L (21-28 mmol/L) (3).

1.1.1 COMPENSATION MECHANISMS

Mainly respiratory disorders are compensated by metabolic compensation mechanisms and vice versa (5). That means that in case of respiratory acidosis, the kidney increases the reabsorption of bicarbonate, leading to an increase in the total buffer bases and a rise in pH: compensated respiratory acidosis (3). During a respiratory alkalosis, the kidney tries to excrete more bicarbonate: compensated respiratory alkalosis (3).

Metabolic acidosis facilitates a strong respiratory drive. By increase in respiration, the pCO_2 in blood decreases and pH value increases: compensated metabolic acidosis (3).

Conversely, in metabolic alkalosis respiration is throttled. The resulting pCO_2 increase lowers the pH value: compensated metabolic alkalosis. Due to the oxygen demand of the organism, the respiratory compensation mechanisms in metabolic alkalosis are limited (3).

1.1.2 BICARBONATE PROTON TRANSPORT IN KIDNEYS

Approx. 80% of the bicarbonate is reabsorbed in the proximal tubule, 20% in der distal tubule and collecting duct system (5,7). The reabsorption of bicarbonate is linked to a secretion of protons. H^+ ions are secreted via an antiport mechanism in exchange for Na^+ out of the tubule cell into the lumen of the tubules (3).

In the lumen, these H^+ ions together with HCO_3^- form carbonic acid, which is cleaved into H_2O and CO_2 by the enzyme carboanhydrase (3,5). The CO_2 passively diffuses back into the tubule cell. Catalyzed by a cytoplasmic carboanhydrase, this CO_2 reacts with H_2O to H_2CO_3, which dissociates into H^+ and HCO_3^- carboanhydrase (5) (3). HCO_3^- is then taken up in a coupled transport symport with Na^+ ions at the basolateral cell membrane into the interstitium. The H^+ ion formed during the dissociation of H_2CO_3 passes through the cycle again (3). Per day, the kidneys regenerate approx. 1 mmol bicarbonate per kilogram of body weight (5).

1.1.3 GAS EXCHANGE

The lung facilitates gas exchange by diffusion. Only 1-1.5% of the oxygen in the blood is present in physical solution. Most of it is bound to hemoglobin. Per liter of blood approximately 200 ml oxygen are bound to hemoglobin and only 3 ml are physically dissolved (3,6).

The reaction kinetics of hemoglobin with oxygen shows an S-shaped curve (O_2 dissociation curve), characterized by a largely linear dependence of O_2 partial pressure and oxygen saturation in an average O_2 partial pressure range between 20 and 40 mmHg (3,6).

Several factors influence the oxygen binding capacity of hemoglobin which results in a shift of the O_2 binding curve. Alkalization of the blood (pH>7.4), decrease in pCO_2, 2,3-diphosphoglycerol (DPG) or temperature leads to a shift of the curve to the left, which means that affinity of oxygen to hemoglobin and O_2 saturation are increased. This enables easier oxygen uptake (3,8,9).

On the other hand, the acidification of the blood (pH<7.4), increase in pCO_2, temperature or 2,3-DPG leads to a shift of the curve to the right. The lower affinity of oxygen to hemoglobin and O_2 saturation facilitates easier release of oxygen into the tissue. This is especially important when tissues have a higher oxygen demand (e.g. during exercise) (3,8,9).

1.1.4 CO_2 TRANSPORT IN THE BLOOD

Approx. 10 % of the CO_2 in the blood are dissolved physically and another 10% bind directly to amino groups of the hemoglobin molecule (carbamino-hemoglobin) (3). The largest proportion of CO_2 is converted to HCO_3^-, which is dissolved in blood (3,9).

1.1.5 REGULATION OF BREATHING

Under normal conditions, the CO_2 partial pressure has the biggest influence on the respiratory drive: an increase leads to an increase in respiratory minute volume (3,10). Increase in H^+ ion concentration or a decrease in O_2 partial pressure triggers increased breathing (10). Additional respiration stimulating factors are low hypothermia, pain, adrenalin and progesterone (pregnancy) (3,8,11).

1.2 STRESS

Daily and seasonal routines or unpredictable events like disease or injuries in the life cycle of all mammals require specific morphological, physiological and behavioral adjustments to maintain homeostasis immediately (12). All those unspecific reactions to maintain the homeostasis were first described by Selye in 1936 and introduced as the term “stress” (13,14). Stressors were defined as events which trigger such reactions. He later defined that stress is always a reaction to a threat and is always accompanied by a physical reaction (13). Newer definitions distinguish between real threats and predicted threats. Real threats like pain, humoral inflammatory signals or stimulation of baroreceptors are recognized by the brain via sensory pathways and directly activate stress centers in the hypothalamus. Predicted threats anticipate danger or physiological challenges via hippocampus, amygdala or prefrontal cortex and lead to activation of stress without previous changes in homeostasis (14,15). Other subclassify stress in internal (e.g. feelings or high cholesterol levels) and external stress (e.g. extreme cold or loss of work) (16).

Although a considerable amount of research has been done in the last years, there is no generally accepted definition of the term stress. Especially in non-scientific journals and in general public there is often no clear discrimination between the term stress and stressors. Further the definition is almost always imprecise and often misleading. This is due to the fact that specific and unspecific stress reactions cannot be distinguished clearly and that there is no clear differentiation between mental or physical stress, regardless, which definition seems to be most appropriate (15,17).

More recently McEwen additionally introduced the term allostasis which basically describes reaction to a stressor in a broader context (18). According to that, stress is not just a short-lasting set-point mechanism but rather a broad range of adaptive responses including regulation of gene expression, protein synthesis and hormone secretion (18). This view allows to explain individual differences and related long term effects in stress (14,17). The latter is the definition of stress in this thesis.
It seems understandable, that the dimension of stress depends on subject's genetic predisposition, physiological and psychological state as well as the type of stressor (16). Studies have shown that relationship-stress and termination of relationship for example lead to increased anxiety, decreased life satisfaction and higher rate of mental illness (19,20). Additionally, interpersonal events like sexual assault trigger psychological distress in a larger extend compared to events caused by nature or accident like natural disaster (21,22).
Psychosocial stress is increasing in Western societies and more and more people have severe problems dealing with it (16,23). Table 1.2.1 shows examples of different types of stressors.

Table 1.2.1 Examples of different stressors (14,24)

STRESSOR	EXAMPLES OF STRESSORS
DAILY HASSELS	TRAFFIC, CONFLICTS WITH COLLEAGUES, WORK DEADLINES
LIFE CHANGES	DEATH, DIVORCE, JOB LOSS, VACATION
ENVIRONMENTAL INFLUENCES	NATURAL DISASTER, WAR, TEMPERATURE CHANGES, WEATHER CHANGES, POLLUTION, SOCIOECONOMIC STATUS
PSYCHOLOGICAL DETERMINANTS	SELF-PERCEPTION, OPTIMISM, ANTICIPATION, TRAVELLING IN AN UNFAMILIAR CITY
PHYSICAL FACTORS	ILLNESS, INJURIES, ACCIDENT,

1.3 RESPONSE TO A STRESSOR

In general two at several levels tightly connected neuroendocrine systems mediate stress: The sympatho-adrenal system (SAS) and the hypatholamic-pituitary-adrenal (HPA) axis (Fig. 1.3.1) (15,25). Both mediate a bi-directional communication between the body and the brain and ensure adequate adaption to stressors and associated disturbances in homeostasis (14) (15). They are often activated simultaneously (16,26).

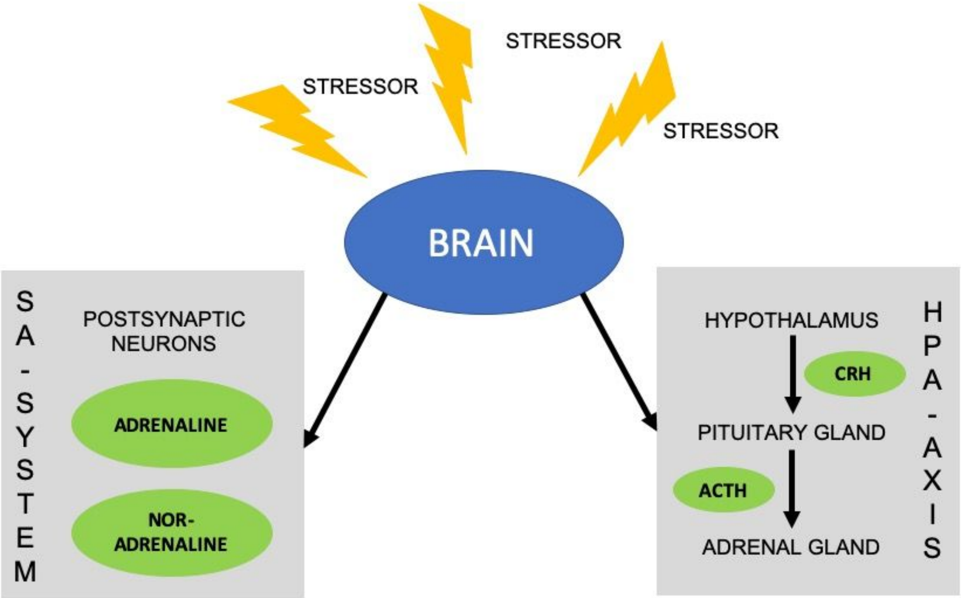

Figure 1.3.1 Schematic representation of the hypothalamic-pituitary-adrenal (HPA) axis and the sympatho-adrenal system (SAS), as main parts of the stress system. CRH: corticotropin-releasing hormone; ACTH: adrenocorticotropin hormone (15,16,27)

Table 1.3.1 Effects of stress and activation of the sympatho-adrenal (SA)-System or the hypothalamic-pituitary-adrenal (HPA)-axis; (3,15,16,26–28)

EFFECTS OF STRESS	
SA-SYSTEM	**HPA-AXIS**
INCREASED OXYGEN TRANSPORT TO HEART, BRAIN AND SCELETAL MUSCLES	DIRECTION OF OXYGEN AND NUTRIENTS TO THE BRAIN
VASODILATATION (MUSCLE, HEART)	GLUCONEOGENESIS
BRONCHODILATATION	LIPOLYSIS
VENOCONSTRICTION	INHIBITION OF GROWTH AND REPRODUCTIVE SYSTEMS
LEUCOYTOSIS	CONTAINMENT OF INFLAMMATORY RESPONSES
	INCREASED AROUSAL
	VIGILANCE AND COGNITION
	SUPPRESSION OF FEEDING AND REPRODUCTIVE BEHAVIOR
	EUPHORIA (OR DYSPHORIA)
	HEIGHTENED ANALGESIA

1.3.1 THE SYMPATHO-ADRENAL SYSTEM

The SAS is responsible for mediating the immediate stress reaction or the so called “fight-or-flight”-response (15). Activation (primarily alarm reactions) of the SAS leads to the immediate release of the catecholamines noradrenaline (norepinephrine) and adrenaline (epinephrine) and activation of peripheral organs via direct neural innervation (25,29). The plasma concentration of catecholamines peaks within one to five minutes and promotes, beside a vasodilation in muscles and heart, hepatic glycogenolysis, increase in blood pressure and heart rate (30).

Those mechanisms prepare the organism to control or fight the (potential) threat by active coping strategies (Table 1.3.1) (15).

1.3.2 HPA AXIS

If a specific situation cannot be controlled by the SAS, cortisol is released by activation of the HPA axis (28,31). The hormonal changes facilitate passive coping strategies which influence energy metabolism and behavior (25). Therefore e.g. oxygen and nutrients are directed to the brain, gluconeogenesis and lipolysis increase and reproductive systems, growth and inflammatory responses are inhibited. Furthermore the hormonal changes go along with increased arousal, cognition and vigilance (Table 1.3.1) (15).

The brainstem and the forebrain limbic structures (prefrontal cortex, hippocampus and amygdala) react upon exposure to physical and/or mental stressors and send signals to the hypothalamic paraventricular nucleus (PVN) (32). The PVN in turn activates the sympathetic nervous system and secretes corticotropin-releasing hormone (CRH) and arginine vasopressin. CRH stimulates the secretion of adrenocorticotropic hormone/corticotropin (ACTH) from corticotrope cells of the anterior pituitary gland which in turn controls the adrenal secretion of corticosterone/cortisol from the adrenal cortex into the blood (15,29,33).

Via hippocampus the glucocorticoids have an inhibitory effect on the activity of the HPA axis. This negative feedback loop limits the duration of the response and prevents tissues from glucocorticoid-related damage (29,34,35).

1.4 EUSTRESS, DISTRESS AND DISEASE

In case of a well-regulated (sufficient activation and tightly controlled termination) and successful adaptive response to handle changes in homeostasis, stress is termed “eustress” and can be beneficial for the organism, when homeostasis is re-balanced (15). An example for eustress is regular moderate physical activity, which is known to reduce the risk for many diseases like cardiovascular disorders (36).

Dependent on quality, intensity and duration of the stressor and subjects genetic predisposition as well as physiological and psychological state, the reactions of the organism can be harmful (15). The so called distress causes a chronic hypo- or hyperactivation of the HPA-axis, which is thought to be causative for many pathological changes (15,29,37).

The hyperactivation of the HPA axis over a longer period of time for example suppresses growth hormone secretion responsible for delayed growth in children (26,38,39). Moreover, thyroid function and the reproductive axis' activity is decreased during chronic HPA-axis activation (26,40–43). Further, an association between HPA axis activation and disturbances in metabolism, immune function and psychiatric disorders was reported (26,33,34,44–47). With increased glucocorticoid synthesis, accumulation of body fat is dysregulated and increase in food intake and body weight gain is more likely (48). Also, the intensity of calorie uptake is altered during acute and chronic stress. Studies report that people who perceive stress tend to prefer tasty and calorically-dense foods independently from total calorie intake (48–54). The linking mechanisms between stress and food intake are poorly understood.

Some studies even showed, that prolonged stress and related hormonal changes lead to structural changes in different parts of human brains, like atrophy of the brain mass or brain weight loss (37,55,56). Those changes in turn lead to differences in stress response, cognition and memory amplifying further reactions to stressors (37,56). Other organic changes include thymus weight loss, stomach ulcerations and adrenal tissue proliferation (15).

Not only hyperactivation, but also hypoactivation of the stress system is associated with syndromes like chronic fatigue syndrome or seasonal depression (26). Changes in HPA-axis activity also occur during aging, where they are more prominent in women than in men, showing, that there are also gender related differences (55). This was also confirmed by De Vriendt et al. (57), who showed that adolescent girls perceive higher levels of stress than boys (Huybrechts *et al.*, 2014).

Whether and how stress can be influenced is still unclear. Physical activity seems to play an important role in modulating stress and resulting diseases like headache, feeling nervous, abdominal pain and sleeping difficulties (16,59–62).

1.5 ASSESSMENT OF STRESS

Due to the intraindividual differences in extend and nature of stress, quantification and objective evaluation of perceived stress is challenging. Additionally, levels of biomarkers depend on gender, weight and age or on anxiety to the test themselves. In parallel almost all diseases affect the biomarker level (16,24).

There are several ways to quantify stress including psychological changes via questionnaires or physiological changes via a broad range of physiologic parameters or biomarkers in blood, hair and urine (24).

Most common direct biomarkers for evaluation of HPA axis activation are direct measurements of prolactin, cortisol, epinephrine and norepinephrine in blood, urine and saliva. Similar to that, catecholamine levels are also important biomarker for assessment of SAS activation (58). Other methods like microneurography make it possible to record sympathetic nerve activity via microelectrodes in skin and muscle fibers (63).

Although many studies showed a good reliability of various biomarkers there are also conflicting results (64,65). This for example is the case for cortisol levels in blood, hair, saliva and urine (66). As one of the most investigated biomarkers of stress, cortisol is known for its circadian cycle and its individual changes upon eating, sleeping and certain medications. This makes it hard to compare absolute levels throughout the day and interindividually (66). This is probably the reason, why there are no widely accepted reference values for cortisol levels. A non-invasive marker for cortisol measurement is free cortisol in 24h urine but it could be difficult to obtain specimen in a daily routine setting (16). Stalder et al showed in a meta-analysis that self-reported stress is unrelated to hair cortisol concentrations (67). Moreover, both cortisol- and catecholamine effects seem to depend upon the momentary receptor situation, which is hardly ever assessed.

Measurements of indirect biomarkers which change upon stress, like blood pressure, heart rate or peripheral blood flow became very popular (24,63). There are a lot of studies which describe an association between stress and cardiovascular parameters (16,68,69).

A large number of studies investigated the metabolic and electrolyte changes during increased sympathetic activity (70–76). The pH-, oxygen- and electrolyte changes during chronic mental and physical workloads showed a similar pattern indicating a profound relationship between blood gases, electrolytes and perceived stress (70–76). Probably one of the most popular indirect stress assessment parameters, in past mainly used to quantify effort during sports, is lactate. The well investigated parameter allows reliable quantification of muscular effort and makes conclusions on particular muscular stress possible (3). Concentrations up to 2 mmol/L mark an aerobic metabolism meaning that most of the energy consumed is obtained from aerobic glycolysis and that continuous performance is possible. Lactate levels in blood higher than 4 mmol/L indicate an anaerobic metabolism and limited performance capabilities (3). Using those levels as cut-off values, recent studies investigated lactate as a marker for high risk of mortality in patients with suspected sepsis or cancer (77–80).

1.6 ELECTROLYTES AND STRESS

Many studies, predominantly in athletes investigating performance in sports, revealed a tight relationship between electrolyte levels in saliva and blood and stress or physical effort (70–76,81). Hinton reported, that the potassium (K)/sodium (Na) ratio increases during mental stress (43,82). Saliva concentrations of Na and chloride (Cl) increase during exercise and with increased sympathetic activity (83). It is known that the activity of the autonomic nervous system influences the activity of salivary glands, with changes in secretion and reabsorption of electrolytes (84–86).

As shown in several studies, K concentrations in plasma increase during exercise. This K is most probably derived from working muscles (87–94).

Na and K plasma concentrations in long-distance runners during high temperatures and humidity significantly increased and magnesium (Mg) concentrations decreased after the run (95,96).

Hazar et al. showed that serum K levels decreased directly after a training in male hockey players. After 1 hour higher K levels were observed (96). This indicates, that those fluctuations in electrolytes are not only intensity but also time-dependent (81).

Nevertheless, exact mechanisms behind those electrolyte changes are not clear yet. In case of K some studies report e.g. changes in Na-K-ATPase activity with electrolyte changes while others didn't find any fluctuations (95,97–99). Another possible explanation for increased K levels after exercise is that K has a functional role in controlling circulation (94,95,100–106).

Although all those explanations are inconclusive, the tight relationship doesn't seem surprising due to the importance of electrolytes in many physiologic processes and acid-base regulation (95,107). This fact led to the hypothesis, that electrolytes are one possible limiting factor during stress. Performance in sports or the reaction to a stressor could be influenced by supplementation of electrolytes. Similar to the general understanding of electrolytes and their role during stress, results regarding electrolyte supplementation and subsequent effects on performance in sports are conflicting. Hence, Mg supplementation could not prevent a negative Mg balance in athletes over a period of one year (95,108). On the other hand, Kara et al. suggested that zinc (Zn) supplementation could be beneficial to antioxidant systems and performance in sports (81,109). In a study from Knechtle et al. a 4-week supplementation of a mixture minerals and vitamins did not improve race performance in ultra-endurance runners (110). Conflicting results may result because serum blood levels of electrolytes do not necessarily correlate with body levels (95). As supplementation is probably most beneficial in persons with chronical low electrolyte levels, reliable determination of the status is crucial.

1.7 MAGNESIUM AND STRESS

Mg is one of the most important intracellular cations and a cofactor for more than 600 enzymatic reactions in the body, which include protein synthesis, DNA and RNA synthesis, and energy metabolism (111,112). In human, approximately 24 g of Mg can be found in the body, of which 99% are stored intracellularly in bone, muscle and soft tissue, and only 1% is in the extracellular space. About 40% of the extracellular Mg is bound to albumin and complexed to serum anions whereas 60% exist in the ionized, free and physiologically active form (113).

The daily Mg ingestion is approx. 300mg. Interestingly, the absorption varies from 25% when having a Mg-rich diet to 75%, when diet is Mg-depleted (113). Some studies suggest that 75% of U.S. citizens do not meet the dietary suggestions (112), probably because among other things Mg-content in fruits and vegetables decreased by 20-30% over the last 60 years (111).

At least 50% of total body Mg is stored in bone, dynamically exchanged via transporters that mediate Mg-flux, however, they have not been determined yet (113).

Many mechanisms where Mg plays an important role have been discovered in the last years. In the brain, low extracellular Mg levels contribute to increased intracellular calcium (Ca) levels via the N-methyl-D-aspartate (NMDA) receptors. This may leads to reactive oxygen species (ROS) production and probably to neuronal cell death (111). In asthmatic patients a low Mg level in serum and erythrocytes is observed (114–117). It is also known that low Mg levels cause broncho- and vasoconstriction (111,118). In heart Mg plays a crucial role as a regulator of ion channel activity and myocardial contractility (111,119). Additionally high Mg levels result in an improved lipid profile and endothelial function (111,120,121). The connection between Mg and muscle cramps is not completely understood yet but as muscle contraction is a highly Ca-dependent process and Mg serves as a Ca-antagonist there seems to be a tight connection (111). Recent studies claim, that Mg is probably a key player in glucose and insulin metabolism and could play a major role in developing diabetes mellitus, as diabetes mellitus type 2 patients often have low serum Mg levels (112,122–125).

Yet, the exact mechanisms for developing a Mg-deficiency are not understood but a poorly controlled diabetes mellitus, chronic malabsorptive problems, medication use, alcoholism and older age are thought to be crucial factors (112).

Mg supplementation is used as a therapeutic strategy for a broad range of various diseases including asthma, low bone mineral density, headache and migraine as well as arrythmia, especially torsade de pointes (112).

Although a lot of symptoms are connected to early signs of Mg deficiency which include loss of appetite, nausea, vomiting, fatigue or weakness, diagnosing a deficiency is challenging due to its distribution in the body (112,126,127). In addition because approx. one third of Mg in bone is freely exchangeable, the Mg level seems to be very dynamic (128). Reviews found, that stress like physical exercise results in redistribution of Mg within the body. Deuster et al. observed a shift of Mg from the plasma into red blood cells (127,129). Other studies reported Mg loss during exercise via sweat and cellular exfoliation (127).

Redistribution is influenced by type, intensity and duration of exercise and probably by Mg status itself and therefore it is a promising tool for Mg status assessment (130). Bohl and Volpe suggested, that short term high-intensity exercise and sustained moderate physical exercise increased serum Mg concentration (131). A concomitant increase in serum creatine kinase activity suggested a muscle breakdown as a cause (132,133). In contrast to that, prolonged endurance exercise decreased serum Mg level (130). Acidosis and aldosterone secretion were shown to cause a hypomagnesaemic state (131). On the other hand, cortisol, catecholamines, glucagon and PTH cause a hypermagnesaemic state (131). Porta et al. showed in several studies that Mg dynamics varied dependent on the type of exercise (70–76).

Furthermore, some studies suggest, that slight fluctuations in plasma Mg may indicate a deficient Mg status, whereas a relatively large decrease indicates a sufficient Mg status (130). When assessing those Mg shifts it is hypothesized that ionized Mg seems to be much more sensitive compared to total Mg in blood. In fact, Barrera et al found no relationship between total and ionized Mg after supplementation in critically ill patients (134).

Yet, the exact mechanisms of the effect of Mg and about supplementation-impact on performance are not clearly understood. Brilla and Haley showed that Mg supplementation improves muscle function (135). But not only metabolic parameters react upon Mg supplementation. It was shown that Mg deficiency leads to increased inflammation and change of the HPA axis activity with increase in ACTH levels in supplemented subjects (136) (137)(138).

Although Mg supplementation seems to be beneficial in certain situations, there are also some conflicting results (130). Studies reported that Mg supplementation failed to promote enhanced performance in different physical training types (127). Some studies suggest that actually Mg status is the most important factor when assessing success of a Mg supplementation (130).

2 AIM AND HYPOTHESIS

The quantification of stress still remains difficult due to huge differences in interindividual stress as seen by many conflicting results in recent literature. The tight relationship between stress and disease is the main driver for research to quantify (pre)loads in an objective manner. Previous studies by Porta et al. showed, that this could be done by investigation of changes in electrolyte levels and metabolic parameters like lactate or blood glucose levels (70,72,139–146).

The aim of the present studies was to investigate, if reliable quantification in terms of intensity and duration of stress based on electrolyte and metabolic changes in capillary blood is possible and if stress can be influenced by electrolyte supplementation.

Therefore, data of several 2400 m runs were combined and analyzed to gain a deep understanding on electrolyte and metabolic changes upon a mainly physical stressor. Additionally, if mental preloads are detectable, how do they influence stress during the run? A randomized, double-blind, placebo-controlled study with a cross-over design was facilitated to investigate the impact of acute Mg supplementation on electrolyte levels and metabolic parameters in blood and effort during a subsequent 2400 m run. Analysis of data from a mental coaching weekend was performed to investigate changes in electrolytes and metabolic parameters upon mental relaxation exercises. Finally, analysis of data from accordion students should reveal, if stress due to the combination of strenuous but mentally positive tasks differ from just strenuous tasks like a 2400 m run.

Evaluations of such homeostatic changes upon stress may allow a very sensitive and objective assessment of the current physiological state, even before physical symptoms arise. A deep understanding of homeostatic reactions could make it possible to influence stress by relaxation or supplementation of metabolically required electrolytes. Objective quantification of stress could allow more sophisticated study designs when assessing the impact of various stressors in future studies. Furthermore, such quantifications could help to detect stress related diseases earlier and increase awareness for stress and its consequences in general population.

3 MATERIAL AND METHODS

3.1 STUDY DESIGN

3.1.1 ANALYSIS OF 2400 M RUNNERS

For the analysis, data from all 2400 m runs with the same experimental settings conducted within the last 10 years were combined retrospectively. All runs were part of the training program for officer trainees in the bachelor's program Military Leadership at the Theresian Military Academy in Wiener Neustadt, Austria. Blood sampling for stress assessment (SA, see chapter 3.3) was performed directly before and after the run (147).

3.1.2 STRESS WEEK / PLACEBO CONTROLLED MAGNESIUM SUPPLEMENTATION

To investigate the Mg dynamics over a longer period of time and examine the influence of Mg supplementation on subsequent performance and effort in sports, a three week, randomized, double-blind, placebo-controlled Mg supplementation study with a cross-over design at the Theresian Military Academy in Wiener Neustadt, Austria was conducted. Only healthy (according to WHO criteria) officer trainees between 20 and 40 years of age, without taking co-medication were allowed to participate. The study was divided into three separate blocks were various scenarios were analyzed. Each block is described in detail below.

In this thesis, only data from block 1 and block 3 was analyzed as there was a clear focus on the effect of Mg supplementation on subsequent effort during a 2400 m run. Nonetheless, it is crucial to present the predefined tasks for all participants in the week between block 1 and block 3 as this is important for the interpretation of the data in this thesis. The equal treatment of all participants within the study period was an attempt to reduce bias of preloads from individual military exercises. However, as stress in response to various tasks is subjective, differences in preloads between all participants cannot be excluded.

3.1.2.1 BLOCK 1

The study started one week before the beginning of the stress week with a randomized, double-blind, placebo-controlled Mg supplementation and a first 2400 m run (see Figure 3.1.1). Already before the start of the study, participants were randomized 1:1 into a placebo group or a Mg group by flipping a coin. The participants started the run in pairs of 2 at 10-minutes intervals. One hour before the run, participants of the verum group were asked to take two tablets of Dr. Böhm Magnesium Sport (Apomedica GmbH, Graz, Austria) containing 300 mg Mg and 500 mg K as well as 100 mg vitamin C in total, together with 125 ml of tap water. Dr. Böhm Magnesium Sport is authorized as a food supplement in Austria. Ingredients of two tablets correspond to the recommended daily dose for each K as well as Mg. Participants of the placebo group were asked to take two tablets without Mg or potassium together with 125ml of tap water. The tablets were not distinguishable in regards of taste and visual appearance. Exactly one hour after ingestion of Mg or placebo, capillary blood samples from the fingertip for SA (see chapter 3.3) were drawn and blood pressure and heart rate were determined (see chapter 3.4). Immediately after all data has been collected, the participants were asked to run 2400 m as fast as they can or under 11 min 30 sec, which is the usual time limit for such runs at the Theresian Military Academy. After the run, blood sampling was done, and cardiovascular parameters were determined again.

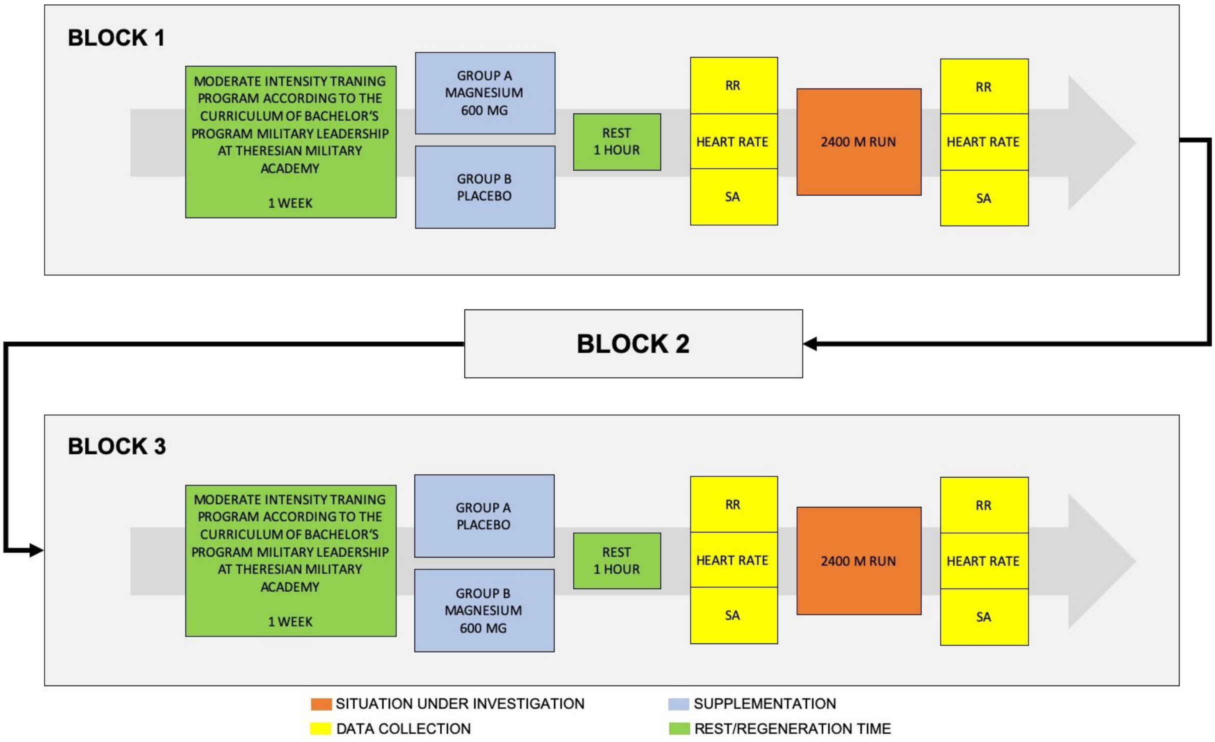

Figure 3.1.1 Schematic diagram of the study design of the stress week. RR = Systolic and diastolic blood pressure; SA = Stress Assessment

3.1.2.2 BLOCK 2

The second part of the study (see Fig. 3.1.2) started with a moderate intensity training program. After detailed instructions regarding all study procedures capillary blood samples were drawn for SA as well as blood pressure and heart rate recorded. Next, the participants were prepared for the decision-box (D-Box) which took approx. one hour. The D-box is a military training tool to practice decision making in a specified time. At the end of the exercises, capillary blood samples were drawn again and blood pressure as well as heart rate recorded. The rest of the day, military exercises and situations were trained. During the night, participants were not allowed to sleep and all of them had to absolve a nightly march to simulate severe mental and physical stressors. Next day, exactly the same procedure with the D-Box was repeated. After the second D-Box, participants were allowed to relax until the next day were the stress week was accomplished by a 2400 m run. Similar to BLOCK 1, all participants were rerandomized 1:1 and were asked to take two tablets of Dr. Böhm Magnesium Sport (Apomedica GmbH, Graz, Austria) or placebo one hour before the run to test the influence of Mg supplementation in exhausted persons due to severe stress the days before. Capillary blood samples were drawn (see chapter 3.3), and cardiovascular markers recorded (see chapter 3.4) directly before and after the run.

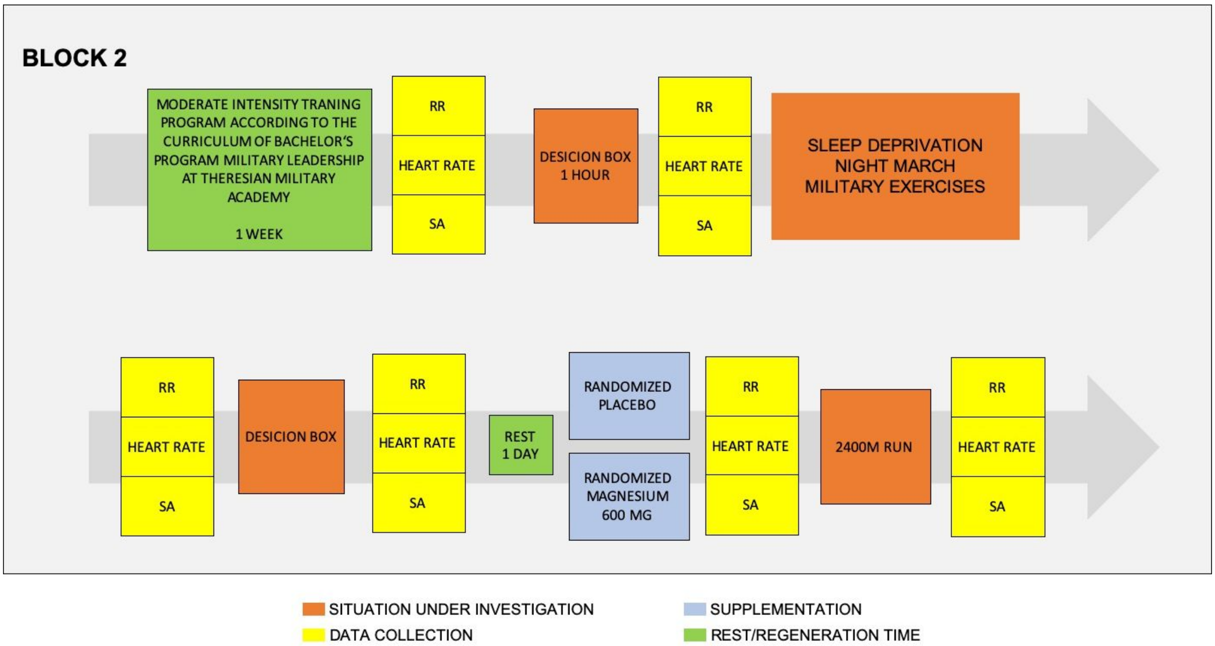

Figure 3.1.2 Schematic diagram of the study design of Block 2 of the stress week. RR = Systolic and diastolic blood pressure; SA = Stress Assessment

3.1.2.3 BLOCK 3

One week after the stress week mentioned above, participants had to run 2400 m again, once more including a Mg supplementation (see Fig 3.1.1). The randomization was the same as in BLOCK 1 but this time placebo and Mg groups were switched. Participants who took Dr. Böhm Magnesium Sport before the first run in BLOCK 1 this time were asked to take placebo and vice versa. To be in line with BLOCK 1 and BLOCK 2, supplementation was done exactly one hour before the run. SA and determination of cardiovascular markers were done in line with previous runs directly before and after the run.

3.1.3 MENTAL COACHING WEEKEND

To investigate the influence of an intensive coaching and relaxing weekend on metabolic and respiratory parameters, participants were coached intensively for three days in a country resort in Bad Gleichenberg, Austria and voluntarily underwent SA before and after that period (148). The goal of this seminar was to help the participants to relax and provoke a shift in mindset towards personal expectations in work and life. Data from all participants who wanted to participate in the investigation of this weekend were analyzed retrospectively in this study.

At the beginning of the seminar, participants were asked to rate their physical, mental and overall state of mind on a scale from 0 to 10 points (10 points optimum), followed by determination of blood pressure and heart rate as well as capillary blood sampling for SA. During the seminar, participants were coached in several single and group sessions combined with low intensity hiking, breathing exercises, wellness and meditation. At the end of the three days, participants rated their state of mind again, cardiovascular parameters were determined (see chapter 3.4), and capillary blood samples were drawn for SA (see chapter 3.3). Blood analysis was performed immediately after blood sampling. Figure 3.1.4 shows a graphical representation of the study design.

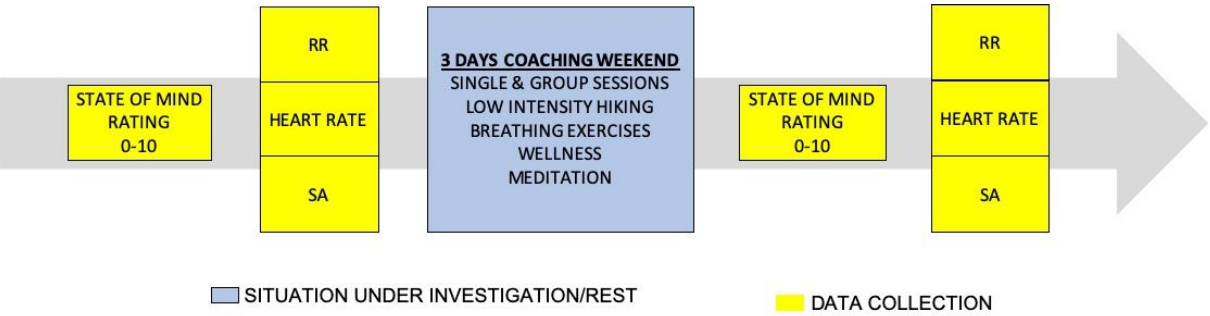

Figure 3.1.3 Schematic diagram of the study design of the mental coaching weekend. RR = Systolic and diastolic blood pressure; SA = Stress Assessment

3.1.4 COMBINATION OF PHYSICAL AND MENTAL STRESSORS – ACCORDION CLASS

To investigate the influence of music and especially training on a music instrument on respiratory and metabolic parameters, accordion students from the accordion teaching center in Gasen (“Stoanihaus”), Styria, Austria were invited to voluntarily undergo stress assessments (SA) and to complete the MMSQ – a psychological questionnaire (see chapter 3.5). Data from all students who wanted to participate in the investigation from two separate days were analyzed retrospectively in this study (149,150). To exclude possible day-dependent effects on individual reactions to the lesson, only participants from one day were analyzed in this thesis.

The students arrived at scheduled time points, depending on the appointment for the accordion lesson. After arrival, students were asked to rest a view minutes to exclude effects from travel related efforts. After completion of the MMSQ, blood pressure and heart rate were measured as described in chapter 3.4. 100 µl of capillary blood for stress assessment (SA) were drawn from the fingertip after recording of cardiovascular parameters. Height, weight and age were recorded in parallel. After collection of all information, the students started their 30 minutes accordion lesson.

Immediately after completion of the class, blood pressure and heart rate were measured again followed by capillary blood sampling for immediate SA. The study was finalized by completion of the MMSQ. Figure 3.1.4 shows a graphical representation of the study design.

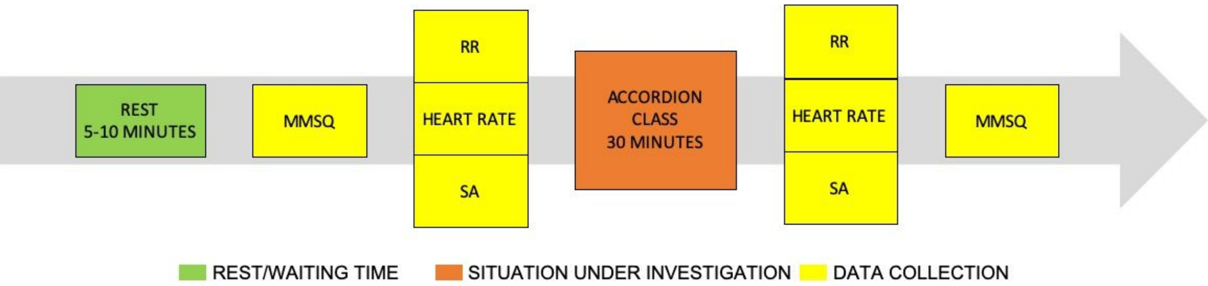

Figure 3.1.4 Schematic diagram of the study design of the accordion class. MMSQ = Multidimensional Mood State Questionnaire; RR = Systolic and diastolic blood pressure; SA = Stress Assessment

3.2 2400 METER RUN

To determine the impact of physical effort on the organism, a 2400 meter run was facilitated as a well-defined and standardized workload. The main advantage of the run is, that it is well known by the participants of the Theresian Military Academy as a routine sports program and part of the bachelor's degree study program Military Leadership. Although mental and physical stress cannot be clearly distinguished, we assumed that its influence as a mental stressor is marginal. Blood sampling and SA (see chapter 3.3) always were performed directly before and after the run on a voluntarily basis.

Participants were asked to run 2400m as fast as possible. In all our experiments, the individual running times basically varied between 520 and 750 seconds, outliers not taken into account.

3.3 CAPILLARY BLOOD SAMPLING AND STRESS ASSESSMENT

Capillary blood samples (100µl) from the fingertip were drawn at several predefined timepoints (before and after mental or physical workloads) by punctuation with automatic lancets. The samples were analyzed immediately using a pHOx-M blood analysis device (Nova Biomedical, Waltham, USA; distributed by TECOM GmbH, Wiener Neudorf, Austria) and a broad range of metabolic parameters as well as electrolytes (see table 3.3.1) was determined within approx. two minutes. Calibration was done according to manufacturer's instructions. The main advantage of the pHOx-M device is that it measures ionized Mg instead of total Mg, which is thought to be much more sensitive to evaluate Mg dynamics (151,152). Further, its mobility makes it possible to analyze the blood samples at random places, dependent on the experimental setting. Table 3.3.1 gives an overview of analyzed parameters and respective methodologies.

Table 3.3.1 Methodologies of measurements of the pHOx-M blood analysis device.

MEASURED PARAMETER	METHODOLOGY	REFERENCE RANGE	UNIT
pH	DIRECT ION-SELECTIVE ELECTRODE	7.35 – 7.45	–
Ionized Mg	DIRECT ION-SELECTIVE ELECTRODE	0.44–0.59	mmol/L
Ionized Ca	DIRECT ION-SELECTIVE ELECTRODE	1.09–1.30	mmol/L
Ionized Na	DIRECT ION-SELECTIVE ELECTRODE	136–146	mmol/L
Ionized K	DIRECT ION-SELECTIVE ELECTRODE	3.5–5.1	mmol/L
pCO_2	SEVERINGHAUS ELECTRODE	35–45	mmHg
pO_2	AMPEROMETRIC	> 40	mmHg
Glucose	ENZYME/AMPEROMETRIC	<110	mg/dL
Lactate	ENZYME/AMPEROMETRIC	0.7–2.5	mmol/L
HCO_3	CALCULATED	21–28	mmol/L
Base excess	CALCULATED	(-2) – (+2)	mmol/L
O_2 Saturation	OPTICAL, REFLECTANCE	94–98	%

Estimated non lactic acidity (ENLA) was calculated by the following formula:

$$\text{ENLA (mmol/L)} = (-1) \times \text{BE (mmol/L)} - \text{lactate (mmol/L)}$$

3.4 BLOOD PRESSURE AND HEART RATE MEASUREMENT

Systolic and diastolic blood pressure, as well as heart rate were determined by using a BM 40 upper arm blood pressure monitor (Beurer GmbH, Ulm, Germany) according to manufacturer's instructions.

3.5 MULTIDIMENSIONAL MOOD STATE QUESTIONNAIRE (MMSQ)

The original German Version of the Multidimensional Mood State Questionnaire consists of 24 items (each with a five-step answer scale) and was used to determine three bipolar dimensions of current mental well-being: good-bad mood, awake-tired and calm-nervous (153). All three scales can be divided into two parallel test halves, which can be used to measure the progress of the mental state.

The MMSQ is described to be used for adolescents and adults, in therapy evaluation and basic research, especially emotional psychology, psychophysiology and psychopharmacology, but also in applied research in health psychology, sports psychology, ecopsychology and more.

The internal consistency (Cronbach's Alpha) of the scales lies between $\alpha = 0.86$ and $\alpha = 0.94$. It takes approx. 4 to 8 minutes to complete the questionnaire.

3.6 STATISTICAL ANALYSIS

All calculations in this thesis as well as charts and figures have been done by using the statistics program SPSS 25 (IBM Corp., USA). Statistical analysis was based on descriptive statistics, which was initially planned as the only method for data evaluation. Therefore, only this analysis was included in ethics proposals. It was later decided to use other statistical methods to gain better insights into stress-related dynamics and to compare different subgroups in regards of their reactions.

Depending on the distribution, mean values and standard deviations or medians and quartiles were calculated (for numerical data). For normal distributed data sets paired-samples or independent-samples t-tests were conducted to compare means of measured metabolic parameters and electrolyte levels. For small sample sizes, Wilcoxon signed-rank test was performed to compare means. Pearson product-moment correlation coefficients were computed to assess the relationship between different metabolic parameters and electrolyte levels. Changes in investigated parameters during specific situations were computed by subtraction of the value before from the value after the situation. Cases with missing variables were excluded analysis by analysis. In bar charts, statistical results are presented using the 95% confidence interval.

3.7 ETHICS AND RECRUITMENT

In order to study even the smallest changes in metabolic parameters and electrolyte levels, as homogeneous groups as possible were investigated. This was especially the case when stress was investigated in officer trainees of the Theresian Military Academy. Recruitment was dependent on each particular situation under investigation. As all situations were either part of the bachelor’s program Military Leadership at the Theresian Military Academy or specific sessions or trainings, all participants of each particular situation under investigation were asked to participate in the study.

All participants of all studies in this thesis were instructed according to the Declaration of Helsinki (154) and both retrospective as well as prospective investigations were approved by the ethical commission of the Theresian Military Academy in Wiener Neustadt, Austria. Almost all studies in this thesis were observational studies and all exercises were part of the training program or part of activities not required by the study protocol. Participants were told several times that their participation is on a voluntary basis and that they are able to withdraw from the studies at any time without giving reasons. The Mg supplementation study was carried out as a blinded, randomized controlled trial. Randomization was done by flipping a coin. All collected data were anonymized (see chapter 3.8) and stored on separate password-protected hard disks.

For prospectively collected data, all participants gave informed consent prior to study enrolment.

3.8 ANONYMIZATION OF DATA

All data were anonymized by irreversibly encoding ID-numbers to allow grouping of individual results without retaining personally identifying information. Dates and times were converted to relative values, just for identification of temporal spacing between measurements.

4 RESULTS

4.1 ANALYSIS OF 2400 M RUNNERS

Blood samples of 320 officer trainees (6 female) from a total of seventeen 2400 m runs at the Theresian Military Academy were analyzed (see chapter 3.3) to better understand Mg dynamics upon exercise in dependence of metabolic and respiratory effort (147). Only blood parameters were available for this analysis, as additional information like age, weight or height have not been recorded. Data from participants, who withdrew their informed consent were not available for this analysis. Due to very rare technical malfunctions of the pHOx-M blood analysis device, it could happen, that single parameters could not be measured. Cases with one missing variable were excluded analysis by analysis. Cases with two or more missing values were excluded from all analyses (n=10). Table 4.1.1 gives an overview of all runs, included participants and missing variables.

Table 4.1.1 Overview of all 2400 m runs, included participants and missing variables

RUN	N	Missing variables	EXCLUDED FROM ANALYSIS (>1 missing value)
No. 1	16	complete dataset	–
No. 2	24	complete dataset	–
No. 3	15	Lactate after the run (n=1)	n=1
No. 4	24	complete dataset	–
No. 5	19	complete dataset	–
No. 6	22	BG before the run (n=1)	n=1
No. 7	25	BG before the run (n=1)	n=8
No. 8	25	iMg after the run (n=1)	–
No. 9	16	complete dataset	–
No. 10	8	complete dataset	–
No. 11	15	BG before the run (n=2)	–
No. 12	25	iMg before the run (n=25)	–
No. 13	16	complete dataset	–
No. 14	25	complete dataset	–
No. 15	20	complete dataset	–
No. 16	9	iCa after the run (n=1)	–
No. 17	16	complete dataset	–
TOTAL	310	iMg = 26; BG = 4; iCa=1; Lactate=1	n=10

A paired-samples t-test was conducted to compare metabolic and electrolyte changes before and after a 2400 m run. Table 4.1.2 shows differences in all measured parameters.

Table 4.1.2 Significant differences in all analyzed parameters before and after the 2400 m run (paired samples t-test); mean values outside the reference range are marked with an asterisk; BE = base excess; BG = blood glucose

PARAMETER (REF. & UNIT)	**BEFORE THE RUN**			**AFTER THE RUN**		**T-VALUE**	**P-VALUE**
	N	**MEAN**	**SD**	**MEAN**	**SD**		
pH (7.35–7.45)	310	7.43	0.021	7.42	0.046	1.918	0.056
pCO_2 (35–45 mmHg)	310	35.10	3.031	33.26*	3.316	11.557	0.000
BE (-2–+2 mmol/L)	310	-1.05	1.856	-2.43*	3.827	6.804	0.000
HCO_3^- (21–28 mmol/L)	310	23.47	1.791	22.20	3.233	7.646	0.000
pO_2 (>40 mmHg)	310	72.32	7.221	75.43	8.897	-5.593	0.000
O_2sat (94–98 %)	310	94.61	1.730	95.17	1.485	-5.201	0.000
iNa (136–146 mmol/L)	310	144.25	3.534	144.77	3.662	-3.038	0.003
iCa (1.09–1.30 mmol/L)	309	1.10	0.066	1.11	0.077	-2.543	0.011
iMg (0.44–0.59 mmol/L)	284	0.51	0.053	0.49	0.055	8.039	0.000
iK (3.5–5.1 mmol/L)	310	4.22	0.444	4.41	0.534	-7.248	0.000
Lactate (0.7–2.5 mmol/L)	309	2.17	0.896	3.42*	2.705	-8.046	0.000
BG (<110 mg/dl)	306	111.91*	18.354	108.29	18.606	2.463	0.014

Significant decreases in pCO_2, BE and HCO_3^- as well as significant increases in pO_2, O_2sat and lactate level indicate high respiratory and metabolic effort as a reaction to the run. Overall there was a significant decrease in ionized Mg level.

The comparatively large number of participants in this analysis allowed to select specific subgroups with predefined characteristics in regards of metabolism before, during and after the run. The selected subgroups where compared by independent sample t-test and by Pearson product-moment correlation coefficients. The latter give an indication on how Mg-dynamics change with metabolic effort.

To investigate differences in ionized Mg level dynamics between preloaded and not preloaded participants, a subgroup with slightly increased muscular metabolism already before the run was selected. Therefore, all participants with a lactate level of more than 2 mmol/L already before the run were compared with all other runners. As already mentioned in the introduction, a lactate level in blood of 2 mmol/L is thought to be a critical threshold and was investigated in several studies. To investigate only effort due to the run and exclude effects of possible preloads, a second subgroup with an increase in lactate level during the run of more than 2 mmol/L was selected. The third subgroup which was selected consisted of participants with an increase in lactate level of more than 7 mmol/L. This threshold represents the upper 5% of the investigated runners. Figure 4.1.1 shows lactate levels before and after the run. Colors indicate the selected subgroups.

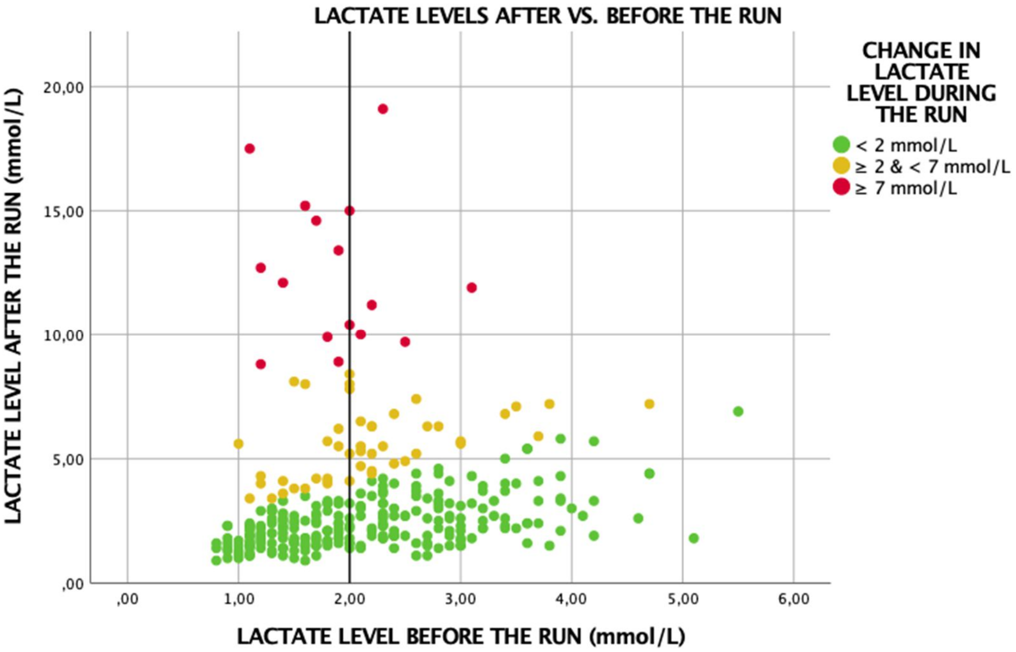

Figure 4.1.1 Lactate levels in blood before and after the 2400 m run. Colors indicate selected subgroups dependent on change in lactate level during the run. Vertical line represents the threshold of 2 mmol/L.

As seen in Fig 4.1.2, mean values of ionized Mg levels were not statistically different before the run in preloaded (n=153, M=0.5051 ±0.05719 mmol/L) vs. not preloaded participants (n= 131, M=0.5082 ±0.04777 mmol/L). After the run, ionized Mg levels were significantly decreased in the preloaded group (n=153, M=0.4759 ±0.05413 mmol/L), compared to the not preloaded group (n=131, M=0.4979 ±0.05449 mmol/L); t=-3.401, p=0.001.

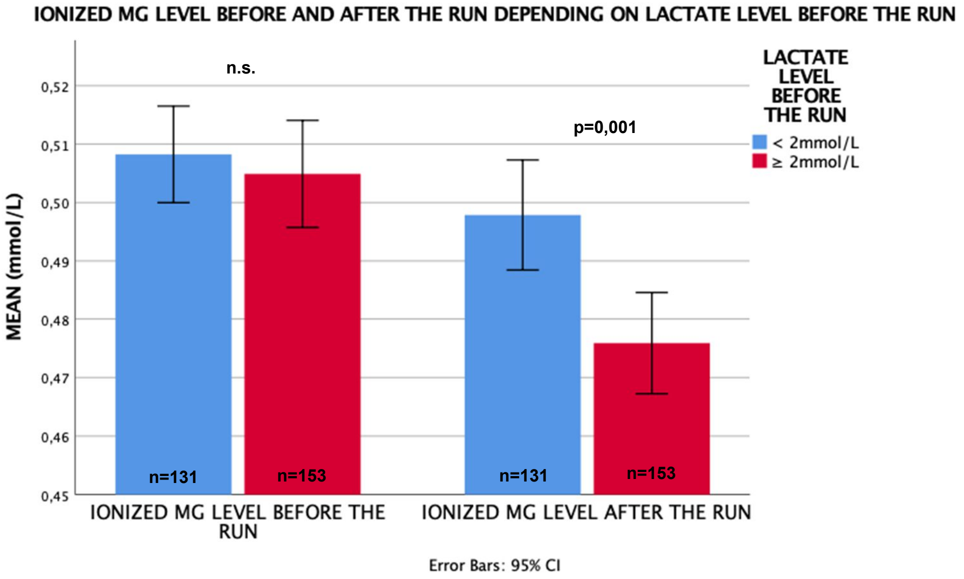

Figure 4.1.2 Ionized Mg level before and after the run depending on lactate level before the run; ionized Mg before the run: 0.5082 (± 0.04777) vs. 0.5051 (± 0.05719) mmol/L, n.s.; ionized Mg after the run: 0.4979 (± 0.05449) vs. 0.4759 (± 0.05413) mmol/L, t=-3.401, p=0.001

Therefore, it is not surprising that decrease in ionized Mg level during the run was significantly higher in the preloaded group (n=153, M=-0.0290 ±0.04985 mmol/L) compared to the not preloaded group (n=131, M=-0.0104 ±0.02993 mmol/L), t=-3.879, p=0.000 (Fig. 4.1.3).

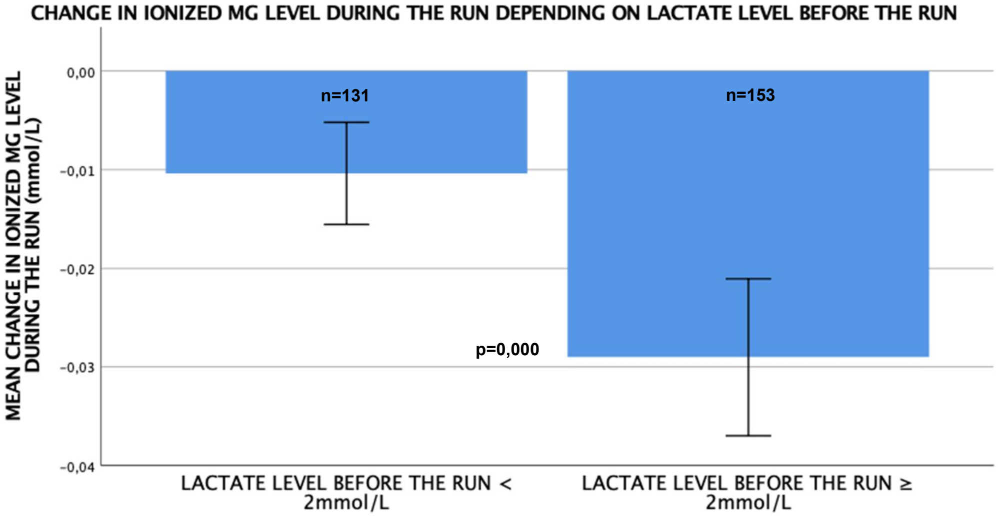

Figure 4.1.3 Change in ionized Mg level during the run depending on lactate level before the run; -0.0104 (± 0.02993) vs. -0.0290 (± 0.04985) mmol/L, t=-3.879, p=0.000

Due to the selection criteria, mean lactate level after the run was still higher in the preloaded group (n= 163, M=3.8160 ±2.53939 mmol/L) compared to others (n=146, M=2.9863 ±2.82329 mmol/L), t=-3.401, p=0.001, whereas changes in mean lactate level tended to be higher in the not preloaded group (0.9847 ± 2.78668 vs 1.5353 ± 2.67961 mmol/L), but this difference did not reach statistical significance (Fig 4.1.4).

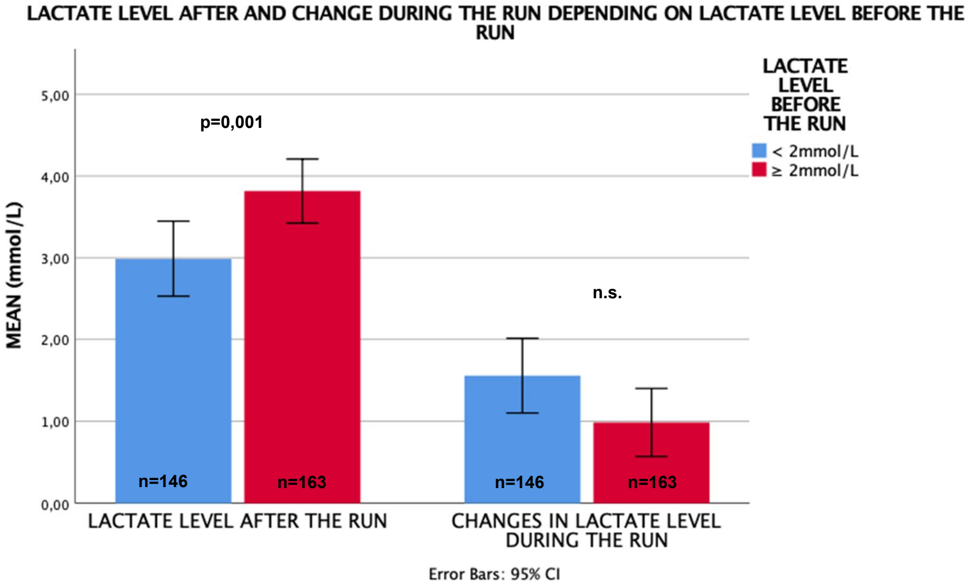

Figure 4.1.4 Lactate level after and change during the run depending on lactate level before the run; lactate level after the run: 2,9863 (± 2,82329) vs. 3.8160 (± 2.53939) mmol/L, t=-3.401, p=0.001; changes in lactate level during the run: 1.5353 (± 2.67961) vs. 0.9847 (± 2.78668) mmol/L, n.s.

A multiple regression analysis was calculated to predict decrease in ionized Mg based on HCO_3^-, pCO_2, BE, lactate level and blood glucose level before the run. A significant regression equation was found, F = 9.725, p = 0.000, with an adjusted R^2 of 0.136. According to the calculated model, ionized Mg level decrease during the run was highest in participants with a high lactate level (unstandardized coefficient=-0.013, t=-4.535, sig.=0.000), high BE (unstandardized coefficient=-0.032, t=-2.352, sig.=0.019) and high pCO_2 (unstandardized coefficient=-0.010, t=-3.136, sig.=0.002) and a low blood glucose level (unstandardized coefficient=0.000, t=2.449, sig.=0.015) and low HCO_3^- (unstandardized coefficient=0.044, t=2.493, sig.=0.013) before the run.

As the changes in ionized Mg levels correlate with the ionized Mg levels before the run, a further analysis was carried out using residualized change scores. The regression analysis still gave the same results, still allowing to predict changes in ionized Mg levels from lactate level, BE, pCO_2, blood glucose level and HCO_3^- before the run.

In the next step a subgroup of runners was selected, who showed an increase in lactate level >2 mmol/L during the run, to investigate differences in ionized Mg dynamics in participants, for whom the run was an strenuous effort (strained) and those, for whom the run was just a moderate exercise (less strained).

Mean ionized Mg level was significantly lower in strained persons before (n=60, M=0.4913 ±0.05322 mmol/L) and after (n=60, M=0.4723 ±0.04767 mmol/L) the run compared to less strained persons (n=225, M=0.5106 ±0.05232 and n=225, M=0.4897 ±0.05671mmol/L, respectively), t=-2.522, p=0.012 and t=-2.173, p=0.031, respectively (Fig. 4.1.5).

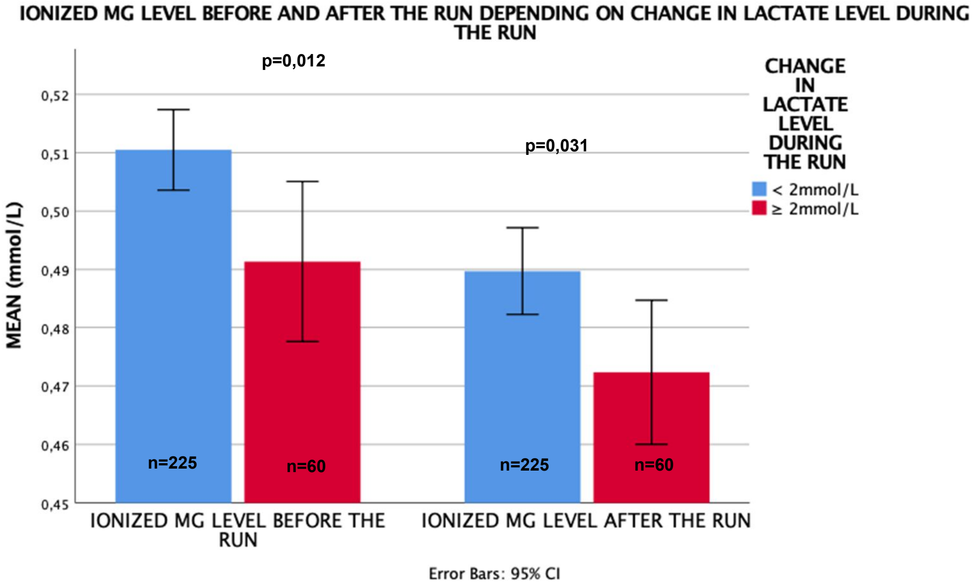

Figure 4.1.5 Ionized Mg level before and after the run depending on change in lactate level during the run; ionized Mg before the run: 0.5106 (± 0.05232) vs. 0.4913 (± 0.05322) mmol/L, t=-2.522, p=0.012; ionized Mg after the run: 0.4897 (± 0.05671) vs. 0.4723 (± 0.04767) mmol/L, t=-2,173, p=0.031

Changes in ionized Mg levels were not different between strained and less strained persons (Fig. 4.1.6). In both groups the Mg level decreased significantly (ionized Mg level before vs. after; p=0.000 in both groups).

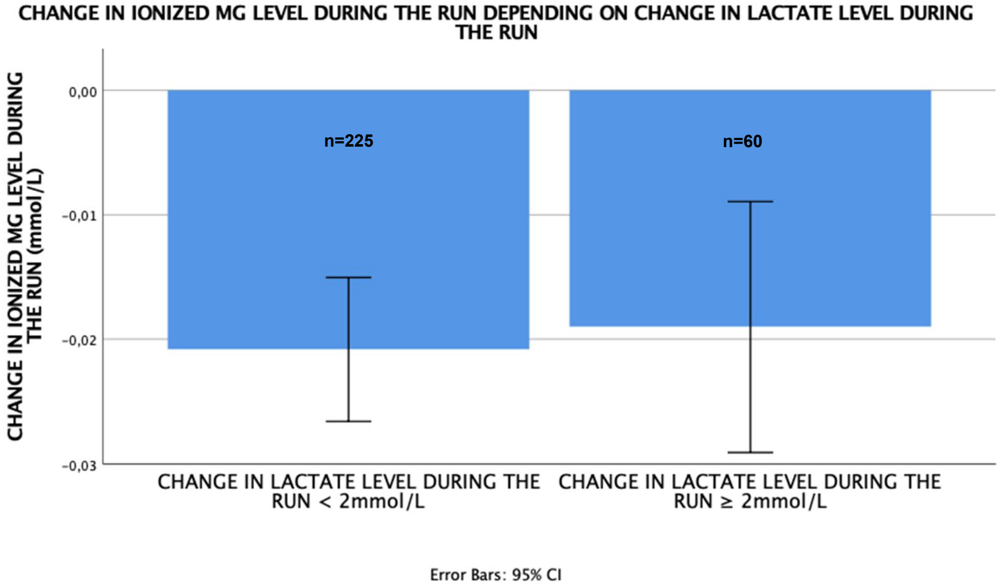

Figure 4.1.6 Change in ionized Mg level during the run depending on change in lactate level during the run; -0.0208 (± 0.04386) vs. -0.0190 (± 0.03896) mmol/L, n.s.

The lactate level after the run was significantly higher in strained (n=63, M=7.3222 ±3.61874 mmol/L) vs. less strained (n=246, M=2.4256 ±0.98766 mmol/L) persons, t=10.639, p=0.000. Nevertheless, mean lactate level before the run was not statistically different between the compared groups (Fig. 4.1.7).

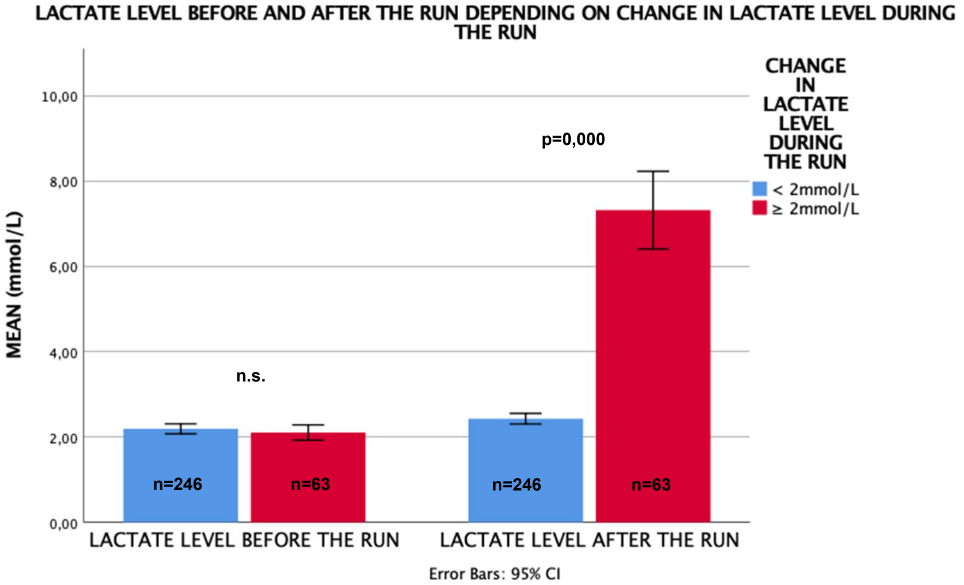

Figure 4.1.7 Lactate level before and after the run depending on change in lactate level during the run; lactate level before the run: 2.1842 (± 0.93741) vs. 2.1000 (± 0.71279) mmol/L, n.s.; lactate level after the run: 2.4256 (± 0.98766) vs. 7.3222 (± 3.61874) mmol/L, t=10.639, p=0.000

Next, the differences were assessed in persons with extremely high muscular effort during the run, defined as an increase in lactate level of more than 7 mmol/L. 15 persons (upper 5 % of all participants) fulfilled these criteria and were compared to those with lactate level increase during the run up to 2 mmol/L.

Before the run, mean ionized Mg level was not different between those groups. However, after the run, the mean ionized Mg level was numerically but not significantly higher in the group with extreme muscular effort (n=15, M=0.4967 ±0.04562 mmol/L) compared to others (n=225, M=0.4897 ±0.05671 mmol/L) (Fig. 4.1.8).

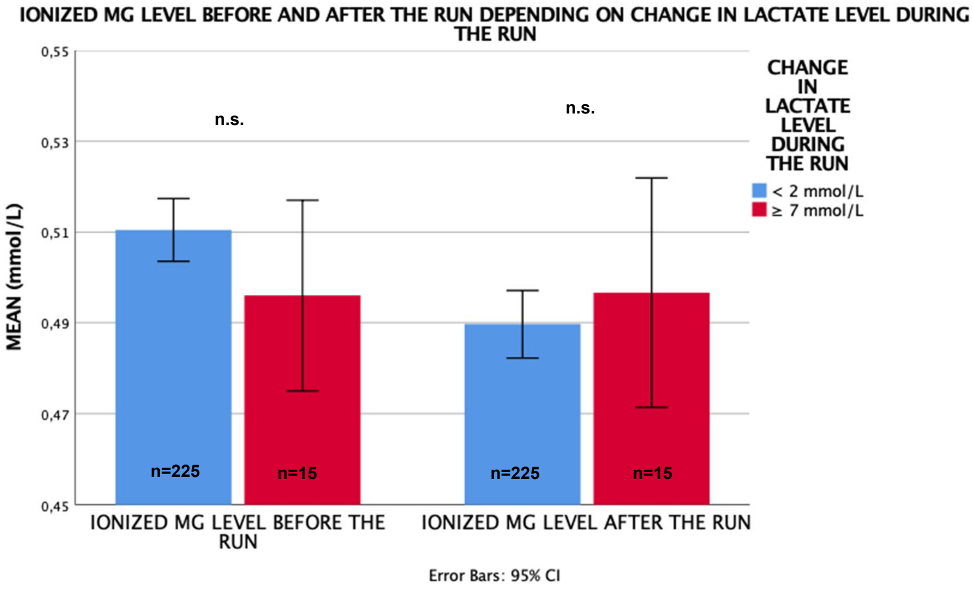

Figure 4.1.8 Ionized Mg level before and after the run depending on change in lactate level during the run; ionized Mg before the run: 0.5106 (± 0.05232) vs. 0.4960 (± 0.03795) mmol/L, n.s.; ionized Mg after the run: 0.4897 (± 0.05671) vs. 0.4967 (± 0.04562) mmol/L, n.s.

Changes in ionized Mg level during the run were numerically different in persons with high muscular effort (n=15, M=0.0007 ±0.03195 mmol/L) compared to others (n=225, M=-0.0208 ±0.04386 mmol/L), t=-1.861, p=0.064, with even slight increases in ionized Mg level (Fig. 4.1.9).

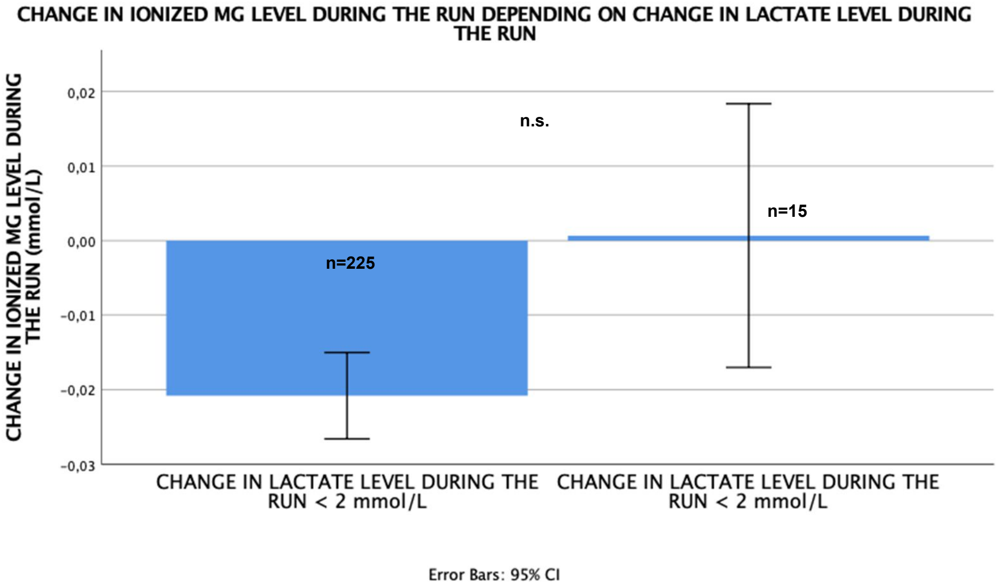

Figure 4.1.9 Change in ionized Mg level during the run depending on change in lactate level during the run; -0.0208 (±0.04386) vs. 0.0007 (±0.03195) mmol/L; t=-1.861, p=0.064

The mean lactate level after the run was significantly higher in persons with high muscular effort (n=16, M=12.5250 ±3.07105 mmol/L) compared to others (n=247, M=2.4256 ±0.98766 mmol/L), t=-13.110, p=0.000. Mean lactate level before the run was significantly lower in runners with increase in lactate level > 7 mmol/L (Fig. 4.1.10).

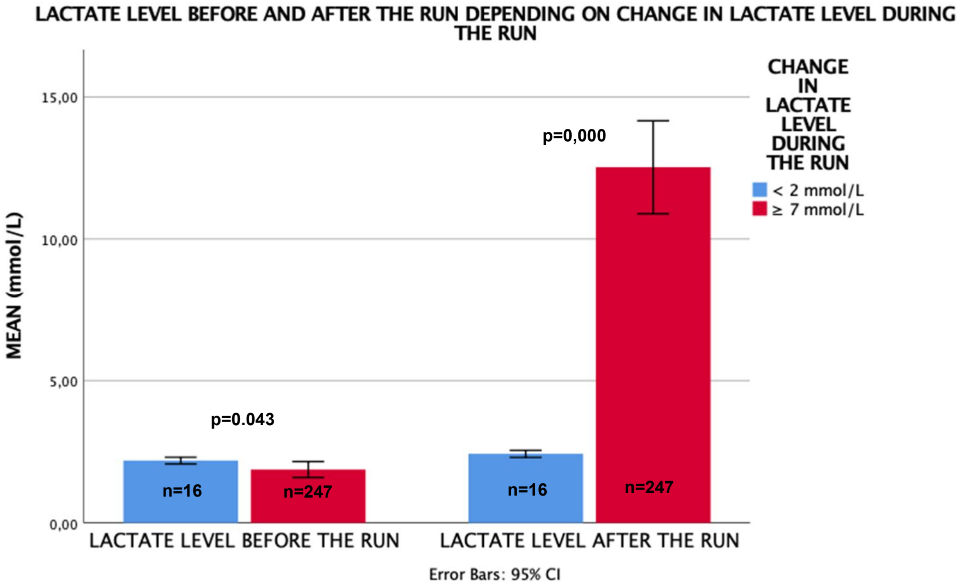

Figure 4.1.10 Lactate level before and after the run depending on change in lactate level during the run; lactate level before the run: 2.1842 (± 0.93741) vs. 1.8750 (± 0.52345) mmol/L, t=2,150, p=0.043; lactate level after the run: 3.0135 (± 1.72247) vs. 13.5833 (± 2.80676) mmol/L, t=12.947, p=0.000

In a final step, runners with higher muscular effort during the run (increase in lactate level >2 mmol/L) as well as increase in blood glucose level (>15 mg/dl) as a marker for increased sympatho-adrenal activity were selected (n=21) and compared to all others. The threshold of >15 mg/dl corresponds to the upper 15 % of all runners.

Independent samples t-test and Pearson product-moment correlation coefficients revealed significant differences between those with high effort and those without measurable effort (Table 4.1.2). Respective scatterplots summarize the results and give an overview of significant correlations. Analysis revealed distinct differences between those two groups.

Table 4.1.3 Comparison of changes in analyzed parameters during the run between the high effort and low effort group (independent samples t-test). High effort = change in lactate level during the run > 2 mmol/L & increase in blood glucose level during the run > 15 mg/dl; BE = base excess; BG = blood glucose

	MEAN CHANGE DURING THE RUN							
PARAMETER (REF. & UNIT)	**LOW EFFORT**			**HIGH EFFORT**			**T-VALUE**	**P-VALUE**
	N	**MEAN**	**SD**	**N**	**MEAN**	**SD**		
pH (7.35–7.45)	289	0.003	0.0291	21	-0.120	0.0925	6.066	0.000
pCO_2 (35–45 mmHg)	289	-1.470	2.4556	21	-6.900	2.3345	9.814	0.000
BE (-2–+2 mmol/L)	289	-0.717	2.2619	21	-10.562	5.4415	8.239	0.000
HCO_3 (21–28 mmol/L)	289	-0.742	1.9844	21	-8.614	3.9796	8.984	0.000
pO_2 (83–108 mmHg)	289	2.064	8.6995	21	17.462	12.5098	-5.544	0.000
O_2sat (94–98%)	289	0.484	1.8666	21	1.605	2.0166	-2.643	0.009
iNa (136–146 mmol/L)	289	0.520	3.0706	21	0.500	2.0065	0.030	0.976
iCa (1.09–1.30 mmol/L)	289	0.014	0.0906	20	0.003	0.0466	0.940	0.355
iMg (0.44–0.59 mmol/L)	265	-0.021	0.0429	18	-0.016	0.0390	-0.472	0.637
iK (3.5–5.1 mmol/L)	289	0.227	0.4388	21	-0.347	0.3594	5.847	0.000
Lactate (0.7–2.5 mmol/L)	289	0.713	1.6086	21	8.581	4.2614	-8.418	0.000
BG (<110 mg/dl)	289	-7.130	22.1226	21	44.095	23.0215	-10.213	0.000

Runners with high effort during the run showed significantly larger decreases in pH, pCO_2, base excess, HCO_3 and K as well as significantly higher increases pO_2, lactate level and blood glucose level indicating high effort and respective changes in all analyzed parameters. Ionized Mg levels before and after the run were not statistically different (Fig. 4.1.11).

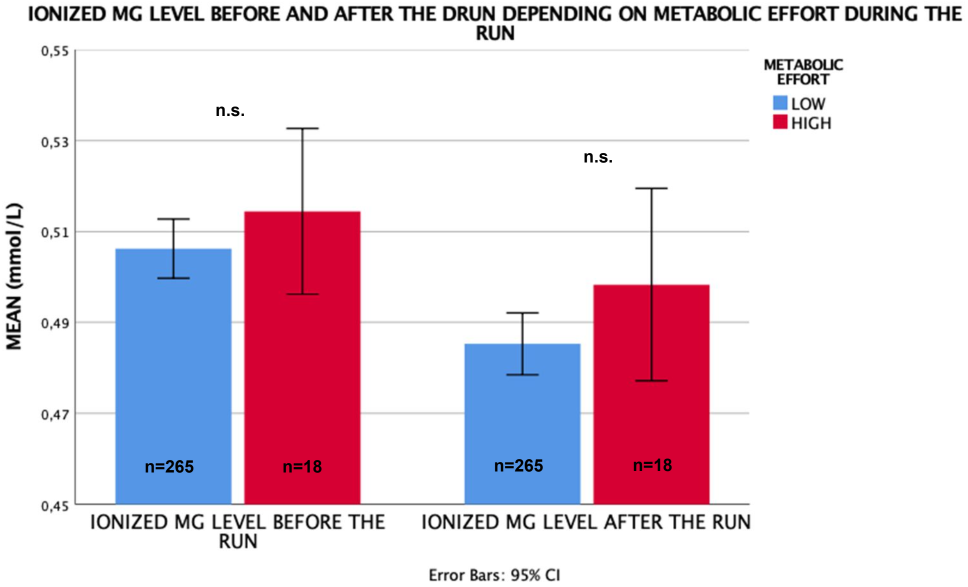

Figure 4.1.11 Ionized Mg level before and after the run depending on metabolic effort during the run; ionized Mg before the run: 0.5064 (± 0.05371) vs. 0.5144 (± 0.03666) mmol/L, n.s.; ionized Mg after the run: 0.4852 (± 0.05612) vs. 0.4983 (± 0.04260) mmol/L, n.s.; High metabolic effort = change in lactate level during the run > 2 mmol/L & increase in blood glucose level during the run > 15 mg/dl

Change in ionized Mg levels was not significantly different between both groups (graph not shown).

Mean lactate levels before the run were not statistically and numerically different between both groups (Fig. 4.1.12). In contrast, change in mean lactate level was approximately 10 times higher in the high effort group (n=21, M=8.5810 ±4.26141 mmol/L) compared to others (n=289, M=0.7128 ±1.60862 mmol/L), t=-8.418, p=0.000, leading to a significantly higher mean lactate level after the run in high effort group (n=21, M=10.5143 ±4.06353 mmol/L vs. n=289, M=2.9069 ±1.65963 mmol/L), t=-8.527, p=0.000.

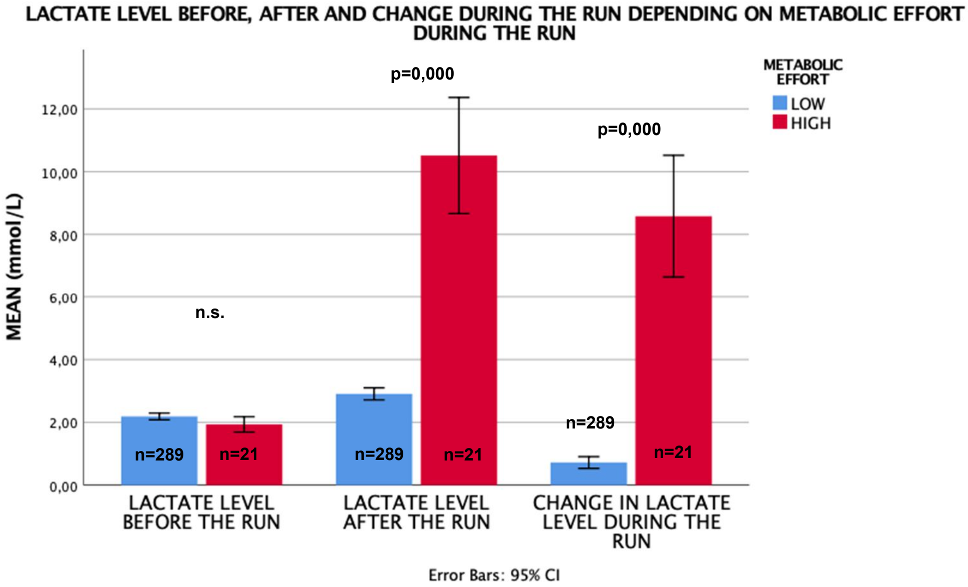

Figure 4.1.12 Lactate level before, after and changes during the run depending on metabolic effort during the run; lactate level before the run: 2.1841 (± 0.91478) vs. 1.9333 (± 0.53790) mmol/L, n.s.; lactate level after the run: 2.9069 (± 1.65963) vs. 10.5143 (±4.26141) mmol/L, t=-8.418, p=0.000; change in lactate during the run: 0.7128 (± 1.60862) vs. 8.5810 (±4.94863) mmol/L, t=-6.147, p=0.000

Pearson product-moment correlation coefficients were calculated and differences between those participants with high effort and those without measurable effort could be observed. Respective scatterplots summarize the results and give an overview of significant correlations. There was a negative correlation between change in ionized Mg level and change in pCO_2 during the run (Fig. 4.1.13, r=-0,631, n=18, p=0,005). Similar findings could be observed with a correlation between change in base excess as a marker for metabolic effort and change in ionized Mg levels (graph not shown, r=-0,680, n=18, p=0,002). Participants with the highest increase in respiratory minute volume, show slight increases in ionized Mg level whereas ionized Mg level in respiratory calmer persons seems to decrease.

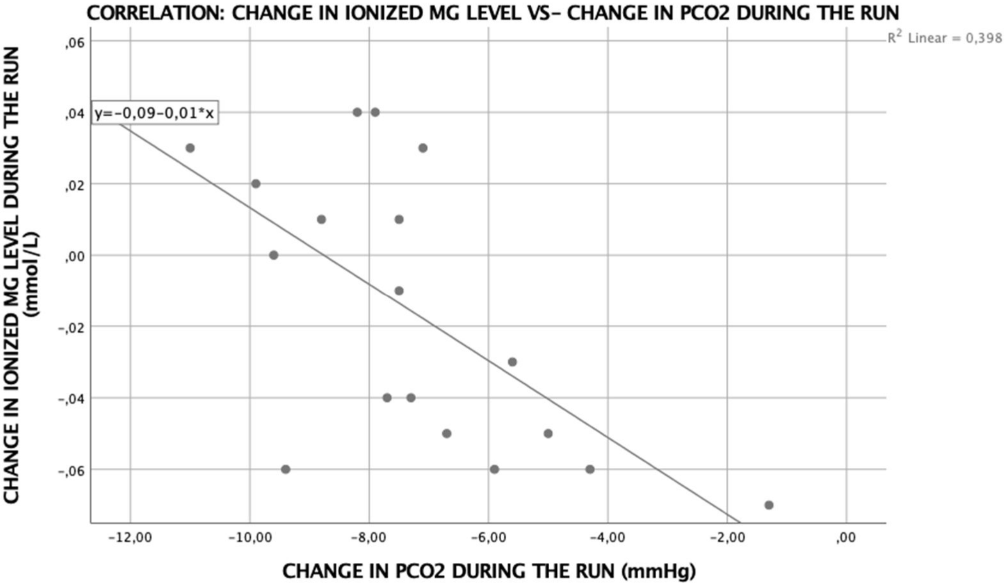

Figure 4.1.13 Correlation: Change in ionized Mg level vs. change in pCO_2 during the run in participants with high metabolic effort (increase in lactate level > 2 mmol/L & increase in blood glucose level > 15 mg/dl during the run); r=-0,631, n=18, p=0,005.

A significant positive correlation of changes in ionized Mg levels and changes in lactate levels during the run (Fig. 4.1.14, r=0.819, n=18, p=0.000) indicates, that also muscular effort drives Mg dynamics. Mg levels tend to increase in persons with high lactate increases during the run and decreases in persons with low lactate increases.

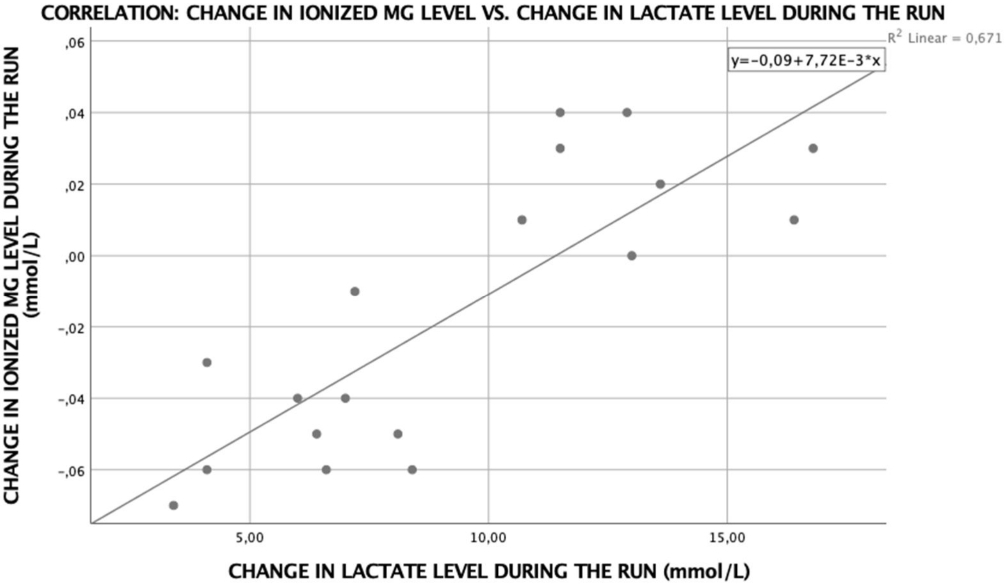

Figure 4.1.14 Correlation: Change in ionized Mg level vs. change in lactate level during the run in participants with high metabolic effort (increase in lactate level > 2 mmol/L & increase in blood glucose level > 15 mg/dl during the run); r=0.819, n=18, p=0.000

Similar findings could be observed with a correlation between change in base excess as a marker for acid production and change in ionized Mg levels (Fig. 4.1.15, r=-0.798, n=18, p=0.000).

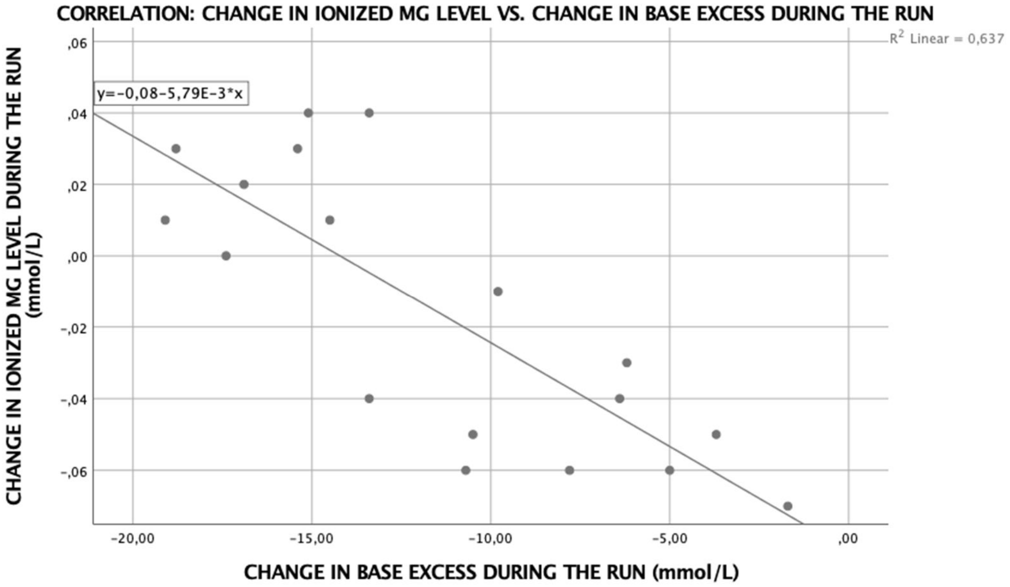

Figure 4.1.15 Correlation: Change in ionized Mg level vs. change in base excess during the run in participants with high metabolic effort (increase in lactate level > 2 mmol/L & increase in blood glucose level > 15 mg/dl during the run); r=-0.798, n=18, p=0.000

The higher metabolic and respiratory effort seemed to affect the pH value during the run. There was a negative correlation between changes in pH value and changes in ionized Mg level during the run (Fig. 4.1.16, r=-0.801, n=18, p=0.000). Participants with a large decrease in pH value showed an increase in ionized Mg level, whereas ionized Mg level decreased most when pH value did not change during the run.

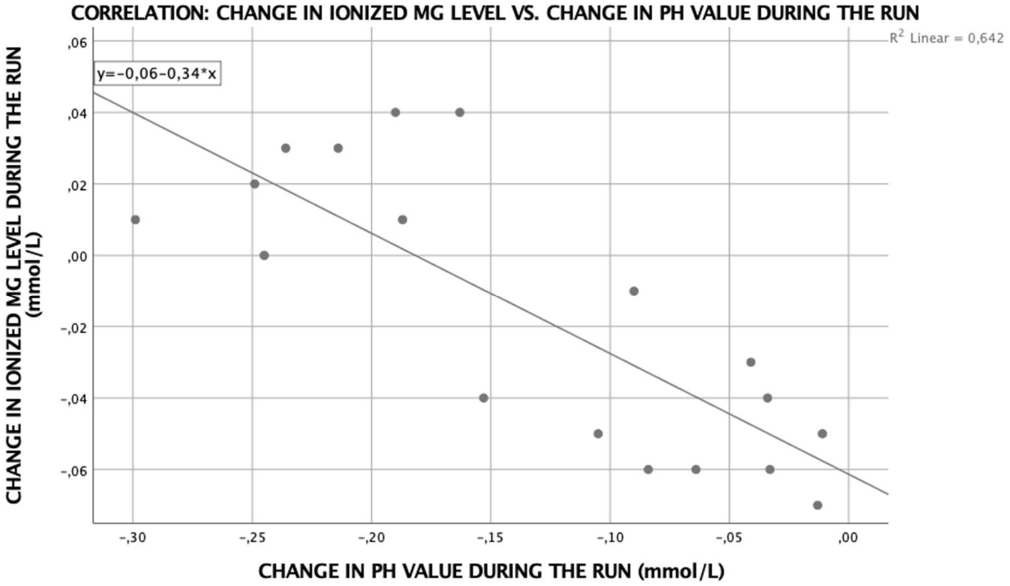

Figure 4.1.16 Correlation: Change in ionized Mg level vs. change in pH value during the run in participants with high metabolic effort (increase in lactate level > 2 mmol/L & increase in blood glucose level > 15 mg/dl during the run); r=-0.801, n=18, p=0.000

None of those correlations could be observed in the group with less effort.

4.2 STRESS WEEK / PLACEBO CONTROLLED MAGNESIUM SUPPLEMENTATION

To investigate the impact of an acute Mg supplementation on subsequent effort and performance in sport, a randomized, double-blind, placebo-controlled study with a crossover design was conducted (see chapter 3.1.2). In total 26 persons (1 female) with a mean age of 24.2 (±2.5) years, a mean weight and height of 80.6 (±10.17) kg and 179.9 (±7.34) cm, respectively and a mean BMI of 24.87 (±2.491) were included in the study. Additional characteristics of the study population have not been collected. At the first 2400 m run 13 participants were randomized to the placebo and 13 to the Mg group. During the study, 7 persons withdraw their informed consent or were lost to follow up. At the last run, of the remaining 19 participants, 6 persons of the Placebo group and all 13 participants of the Mg group remained in the study. As a result of the switch-design, at the last run 6 participants were given Mg and 13 participants were given placebo. For final analysis, results from both runs were pooled resulting in 19 participants in the Mg group and 26 participants in the placebo group. As described in chapter 3.3, capillary blood samples were drawn one hour after supplementation which was immediately before the run as well as directly after the run and analyzed immediately. The results of both days were pooled and split depending on the respective intervention.

Due to a malfunction of the pHOx-M blood analysis device, pCO_2, BE, HCO_3^- and O_2sat-parameters were not available from one participant before and one participant after the run after placebo supplementation, and pCO_2, BE, HCO_3^- and O_2sat after the run after placebo supplementation as well as the potassium level, systolic blood pressure, diastolic blood pressure and heart rate before the run after Mg supplementation from another participant. Cases with missing variables were excluded analysis by analysis.

A paired-samples t-test was conducted to compare metabolic and electrolyte changes before and after the 2400 m run between the two groups and Pearson product-moment correlation coefficients were computed to assess correlations before and after the run. Looking at both groups separately, almost all analyzed parameters after the run were significantly different compared to the values before the run. In the Mg group only ionized Ca and the diastolic blood pressure did not differ significantly before and after the run. In the placebo group the diastolic blood pressure, ionized Ca and ionized Mg level did not differ significantly. The ionized Mg level in the Mg group significantly decreases (n=19, M=0.5126 ±0.04107 mmol/L before vs. M=0.4895 ±0.03171 mmol/L after; t=2.357, p=0.030) as a reaction to the run whereas in the placebo group the ionized Mg level stays more or less the same (n=26, M=0.4973 ±0.04750 mmol/L before vs. M=0.4885 ±0.03674 mmol/L after; t=1.244, p=0.225).

Table 4.2.1 and table 4.2.2 show a comparison of all analyzed parameters before vs. after the run in the Mg supplemented and placebo supplemented group, respectively.

Table 4.2.1 Comparison of analyzed parameters before and after the 2400 m run in the Mg supplemented group (paired samples t-test); BE = base excess; BG = blood glucose; RRsys = systolic blood pressure, RRdia = diastolic blood pressure; mean values outside the reference range are marked with an asterisk

MAGNESIUM GROUP							
PARAMETER (REF. & UNIT)	**BEFORE THE RUN**			**AFTER THE RUN**		**T-VALUE**	**P-VALUE**
	N	**MEAN**	**SD**	**MEAN**	**SD**		
pH (7.35–7.45)	19	7.44	0.017	7.34*	0.054	8.368	0.000
pCO$_2$ (35–45 mmHg)	19	34.40*	2.016	29.30*	2.248	10.488	0.000
BE (-2–+2 mmol/L)	19	-0.60	1.255	-10.23*	2.667	13.609	0.000
HCO$_3$ (21–28 mmol/L)	19	23.71	1.185	15.84*	1.922	15.125	0.000
pO$_2$ (>40 mmHg)	19	76.28	4.134	93.93	4.795	-10.256	0.000
O$_2$sat (94–98 %)	19	95.70	0.663	96.88	0.511	-6.650	0.000
iNa (136–146 mmol/L)	19	149.87*	2.030	150.88*	2.589	-2.383	0.028
iCa (1.09–1.30 mmol/L)	19	1.21	0.051	1.18	0.056	1.663	0.114
iMg (0.44–0.59 mmol/L)	19	0.51	0.041	0.49	0.032	2.357	0.030
iK (3.5–5.1 mmol/L)	18	4.36	0.240	4.21	0.188	3.611	0.002
Lactate (0.7–2.5 mmol/L)	19	1.74	0.653	9.83*	2.883	-13.584	0.000
BG (<110 mg/dl)	19	110.21*	7.627	143.68*	34.254	-4.221	0.001
RRsys (<120 mmHg)	18	142.00*	16.553	159.28*	18.948	-4.691	0.000
RRdia (<80 mmHg)	18	76.44	8.535	80.28*	11.509	-1.374	0.187
HEART RATE	18	75.28	10.932	115.89	13.297	-15.166	0.000

Table 4.2.2 Comparison of analyzed parameters before and after the 2400 m run in the placebo supplemented group (paired samples t-test); BE = base excess; BG = blood glucose; RRsys = systolic blood pressure, RRdia = diastolic blood pressure; mean values outside the reference range are marked with an asterisk

PLACEBO GROUP							
PARAMETER (REF. & UNIT)	**BEFORE THE RUN**			**AFTER THE RUN**		**T-VALUE**	**P-VALUE**
	N	**MEAN**	**SD**	**MEAN**	**SD**		
pH (7.35–7.45)	26	7.43	0.0287	7.31*	0.083	8.217	0.000
pCO$_2$ (35–45 mmHg)	23	34.45*	2.6510	28.43*	2.554	14.312	0.000
BE (-2–+2 mmol/L)	23	-1.47	2.5306	-12.70*	4.121	12.066	0.000
HCO$_3$ (21–28 mmol/L)	23	23.07	2.1991	14.06*	3.002	13.135	0.000
pO$_2$ (>40 mmHg)	26	76.29	7.2817	94.24	4.539	-11.330	0.000
O$_2$sat (94–98 %)	23	95.32	1.4575	96.51	0.844	-3.231	0.004
iNa (136–146 mmol/L)	26	149.64*	2.0521	152.00*	2.630	-6.060	0.000
iCa (1.09–1.30 mmol/L)	26	1.21	0.0307	1.20	0.043	0.688	0.498
iMg (0.44–0.59 mmol/L)	26	0.50	0.0275	0.49	0.037	1.244	0.225
iK (3.5–5.1 mmol/L)	26	4.24	0.1771	4.16	0.187	2.465	0.021
Lactate (0.7–2.5 mmol/L)	26	2.45	1.8781	11.33*	4.139	-11.155	0.000
BG (<110 mg/dl)	26	109.31	6.596	161.00*	46.317	-5.936	0.000
RRsys (<120 mmHg)	26	136.12*	13.429	158.58*	17.268	-5.657	0.000
RRdia (<80 mmHg)	26	77.27	12.600	79.31	10.487	-0.780	0.443
HEART RATE	26	80.92	19.285	118.35	14.664	-9.263	0.000

Table 4.2.3 and 4.2.4 show a comparison of all analyzed parameters between the Mg supplemented vs. placebo supplemented group before and after the run, respectively.

Baseline values in both groups did not differ significantly except ionized K levels and the systolic blood pressure, both significantly higher in the Mg supplemented group (Table 4.2.3).

Table 4.2.3 Comparison of analyzed parameters between Mg and placebo supplemented groups before the 2400 m run (paired samples t-test); BE = base excess; BG = blood glucose; RRsys = systolic blood pressure, RRdia = diastolic blood pressure; mean values outside the reference range are marked with an asterisk

PARAMETER BEFORE THE RUN (REF. & UNIT)	N	MG GROUP		PLACEBO GROUP		T-VALUE	P-VALUE
		MEAN	SD	MEAN	SD		
pH (7.35–7.45)	19	7.44	0.017	7.44	0.032	0.733	0.473
pCO_2 (35–45 mmHg)	18	34.48*	2.036	33.46*	2.551	1.246	0.230
BE (-2–+2 mmol/L)	18	-0.61	1.289	-1.66	2.744	1.567	0.136
HCO_3 (21–28 mmol/L)	18	23.71	1.219	22.78	2.321	1.618	0.124
pO_2 (>40 mmHg)	19	76.28	4.134	77.11	7.718	-0.465	0.647
O_2sat (94–98 %)	18	95.65	0.652	95.61	1.532	0.118	0.907
iNa (136–146 mmol/L)	19	149.87*	2.030	149.44*	2.355	0.718	0.482
iCa (1.09–1.30 mmol/L)	19	1.21	0.051	1.21	0.028	0.328	0.746
iMg (0.44–0.59 mmol/L)	19	0.51	0.042	0.50	0.022	1.069	0.299
iK (3.5–5.1 mmol/L)	18	4.36	0.240	4.24	0.195	2.914	0.010
Lactate (0.7–2.5 mmol/L)	19	1.74	0.653	2.64*	2.155	-1.699	0.106
BG (<110 mg/dl)	19	110.21*	7.627	110.21*	5.978	0.000	1.000
RRsys (<120 mmHg)	18	142.00*	16.553	131.61*	11.602	2.583	0.019
RRdia (<80 mmHg)	18	76.44	8.535	75.28	10.260	0.349	0.731
HEART RATE	18	75.28	10.932	83.17	21.607	-1.504	0.151

After the run, only base excess and HCO_3 differed significantly between both groups (Table 4.2.4)

Table 4.2.4 Comparison of analyzed parameters between Mg and placebo supplemented groups after the 2400 m run (paired samples t-test); BE = base excess; BG = blood glucose; RRsys = systolic blood pressure, RRdia = diastolic blood pressure; mean values outside the reference range are marked with an asterisk

PARAMETER AFTER THE RUN (REF. & UNIT)	N	MG GROUP		PLACEBO GROUP		T-VALUE	P-VALUE
		MEAN	SD	MEAN	SD		
pH (7.35–7.45)	19	7.34*	0.054	7.31*	0.077	1.625	0.121
pCO_2 (35–45 mmHg)	18	29.34*	2.300	27.98*	2.525	2.024	0.059
BE (-2–+2 mmol/L)	18	-10.39*	2.652	-12.42*	4.256	2.116	0.049
HCO_3 (21–28 mmol/L)	18	15.74*	1.929	14.18*	3.177	2.166	0.045
pO_2 (>40 mmHg)	19	93.93	4.795	93.67	4.702	0.232	0.819
O_2sat (94–98 %)	18	96.91	0.505	96.54	0.762	1.910	0.073
iNa (136–146 mmol/L)	19	150.88*	2.589	151.78*	2.692	-1.438	0.168
iCa (1.09–1.30 mmol/L)	19	1.18	0.056	1.20	0.042	-1.273	0.219
iMg (0.44–0.59 mmol/L)	19	0.49	0.032	0.49	0.034	-0.445	0.662
iK (3.5–5.1 mmol/L)	19	4.22	0.185	4.15	0.208	2.106	0.050
Lactate (0.7–2.5 mmol/L)	19	9.83*	2.883	11.22*	3.902	-1.613	0.124
BG (<110 mg/dl)	19	143.68*	34.254	165.21*	45.721	-1.895	0.074
RRsys (<120 mmHg)	19	158.89*	18.490	156.16*	18.237	0.671	0.510
RRdia (<80 mmHg)	19	80.00	11.250	76.68	10.667	1.099	0.286
HEART RATE	19	115.00	13.491	120.68	13.077	-1.294	0.212
RUNNING TIME (sec)	19	636.37	48.165	629.79	79.905	0.395	0.698

A significantly higher base excess and higher HCO_3 level in the Mg supplemented group compared to the placebo group indicates less metabolic effort upon Mg supplementation.

Pearson product-moment correlation coefficients and respective plots revealed huge differences in metabolic and electrolyte changes between the two groups.

As the only objective marker for performance during the run, running time correlated with several metabolic markers for effort only in the placebo group.

There was a positive correlation between running time and change in pCO_2 during the run in the placebo group (Fig 4.2.1, r=0.529, n=24, p=0.008), indicating higher breathing frequency with faster running time. This could not be observed in the Mg supplemented group (graph not shown, r=-0.109, n=19, n.s.)

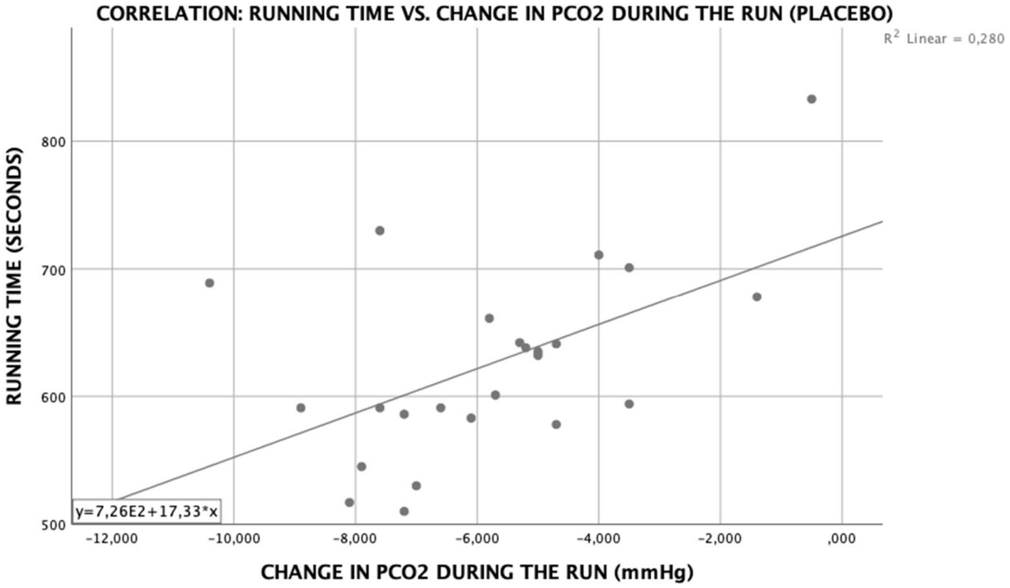

Figure 4.2.1 Correlation: Running time vs. change in pCO_2 *during the run after placebo supplementation; r=0.529, n=24, p=0,008*

As a marker for muscular effort, changes in lactate level during the run negatively correlated with running time in the placebo group (Fig 4.2.2, r=-0.685, n=26, p=0.000). Without Mg supplementation, faster running times go along with a higher increase in lactate level during the run. With supplementation of Mg this linear relationship vanishes (graph not shown, r=-0.274, n=19, p=0.257).

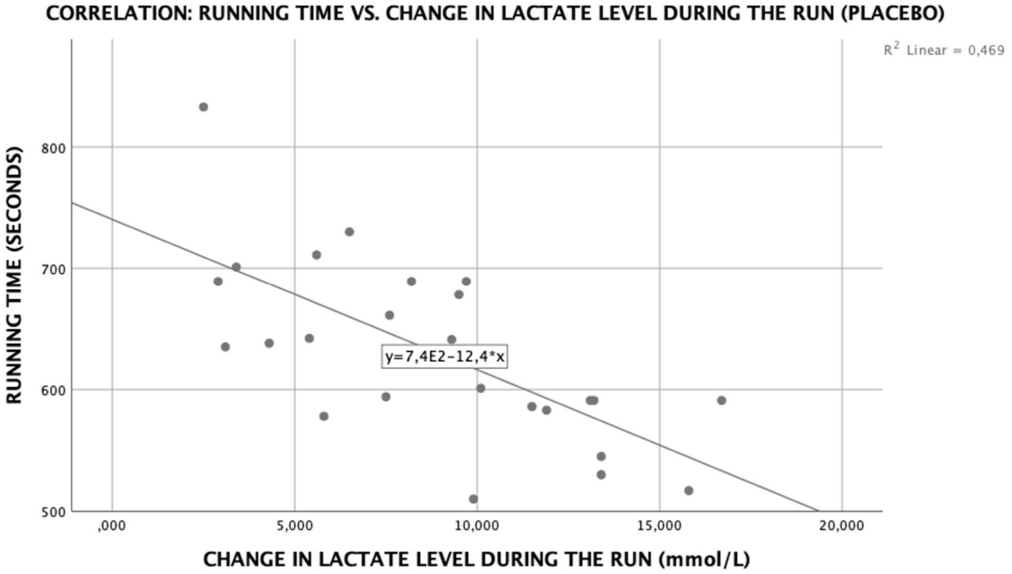

Figure 4.2.2 Correlation: Running time vs. change in lactate level during the run after placebo supplementation; r=-0.685, n=26, p=0.000

In the placebo group, there was also a negative correlation between running time and systolic blood pressure after the run (Suppl. Fig. 8.1, r=-0.601, n=26, p=0.001) as well as changes in systolic blood pressure during the run (Suppl. Fig. 8.2, r=-0.535, n=26, p=0.005). Systolic blood pressure was higher after and increased during the run with faster running time in the placebo group. Again the correlations were not seen in the verum group (graphs not shown; r=0.093, n=19, n.s. for systolic blood pressure after the run, r=-0.154, n=19, n.s. for change in systolic blood pressure during the run).

A similar situation was seen with ionized Mg levels after the run (Fig 4.2.3, r=-0.549, n=26, p=0.004) and changes in ionized Mg levels during the run (Fig 4.2.4, r=-0.427, n=26, p=0.030), which significantly correlated negatively with the running time in the placebo group. Ionized Mg levels were higher after and increased during the run with faster running time in the placebo group. In the Mg group, ionized Mg levels after and respective changes during the run did not correlate with running time (graphs not shown, r=-0.184, n=19, n.s. for ionized Mg level after the run and r=-0.097, n=19, n.s. for change in ionized Mg level during the run).

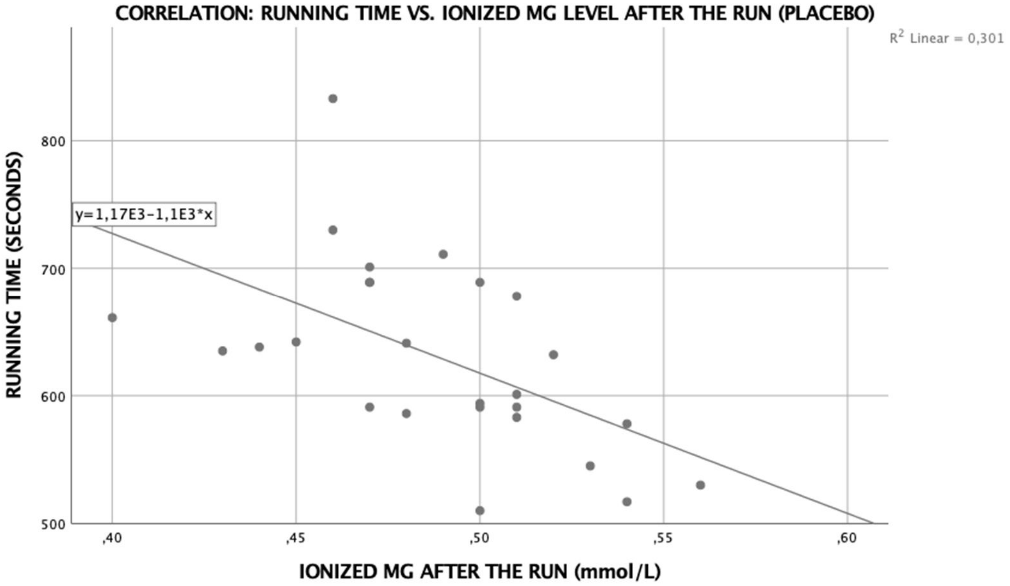

Figure 4.2.3 Correlation: Running time vs. ionized Mg level after the run after placebo supplementation; r=-0.549, n=26, p=0.004

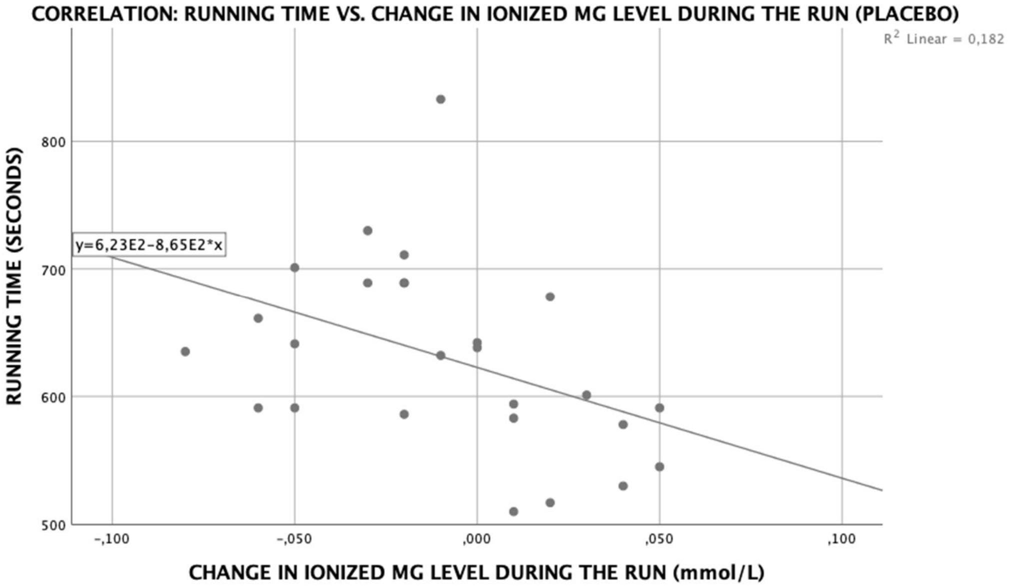

Figure 4.2.4 Correlation: Running time vs. change in ionized Mg level during the run after placebo supplementation; r=-0.427, n=26, p=0.030

The data regarding ionized Mg dynamics reveal, that the absolute levels in ionized Mg seem to change upon metabolic reactions and therefore are not necessarily indicative for Mg deficiency.

Although, in this case running time is probably the most important marker for performance, it is dependent on motivation and of course on personal training status. Therefore, metabolic parameters help to reveal more detailed information on personal effort.

In the placebo group, the ionized Mg level after the run correlated positively with changes in diastolic blood pressure (Fig. 4.2.5, r=0.431, n=26, p=0.028). Runners with high ionized Mg levels after the run showed highest increase in diastolic blood pressure and vice versa. After Mg supplementation, there was no relationship between diastolic blood pressure and ionized Mg level Fig (graph not shown; r=-0.022, n=19, n.s.).

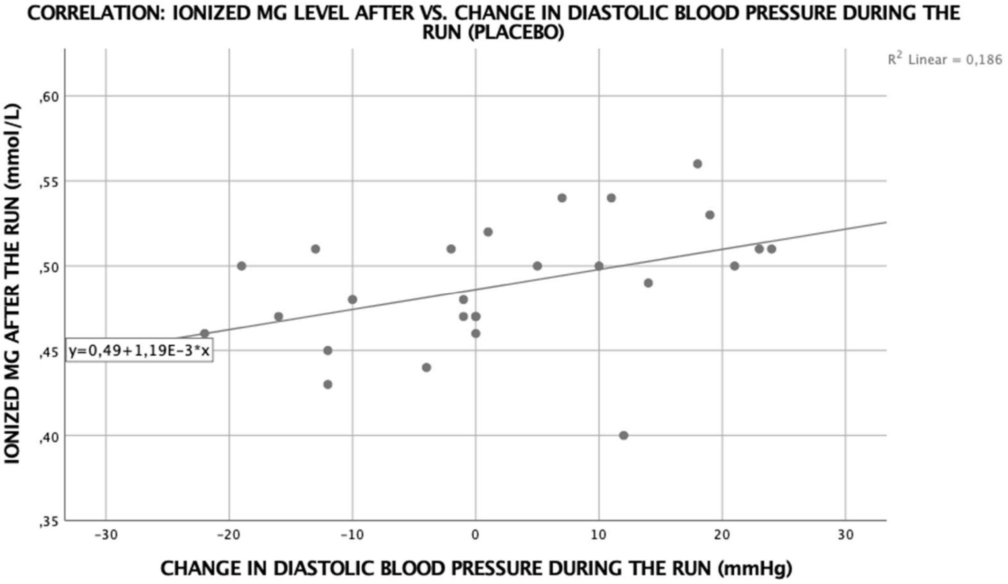

Figure 4.2.5 Correlation: Ionized Mg level after vs. change in diastolic blood pressure during the run after placebo supplementation; r=0.431, n=26, p=0.028

To assess if muscular and metabolic effort have an impact on Mg dynamics, Pearson product-moment correlation coefficients were calculated between changes in ionized Mg levels and diverse metabolic parameters.

There was a significant positive correlation between change in ionized Mg level and change in lactate level during the run in the placebo group (Fig. 4.2.6, r=0.399, n=26, p=0.044). Without Mg supplementation, increase in lactate level goes along with increase in ionized Mg level during the run. This was not seen after Mg supplementation (graph not shown, r=0.006; n=18, n.s.).

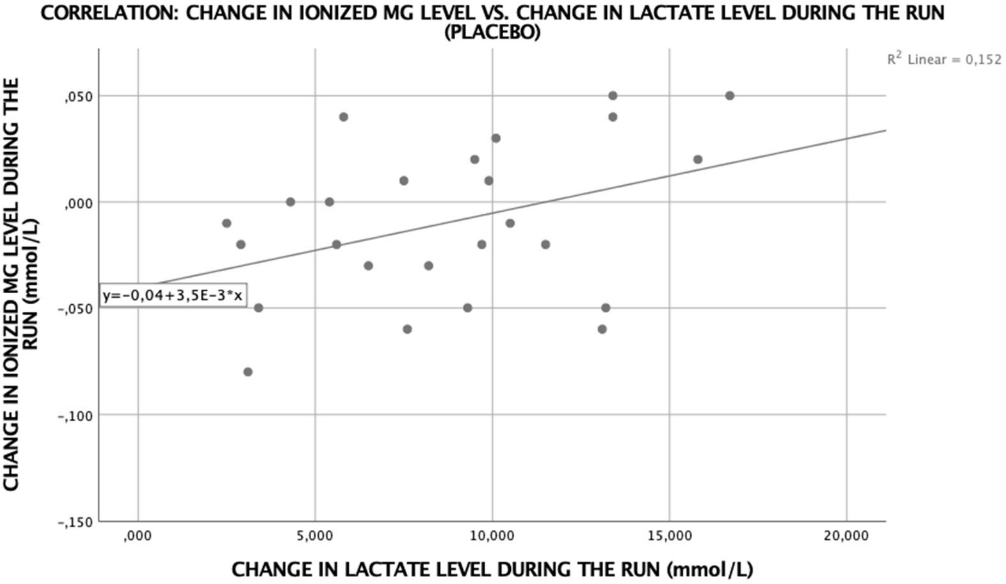

Figure 4.2.6 Correlation: Change in ionized Mg level vs. change in lactate level during the run after placebo supplementation; r=0.399, n=26, p=0.044

In the placebo group, change in ionized Mg level correlated significantly with change in base excess (Fig. 4.2.7, r=-0.451, n=24, p=0.027). Ionized Mg level in blood seems to increase with increased metabolic activity (decrease in base excess). In persons, in whom base excess decreased less than 10 mmol/L, ionized Mg level decreases. Again, this was not seen after Mg supplementation (graph not shown, r=0.042, n=18, n.s.).

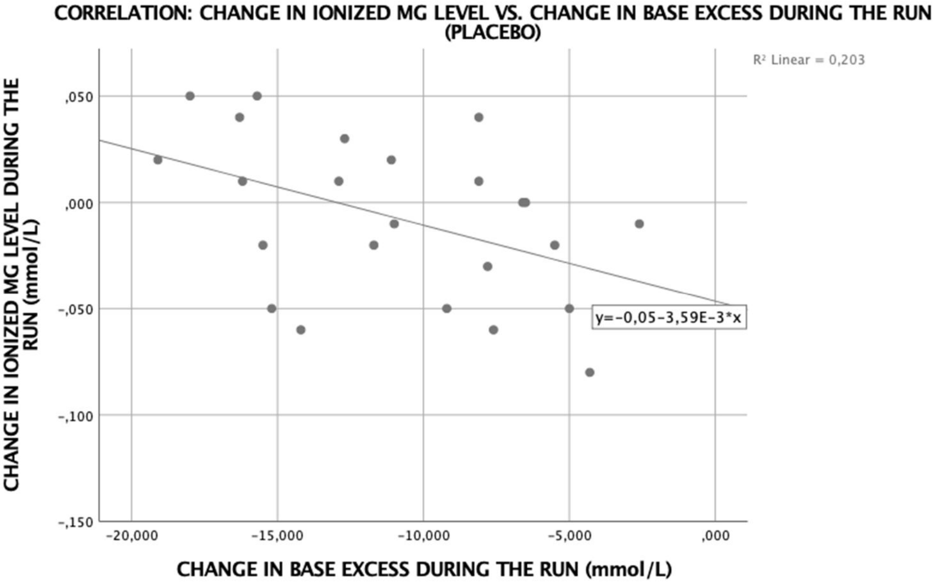

Figure 4.2.7 Correlation: Change in ionized Mg level vs. change in base excess during the run after placebo supplementation; r=-0.451, n=24, p=0.027

Change in estimated non lactic acidity correlated with a change in ionized Mg level in the placebo group (Suppl. Fig. 8.3, r=0.421, n=24, p=0.040) but not after Mg supplementation (graph not shown, r=-0.123, n=18, n.s.). An increase in estimated non lactic acidity above 2 mmol/L during the run goes along with an increase in ionized Mg level. Overall, changes ionized Mg level correlated negatively with changes in pH value during the run in the placebo group (graph not shown, r=-0.582, n=26, p=0.002). Decrease in in pH value goes along with increase in ionized Mg level and vice versa.

Ionized Mg and blood glucose levels seem to be closely related, but only in the placebo group. There was a significant correlation between change in ionized Mg level and change in blood glucose during the run (Fig. 4.2.8, r=0.723, n=26, p=0.000). Very high increases in blood glucose (> 50 mg/dl) go along with increases in ionized Mg level. At the same time there was a correlation between ionized Mg level after the run and changes in blood glucose level after placebo supplementation observable (Suppl. Fig. 8.4, r=0.757, n=26, p=0.000). Both were not seen after Mg supplementation (graphs not shown, r=0.120, n=18, n.s. for change in ionized Mg level during the run and r=0.172, n=18, n.s. for ionized Mg level after the run).

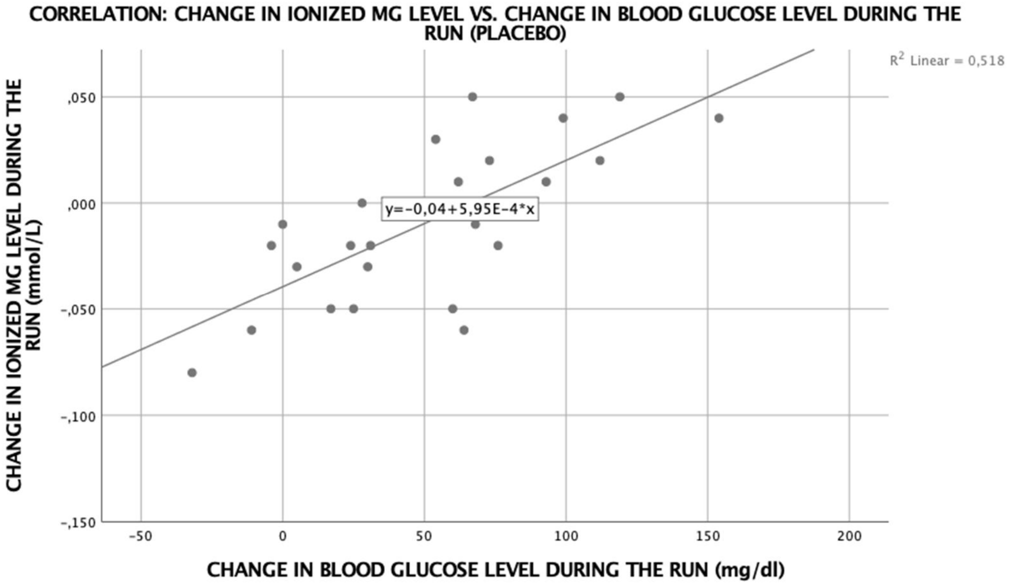

Figure 4.2.8 Correlation: Change in ionized Mg level vs. change in blood glucose level during the run after placebo supplementation; r=0.723, n=26, p=0.000

Runners with high ionized Mg levels after the run were those with the highest increases in blood glucose during the run. Taken all those results together it seems, that high sympatho-adrenal activity (increase in blood glucose) leads to an increase in ionized Mg level. After supplementation, this relationship vanishes. Without supplementation in approx. half of the runners blood glucose level increases more than 50 mg/dl during the run, whereas only a few had such increases after Mg supplementation. Nevertheless, differences in changes in blood glucose levels between both groups were not statistically significant, although blood glucose increase was almost twice as high in the placebo group compared to the Mg group (M=35.5 ±34.393 mg/dl vs. M=58.06 ±44.379 mg/dl, t=-1.914, p=0.073).

The relationship between blood glucose level and ionized Mg level seems to evolve during the effort, as there is no such correlation before the run in both groups (graphs not shown).
Not only changes but also absolute values in blood glucose and ionized Mg levels correlate after the run in the placebo group (Suppl. Fig. 8.5, r=0.755, n=26, p=0.000). This is not seen after Mg supplementation (graph not shown, r=0.141, n=18, n.s.).

4.3 MENTAL COACHING WEEKEND

In this study 8 managers (6 female, 2 male) with a mean age of 57 years (49-85) were included (148). Mental and physical well-being scores were not available from one participant. Additional characteristics of the study population have not been collected. A paired-samples t-test was conducted to compare metabolic and electrolyte changes before and after a three days coaching weekend in a country resort in Bad Gleichenberg, Austria. All significant differences are shown in Table 4.3.1.

Table 4.3.1 Significant differences in all analyzed parameters before and after the mental coaching weekend (Wilcoxon signed-rank test); BG = blood glucose level; HR = heart rate; RRsys = systolic blood pressure, RRdia = diastolic blood pressure; ENLA = estimated non lactic acidity; n=8; mean values outside the reference range are marked with an asterisk (148)

PARAMETER (REF. & UNIT)	**BEFORE THE COACHING WEEKEND**		**AFTER THE COACHING WEEKEND**		**Z-SCORE**	**P-VALUE**
	Mean	**SD**	**Mean**	**SD**		
pH (7.35–7.45)	7.50*	0.023	7.49*	0.018	-1.682	0.102
pCO_2 (35–45 mmHg)	25.06*	3.305	27.70*	2.881	-2.380	0.016
BE (-2–+2 mmol/L)	-3.75*	2.200	-2.45*	1.533	-1.895	0.063
HCO_3 (21–28 mmol/L)	19.64*	2.228	21.12	1.672	-2.243	0.023
pO_2 (>40 mmHg)	77.58	4.971	75.73	6.274	-1.400	0.195
O_2sat (94–98 %)	96.63	0.855	96.20	0.956	-1.266	0.250
iNa (136–146 mmol/L)	134.65*	2.526	144.36	3.566	-2.383	0.016
iCa (1.09–1.30 mmol/L)	1.12	0.048	1.17	0.040	-2.254	0.031
iMg (0.44–0.59 mmol/L)	0.49	0.021	0.53	0.037	-2.527	0.008
iK (3.5–5.1 mmol/L)	3.72	0.212	4.12	0.284	-2.380	0.016
Lactate (0.7–2.5 mmol/L)	1.55	0.754	1.36	0.498	-0.315	0.813
BG (<110 mg/dl)	151.38*	49.083	122.25*	30.480	-2.100	0.039
RRsys (<120 mmHg)	140.50*	21.287	140.25*	23.741	-0.140	0.922
RRdia (<80 mmHg)	82.50*	12.862	82.13*	11.064	-0.338	0.813
HEART RATE	82.75	8.120	72.50	7.597	-2.527	0.008
PHYSICAL WELL-BEING SCORE	5.15	1.574	6.71	0.756	-2.232	0.031
MENTAL WELL-BEING SCORE	5.43	1.618	6.14	2.478	-0.954	0.438
GENERAL WELL-BEING SCORE	7.57	0.787	7.14	2.734	-0.272	0.938
ENLA	2.20	2.194	1.09	1.623	-1.823	0.078

Significant increases in pCO_2, BE, HCO_3 and physical well-being score as well as significant decreases in blood glucose level and heart rate indicate a physical and mental relaxation due to the weekend.

To understand mental and physical states of the participants before the weekend, Pearson product-moment correlation coefficients were computed between measured metabolic markers, electrolytes and respective changes.

pCO_2 – as a marker for the respiratory minute volume – correlated with base excess before the coaching weekend (graph not shown; r=0.832, n=8, p=0.010) (148). Participants with lower base excess, indicating a calmer metabolism, have a lower breathing frequency. Similar was the situation with ENLA (Suppl. Fig. 8.6, r=-0.846, N=8, p=0.008). As base excess and ENLA are markers for acutely increased metabolic processes, it seems that some participants already had increased metabolism in anticipation of the weekend, which goes along with increased respiratory minute volume.

Although base excess and pCO_2 increased significantly during the weekend, there is still a correlation after the weekend between base excess and pCO_2 (graph not shown; r=0.877, n=8, p=0.004) and ENLA and pCO_2 (Suppl. Fig. 8.7; r=-0.770, n=8, p=0.025).

Similar correlations were observed between pCO_2 and HCO_3 before and after the coaching weekend (Suppl. Fig. 8.8; r=0.920, n=8, p=0.001 and Suppl. Fig. 8.9, r=0.941, n=8, p=0.000, before and after respectively). The correlations between pCO_2, HCO_3, ENLA and BE indicate that decreased pCO_2 is a reliable marker for metabolic excitement.

pCO_2 not only correlated well with metabolic parameters, but also with systolic and diastolic blood pressure. Participants with increased respiratory minute volume (decreased pCO_2) showed higher systolic (Fig. 4.3.1, r=-0.868, n=8, p=0.005) and higher diastolic (Suppl. Fig. 8.11, r=-0.868, n=8, p=0.005) blood pressure before the coaching weekend.

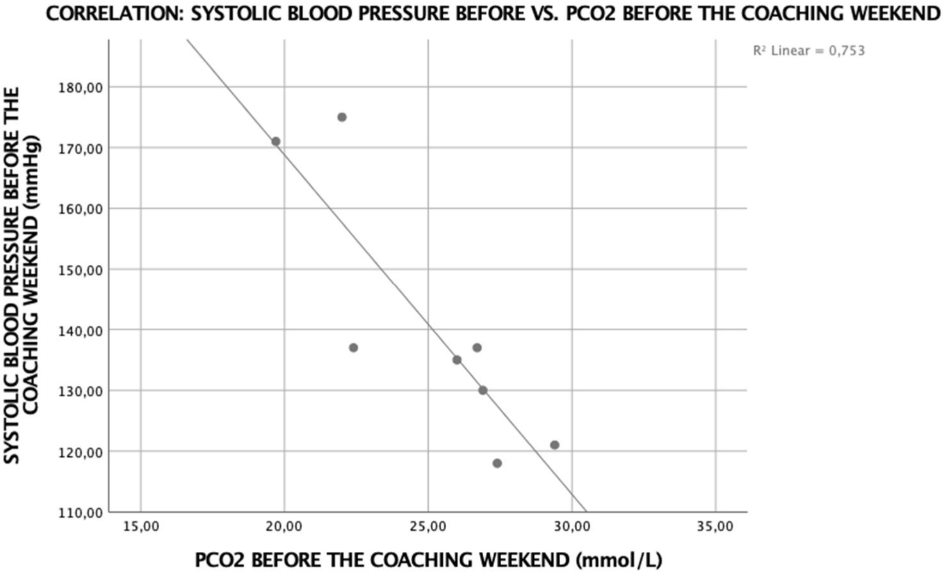

Figure 4.3.1 Correlation: Systolic blood pressure before vs. pCO$_2$ before the coaching weekend; r=-0.868, n=8, p=0.005

Also after the weekend the linear relationship between blood pressure and pCO$_2$ was still observable for systolic blood pressure (Suppl. Fig. 8.10; r=-0.941, n=8, p=0.001), although correlation for diastolic blood pressure did not reach statistical significance (Suppl. Fig. 8.12, r=-0.458, n=8, p=0.253).

All those correlations suggest, that due to low metabolic effort and requirements, respiration is the main regulator for maintaining acid base homeostasis. This is underlined by a significant negative correlation between pCO$_2$ and pH value after the weekend (Suppl. Fig. 8.13, r=-0.761, n=8, p=0.028).

Already a very small increase in respiratory frequency leads to an increased pH-value. With a mean pH value of 7.499 (±0.02338) before the weekend, and 7.4875 (±0.01794) after the weekend, the pH values are in the alkaline range in all participants. Before the weekend, the correlation between pH value and pCO$_2$ did not reach statistical significance (graph not shown).

The diastolic blood pressure seems to decrease more during the weekend in participants with a high, alcaline pH value and even increase when pH value is lower (Fig. 4.3.2, r=-0.827, n=8, p=0.011) before the weekend. This fits in with the other observations: Before the weekend, the participants seem to be excited, as seen in an increased breathing frequency which goes along with a subsequent increase in pH. The nervous persons get calmer during the weekend, indicated by a decrease in diastolic blood pressure. In calm persons before the weekend, the diastolic blood pressure even increases during the weekend

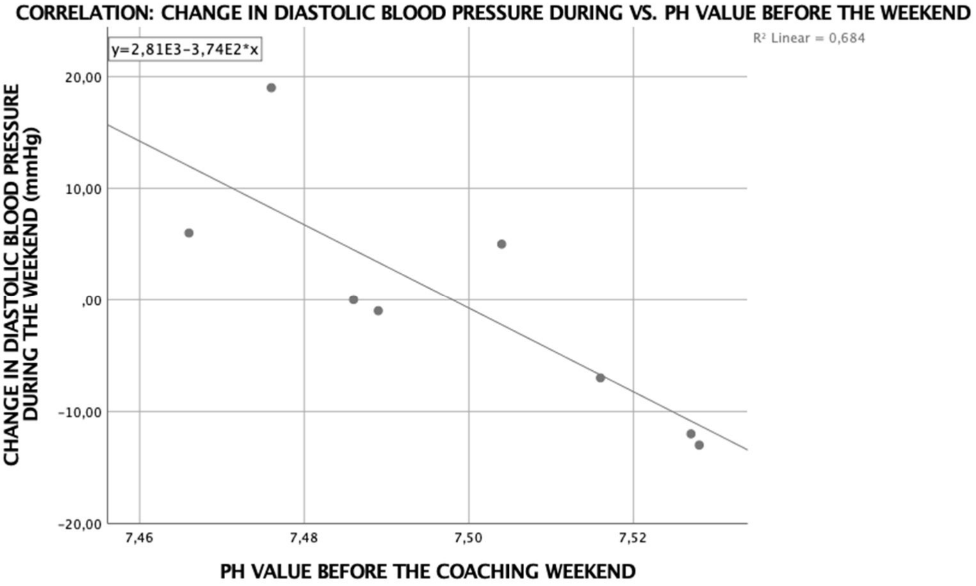

Figure 4.3.2 Correlation: pH value before vs. change in diastolic blood pressure during the coaching weekend; r=-0.827, N=8, p=0.011

To investigate the relationship between subjective feelings and objectively measureable parameters, correlation coefficients were calculated for scores for mental and physical well-being (10 points optimum) and metabolic parameters in blood. Before the coaching weekend, both the physical (Fig. 4.3.3, r=-0.802, n=7, p=0.030) as well as the mental well-being score (Suppl. Fig. 8.14, r=-0.797, n=7, p=0.032) correlated negatively with the pO_2 – besides pCO_2 a marker for respiratory minute volume. Persons who claimed to be in a good physical and mental state of mind seem to have a calmer breathing.

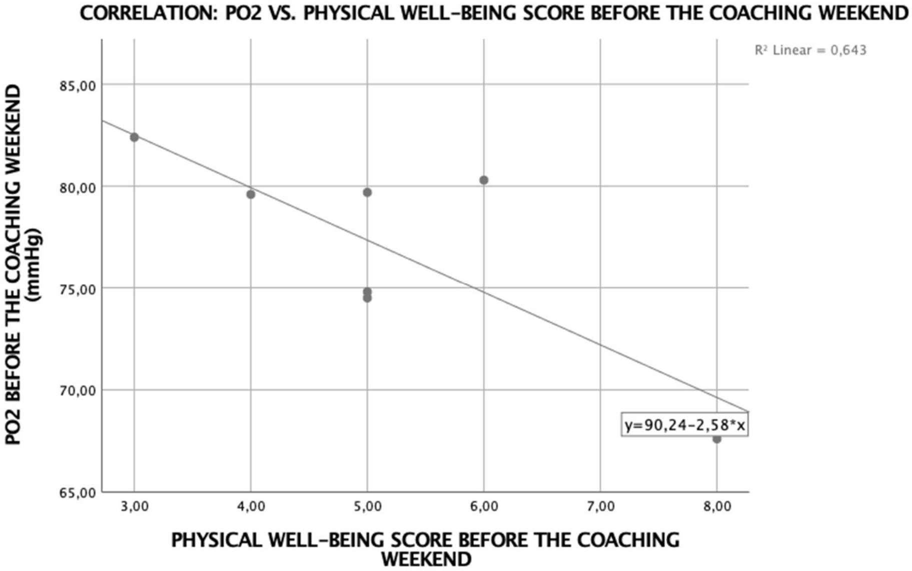

Figure 4.3.3 Correlation: Physical well-being score before vs. pO_2 before the coaching weekend; r=-0.802, n=7, p=0.030

Of special interest are the Mg dynamics in participants over the weekend. Therefore correlation coefficients were calculated for ionized Mg levels, change in ionized Mg levels and base excess as well as the general well-being score (10-point optimum).

In all participants, ionized Mg level increased during the weekend. Increase correlated positively with increase in base excess (Fig 4.3.4, r=0.732, n=8, p=0.039). Mg level increased more in participants with higher increase in BE. Ionized Mg levels before the weekend correlated with changes in general well-being score (Fig. 4.3.5, r=-0.801, n=8, p=0.017). Persons with a low ionized Mg level before the weekend, felt the best after the weekend. This probably indicates a decreased Mg level due to preloads or nervous anticipation before the weekend. After the weekend those particpants, who had the highest preload (lowest Mg level) profited the most from the weekend.

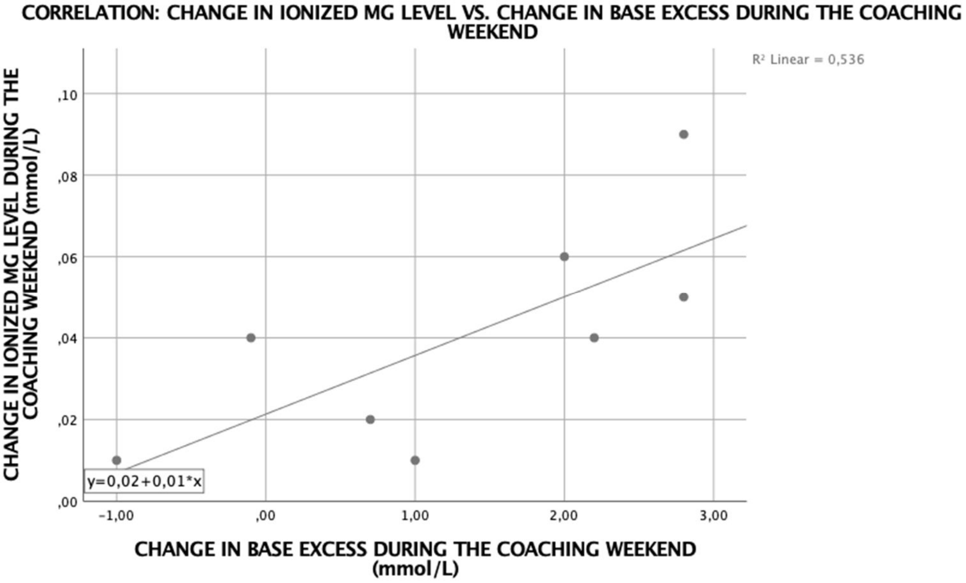

Figure 4.3.4 Correlation: Change in ionized Mg level vs. change in base excess during the coaching weekend; r=0.732, n=8, p=0.039

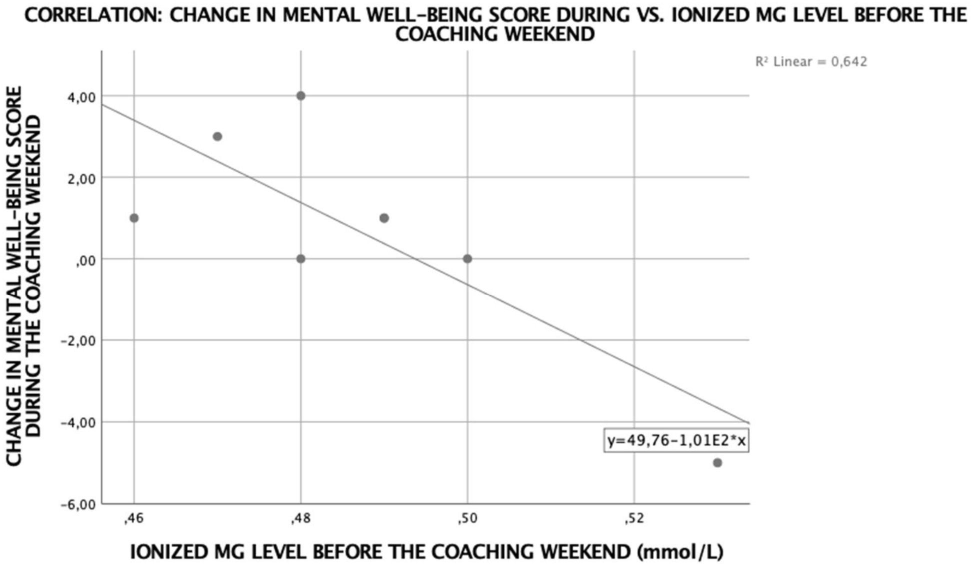

Figure 4.3.5 Correlation: Change in mental well-being score during vs. ionized Mg level before the coaching weekend; r=-0.801, n=8, p=0.017

4.4 COMBINATION OF PHYSICAL AND MENTAL STRESSORS – ACCORDION CLASS

In this study 32 accordion students (18 female) with a mean age of 62 years (44-78) were included (150). A paired-samples t-test was conducted to compare metabolic and electrolyte changes before and after a 30 minutes accordion lesson. Table 4.4.1 shows all statistically significant differences. The increase in BE and HCO_3 and the decrease in lactate, systolic blood pressure and heart rate suggest, that the participants get calmer during the lesson in regards of metabolism. The significant increase in all three dimensions of the MMSQ (see chapter 3.5) indicate, that participants feel calmer, more awake and better after the lesson. Additionally, Mg and Ca increased significantly during the lesson. An independent samples t-test revealed no statistically significant differences between men and women regarding all analyzed variables before and after the lesson as well as changes during the lesson.

Table 4.4.1 Significant differences in analyzed parameters before and after the accordion lesson (paired samples t-test), BE = base excess; RRSys = systolic blood pressure; RRdia = diastolic blood pressure; HR = heart rate; GB = Good/Bad; AT=Awake/Tired; CN=Calm/Nervous; ENLA = estimated non lactic acidity; mean values outside the reference range are marked with an asterisk

Parameter (REF. & UNIT)	**Before the lesson**			**After the lesson**		**t-value**	**p-value**
	N	**Mean**	**SD**	**Mean**	**SD**		
pH (7.35–7.45)	32	7.49*	0.023	7.50*	0.019	-1.519	0.139
pCO_2 (35–45 mmHg)	32	29.84*	2.035	30.12*	1.852	-1.284	0.209
BE (-2–+2 mmol/L)	32	-0.46	1.827	0.03	1.362	-2.888	0.007
HCO_3 (21–28 mmol/L)	32	23.04	1.637	23.48	1.263	-2.885	0.007
pO_2 (>40 mmHg)	32	66.77	5.855	67.04	5.042	-0.317	0.753
O_2sat (94–98 %)	32	94.53	1.439	94.68	1.305	-0.743	0.463
iNa (136–146 mmol/L)	32	144.60	1.722	144.81	2.029	-0.948	0.350
iCa (1.09–1.30 mmol/L)	32	1.16	0.036	1.19	0.041	-3.685	0.001
iMg (0.44–0.59 mmol/L)	32	0.55	0.049	0.56	0.043	-2.465	0.019
iK (3.5–5.1 mmol/L)	32	4.27	0.282	4.35	0.281	-1.717	0.096
Lactate (0.7–2.5 mmol/L)	32	2.20	0.686	1.88	0.525	2.877	0.007
BG (<110 mg/dl)	32	142.66*	49.786	137.06*	43.181	1.233	0.227
RRsys (<120 mmHg)	31	155.23*	27.467	148.71*	24.954	2.729	0.011
RRdia (<80 mmHg)	31	90.35*	14.247	88.23*	11.051	0.958	0.346
HEART RATE	31	83.16	18.612	79.48	14.233	2.275	0.030
GB-SCORE	32	17.88	2.028	18.75	1.666	-2.441	0.021
AT-SCORE	31	16.03	2.738	17.87	1.910	-3.350	0.002
CU-SCORE	32	15.031	2.456	17.06	2.501	-4.630	0.000
ENLA	32	-1.74	1.568	-1.92	1.409	1.234	0.226

Pearson product-moment correlation coefficients were computed to assess the relationship between the measured metabolic parameters and electrolyte changes. Respective scatterplots summarize the results and give an overview of significant connections.

The metabolic markers ENLA and base excess both correlated with systolic blood pressure before the lesson. Higher ENLA – indicating an increased metabolism – goes along with a higher systolic blood pressure (graph not shown; r=0.364, n=31, p=0.044).

A higher base excess – indicating a calmer metabolism – goes along with a lower systolic blood pressure (Fig. 4.4.1, r=-0.367, n=31, p=0.042).

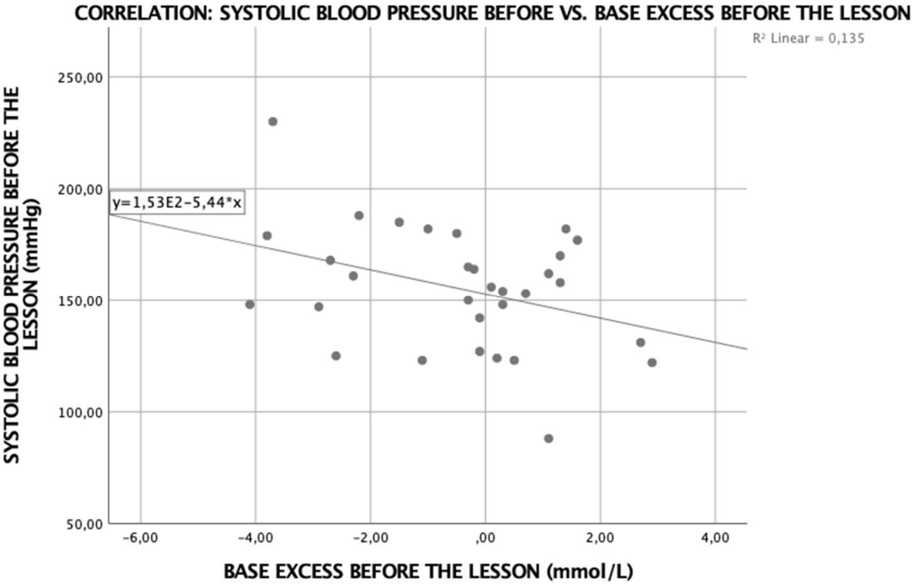

Figure 4.4.1 Correlation: Systolic blood pressure vs. base excess before the lesson; r=-0.367, n=31, p=0.042

Similar findings could be observed when assessing the relationship between lactate level before and after the lesson as well as respective changes during the lesson. Lactate level before the lesson correlated positively with lactate level after the lesson (Suppl Fig. 7.20, r=0.501, n=32, p=0.003).

The significant decrease in lactate level during the lesson correlated negatively with the baseline values, indicating a higher decrease in high lactate levels at baseline (Fig. 4.4.2; r=-0.682, n=32, p=0.000).

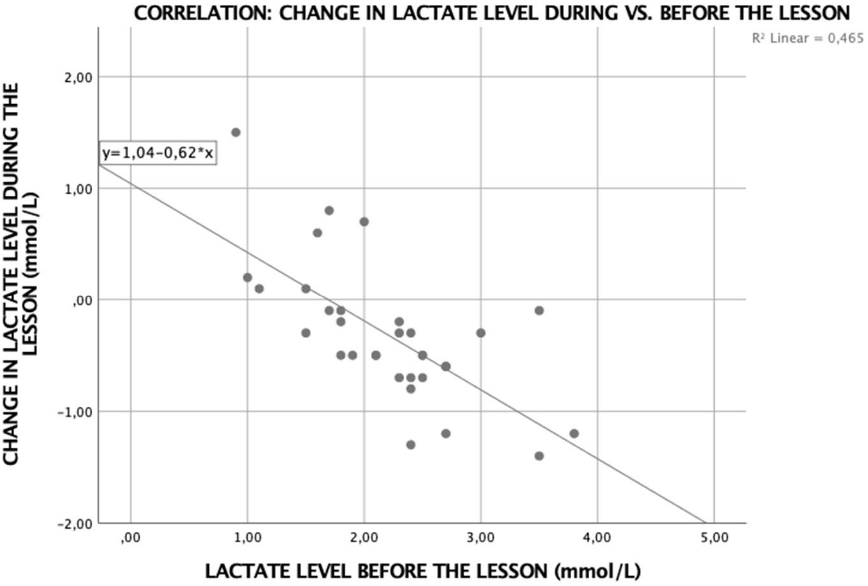

Figure 4.4.2 Correlation: Change in lactate level during vs. before the lesson; r=-0.682, n=32, p=0.000

The correlations observed with lactate levels could be seen in both males and females (graphs not shown, r=-0.689, n=14, p=0.006 for males and r=-0.690; n=18, p=0.002 for females). Lactate level – as a marker for muscular metabolism – before the lesson correlated negatively with ionized Mg level before the lesson (graph not shown, r=-0.378, n=32, p=0.033). Ionized Mg levels before the lesson also correlated negatively with lactate levels after the lesson (Fig. 4.4.3, r=-0.486, n=32, p=0.005). Same could be observed for blood glucose level – as a marker for sympatho-adrenal activity – before (graph not shown, r=-0.356, n=32, p=0.46) as well as after the lesson (Fig. 4.4.4, r=-0.365, n=32, p=0.040).

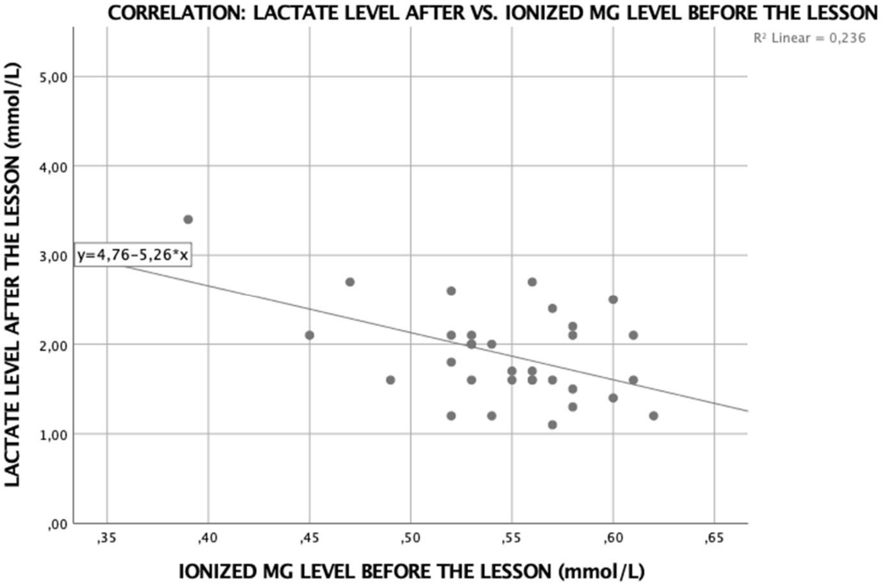

Figure 4.4.3 Correlation: Lactate level after vs. ionized Mg before the lesson; r=-0.486, n=32, p=0.005

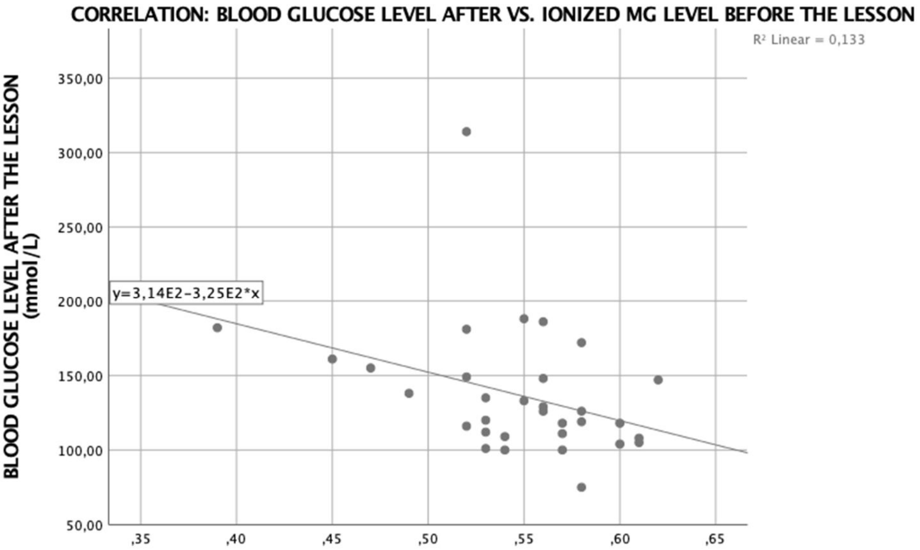

Figure 4.4.4 Correlation: Blood glucose level after vs. ionized Mg before the lesson; r=-0.365, n=32, p=0.040

Changes in lactate level during the lesson seem to go along with buffer capacity before the lesson indicated by base excess as there is a positive correlation observed (Fig. 4.4.5, r=0.551, n=32; p=0.001). Lactate tends to increase during the lesson in students with high base excess and vice versa.

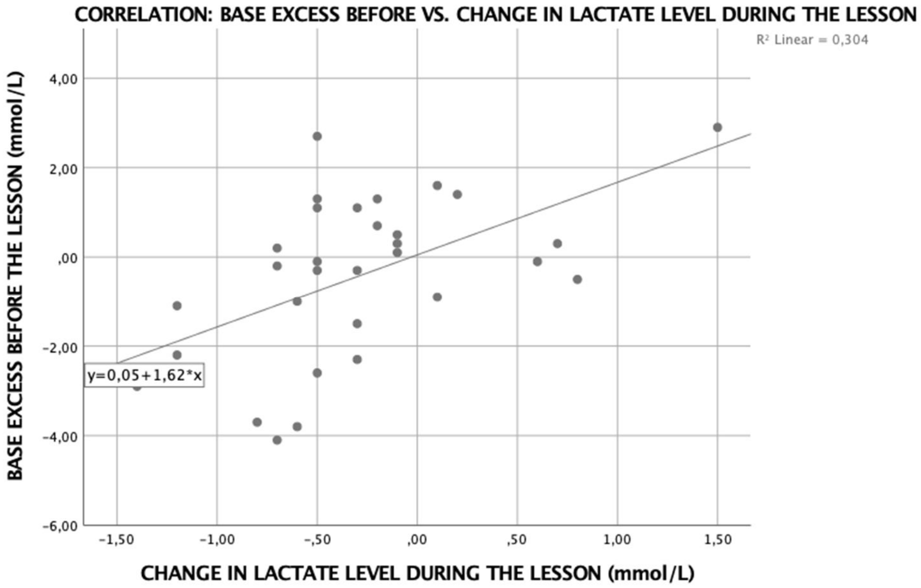

Figure 4.4.5 Correlation: Base excess before vs. change in lactate level during the lesson; r=0.551, n=32; p=0.001

Moreover, a relationship between subjective perceptions and objective parameters in blood could be observed. The awake/tired dimension of the MMSQ before the lesson, correlated negatively with the change in pH values during the lesson (Fig 4.4.6, r=-0.437; n=32, p=0.012). Students who claimed to be more awake before the lesson, seem to have a larger decrease in pH value during the lesson. pH-value however, seems to increase during the lesson in very tired studends.

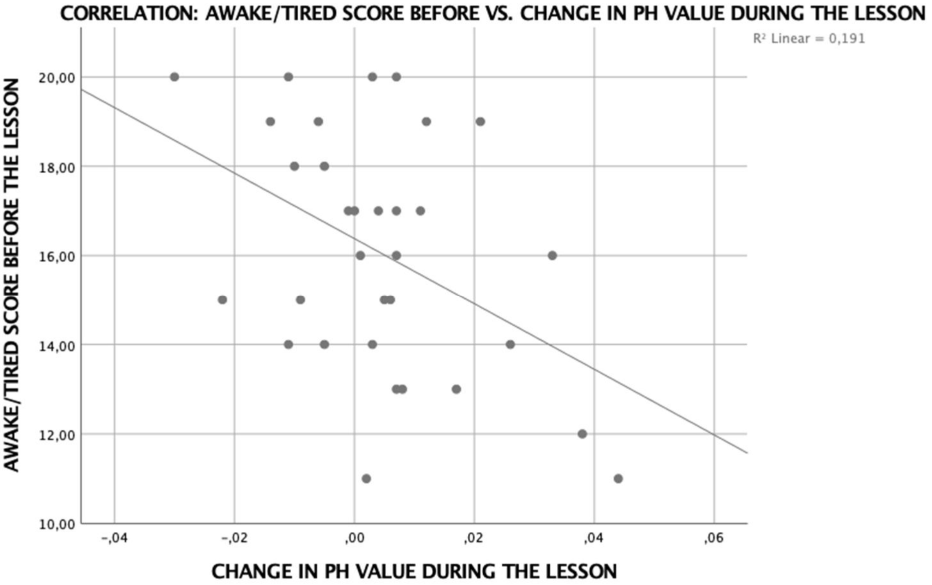

Figure 4.4.6 Correlation: Awake/tired score before vs. change in pH value during the lesson; r=-0.437; n=32, p=0.012

The awake/tired score before the lesson correlated positively with the diastolic blood pressure after the lesson (Fig 4.4.7, r=0.428, n=31; p=0.016). Students who claimed to be awake before the lesson, had a higher diastolic blood pressure after the lesson.

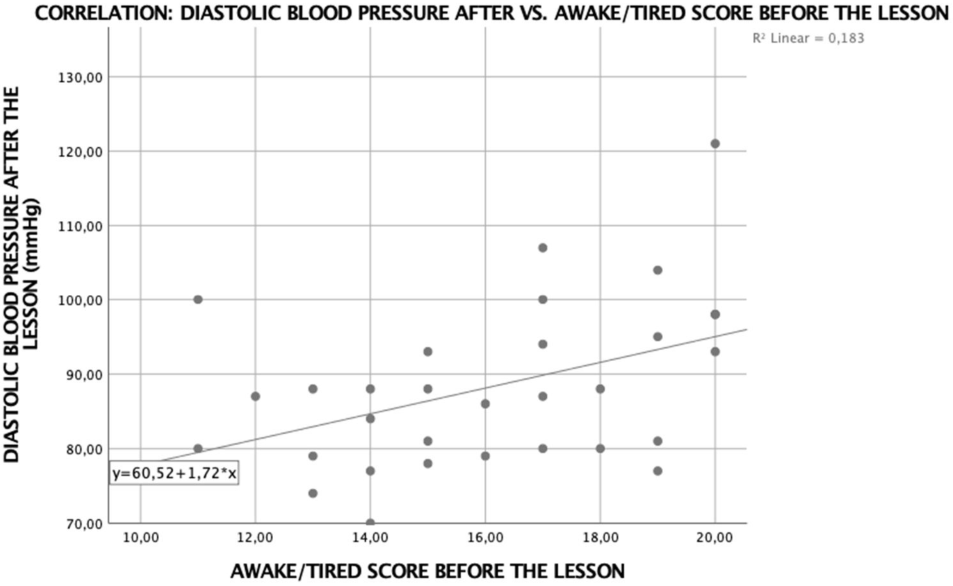

Figure 4.4.7 Correlation: Diastolic blood pressure after vs. awake/tired score before the lesson; r=0.428, n=31; p=0.016

The subjective awake/tired score given by students after the lesson, correlated positively with change in lactate level during the lesson (graph not shown, r=0.525, n=31, p=0.002). Students who were tired after the lesson showed a decrease in lactate level during the lesson, students who were awake, showed an increase in lactate level during the lesson.

In general, systolic blood pressure after the lesson correlated with the systolic blood pressure before the lesson (graph not shown; r=0.876, n=31, p=0.000).
In contrast to females (graph not shown; r=-0.105, n=17, n.s.) the change in systolic blood pressure during the lesson correlated with systolic blood pressure before the lesson in males (graph not shown, r= -0.792, n=14, p=0.001). The higher the blood pressure before the lesson, the higher the decrease during the lesson.

Similar to systolic blood pressure, the diastolic blood pressure before correlated with the diastolic blood pressure after the lesson, with lower pressures afterwards (graph not shown, r=0.546, n=31, p=0.001).
Changes in diastolic blood pressure during the lesson correlated negatively with diastolic blood pressure before the lesson, indicating higher decreases in persons with higher levels at the beginning, and even slight increases with lower pressure (graph not shown; r=-0.664, n=31, p=0,000).
In contrast to systolic blood pressure, correlation between baseline values in diastolic blood pressure and respective changes during the lesson was observed in males and females (graph not shown; r=-0.710, n=14, p=0.004 for males and r=-0.630, n=17, p=0.007 for females).

Ionized Mg levels before and after the lesson as well as systolic blood pressure before and after the lesson correlated with age, whereas older participants seem to have lower Mg levels (graph not shown; r=-0.393, n=32, p=0.026 before and graph not shown; r=-0.420, n=32, p=0.017 after) and higher systolic blood pressure (graphs not shown; r=0.519, n=31, p=0.003 before and r=0.377, n=31; p=0.037 after). Changes in Mg during the lesson did not correlate with age.

A negative correlation between baseline ionized Mg level and change in diastolic blood pressure during the lesson could be observed (Fig. 4.4.8; r=0.393, n=31, p=0.029). Diastolic blood pressure seems to increase in accordion students with lower ionized Mg levels and vice versa.

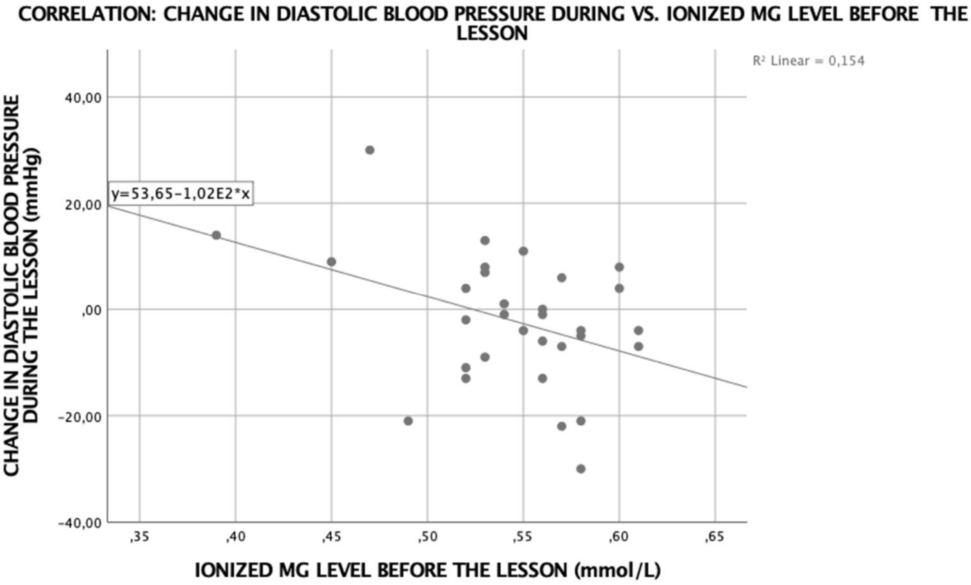

Figure 4.4.8 Correlation: Change in diastolic blood pressure vs. ionized Mg level before the lesson; r=-0.393, n=31, p=0.029

Ionized Mg level correlated negatively with the systolic blood pressure after the lesson (Fig. 4.4.9, r=-0.373, n=31, p=0.038). A higher Mg level after the lesson goes along with a decreased systolic blood pressure indicating less excitement with higher Mg levels.

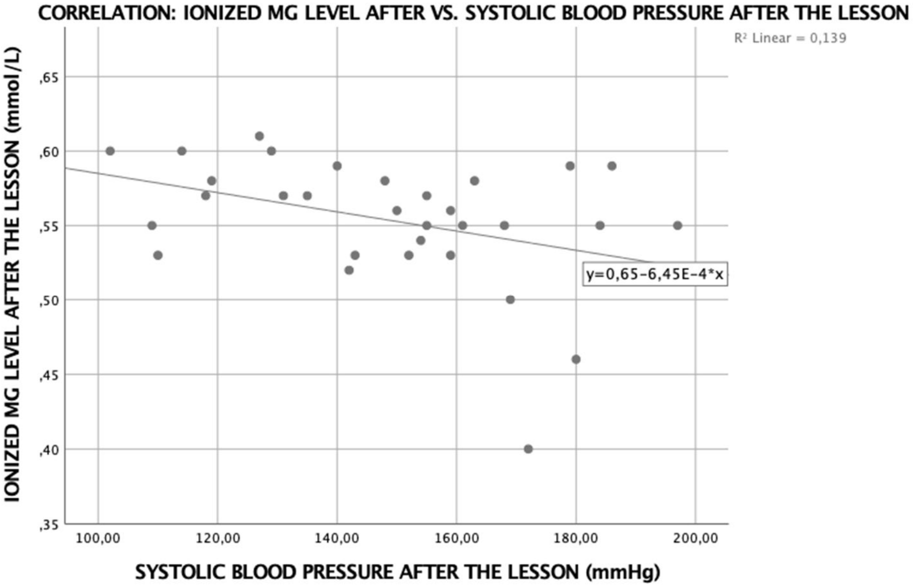

Figure 4.4.9 Correlation: Ionized Mg level after vs. systolic blood pressure after the lesson; r=-0.373, n=31, p=0.038

5 DISCUSSION

5.1 ANALYSIS OF 2400 M RUNNERS

The analysis with data of more than 300 persons before and after a 2400 m run, to describe stress during a standardized physical load showed changes in all analyzed parameters after the run compared to the situation before the run. The significant decrease in base excess and the increase in respiration depth and/or frequency and lactate point towards increased acid production and increased muscular metabolism. Correlative statistics and regression analysis provide further information on metabolic processes and enable an explanation of Mg shifts and load-dependent changes in homeostasis. The large number of test persons examined makes it possible to pinpoint even small Mg changes and to understand some mechanisms of Mg fluctuations for the first time (147).

For the detailed assessment of performance-specific reactions of the body or its response to stress, a considerable number of stress situations have been investigated in recent years (70,71,73,74,141–144,146,155–159). Many studies investigated changes during a 2400 m run. On the one hand, because such a run is a standardized procedure, on the other hand, because it is part of the training program at the Theresian Military Academy and there it is essential for the assessment of athletic performance. As the test persons were familiar with the procedures of a 2400 m run, effects of mental stress were expected to be minimal. However, a clear distinction between metabolic effects of mental and physical stress still remains difficult.

Due to the standardized measurement protocol and the identical procedures before, after and during a 2400 m run, data from a total of 320 runners could be collected in recent years, with one measurement before and one measurement after the run. Since even the blood gas analyzer was identical, it was decided to perform a large analysis to better describe physiological relationships (147).

The significant changes of all measured parameters after the run seem plausible. The respiratory minute volume increased due to the higher oxygen demand; the lactate level increased due to the muscular strain. At the same time, the increased metabolism lead to the increased formation of acids that must be buffered - this in turn triggered respiration and an expiration of CO_2.

Concerning the 2400 m run, a relatively short intensive load was examined and large interindividual differences in changes in homeostasis could be observed. In contrast to an ergometer with a defined performance setting, no defined performance could be expected when running, mainly for reasons of motivation and personal training status. Thus, it could have happened that a highly motivated test person would have reached his/her performance limit, whereas another test person perceived the 2400 m run just as a relaxed sports unit. For some, the 2400 m was a short, intensive, challenging exercise, for others "only" a moderate, short exercise. This is the reason, why the term “performance” in this thesis under such experimental settings (without measuring the exact time or workload like on an ergometer) can only be assumed by looking at metabolic changes. Performance therefore has to be considered as something subjective.

Another aspect that needs to be considered is the impossibility of separating metabolic effects of psychological and physical stress. Although, the 2400 m is well known to all participants of the study, it was still impossible to exclude mental stress and reactions already before the run, as it could be seen by the measurements. For some test persons the 2400m run was a pleasant sporting activity, for others the run was associated with pressure to perform, nervousness and tension. It is assumed that even before physical exertion - in the so-called sympatho-adrenal anticipation phase - the body reacts to increased anticipatory strain in preparation for the upcoming exertion. Adrenaline triggers for example an increase in blood glucose level or the production of lactate, even though there is no muscular activity. In fact, very high blood glucose levels in the expectation phase of bungee jumpers, who were exposed to fear and massive psychological stress were observed (158).

Also, in this cohort with 310 analyzed runners, 163 runners had a lactate level of ≥2 mmol/L already before the run. The half-life of lactate in the body varies and is dependent on training status, substrate availability and elimination and conversion rate and there is no clear data on that. There are some studies which report half-lifes between approx. 5 to 20 minutes (160,161). As all participants didn’t exercised directly before the first measurement, the most obvious explanation for higher lactate levels already before the run is nervousness and sympatho-adrenal anticipation. Fast secretion of adrenalin and noradrenalin upon psychological stressors lead to increased muscular metabolism with subsequent increases in lactate levels. This could be shown in several studies, by investigating the effect of adrenalin infusions (162–164).

By selecting different sub-groups, detailed analysis of strained or less strained runners was possible, showing significant differences in regards of Mg dynamics and metabolic markers.

Although, many studies report changes in the electrolyte homeostasis after exercise (130,142,143,146,159,165–167), electrolyte shifts are not always plausible and the results are not altogether conclusive (95,147,168,169). It was hypothesized, that during exertion, for example Mg is transferred into tissue with acutely high energy turnover, where Mg for example serves as a co-factor for many enzymes important in metabolism. Especially the fat tissue, which provides the body with free fatty acids in case of increased energy requirements, as well as the muscles, which utilize fatty acids and sugars in order to be able to perform, profit from that reallocation. In this study, overall a significant decrease of ionized Mg in blood was observed which fits in with results from preceding studies and confirms the hypothesis.

In former investigations it was puzzling, that, depending on the initial situation, sometimes an increase and sometimes a decrease of the ionized Mg was observed (74,139,141,143,146,159). Therefore, it was suspected, that Mg shift is subject to other physiological processes and that, depending on the type of load and pre-loads, there also may be an increase in the ionized Mg level in blood. As reviewed by Nielsen and Lukaski, Mg levels in blood increase after a short intensive workload, and decrease after long moderate exercises (130).

To understand the dependencies of changes in Mg levels on metabolic markers, a regression analysis was calculated. The significant regression model implicates, that changes in ionized Mg levels in this cohort can be described with HCO_3^-, pCO_2, BE, lactate level and blood glucose level before the run.

According to that, participants with high lactate levels before the run, have the highest decrease in ionized Mg levels during the run. This is in line with the sympatho-adrenal anticipation and the transfer of ionized Mg into tissue with acutely high energy turnover. If energy-turnover is higher before the run already, it is obvious that it is even higher during the run and more ionized Mg is needed.

Next the results from the regression analysis imply that ionized Mg levels during the run decrease most in participants with a particularly low respiratory minute volume (high pCO_2) and high BE. The shift of ionized Mg into body compartments with high energy turnover is most probably a time dependent process. In respiratory calm participants, the shift of ionized Mg has not yet started, consequently those runners have the highest basal ionized Mg levels. This could be the reason, why many studies report the highest decreases in ionized Mg levels in people with highest basal levels (74,139,141,143,146,159). The base excess as an indicator for a metabolic acidosis or alkalosis points more or less in the same direction. A high BE, indicating a well-compensated and in fact a calm metabolism, is predictive for a high decrease in ionized Mg levels.

When considering HCO_3^- the situation looks different, as low HCO_3^--levels predict a decrease in ionized Mg levels. This outcome is most likely to be interpreted together with lactate levels. Increased (muscular) metabolism upon sympatho-adrenal anticipation leads to increased acid

production, which is predominantly buffered by HCO_3^-. As a result, low levels already indicate the beginning of an increased metabolism resulting in larger shifts upon the subsequent run.
As stated before, the contradictory findings of the analysis that on the one hand, respiratory calm (indicated by high pCO_2) and metabolically calm (indicated by high BE) as well as higher muscular activity (indicated by higher lactate levels) and acid compensation (indicated by low HCO_3^-) predict a larger decrease in ionized Mg level could be explained by a time dependence of the compensation mechanisms. With the beginning of adrenalin and noradrenalin excretion, muscular activity increases, leading to increased lactate levels. Increased acid production requires buffering by HCO_3^- leading to decreased HCO_3^--levels. In further consequence, pCO_2 has to be removed by increased respiratory minute volume. With increased acid production, BE starts to decrease. Dependent on the timepoint of the first measurement and the dynamics of the individual stress, different parameters predict decreases in ionized Mg levels the most.
Finally, the regression analysis shows, that a higher blood glucose level before the run goes along with an increase in ionized Mg level during the run. Indeed, there are hints in literature, that ionized Mg is needed for adequate intracellular glucose uptake and the action of insulin (112,122–125). High glucose levels therefore require higher amounts of ionized Mg in the blood, which is why it prevents shifting of ionized Mg into the muscle. Nonetheless, it should be considered, that the secretion of adrenalin and noradrenalin facilitate an increase in blood glucose level. The need of ionized Mg in blood due to high blood glucose levels could be stronger than the adrenaline and noradrenalin mediated shift into body compartments with high energy turnover. Studies with more specific study designs addressing those questions are needed.

To underline the results of the regression analysis, subgroup analysis was done to reveal significant differences in Mg changes, depending on perceived stress during the run and mental and/or physical preloads.
By selecting runners with higher lactate levels (>2mmol/L) already before the run to select mainly for muscular preload, analysis of preloaded participants was possible. Significant larger decrease in ionized Mg level in blood during the run compared to runners without a preload could be observed. Participants with a lactate increase of >2 mmol/L during the run had a significantly lower Mg level before and after exercise compared to persons with a lower lactate increase. It is difficult to judge whether the increase in lactate is due to the low Mg level (Mg deficiency) or whether the Mg level was already lowered due to prior exercise or stress. However, change in ionized Mg during the run was not different in both groups. When defining Mg deficiency as a reduced capacity for redistribution in body compartments with high energy

turnover, it has to be assumed that there was sufficient Mg and thus sufficient reserves for redistribution. On the other hand, ionized Mg level in blood even increased in runners with lactate increase of more than 7 mmol/L, which could be a sign for muscular damage-related Mg increases.

Additionally, strained runners were selected who showed an increase in lactate level of >2 mmol/L and an increase in blood glucose level of >15 mg/dl. During moderate physical stress, utilization of blood glucose can be assumed, as the muscles metabolize glucose for energy production. It therefore can be assumed that an increase in blood glucose level during physical activity is adrenalin mediated.

It turned out, that an increase in lactate level with a simultaneous increase in blood glucose level was about 10 times higher (!) compared to participants without increase in blood glucose level during the run. However, no significant differences could be observed in ionized Mg levels between exhausted and less strained persons. Again, high increases in blood glucose levels could counteract stress-related ionized Mg-shifts into muscle.

Correlative statistics provide information about the redistribution of ionized Mg in strained runners. The change in ionized Mg levels during the run correlated negatively with the changes in pCO_2. Mg increased in runners with the largest pCO_2 decrease, meaning the highest respiratory effort. The same could be confirmed by correlating the changes in ionized Mg levels with the changes in pO_2 and other metabolic parameters such as BE, HCO_3^-, pH or lactate level. It could be observed that, especially in persons with high effort, an increase in ionized Mg level during the run is more likely than a decrease. It seems that especially with higher effort, redistribution of ionized Mg cannot be facilitated anymore. Muscular damage and turnover of ionized Mg finally leads to an increase in ionized Mg levels in blood. This could be an indication that ionized Mg is one of many limiting factors in maintaining performance. These correlations are no longer detectable in runners with less effort during the run.

Therefore, reliable quantification of previous stress based on Mg dynamics could be possible (147). In the case of previous stress and the associated changes in homeostasis, the ability to react to further stressors could be significantly reduced. If, for example, Mg has already been shifted into the tissue, the level of ionized Mg in the blood drops and the reserves for a further shift are reduced. If further stress follows, muscles and fat tissue are insufficiently supplied with free Mg. The result is an inadequate energy supply or energy carrier utilization and thus an early exhaustion. Consequently, muscular damage and regulatory mechanisms to maintain Mg homeostasis in blood could lead to a Mg increase in blood.

A mixture of preload, anticipation and training status therefore makes it difficult to interpret metabolic parameters and especially the Mg level exclusively which might be a limitation factor of this study (147). This could also be one reason why many Mg supplementation studies are inconclusive, as always absolute Mg levels are compared (76,110,134,142,170–173). Further it is hypothesized that Mg supplementation is more beneficial in Mg deficient persons, and therefore effect dependent on Mg status.

As known so far, this is the largest investigation of changes in metabolic parameters and ionized Mg dynamics during physical effort. The homogenous study population (age, training status) make it possible to characterize even small reactions to physical stressors.
However, this study has potential limitations. As investigated participants were young officer trainees at the Theresian Military Academy, different physical and mental preloads from military exercises the days before cannot be excluded. In addition, mental excitement in expectation to the run could by very different from person to person. Furthermore, selection criteria for each investigated group are rigid and definitive thresholds are not well characterized in regards of physical and mental effort. As mentioned above, performance has to be considered as something subjective in this study, as no objective performance parameters have been recorded. In future studies, exact and objectively measurable definitions of performance are essential. Nevertheless, possible preloads and subjective mental excitement reflect everyday stress and therefore may allow conclusions for the general population.

Ionized Mg, in combination with metabolic parameters, seems to indicate the excitatory and pre-load status of the participants (147). Only the running time during a 2400 m run is used to assess the fitness level of officer trainees. As seen in the data, metabolic markers are crucial to assess performance capabilities and training status and should play a major role in future assessments. Further, it is difficult to assess the Mg status based on ionized Mg blood level alone without taking metabolic parameters into account, as its dynamics and levels depend on many factors. This should be considered in further studies and in the assessment of Mg status. It has to be noted, that nervousness, especially in the clinical setting due to e.g. blood sampling, can cause stress and significantly affect Mg levels.

5.2 STRESS WEEK / PLACEBO CONTROLLED MAGNESIUM SUPPLEMENTATION

To assess whether Mg supplementation could influence effort and performance during a 2400 m run, Mg or placebo was administered to 26 runners 1 hour before the run. 2 weeks later the procedure was repeated, but this time those who received Mg first received placebo and vice versa. The determined parameters did not differ significantly between Mg and placebo, the K level as well as the systolic blood pressure were significantly higher after Mg supplementation. Correlations of the ionized Mg level with other metabolic parameters showed clear differences between the two groups and give an indication of a more individual energy supply after Mg supplementation. The results underline the importance of Mg during stress. Mg could be a limiting factor when organism is regulating stress-related changes in homeostasis and supplementation could therefore be beneficial.

The mean values of almost all analyzed parameters before, after and changes during the run were not significantly different after Mg supplementation. The increased K level before the run can be explained as the “Dr. Böhm Magnesium Sport” tablets contained K besides Mg. K levels seem to increase immediately after supplementation whereas Mg levels did not.

In both groups, the systolic blood pressure and the ionized Na was increased and higher than the reference level in both groups. As all participants were young and well trained without any known sign of hypertensive disorders, the most likely explanation for an increased systolic blood pressure is that many of them have been nervous in expectation to the run. This is in line with slightly increased respiratory minute volume (indicated by decreased pCO_2) and slightly increased blood glucose levels. Dehydration is best known for an increased ionized Na level, however, there was no sign for less hydration of the participants. As the study was done in summer and water consumption was not recorded, dehydration cannot be excluded.

Runing time, the only objective parameter for performance in this study, did not differ significantly between both groups. Nonetheless, the running time was correlated with various metabolic parameters. The two groups showed clear differences in this respect: After placebo supplementation, the running time correlated significantly with the respiratory minute volume. Respiration was lower with longer running times. This can be explained by a lower oxygen and metabolic demands. Also increase in lactate level during the run correlated with the running time only in the placebo-group. Both correlations disappear after Mg supplementation. As already shown in previous studies (76,174), it seems, that Mg is no longer a limiting factor in energy supply after supplementation and that individual fitness plays a more important role.

After placebo supplementation, the decrease or increase in ionized Mg level during the run correlates with the slower or faster running times, respectively. This is in line with previous work and the analysis, that short intensive loads tend to lead to an increase in Mg level and longer or less intensive efforts tend to lead to a decrease (174). After Mg supplementation, however, this relationship is no longer observable. It is possible that the Mg dynamics are significantly influenced by supplementation due to the replenishment of reserves and even acute availability.

Correlations with metabolic parameters indicate that the ionized Mg tends to decrease in calmer participants during the run and increases in more stressed participants. This applies to both groups and is a further indication of the importance of Mg in energy metabolism.

The relationship between glucose and Mg and the difference in both groups was striking. While the changes in blood glucose levels during and blood glucose levels after the run correlate with changes in Mg levels during and Mg levels after the run in the placebo group, there are no correlations in the Mg group observable. As mentioned before, many studies have shown that Mg is closely related to insulin and glucose metabolism, as Mg is necessary for glucose transport into the cell (112,122,123,125). Therefore, more Mg has to be shifted into the blood if blood glucose levels increase to facilitate sufficient glucose transport. This is for example the case when high levels of adrenaline are excreted and as a result, glucose levels in blood increase. In the Mg group, the increase in Mg level was significantly less dependent on glucose levels as saturated Mg stores obviously react different to these changes. Further this could be an explanation why Mg levels seem to be increased after short, high intensity exercises, as adrenalin-mediated glucose increases in blood drive Mg shifts.

To our knowledge, there is no randomized, placebo-controlled, double-blind study to assess the influence of acute Mg supplementation on effort and performance in sport. Some studies assessed long term effects of Mg supplementation, others reported conflicting results when assessing improvement in performance (76,133,142,170,174,175). This study was designed as a double-blinded switching study to investigate acute effects of Mg-supplementation and to better understand Mg dynamics.

Nevertheless, this study has three major limitations. The first is, that dietary intake of Mg was not assessed prior the investigation. Therefore, it could be possible, that some participants supplemented Mg days before the start of the study or had a diet rich in Mg. The second is the fact, that a tablet of Dr. Böhm Magnesium Sport, besides Mg also contained K. There is a possibility, that K and not Mg influences effort and performance in sports. Finally, performance is not well defined as only running time was recorded as its only objective marker. Although, participants were asked to run as fast as possible or under 11 minutes 30 seconds, it could be that some of them were not willing to go to their limit. This could explain why there are no significant differences in running time between the Mg- and Placebo-group.

It can be concluded that acute Mg and K supplementation, just one hour before the exercise has a positive impact on effort during a 2400 m run. The absolute Mg level alone is not indicative for reliable Mg status assessments and always has to be interpreted together with metabolic markers. Mg deficiency is one of many limiting factors for performance capabilities and probably can be influenced by acute Mg supplementation. Larger studies with better defined performance markers as well as documented dietary Mg intake prior the study are needed to better understand impact of Mg supplementation on effort and performance and to clarify, if observed differences in blood pressure and blood glucose level can be confirmed.

5.3 MENTAL COACHING WEEKEND

In order to demonstrate the relaxing effect of a guided coaching weekend on subjective calming, metabolic markers and psychological scores were collected before and after a 3-day weekend to assess well-being. After the weekend, mental relaxation resulted in a significantly decreased respiratory minute volume (indicated by increase in pCO_2), heart rate and blood glucose levels as well as higher electrolyte levels (iNa, iCa, iMg and iK) and a higher physical well-being scores (148).

At the beginning of the weekend, very low pCO_2 and HCO_3^- levels and consequently high pH values have been measured. This was probably due to increased nervousness in expectation to the weekend. Higher blood glucose levels are in line with that. Nevertheless, food uptake was not prohibited before the measurement and could be a reason for higher levels as well.

The linear correlations between BE, and the estimated non lactic acidity with pCO_2 (respiratory minute volume) both before and after the weekend indicate, that respiratory compensation mechanisms, are sufficient to maintain slight changes in homeostasis (148). Nevertheless, several persons seem to suffer an increased metabolism before the weekend which is indicated by a decreased pCO_2 and BE and increased ENLA. All participants had a pH value higher than 7,44 which - together with decreased pCO_2 - indicates respiratory overcompensation already before the weekend (148).

The correlation between systolic blood pressure and estimated non lactic acidity indicates an acutely increased metabolism, as cardiovascular parameters follow metabolic excitement and vice versa. This could also be observed with pCO_2 and diastolic as well as systolic blood pressure: Participants with the calmest breathing, consequently, had the lowest blood pressure. In participants with calmer breathing before the weekend diastolic blood pressure increased during the weekend and vice versa. It could be suggested, that participants, who were nervous or preloaded before the weekend, profited most from relaxing exercises and got calmer, whereas persons with low blood pressure and metabolism before the weekend got activated (up to a certain degree) from group exercises and moderate sports activities.

Changes in ionized Mg levels in the blood as a sensitive marker for metabolic excitement correlated positively with changes in HCO_3 and base excess levels. This is an indication for metabolic calming of the participants with which increased Mg levels seem to be associated (148).

The subjective physical and mental well-being score correlated with the pO_2 before the weekend. Participants who did not feel well before the weekend seem to have a higher respiratory minute volume (indicated by a decreased pO_2). This is another indication of nervousness and therefore an increased metabolism. The mental well-being score increased most for participants with a low Mg level before the weekend. The lowered Mg level could therefore be an indication of a longer lasting mental stress or preloads. Stressed persons will then benefit most from a coaching weekend.

A limitation of this study is the small sample size of 8 participants. Additionally, the situation under investigation was a three-day coaching weekend with many different meditation and relaxing exercises, moderate physical activity and diverse workshops. This makes it hard to identify, which component was most beneficial for the participants.

Nevertheless, this real-world study investigated an inhomogeneous population which allows conclusions on stress and related reactions of the general population.

5.4 COMBINATION OF PHYSICAL AND MENTAL STRESSORS - ACCORDION CLASS

Measurement of metabolic, cardiovascular and psychological parameters before and after 30 minutes of accordion play showed significant increases in base excess, HCO_3 and MMSQ scores as well as significant reductions in lactate and systolic blood pressure (150). Levels of ionized Mg correlated with lactate, blood glucose levels and change in systolic blood pressure before and after the lesson. The awake/tired score correlated with pH changes and diastolic blood pressure. This correlation was more pronounced in men. The data suggest that mentally positive tasks although combined with moderate exercise lead to a significant calming of the metabolism. Ionized Mg, together with other metabolic markers, seems to allow a sensitive assessment of mood (150).

Similar to the observations from the coaching weekend, mean pCO_2 was very low in the study population at baseline resulting in high mean pH values. Again, this is expected to be an effect of the sympatho-adrenal anticipation due to the upcoming lesson.

Several studies show a calming effect of music on the body, which is accompanied by a reduction in blood pressure and heart rate (157,176–179). In addition, it is well known that moderate exercise has a positive effect in hypertensive and border hypertensive patients. Taking into account the high age (median = 62 years) of the participants in this study and the relatively high weight of an accordion of 7-9 kg, the change in metabolic parameters indicate calming of participants despite the physical effort made during the lesson. In mean diastolic blood pressure, there was no significant difference before and after the lesson. It is important to note that in men the drop in systolic blood pressure correlated with the values before the lesson - music and exercise had a particularly calming effect on men with high blood pressure. Diastolic blood pressure, on the other hand, changed in both women and men depending on the baseline value.

The decrease in blood pressure indicates a calming of the test persons, especially those, with high baseline blood pressure (150). Before the lesson, participants with a higher systolic blood pressure also appear to be metabolically more excited, and vice versa, as seen in the correlation between systolic blood pressure and ENLA as well as base excess. Lactate levels were slightly increased already before the stress situation, with levels up to approx. 3,5 mmol/L. The lactate level decreased significantly during the lesson despite the sub-maximal effort. Together with the decreased pCO_2 before the lesson, this also could be an indication of sympatho-adrenal anticipation and adrenalin-induced higher muscular blood glucose turnover or higher preload. Students with highest excitement already before the lesson or highest preloads calm down most during the lesson.

There was a negative correlation between the ionized Mg level before the lesson and blood glucose level as well as lactate level before and after the lesson. As lactate and blood glucose levels both are markers for metabolic excitement, metabolic calm persons seem to have particularly high ionized Mg levels before the lesson. Moreover, the higher the ionized Mg level before the lesson, the greater the drop in diastolic blood pressure during the lesson, and the higher the ionized Mg level after the lesson, the lower the systolic blood pressure after the lesson. It seems that participants are particularly calmer when showing high ionized Mg levels.

In addition to the parameters in the blood, subjective perceptions were also collected in this study using the German “Multidimensional Mood State Questionnaire”. The scores significantly improved in all three dimensions during the lesson, clearly indicating an increased well-being (150).

When taking a look at the relationship between subjective scores and metabolic markers there was a positive correlation between the awake/tired-score before the lesson and changes in pH during the lesson. In participants, who claimed to be particularly awake before the lesson, pH value decreased during the lesson. Conversely, the pH value increased in tired accordion students. As already mentioned above, sympatho-adrenal anticipation could lead to increased respiration leading to an increased pH value. Upon calming, pH value drops during the lesson. Further an increase in lactate levels during the lesson in awake participants before the lesson could be observed which could also be a reason for a decrease in pH during the lesson. Nevertheless, the subjective feeling of being awake before the lesson seems to go along with increased metabolism. Additionally, diastolic blood pressure was higher after the lesson in students who previously indicated that they were particularly awake before the lesson. However, these correlations were no longer observed with scores obtained after the lesson. Possibly also because being awake or tired before the lesson may have a different meaning for the students than after the lesson. Thus, before the lesson it could represent the general daily state of mind, but after the lesson it could represent fatigue or the activation or elevated concentration by the exercises. This should always be taken into account when assessing any psychological questionnaire (150).

The comparatively large number of participants in this study reveal not yet observable relationships between metabolic and cardiovascular parameters and subjective feelings. Although situation under investigation was a 30 min lesson with the accordion and the students were not allowed to do anything else between the two measurements, it cannot be clearly stated that all observed changes and relationships occurred due to the lesson itself as there was no control group. Further studies are needed to investigate, if this effect was observable only because of the moderate exercise or because of mental tasks with experiences of success as well as music.

Further the experience level in accordion playing of the students was not assessed. Reactions due to the lesson could be dependent on experience level. It could be possible that a beginner in accordion playing has very different expectations on the lesson compared to an experienced student who knows the teacher and his/her own skills. This should be taken into account for future studies.

Blood pressure and heart rate was determined by using an upper arm blood pressure monitor. Measurement accuracy could be a limiting factor when concluding on changes in cardiovascular parameters.

Cardiovascular as well as metabolic and psychological parameters point in the same direction of cardiovascular sedation, reduced metabolism and increased well-being after 30 minutes of accordion lesson. It is important to notice, that calming of these physiological parameters after the lesson could be observed despite the submaximal effort during the lesson. Based on previous investigations, it can be concluded that positive mental feelings and enjoyable tasks have a huge impact on the way stress is perceived (150).

6 CONCLUSIONS

Ionized Mg, in combination with metabolic parameters, seems to indicate the excitatory and pre-load status in persons with mental or physical stress. In a 2400 m run, change (predominantly decrease) in ionized Mg level depends on subjective anticipation as characterized by lactate level, blood glucose level, pCO_2, BE and HCO_3^- before the run. During a coaching weekend, ionized Mg levels increased most in participants with strongest calming indicated by metabolic parameters. Sensitive and time dependent fluctuations in ionized Mg level make reliable quantification of reactions to a stressor possible and allow characterization of physical and mental stress.

As it is involved in many enzymatic reactions in the organism, Mg could be crucial for adequate adjustment mechanisms when regulating changes in homeostasis. Therefore, it could be shown that acute Mg supplementation, just one hour before the exercise has a positive impact on effort, compared to placebo.

Assessment of an adequate Mg status is difficult as confirmed by conflicting results in previous studies. Solely the total or ionized Mg level in blood, without consideration of metabolic parameters does not seem to be a reliable marker. As its dynamics and levels depend on many factors including mental and/or physical state, always metabolic markers should be taken into account when assessing Mg status. Therefore, it has to be noted, that nervousness, especially in the clinical setting due to e.g. blood sampling, can cause stress and significantly affect Mg levels. This should be considered in further studies and in the assessment of Mg status.

Positive mental feelings and enjoyable tasks seem to have an impact on the way stress is perceived. Due to its relatively high weight, playing an accordion for 30 minutes can be considered as moderate exercise, especially in elderly participants. Despite the physical activity, investigated parameters in blood indicate relaxation of the metabolism, probably by improvement of the state of mind as indicated by increase in psychologic scores.

Although it remains a challenge to find adequate cohorts, further studies are needed to describe mechanisms, responsible for Mg fluctuations, especially when taking changes upon mental stressors into account. There is a need for standardized protocols in experimental and clinical settings when assessing Mg status.

7 LITERATURE

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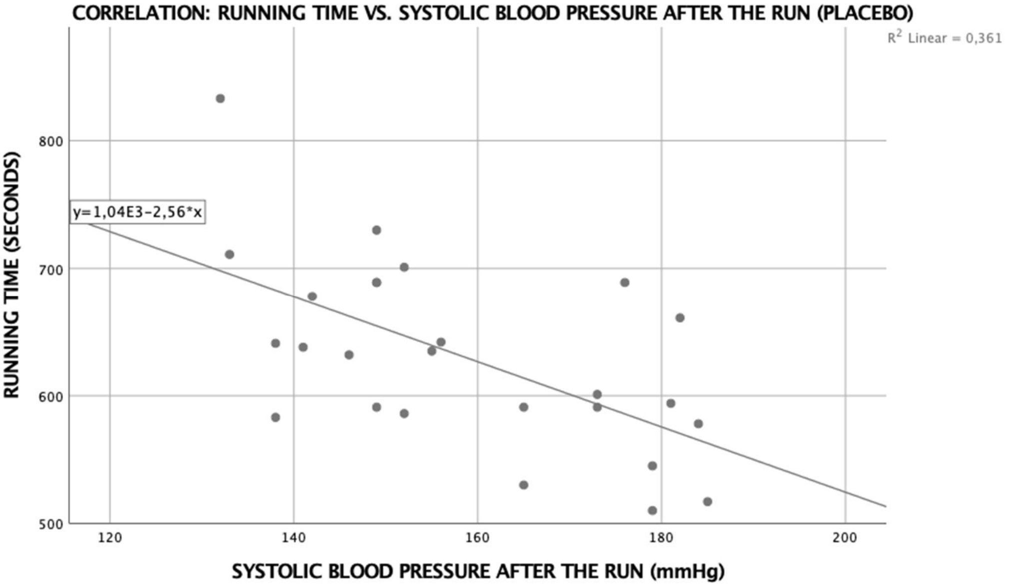
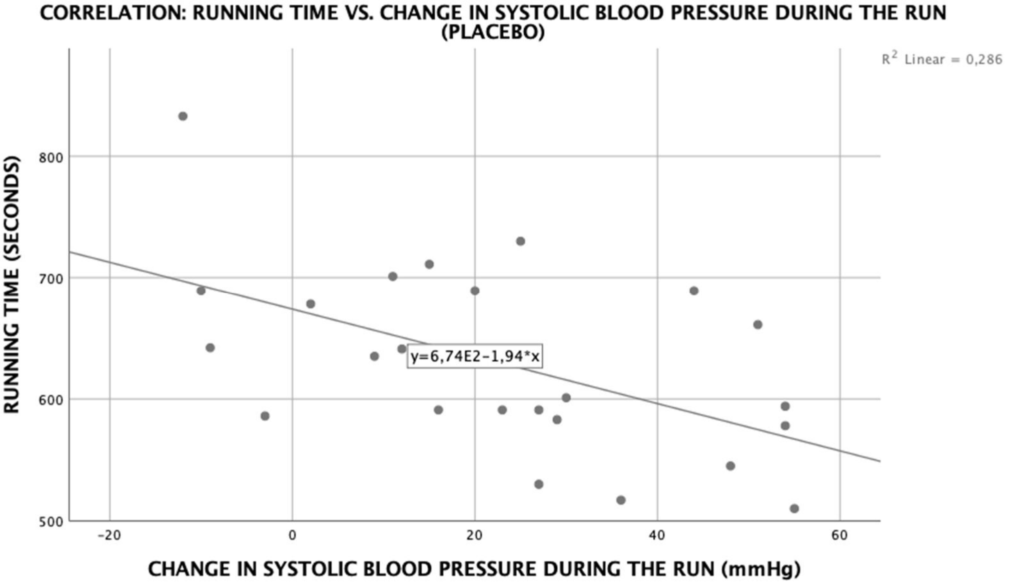
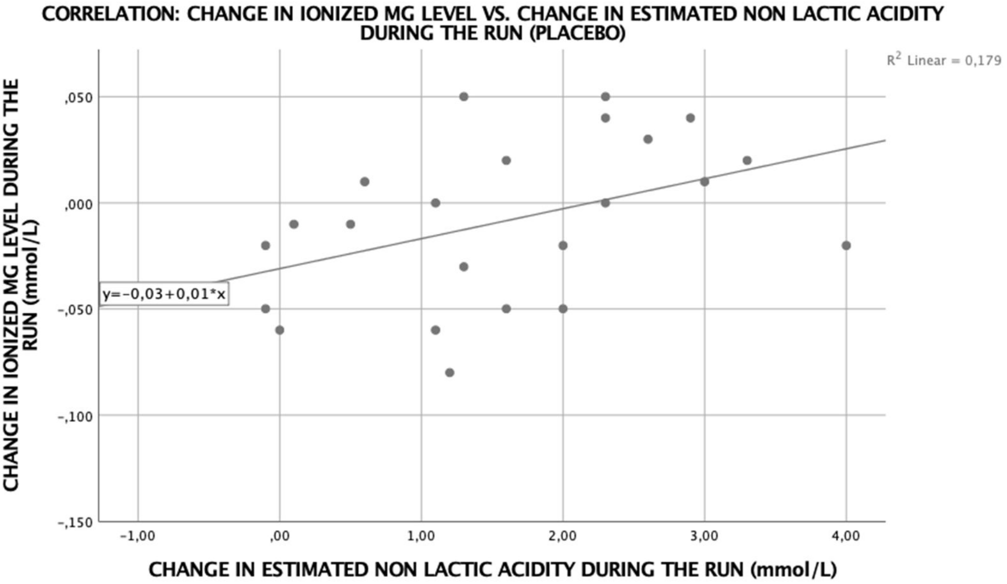
8 SUPPLEMENTAL FIGURES



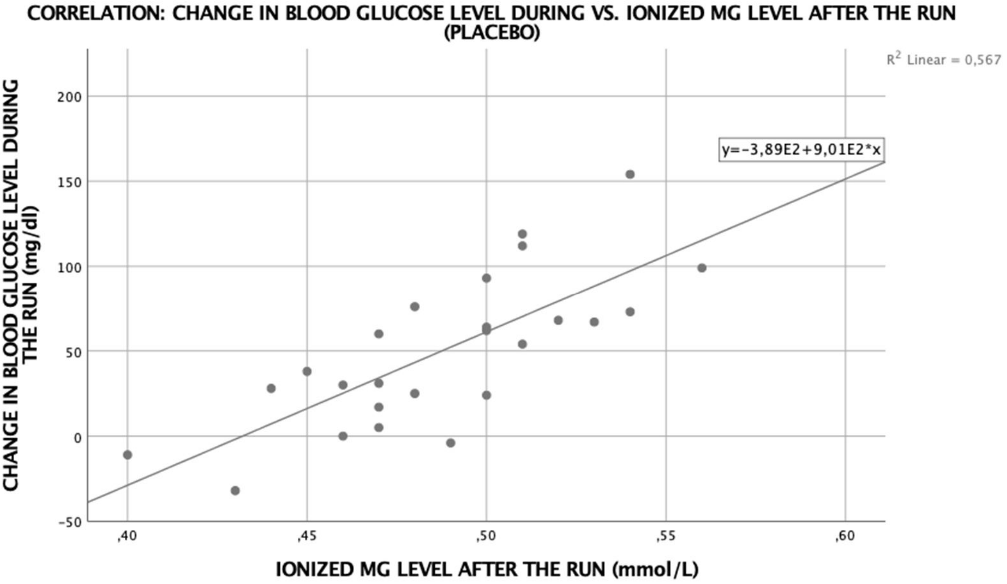
Supplemental Figure 8.1 Correlation: Running time vs. systolic blood pressure after the run after placebo supplementation; r=-0.601, n=26, p=0.001



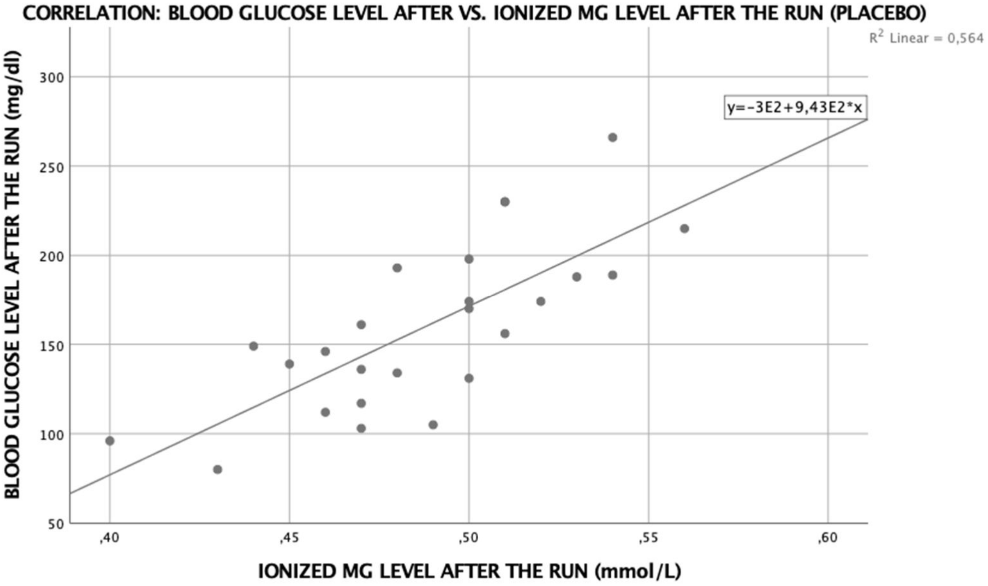
Supplemental Figure 8.2 Correlation: Running time vs. change in systolic blood pressure during the run after placebo supplementation; r=-0.535, n=26, p=0.005



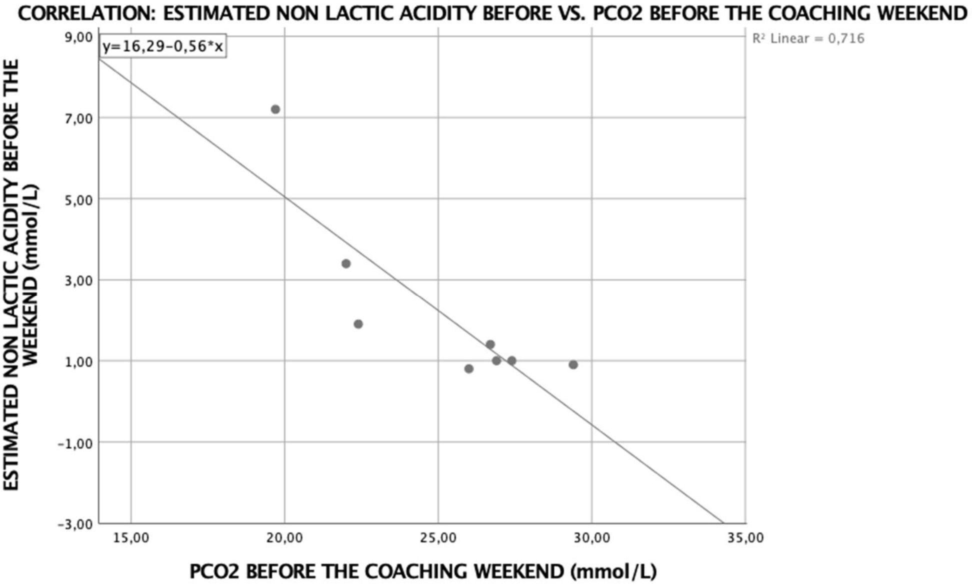
Supplemental Figure 8.3 Correlation: Change in ionized Mg level vs. change in estimated non lactic acidity during the run after Mg supplementation; r=0.421, n=24, p=0.040



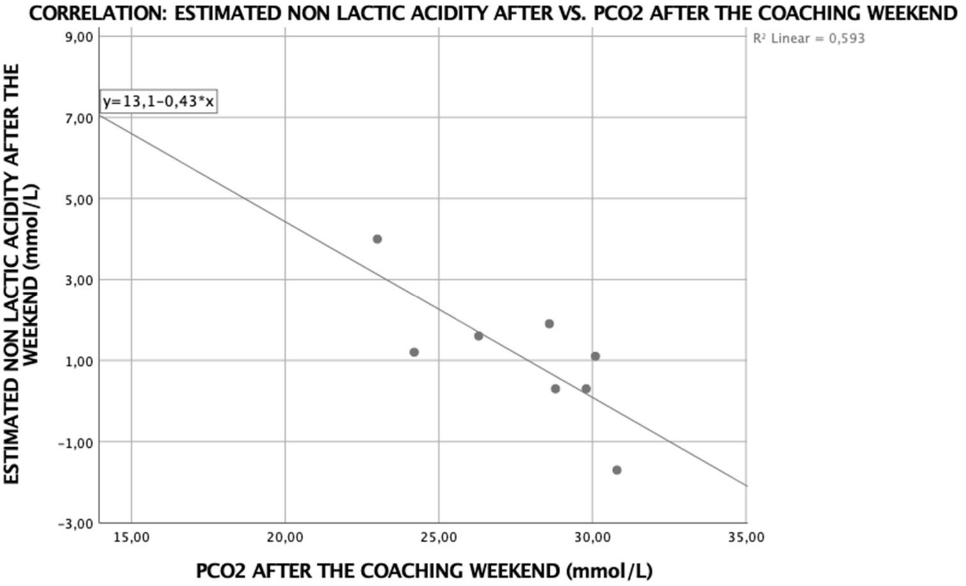
Supplemental Figure 8.4 Correlation: Change in blood glucose level during vs ionized Mg level after the run after placebo supplementation; r=0.757, n=26, p=0.000



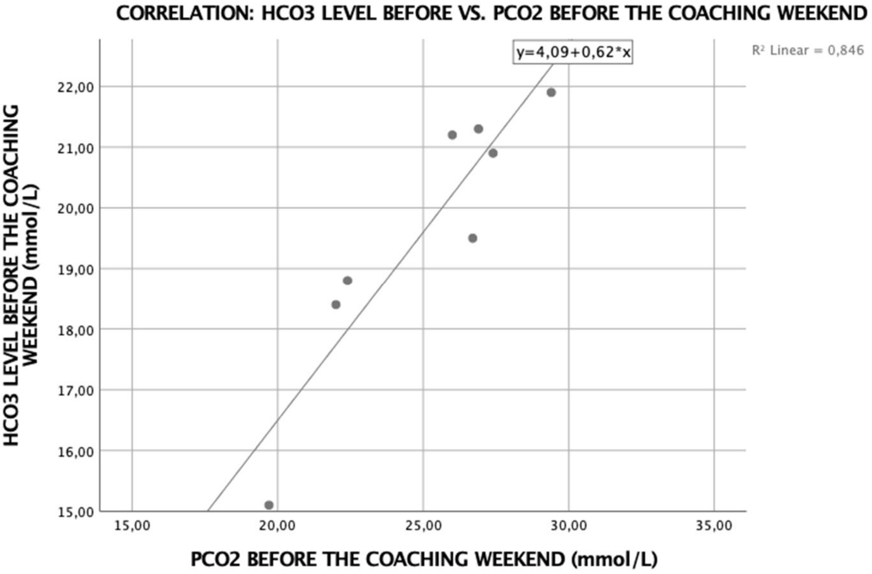
Supplemental Figure 8.5 Correlation: Blood glucose level vs. ionized Mg level after the run after placebo supplementation; r=0.755, n=26, p=0.000



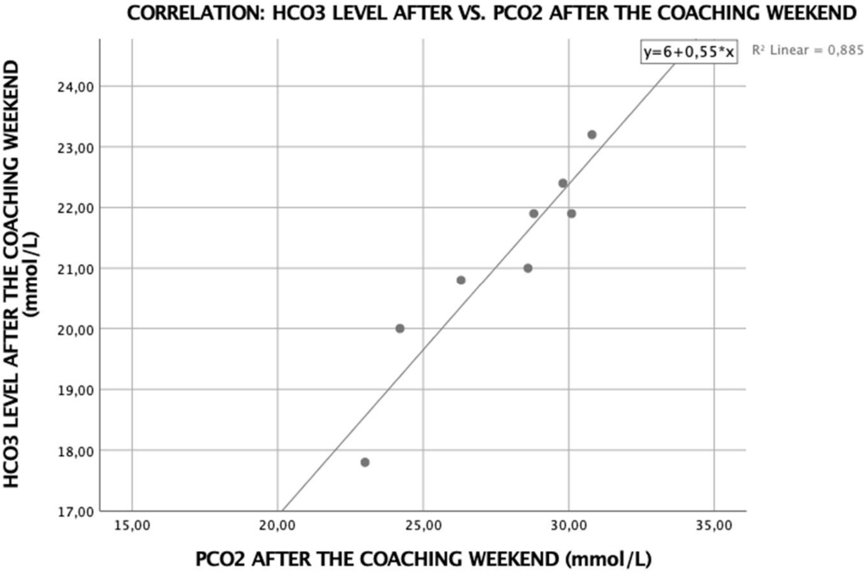
Supplemental Figure 8.6 Correlation: Estimated non lactic acidity before vs. pCO_2 before the coaching weekend; r=-0.846, n=8, p=0.008



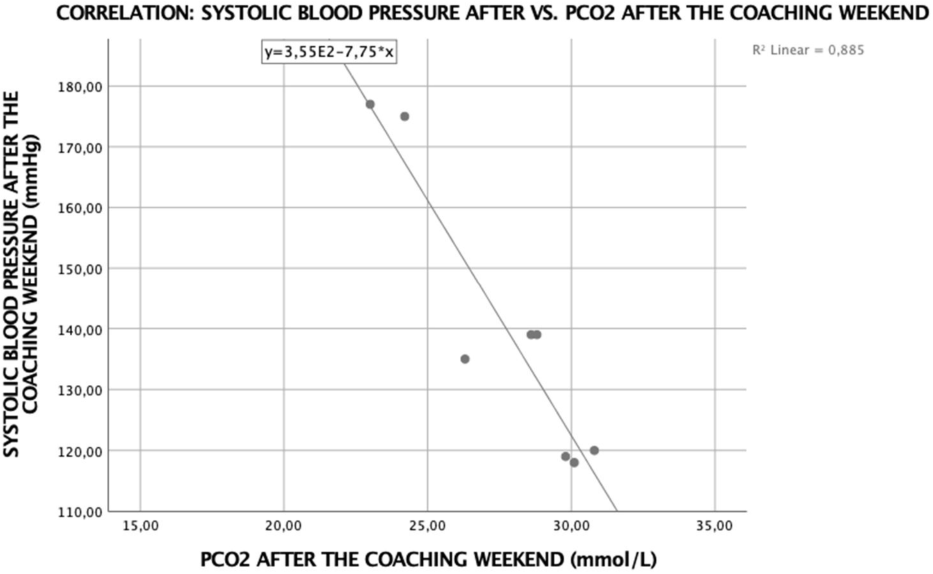
Supplemental Figure 8.7 Correlation: Estimated non lactic acidity after vs. pCO_2 after the coaching weekend; r=-0.770, n=8, p=0.025



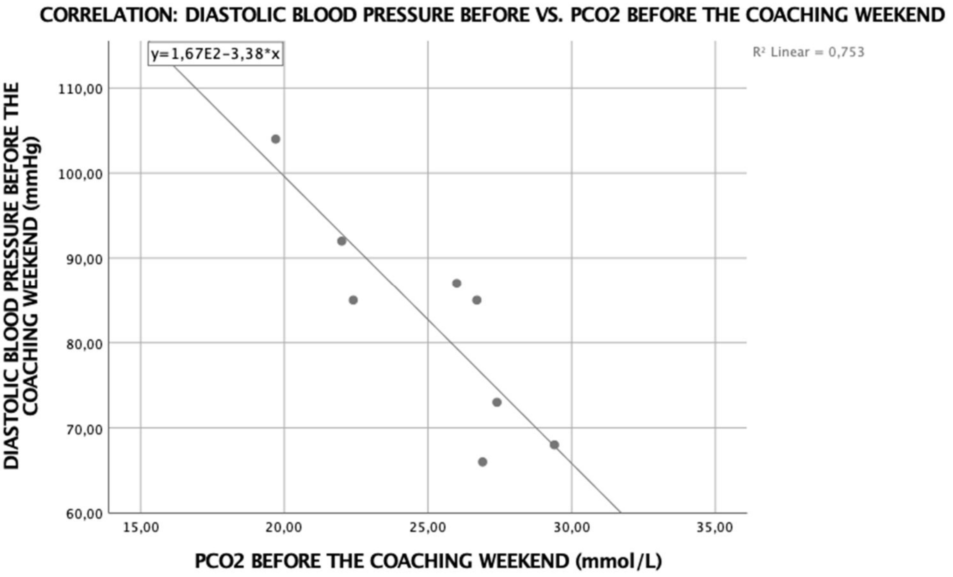
Supplemental Figure 8.8 Correlation: HCO_3 level before vs. pCO_2 before the coaching weekend; r=0.920, n=8, p=0.001



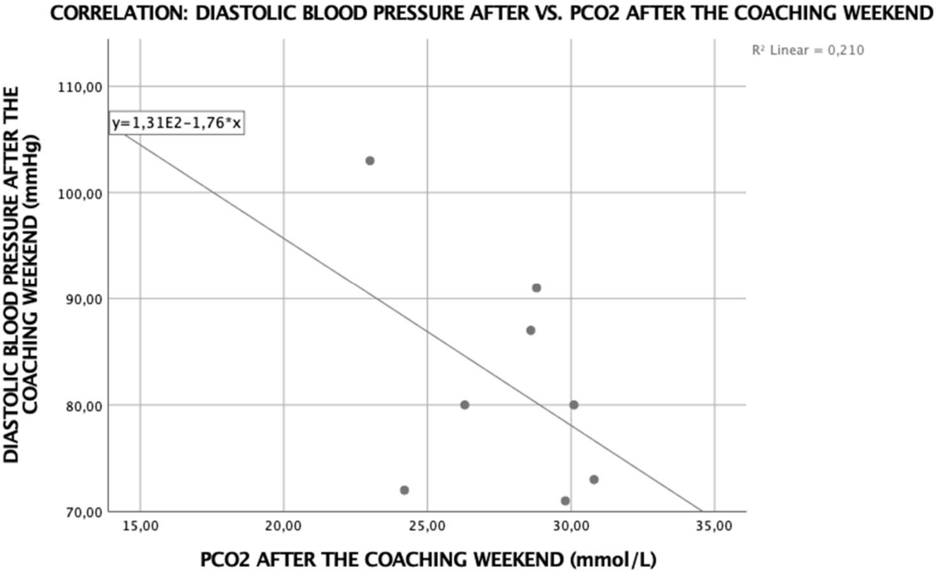
Supplemental Figure 8.9 Correlation: HCO_3 level after vs. pCO_2 after the coaching weekend; r=0.941, n=8, p=0.000



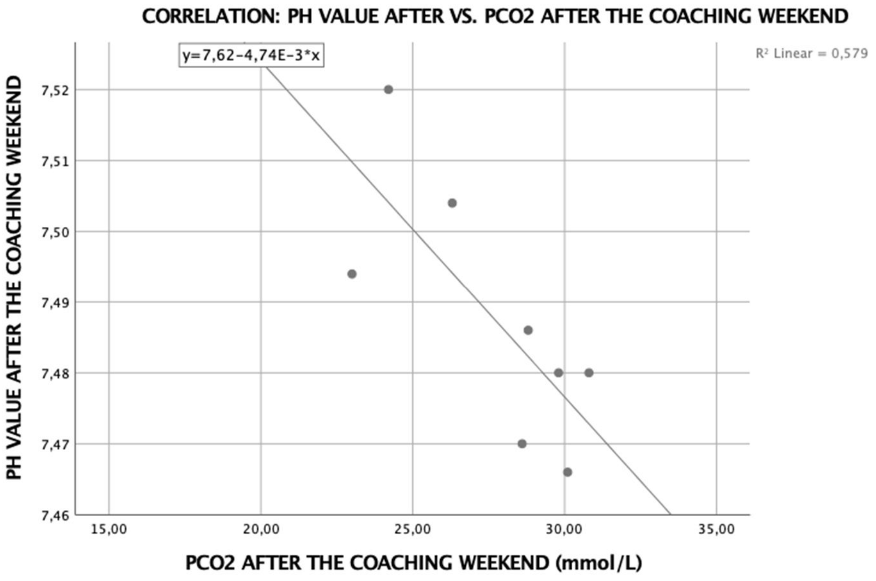
Supplemental Figure 8.10 Correlation: Systolic blood pressure after vs. pCO_2 after the coaching weekend; r=-0.941, n=8, p=0.001



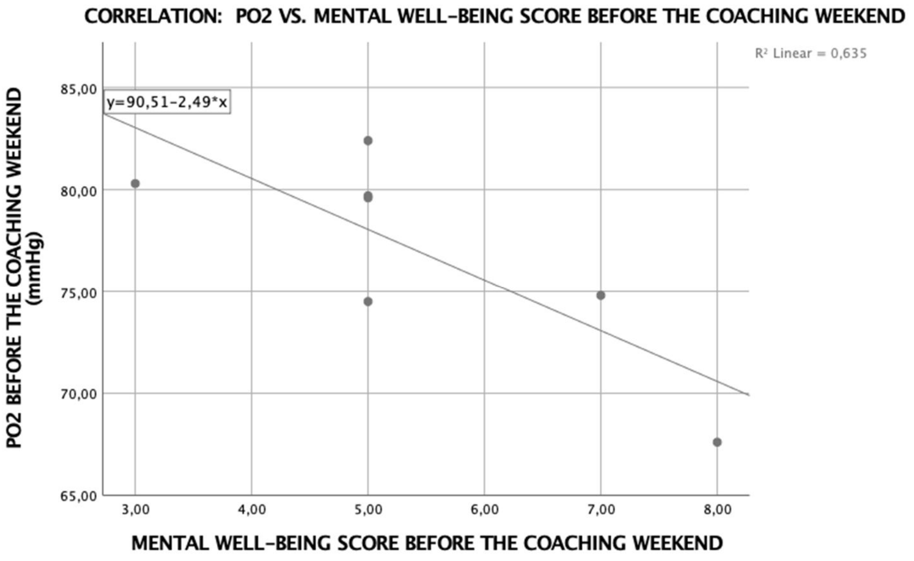
Supplemental Figure 8.11 Correlation: Diastolic blood pressure before vs. pCO$_2$ before the coaching weekend; r=-0.868, n=8, p=0.005



Supplemental Figure 8.12 Correlation: Diastolic blood pressure after vs. pCO$_2$ after the coaching weekend; r=-0.458, n=8, p=0.253



Supplemental Figure 8.13 Correlation: pH value after vs. pCO_2 after the coaching weekend; r=-0.761, n=8, p=0.028



Supplemental Figure 8.14 Correlation: Mental well-being score before vs. pO_2 before the coaching weekend; r=-0.797, n=7, p=0.032