Influence of Whole-Body Electrostimulation on Human Red Blood Cell Deformability

Andre Filipovic,[1,2] Heinz Kleinöder,[1] Denise Plück,[2] Wildor Hollmann,[2] Wilhelm Bloch,[2] and Marijke Grau[2]

[1]*Institute of Sport Science and Sport Informatics, German Sport University Cologne, Cologne, Germany; and* [2]*Institute of Molecular and Cellular Sport Medicine, German Sport University Cologne, Cologne, Germany*

Abstract

Filipovic, A, Kleinöder, H, Plück, D, Hollmann, W, Bloch, W, and Grau, M. Influence of whole-body electrostimulation on human red blood cell deformability. *J Strength Cond Res* 29 (9): 2570–2578, 2015–Red blood cell–nitric oxide synthase (RBC-NOS)–dependent NO production is essential for the maintenance of RBC deformability, which is known to improve oxygen supply to the working tissue. Electrostimulation of the whole body (WB-EMS) has been shown to improve maximal strength, springiness, and jumping power of trained and untrained athletes. To examine whether these 2 parameters are associated, this study, for the first time, aimed to investigate the effects of an 18-week dynamic WB-EMS program on RBC deformability in addition to maximal strength performance (1 repetition maximum [1RM]) in elite soccer players. Fifteen test persons were assigned in either WB-EMS group (EG, $n = 10$) or training group (TG, $n = 5$). Next to their weekly training sessions, EG performed 3 $\times$ 10 squat jumps under the influence of WB-EMS twice per week between weeks 1 and 14 and once per week between weeks 14 and 18. Training group only performed 3 $\times$ 10 squat jumps. Performance was assessed by a maximal strength test on the leg press machine (1RM). Subjects were tested at baseline and after weeks 7, 14, and 18 with blood sampling before (Pre), 15–30 minutes after (Post), and 24 hours after (24-hour Post) the training. The results showed that maximal strength was significantly improved in EG ($p < 0.01$). Maximum RBC deformability (EImax) increased on EMS stimulus in EG while it remained unaffected in the TG. Acute increase in EImax at baseline was explained by an increase in RBC-NOS activation while chronic increase of deformability must be caused by different, yet unknown, mechanisms. EImax decreased between weeks 14 and 18 suggesting that 1 WB-EMS session per week is not sufficient to alter deformability (EImax). In contrast, the deformability at low shear stress (EI 3 Pa), comparable with conditions found in the microcirculation, significantly increased in EG until week 14, whereas in TG deformability only, increased until week 7 due to increasing training volume after the winter break. The results indicate that WB-EMS represents a useful and time-saving addition to conventional training sessions to improve RBC deformability and possibly oxygen supply to the working tissue and thus promoting general force components in high performance sport.

Key Words strength training, RBC deformability, RBC-NOS activation, elite athletes

Introduction

In high-performance field sports, game play speed and intensity increases, and thus athletes' performance levels are constantly increasing. In regard of soccer, the ability to perform repeated high-intensity exercise during the entire 90 minutes is of great importance (35). Modern video analysis systems show that elite players perform 28% more high-intensity running (2.43 vs. 1.90 km) and 58% more sprinting (650 vs. 410 m) during competitive games compared with semiprofessional players (35). In summary, top players perform more high-intensity exercise with shorter rest intervals during the 90 minutes of game play. These intermittent exercises or repeated sprint performances during soccer match-play require a high demand of aerobic and anaerobic endurance what in turn highly depends on the oxygen transport to the muscles. Nielsen et al. (36) assume that the development of local acidosis caused by intense exercise can lead to a reduced affinity of hemoglobin for oxygen, which forwards deoxygenation in the muscles. Several studies showed that muscle (re)oxygenation influences the phosphocreatine resynthesis after intense exercise (cf. (34)). Furthermore, the phosphocreatine resynthesis during short recovery periods between repeated exercise bouts can be crucial and limit the repeated-sprint performance (5). Within the body, the red blood cells (RBCs) deliver oxygen to the muscle tissues through the blood flow through the circulatory system. For optimal oxygen support, the RBCs have to squeeze through the small capillaries that are smaller

Address correspondence to Andre Filipovic, Andre.Filipovic@gmx.net.

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than the RBCs diameter. This requires a high membrane deformability yet remarkably stable membrane. The higher the deformability the less applied force is necessary to allow the cell to pass through capillaries, and the better the blood flow respectively oxygen support (10). According to Connes et al. (11), an increased RBC deformability can improve the blood oxygen content due to an increased oxygen diffusion from alveoli to pulmonary capillaries. Regarding intense exercise, an improved oxygen transport and thus higher muscle oxygen-level can positively influence the reoxygenation in the muscles and thus phosphocreatin resynthesis for a faster recovery. Due to the high demand of muscle oxygenation during and between the repeated sprint actions, improved RBC deformability could be advantageous for the performance of soccer players. Brun et al. (9) support this theory suggesting that increased blood fluidity may improve oxygen delivery to the muscle during exercise in trained athletes. Furthermore, several studies found a positive correlation of RBC deformability to isometric strength as well as between blood fluidity and aerobic endurance capacity and maximum oxygen uptake (cf. (9)).

Several studies showed that nitric oxide (NO) produced by the nitric oxide synthase (NOS) in human RBCs (RBC-NOS) positively influences the deformability and thus the rheological characteristics of the blood (6,26,45,49). Thereby, phosphorylation of the RBC-NOS at its serine 1177 residue (RBC-NOS^{Ser1177}) has been shown to represent increased enzyme activation and thus increased NO production and RBC deformability (23,44). Red blood cell–nitric oxide synthase activation and deformability have been shown to be influenced by physical exercise whereby the intensity of exercise determines the outcome.

Although moderate exercise has been shown to increase deformability and RBC-NOS activation in both healthy subjects and patients with microcirculatory disorders, (1,46), high intensive exercise reduces deformability (1,52) and RBC-NOS activation (45) suggesting that intensive exercise leads to a reduction of proteins within the RBCs (45).

Electrostimulation training (EMS) has been used in rehabilitation for many years. In the last few years, the interest of EMS used for strength training in high-performance sports with elite athletes has grown. Several studies showed that EMS can be an effective method for developing strength qualities especially speed strength respectively movement velocity (9,16,22,23,27,35,36,38). Modern whole-body EMS devices (e.g., miha bodytec, Augsburg, Germany) enable athletes to train a whole muscle chain and thus train dynamically in specific movements, e.g., jumping movement.

So far, it is unknown whether RBC deformability may be influenced by EMS. It is suggested that EMS increases deformability to adapt to changing demands of the muscles due to the EMS stimulus. This may explain possible pathways that improve muscle function by EMS in the human body. The effects of WB-EMS on performance and on rheological qualities could be interesting not only for athletes but also for patients with microcirculatory disturbances such as patients with diabetes mellitus, arteriosclerosis, or sickle-cell disease as it could reduce the risk of clinical complications.

Methods

Experimental Approach to the Problem

This study was undertaken to address the following question: Does WB-EMS influence deformability of RBC, thus possibly explaining increased oxygen transfer to the working muscles under the influence of EMS? For this purpose, a team of professional soccer players were assigned into 2 different groups. The EMS groups (EG) performed dynamic whole-body strength training with EMS accompanied by 3 × 10 squat jumps in addition to the daily soccer routine. To differentiate between the effects caused by EMS and by the squat jumps, a second training group (TG) performed 3 × 10 squat jumps without EMS stimulus on the same days as the EG. The test persons conducted 2 test sessions per week during weeks 1 to 14 to test the effects of the WB-EMS on rheology parameters such as deformability. Subsequently, a 4-week maintenance phase was

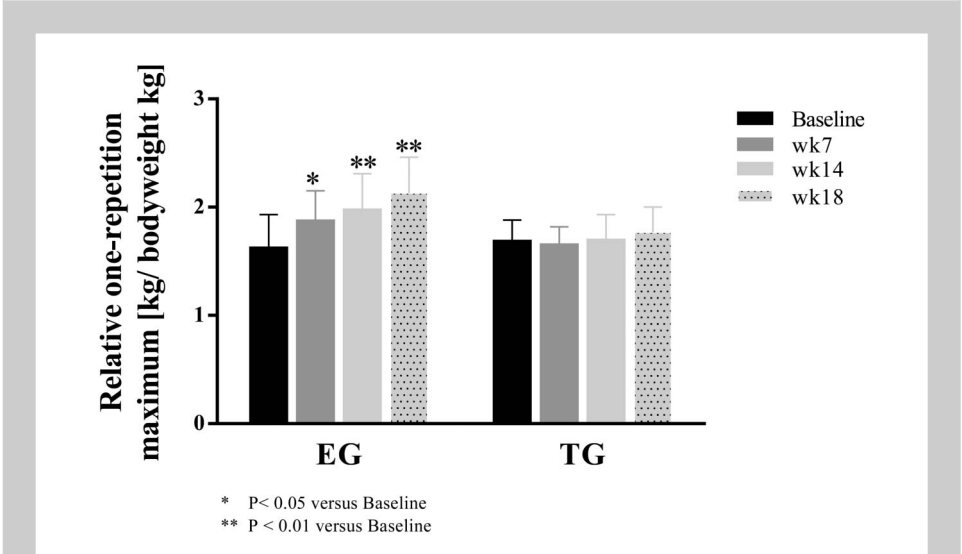

Figure 1. Performance capacity, represented by relative maximal strength (1RM) at the leg press machine (1-legged), significantly increased in EG from baseline to weeks 7, 14, and, 18, respectively. No difference in maximal strength (1RM) was observed in TG. 1RM, 1 repetition maximum; EG, EMS group; TG, training group.

conducted with 1 test session per week to test whether the effects of the EMS period were preserved. Parameters were assessed before (Pre), 15–30 minutes after (Post), and 24 hours after (24-hour Post) the respective test sessions at baseline and after weeks 7, 14, and 18. The changes in the different groups were statistically evaluated.

Subjects

Fifteen male field soccer players, competing in the fourth division of the German Soccer Federation (DFB), voluntarily participated in this study and were assigned either into a WB-EMS group or a training group. Basal anthropometric parameters of the subjects were as follows (mean ± *SD*): WB-EMS group (EG: $n = 10$; age, 24.4 ± 3.3 years; height, 183.1 ± 4.0 cm; mass, 81.2 ± 8.9 kg); training group (TG: $n = 5$; age, 25.6 ± 0.6 years; height, 181.0 ± 5.6 cm; mass, 76.1 ± 5.3 kg). All subjects abstained from alcohol consumption for 24 hours before and during the training intervention and were nonsmokers.

The protocols used in this study were approved by the Ethics Committee of the German Sports University Cologne. These protocols are in line with the Declaration of Helsinki, and all participants gave written informed consent to participate in this study.

Procedures

The test persons were professional soccer players and performed 6–7 training sessions per week and competed once a week in the championships. The standard training sessions lasted 70–90 minutes including technical skill activities, offensive and defensive tactics, athletic components with various intensities, small-sided game plays and 20–30 minutes of continuous play. The playtime of the test persons were recorded during the study period. The players were asked to maintain their usual food intake and hydration. Additional strength training was not allowed during the study.

The 18-week study period started after the midseason break and was divided into EMS phase between weeks 1 and 14 and characterized by 2 WB-EMS sessions per week, and a maintenance phase between weeks 14 and 18 characterized by 1 WB-EMS session per week.

The WB-EMS was performed in addition to the daily soccer routine and the EG performed 3 × 10 squat jumps under the influence of EMS. The TG performed the same jumps at the same time as the EG but without EMS.

Exercise Protocol. WB-EMS training was conducted on Mondays and Thursdays to obtain a rest interval of 48 hours between the 2 sessions and the championship game on Saturdays. The EMS training was conducted with a WB-EMS system by "miha bodytec" (Augsburg, Germany). WB-EMS was applied with an electrode vest to the upper body including the chest, upper and lower back, latissimus, and the abdominals and with a belt system to the lower body including the muscles of the glutes, thighs, and calves. The players started with a 2- to 3-minute warm-up

Table 1. Blood parameters obtained in EG and TG after Pre, Post, and 24-hour Post showed no statistical differences between the time-points at baseline.*†

	Pre EG	Post EG	24-hour post EG	Pre TG	Post TG	24-hour post TG
RBC ($\times 10^6/\mu l$)	4.99 ± 0.24	4.79 ± 0.33	4.76 ± 0.35	5.16 ± 0.37	4.45 ± 0.19	4.70 ± 0.20
Hb (g/dl)	15.55 ± 0.70	14.43 ± 0.80	14.22 ± 0.85	15.60 ± 0.85	13.70 ± 0.64	14.00 ± 0.50
Hct (%)	45.48 ± 1.36	44.32 ± 2.15	44.04 ± 3.00	46.30 ± 2.28	41.54 ± 2.18	47.10 ± 1.27
WBC ($\times 10^3/\mu l$)	5.63 ± 1.61	5.10 ± 0.41	5.40 ± 1.77	4.90 ± 1.01	5.78 ± 1.15	5.85 ± 0.60
Platelets ($\times 10^3/\mu l$)	201.75 ± 33.38	211.82 ± 42.07	199.60 ± 49.26	217.75 ± 38.87	238.40 ± 52.51	200.50 ± 19.12

*RBC = red blood cell; Hb = hemoglobin concentration; Hct = hematocrit; WBC = white blood cell; EG = EMS group; TG = training group (control).
†Data are presented as mean ± *SD*.

with easy movement preparations and jumps at a light to moderate stimulation intensity. The players were told to slowly increase the intensity every few impulses. The training started when the players reached the defined training intensity. The EG performed 3 × 10 maximal squat jumps with a set pause of 60 seconds (no currents) per session. Biphasic rectangular wave pulsed currents (80 Hz) were used with an impulse width of 350 microseconds. Every impulse for a single jump lasted for 4 seconds (range of motion [ROM]: 2 seconds eccentric from standing position to an knee angle of 90°; 1 second isometric; 0.1 seconds explosive concentric; 1 second landing and stabilization) followed by a rest period of 10 seconds (duty cycle approximately 28%). The stimulation intensity was determined and set separately for each muscle group by using a Borg Scale (6–20 [20–100%]). For the first 2 sessions, the subjects started with a moderate to submaximal intensity (13–15 [50–60%]). The stimulation intensity was then constantly increased individually every week. The players were told to maintain a high stimulation intensity (Borg Scale, 18–19, [80–90%]) that still assures a clean dynamic jump movement. The TG performed the same amount of jumps with identical interval and conduction twice per week without EMS.

Strength Tests. Tests were performed on a leg press machine (NORSK, Sequence-training-system, Cologne, Germany). The players were positioned horizontal on the sledge with the hip and knee angle under 90°. The maximal strength of each leg was measured by the 1 repetition maximum (1RM) according to Beachle (3). The players performed 2 series with 10 dynamic repetitions with submaximal weight for warm-up. To determine the 1RM, the players were allowed to use both legs to get in starting position with legs fully extended (180°). For testing, the players performed maximal repetitions with 1 leg (ROM knee angle, 180°–90°, 2 s eccentric/2 s concentric). If a player mastered more than 6 repetitions, a load of 10 kg was added to the sledge for the next set after allowing a recovery interval of about 3 minutes.

Blood Sampling. Blood samples were taken before the first WB-EMS session (baseline) after 7 weeks, 14 weeks, and 18 weeks (maintenance phase) of training. On each day, samples were taken from the vena mediana cubiti before (Pre), 15–30 minutes after (Post), and 24 hours after the interventions (24-hour Post).

Blood was anticoagulated using EDTA vacutainer (BD, Heidelberg, Germany) and immediately processed. To measure RBC deformability, blood was mixed with an isotonic viscous polyvinylpyrrolidone (PVP) solution (0.14 mM, osmolality 300 mOsm·L^{-1}, viscosity 30 mPa·s at 37° C, Mechatronics, the Netherlands) in a 1:250 ratio (vol:vol). For the immunohistochemical staining of the RBC-NOS, blood was fixed with 4% paraformaldehyde (vol/vol; 1/1) immediately after blood sampling (23,44,45).

The remaining blood was used to determine basic blood parameters such as the number of RBC [×10^6/μl], white

Table 2. Blood parameters obtained in EG and TG during the 18-week investigation period showed no statistical differences of the Pre values between the groups.*†

	Baseline EG	TG	Week-7 EG	TG	Week-14 EG	TG	Week-18 EG	TG
RBC (×10^6/μl)	4.99 ± 0.24	5.16 ± 0.37	4.84 ± 0.29	4.58 ± 0.28	4.88 ± 0.34	1.54 ± 0.34	4.95 ± 0.34	4.72 ± 0.29
Hb (g/dl)	15.55 ± 0.70	15.60 ± 0.85	14.70 ± 0.76	14.08 ± 0.86	14.78 ± 0.68	13.95 ± 1.30	14.85 ± 0.95	14.45 ± 1.25
Hct (%)	45.48 ± 1.36	46.30 ± 2.28	45.41 ± 2.01	43.06 ± 3.17	45.01 ± 1.93	42.93 ± 5.43	45.87 ± 2.18	44.63 ± 3.42
WBC (×10^3/μl)	5.63 ± 1.61	4.90 ± 1.01	5.10 ± 0.77	5.88 ± 0.84	5.61 ± 0.53	6.30 ± 1.16	5.35 ± 0.86	5.10 ± 0.88
Platelets (×10^3/μl)	201.75 ± 33.38	217.75 ± 38.87	205.64 ± 36.82	240.40 ± 48.47	221.7 ± 49.86	226.75 ± 19.67	228.36 ± 56.60	235.25 ± 26.11

*RBC = red blood cell; Hb = hemoglobin concentration; Hct = hematocrit; WBC = white blood cell; EG = EMS group; TG = training group (control).
†Data are presented as mean ± *SD*.

blood cells [$\times 10^3/\mu l$], and platelets [$\times 10^3/\mu l$], hemoglobin concentration [in grams per deciliter], and hematocrict [in percentage] using the Sysmex Digitana KX-21N (Norderstedt, Germany).

Red Blood Cell Deformability. The deformability was measured by the laser-assisted optical rotational cell analyzer (LORCA; RR Mechatronics, Hoorn, the Netherlands). The LORCA system has been described in detail elsewhere (24).

The blood/PVP sample was sheared in a Couette system to measure the deformability at various physiological and supraphysiological fluid shear stresses. During the measurements, a laser beam was directed through the sheared sample, and the diffraction pattern produced by the deformed red cells was analyzed. On the basis of the geometry of the elliptical diffraction pattern, an elongation index (EI) was calculated by the software as $EI = (L - W) \times (L + W)^{-1}$, where L and W represent length and width of the diffraction pattern, respectively (44). Elongation index values were calculated for 9 shear rates up to 30 Pa (0.3, 0.53, 0.95, 1.69, 3.00, 5.33, 9.49, 16.87, 30 Pa) and were plotted as a function (4). According to Baskurt et al. (4), the Lineweaver-Burk equation was used to calculate the maximum deformability (EImax) (4), which is defined as the theoretical maximum deformability at infinite shear stress. Additionally, EI values obtained at a shear stress of 3 Pa are separately presented as this represents the shear stress that is predominantly found in the microcirculation (39).

Immunohistochemical Procedure. The applied protocol for the immunostaining has been described in various studies (23,44,45). Red blood cell samples were fixed using 2% paraformaldehyde (end concentration). Samples were then dispersed on a slide and heat fixed. For staining, the RBCs were washed in 0.1 M tris-buffered saline (TBS) and permeabilized for 45 minutes in 0.1% trypsin at 37° C. The slides were covered with a solution of 5% hydrogen peroxide, 15% distilled water, and 80% methanol for 30 minutes at room temperature to inhibit endogenous peroxidase. Unspecific binding sites were then blocked with 3% milk powder in 0.1 M TBS for 30 minutes at room temperature. The test area of each slide was incubated with the primary antibody against eNOSSer^{1177} (dilution 1:500 in 0.3% milk powder and 0.03% tween; Upstate, Lake Placid, NY, USA) for 60 minutes. The control area was incubated with 0.3% milk powder and 0.03% tween in the absence of the first antibody. After TBS washing and blocking of unspecific binding sites using 3% Normal Goat Serum (Dako Agilent Technologies,

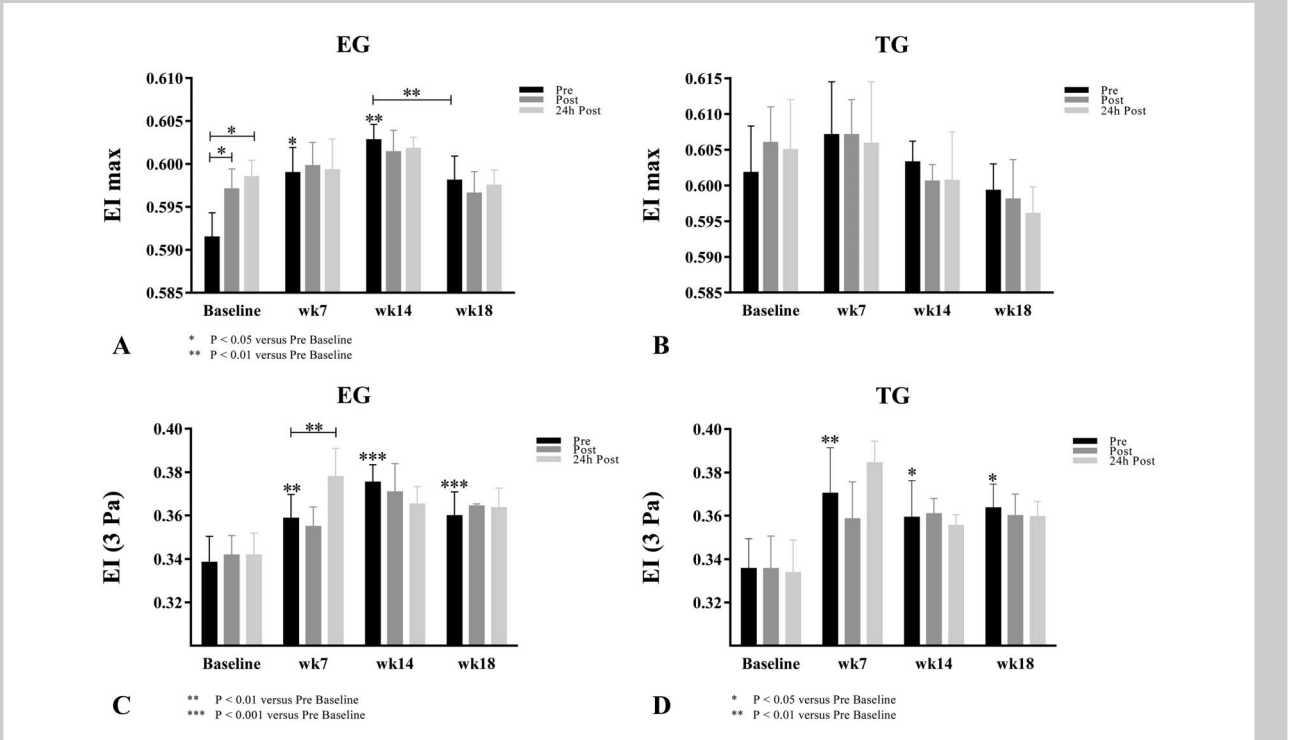

Figure 2. A) In the baseline test, maximum deformability (EImax) significantly increased from Pre to Post and 24-hour Post, respectively in EG. Furthermore, Pre values of EG significantly increased from baseline to week 7 and week 14, respectively and significantly decreased from week 14 to week 18 in EG. B) EImax values obtained in TG showed no significant changes during the study. C) In EG, EI values obtained for 3 Pa significantly increased baseline to week 7 and week 14, respectively. EI values decreased from week 14 to week 18. At week 7, values significantly increased from Pre to 24-hour Post. D) In TG, EI values for 3 Pa significantly increased from baseline to week 7, and then decreased at weeks 14 and 18. EI, elongation index; EG, EMS group; TG, training group.

Glostrup, Denmark), both areas were incubated with the second goat-anti-rabbit antibody (dilution 1:400; Dako) for 60 minutes. A streptavidin-horseradish-peroxidase complex (Amersham, Buckinghamshire, England) was applied as a detection system (dilution 1:150) for 30 minutes. The immunostaining of the RBCs was developed using 3,3-diaminobenzidine-tetrahydrochloride solution (Sigma, St. Louis, MO, USA) in 0.1 M TBS.

The intensity of the immunostained RBCs was determined by measuring gray values (television densitometry) of the RBCs (26,28,44). One hundred RBCs (test area) from at least 4 randomly selected pictures and 50 RBCs (control area) from at least 2 randomly selected pictures of each slide were measured. The intensity of immunostaining was reported as the mean of measuring RBC gray value minus background gray value (44). The background gray value was detected at a cell-free area of the slide. Finally, the gray values of the test area (minus background) and the control area (minus background) were subtracted. The staining intensity was detected using a Leica microscope coupled to a CCD-camera (DXC-1850P; Sony, Cologne, Germany). The pictures were analyzed with the software "ImageJ" (National Institutes of Health, Bethesda, MD, USA). Magnification for all images was 400-fold.

Statistical Analyses

Statistical analyses were performed using Statistical Package for Social Science (SPSS, Version 22.0, Chicago, IL, USA), Origin 8.5 Pro, (Northampton, MA, USA), and GraphPad Prism 5 (La Jolla, CA, USA). Results were presented as mean and *SD*s and were calculated using standard statistical methods. The Kolmogorov-Smirnov test was applied to test for normal distribution, and all the data were normally distributed. Effects related to the training interventions were determined by analysis of variance (ANOVA) with repeated measures (group × time). For each testing, the group differences were determined by a one-way ANOVA. Bonferroni post hoc test was used to calculate significant differences between the tested groups. Statistical differences were considered to be significant for values of $p \leq 0.05$.

Results

One Repetition Maximum Leg Press Machine

A significant increase in maximal strength was observed for the EMS group (EG) at all measuring points compared with the baseline measurement (week 7, $p < 0.05$; week 14, $p < 0.01$, and week 18, $p < 0.01$). The TG showed no changes regarding the maximal strength (Figure 1).

Basal Blood Parameters

No significant acute effect was observed in RBC [$\times 10^6/\mu l$], white blood cells [$\times 10^3/\mu l$], and platelets [$\times 10^3/\mu l$], hemoglobin concentration [in g/dl], and hematocrit [in %] for EG at the baseline test from Pre to Post respectively 24-hour Post (Table 1). Furthermore, Pre values remained unaltered in both groups throughout the investigation period thus showing no long-term effects (Table 2).

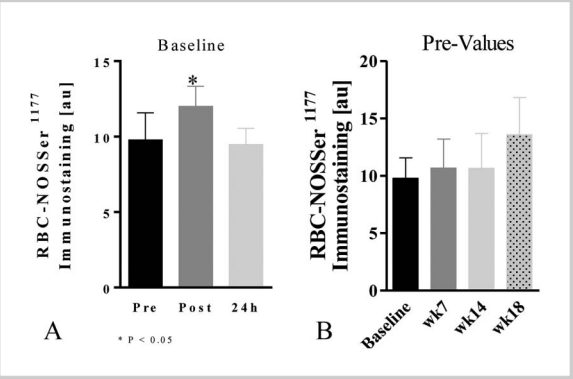

Figure 3. A) In the baseline test, RBC-NOS phosphorylation of the serine 1177 residue significantly increased from Pre to Post in EG. B) Pre values for RBC-NOS phosphorylation of the serine 1177 residue remained unchanged at weeks 7, 14, and 18, respectively. RBS-NOS, red blood cell nitric oxide synthase; EG, EMS group.

Red Blood Cell Deformability

Results showed an acute increase in maximum deformability (EImax) at baseline with significant differences between Pre and Post ($p < 0.05$) as well as Pre and 24-hour Post ($p < 0.05$) in the EG. Moreover, a general increase in EImax was observed until week 14 ($p < 0.05$ week 7; $p < 0.01$ week 14) by comparing respective Pre values. Between weeks 14 and 18, Pre values of EImax significantly decreased ($p < 0.01$), but the values were still above the baseline level (Figure 2A). EImax remained unaltered in TG throughout the investigation period, and differences between Pre, Post, and 24-hour Post were not observed (Figure 2B).

Deformability values obtained in EG for a shear rate of 3 Pa showed no differences between Pre and Post or Pre and 24-hour Post at baseline. But Pre values constantly increased from baseline to week 7 ($p < 0.01$) and week 14 ($p < 0.001$). Elongation index (3 Pa) decreased from week 14 to week 18, but values were still significantly higher compared with baseline ($p < 0.001$). Elongation index (3 Pa) was significantly increased in the TG comparing Pre values of weeks 7 ($p < 0.01$), 14 ($p \leq 0.05$), and 18 ($p \leq 0.05$) with baseline values. Acute changes on squat jumps were not observed.

Red Blood Cell–Nitric Oxide Synthase

Results revealed a significantly increased RBC-NOS^{Ser1177} signal at baseline after the first WB-EMS session (Post) compared with Pre value ($p < 0.05$; Figure 3A). Comparing Pre values of all investigation days showed no chronic effect of WB-EMS on RBC-NOS^{Ser1177} (Figure 3B).

Discussion

The results of this study reveal that 2 dynamic WB-EMS sessions concurrent with 6–7 soccer training sessions per

week can significantly increase maximal strength (1RM) in the lower-body muscles in elite soccer players. Furthermore, the results show that WB-EMS significantly enhances RBC deformability and thus may improve blood circulation and oxygen transport to the muscles and organs of elite soccer players.

The maximal strength (1RM) of professional soccer players investigated in this study significantly increased up to +33% (+16.83 ± 13.06%) after 7 weeks (14 sessions) and up to +43% (+22.42 ± 12.79%) after 14 weeks (28 sessions) of dynamic WB-EMS. These results are in line with the findings of studies using local EMS training (12–28 sessions) on the lower-body muscles in trained and elite athletes (3,17,23,36). Previous investigations concluded that gain in maximal strength was predominantly due to neuronal adaptations that result in an increased activation of the fast-twitch fibers (12,17,18,29–31,34,38). The results reveal that EMS can be an effective alternative to traditional strength training methods for enhancing maximal strength and motor abilities. Improving maximal strength and motor abilities such as sprinting and jumping in elite athletes often requires heavy loads and or high volume training to initiate further adaptations. Considering the basal weekly training volume, additional training can cause heavy stress on the muscle tendon complex that can overstress the muscular system and increase the risk of injuries. Also, in contrast to voluntary exercise, EMS artificially activates muscle contraction without resistance load. Consequently, the stress on the joints and muscle tendon complex is lower. Furthermore, studies investigating the stimulation intensity and the responds of creatine kinase activity to EMS have shown that the electrical stimulus can be more intense than voluntary stimulus (8,19,26,27,43). Jubeau et al. (25) assumes that this could be due to the different recruitment of motor units during EMS compared with voluntary contractions. Accordingly, WB-EMS training, as new/different stimulus for the muscular system, could complement or modify the common training structure and thus enable further adaptations even in highly trained athletes. WB-EMS training would also be advantageously for patients with, e.g., cardiovascular diseases to improve muscle strength as they are not capable of physical exercise such as resistance training or cardio training with higher intensity respectively load. Furthermore, WB-EMS could be used in strength training with immobilized patients.

Red blood cell deformability (EImax and EI 3 Pa) significantly increased in the players of the EG from baseline until week 14. After week 14 WB-EMS sessions were reduced to 1 session per week to maintain deformability but in contrast, a significant decrease in EImax and EI 3 Pa were observed at week 18. Accordingly, one additional WB-EMS session per week in addition to the soccer training seemed to be insufficient to produce an adequate stimulus to enhance or maintain RBC deformability in elite athletes. The TG showed no significant changes in EImax compared with baseline value until week 18. For EI 3 Pa, Pre values significantly increased in the TG from baseline to week 7 but decreased from week 7 to weeks 14 and 18, respectively. The baseline values were measured after the midseason break with the start of the season preparation. Thus, the players were inactive what might have lowered the RBC deformability at low shear stress (EI 3 Pa). With returning to the usual training intensity and volume, the EI (3 Pa) increased in both groups suggesting a positive influence of soccer training on RBC deformability. However, the development of RBC deformability throughout the study differs in the 2 groups, which indicates a positively effect of WB-EMS on RBC deformability. An acute effect of increased deformability was paralleled with increased RBC-NOS activation observed 15–30 minutes after the WB-EMS session (Post) at baseline in EG. This effect of increased RBC-NOS activation simultaneously to an increased deformability supports the findings of previous investigations (7,23,24,42,44,48). Suhr et al. (44) investigated the influence of a moderate endurance training on RBC-NOS activation and deformability. The authors observed a significant increase of RBC-NOS activation directly after a 60-minute endurance run on a treadmill and also documented significant increase in NO production and deformability.

Several studies conclude that shear stress induced by moderate physical exercise activates the PI3 Kinase/Akt Kinase pathway in RBC that in turn activates RBC-NOS by phosphorylation at the Ser^{1177} residue. The activated RBC-NOS then generates NO which is a precondition for enhancing RBC deformability (14,16,44,53). Red blood cell–nitric oxide synthase generated NO has been shown to bind to free thiol groups of RBC proteins forming S-nitrosthiols. Grau et al. identified α- and β-spectrins as most likely targets of S-nitrosylation in RBCs (23). Thereby, RBC-NOS phosphorylation and RBC deformability show a strong positive correlation (22). It is also suggested that RBC-NOS–produced NO is exported from the RBC and may play a role in vascular biology including regulation of blood flow and blood pressure (40,47,51), but this mechanism remains controversial, and from the data presented here, it rather seems that RBC-NOS–produced NO primarily regulates RBC deformability that may positively influence maximal strength. At week 7 and week 14, no further acute effect of WB-EMS on RBC-NOS activation, and consequently, no acute effect on RBC deformability (EImax) were measured Post and 24-hour Post WB-EMS. Also, comparison of Pre values for RBC-NOS activation rejected the hypothesis that successive increase in EImax and EI 3 Pa were caused by higher RBC-NOS activation as no significant difference was observed between the Pre values of RBC-$NOS^{Ser1177}$. Thus, other yet unidentified mechanisms must be responsible for chronic increased deformability on WB-EMS stimulus.

Research studies have investigated further factors such as exercise intensity, lactate acid, and fitness level that can influence blood rheology and especially modulate RBC deformability (9). It is well known that lactate production is high during training and match-play, which increases

plasma lactate concentrations. Connes et al. (11) assume that the lactate anion could increase deformability in aerobically trained athletes, whereas deformability can decrease in untrained subjects (52). Investigations by Brun et al. also support this theory showing that exercise and subsequent lactate production can decrease RBC rigidity and increase RBC deformability in highly trained athletes (cf. (9)). Several studies have shown that local EMS induces a significant increase in lactate concentrations (15,25) and also greater increase compared with voluntary exercise with same intensity (25). Furthermore, physical exercise such as strength training increases the density of the lactate monocarboxylate transporter 1 (MTC1) in the red blood cell membrane (37). In the RBCs, the MCT1 absorbs and releases lactate to avoid intracellular acidification (50). It can be assumed that WB-EMS in addition to 6–7 soccer training session per week have increased the total lactate production in the tested highly trained subjects and thus might have positively influenced RBC deformability.

Taken together, our results revealed that WB-EMS in combination with soccer training improves RBC deformability and thus oxygen supply to the muscles that might have also improved maximal strength and soccer-specific endurance parameters such as repeated sprint ability. An acute increase in RBC deformability can be explained by improved RBC-NOS activation, but further investigations are needed to investigate the mechanisms involved in the chronic increase of RBC deformability as a role of RBC-NOS seems to be excluded.

WB-EMS seems to offer an adequate training method to improve RBC deformability and thus positively influence strength adaptations in professional soccer players during competitive season. It can be also beneficial for patients who are not capable of physical exercise with high load or volume or patients with immobility to enhance strength performance, improve rheological qualities or avoid muscle atrophy.

Practical Applications

This study indicated that a WB-EMS training with 2 sessions per week improved RBC deformability. To sustain RBC deformability, 1 WB-EMS session per week was not sufficient. For practical purposes, EMS training represents a useful and promising alternative to conventional strength training possibly leading to improved blood flow to the working muscle thereby preventing or delaying acidification of the muscles. The underlying mechanism on how EMS affects RBC deformability without affecting RBC-NOS activation has to be examined and necessitate further investigation. It is here suggested that WB-EMS improves tissue oxygenation and may lead to increases in maximum oxygen uptake but studies are needed to prove this hypothesis.

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