Effect of Additional Speed Endurance Training on Performance and Muscle Adaptations

THOMAS PETURSSON GUNNARSSON, PETER MØLLER CHRISTENSEN, KRIS HOLSE, DANNY CHRISTIANSEN, and JENS BANGSBO

Department of Exercise and Sport Sciences, Section of Human Physiology, University of Copenhagen, DENMARK

ABSTRACT

GUNNARSSON, T. P., P. M. CHRISTENSEN, K. HOLSE, D. CHRISTIANSEN, and J. BANGSBO. Effect of Additional Speed Endurance Training on Performance and Muscle Adaptations. *Med. Sci. Sports Exerc.*, Vol. 44, No. 10, pp. 1942–1948, 2012. **Purpose**: The present study examined the effect of additional speed endurance training (SET) during the season on muscle adaptations and performance of trained soccer players. **Methods**: Eighteen subelite soccer players performed one session with six to nine 30-s intervals at an intensity of 90%–95% of maximal intensity (SET) a week for 5 wk (SET intervention). Before and after the SET intervention, the players carried out the Yo-Yo intermittent recovery level 2 (Yo-Yo IR2) test, a sprint test (10 and 30 m), and an agility test. In addition, seven of the players had a resting muscle biopsy specimen taken and they carried out a running protocol on a motorized treadmill before and after the SET intervention. **Results**: After the SET intervention, the Yo-Yo IR2 test ($n = 13$) performance was 11% better ($P < 0.05$), whereas sprint ($n = 15$) and agility ($n = 13$) performances were unchanged. The expression of the monocarboxylate transporter 1 ($n = 6$) was 9% higher ($P < 0.05$). and the expression of the Na^+/K^+ pump subunit β_1 ($n = 6$) was 13% lower ($P < 0.05$) after the SET intervention. The Na^+/K^+ pump subunits α_1, α_2, as well as the monocarboxylate transporter 4 and the Na^+/H^+ exchanger 1 ($n = 6$) were unchanged. After the SET intervention, the relative number of Type IIx fibers and oxygen consumption at 10 km·h^{-1} were lower ($P < 0.05$), whereas $\dot{V}O_{2max}$ was unchanged. **Conclusions**: In conclusion, adding ~30 min of SET once a week during the season for trained soccer players did lead to an improved ability to perform repeated high-intensity exercise, with a concomitant increase in the expression of monocarboxylate transporter 1 and an improved running economy. **Key Words**: MCT1, YO-YO INTERMITTENT RECOVERY TEST LEVEL 2, RUNNING ECONOMY, SOCCER

Intensified training has been shown to effectively enhance performance of trained people (11,19). Esfarjani and Laursen (9) observed in moderately trained runners that 3-km performance and maximum oxygen uptake ($\dot{V}O_{2max}$) were elevated after 10 wk of substituting two of four 60-min endurance training sessions with training at an intensity corresponding to 100% or 130% of a running speed eliciting $\dot{V}O_{2max}$. In addition, training at near-maximal intensity in 30-s exercise periods (speed endurance training [SET]) has been observed to increase short-term and repeated high-intensity exercise performance in well-trained runners despite a marked reduction in training volume (14). Combined with aerobic high-intensity training speed, endurance training also improved the long-term capacity in endurance-trained athletes (2). A few studies have focused on the effect of intensified training of soccer players (15,26). In well-trained soccer players, one aerobic high-intensity training session per week for 12 wk improved performance in the Yo-Yo intermittent recovery level 2 (Yo-Yo IR2) test by 15% (15). Moreover, Thomassen et al. (26) showed an increase in performance of repeated sprint exercise and a nonsignificant increase (6%) in the Yo-Yo IR2 test when the normal training was substituted with SET and aerobic high-intensity training for a 2-wk period after the season. However, it is unclear what happens in trained soccer players when SET is implemented in addition to the normal training during the season.

SET has been shown to induce changes in the muscular ion transport systems. Mohr et al. (22) showed an improved repeated high-intensity performance after 8 wk of SET in normally active males, which was associated with an increase in the expression of the Na^+/K^+ pump subunits α_2 and β_1 as well as a higher expression of the monocarboxylate transporter 1 (MCT1) and Na^+/H^+ exchanger (NHE1). In trained subjects, Iaia et al. (14) and Bangsbo et al. (3) found increases in the expression of the Na^+/K^+ pump subunits α_1, α_2, and β_1 after 4–9 wk of SET in endurance-trained runners, and the changes were related to an improved intense intermittent exercise capacity. Furthermore, in soccer players, Thomassen et al. (26) found a 14% increase in the amount of the α_2 subunit and a nonsignificant increase (13%) in the expression of MCT1 after 2 wk of intensified training. It

APPLIED SCIENCES

1942

is, however, not known how additional SET in soccer players affects the muscular adaptations and how it is related to performance.

SET of endurance-trained runners has been reported to cause a lowering of the oxygen uptake during submaximal running (3,14) and Christensen et al. (6) also found an improved running economy in soccer players after 2 wk of SET and aerobic high-intensity training. Maximum oxygen uptake has been shown to increase after a period with SET in some (20) but not all (3,14) studies with endurance-trained subjects. In soccer players, Jensen et al. (15) found an increase in $\dot{V}O_{2max}$ (5%) after 12 wk of additional aerobic high-intensity training during the season. However, to what extent SET during the season affects $\dot{V}O_2$ during submaximal and maximal running is not established.

Thus, the aim of the present study was to examine whether one session of SET a week during the season of trained soccer players could cause physiological adaptations and improve the ability to perform intense intermittent exercise.

METHODS

Subjects

Eighteen male soccer players from a team in the Danish Second Division with an age, height, and weight of 23.9 ± 0.1 yr, 1.84 ± 0.02 m, and 77.2 ± 1.9 kg, respectively, participated in the study. The players performed various performance tests before and after a 5-wk intervention period during the season. Seven of the players went through additional tests (see Experimental Design). Age, height, weight, and maximal oxygen uptake of these players were 23.3 ± 0.9 yr, 1.84 ± 0.02 m, 79.5 ± 1.9 kg, and 60.6 ± 1.1 mL·kg^{-1}·min^{-1}, respectively. All participants were fully informed of the experimental procedures and any discomforts associated with participating in the study before signing a written informed consent. This study conformed to the Code of Ethics of the World Medical Association (Declaration of Helsinki) and was approved by the Ethics Committee of Copenhagen and Frederiksberg communities.

Experimental Design

During the first 4 wk of the season, the players followed the normal training planned by the coach. In the following 5 wk, the players performed one weekly SET session (SET intervention). Before and after the SET intervention, the players were tested on two separate days. Seven of the players had a muscle biopsy taken and also underwent laboratory testing before and after the SET intervention.

Training

The players had, on average, 2.7 training sessions lasting 3.6 h and 1 match per week before the SET intervention, and during the SET intervention, the players had 2.8 training sessions lasting 4.0 h and 1.8 matches per week. The SET was carried out as drills with and without the ball and consisted of six to nine 30-s intervals per week at an intensity of 90%–95% of max intensity, interspersed with 3 min of rest. In the first week of the SET intervention, the players performed five 30-s intervals and one interval was added every week.

Testing

Before all testing, players refrained from severe physical activity for at least 48 h and at least 3 h after ingestion of a meal. The players performed various field tests (see below) twice: once in the week before the SET intervention and once in the week after the SET intervention. Before the testing, the players were familiarized with the testing procedures on one to three separate occasions. The players performed a 10- and 30-m sprint (SP) test and an agility (AG) test as well as the Yo-Yo IR2 test (17) on a separate day. All tests were performed on artificial grass and preceded by a thorough and standardized 15-min warm-up program.

SP test. This consisted of 3×30-m maximal sprints with at least 2 min of recovery between sprints. All sprints started from a standing position between two markers placed 30 cm in front of the starting line. Sprint time was measured with three ports of light sensors (Newtest Powertimers; Newtest Oy, Oulu, Finland) placed at 0, 10, and 30 m, respectively. The best time recorded was the test result.

AG test. This consisted of three agility runs interspersed by at least 2.5 min of recovery. The players started from a standing position between two markers placed 30 cm from the first light sensor. The test was ~31 m and consisted of a 2×5-m sprint with a 180° turn followed by four high-speed turns around four markers. The first high-speed turn was ~40° and occurred 2 m after the starting line. The second (~80°), third (~40°), and fourth (~80°) turns all occurred 5 m apart in the length of the course. The finish line was placed 2 m after the last high-speed turn. The best time recorded was used as the test result.

Yo-Yo IR2 test. The Yo-Yo IR2 test consisted of repeated 20-m shuttles at a progressively increasing speed controlled by audio bleeps from a CD player. The runs were separated by an active recovery period lasting 10 s. The second time the players failed to reach the finish line in time, the total distance covered was recorded as the test result. Because of injuries, only 13 players completed the Yo-Yo IR2 and the AG tests before and after the SET intervention, whereas 15 players completed the SP test before and after the intervention, respectively.

Seven players took part in laboratory testing before and after the SET intervention period. The players reported to the laboratory ~1 h before the testing. After 20 min of rest in the supine position, a biopsy from musculus vastus lateralis was collected through an incision made in the skin under local anesthesia (20 mg·mL^{-1} lidocaine without adrenalin), and a catheter (18-gauge, 32 mm) was placed in an antecubital vein. In addition, an HR monitor (Polar, Electro Oy,

Kempele, Finland) was placed on the chest of the player and HR was recorded in 5-s intervals. Then, the players were running for 4 min at a speed of 10 km·h^{-1} followed by 2 min of rest and for 4 min at a speed of 14 km·h^{-1} on a motorized treadmill. After 3 min of rest, the players performed an incremental test starting at 14 km·h^{-1} for 3 min followed by an increase in running speed of 1 km·h^{-1}·min^{-1} until volitional fatigue. $\dot{V}O_2$ was measured throughout the whole protocol by a breath-by-breath gas-analyzing system (Oxycon Pro; VIASYS Healthcare, Hoechberg, Germany) that was calibrated before each test. $\dot{V}O_{2max}$ was determined as the highest value achieved during a 30-s period. A plateau in $\dot{V}O_2$ despite an increased running speed combined with an RER > 1.15 were used as criteria for achievement of $\dot{V}O_{2max}$. Blood samples were collected in heparinized 2-mL syringes before and immediately after each of the running bouts as well as 3 and 5 min after the incremental test.

Muscle Analysis

A part of the muscle sample was immediately frozen in liquid N_2 and stored at $-80°C$. The frozen muscle tissue samples were weighed before and after freeze-drying to determine the water content. After freeze-drying the samples, connective tissue, visible fat, and blood were carefully dissected away. Dissecting was done under a stereomicroscope in a room with a temperature of $\sim18°C$ and a relative humidity below 30%. Another part of the biopsy was mounted in an embedded medium (OCT Compound Tissue-Tek; Sakura Finetek, Zoeterwoude, The Netherlands) and frozen in isopentane cooled to the freezing point in liquid N_2. These samples were stored at $-80°C$ until analyzed for fiber type distribution and capillary density by histochemical analysis.

Muscle in Transport Proteins

Muscle samples taken at rest (~4–5 mg dry weight) was homogenized on ice in a fresh batch of buffer (10% glycerol, 20 mM Na-pyrophosphate, 150 mM NaCl, 50 mM HEPES, 1% Nonidet P-40, 20 mM β-glycerophosphate, 10 mM NaF, 2 mM phenylmethanesulfonyl fluoride, 1 mM each of EDTA and EGTA, 10 μg·mL^{-1} each of aprotinin and leupeptin, and 3 mM benzamidine) with a Polytron 3100 (Kinematica, Littau-Lucerne, Switzerland) for not more than 30 s. After rotation end over end for ~1 h, the samples were centrifuged for 30 min at 17,500g at 4°C, and lysates were collected as the supernatant. Protein concentrations were determined in the lysates using bovine serum albumin (BSA) standards (Pierce Biotechnology, Inc., Rockford, IL). The lysates were diluted to appropriate protein concentrations in a 6× sample buffer (0.5 M Tris base, dithiothreitol, SDS, glycerol, and bromophenol blue), and equal amounts of total protein (5–15 μg in accordance with the antibody optimization) were loaded for each sample in different wells on 10% precasted Tris–HCl gels (Bio-Rad Laboratories, Hercules, CA). For comparisons, samples from the same subject were always loaded on the same gel. The gel electrophoresis ran

for ~80–100 min with 55 mA and a maximum of 150 V per gel. Afterward, proteins were blotted to a polyvinylidene difluoride membrane using 70 mA and a maximum of 25 V per gel in 2 h. The membranes were incubated overnight with ~10 mL of primary antibody diluted in either 2% nonfat milk (monoclonal Na$^+$/K$^+$ pump α_1-subunit [~100 kDa], 1:500 dilution [C464.6, no. 05-369; Millipore]; polyclonal α_2-subunit [~100 kDa], 1:500 dilution [no. 07-674; Millipore Corporation, Billerica, MA]; and monoclonal β_1-subunit [~50 kDa], 1:1000 dilution [MA3-930; Affinity BioReagents, Golden, CO]) or 3% BSA (monoclonal NHE1 [~100 kDa], 1:500 dilution; polyclonal MCT1 [~50 kDa], 1:1000 dilution; and polyclonal monocarboxylate transporter 4 [MCT4, 50 kDa], 1:1000 dilution [MAB3140, AB3538P, and AB3316P; Millipore]). After being washed briefly in a Tris-buffered saline, Tween, membranes were incubated with secondary antibody for ~1 hat room temperature. The secondary horseradish peroxidase–conjugated antibodies used were diluted 1:5000 in 2% nonfat milk or 3% BSA depending on the primary antibody (P-0447, P-0448, and P-0449; DakoCytomation, Glostrup, Denmark). Membrane staining was visualized by incubation with a chemiluminescent horseradish peroxidase substrate (Millipore) immediately before the image was digitalized (Image Station 2000MM; KODAK, Rochester, NY). The net band intensities were quantified as the total intensity minus the background intensity (Molecular Imaging Software; KODAK).

Data Treatment

Double determinations were made for the muscle samples, i.e., the biopsies were divided and kept in two parts before freeze-drying, resulting in two results for the same time point. The mean signal intensity of the two samples was used as the result for the individual time point. The intensity of the individual time points was converted into ratios (post/pre), and each ratio was logarithm transformed ($\log(x)$) to evenly distribute the data points. Data points for all subjects were averaged and calculated as geometric means ($10^{\log(x)}$) ± 95% confidence intervals. The mean values from the two separate analyses were used for statistical examination.

Muscle Enzymes

About 2 mg of dry weight was homogenized (1:400) in a 0.3 M phosphate BSA buffer adjusted to pH 7.7, and phosphofructokinase (PFK), hydroxyacyl-CoA dehydrogenase (HAD), and citrate synthase (CS) muscle enzyme activity were determined fluorometrically as described by Lowry and Passonneau (21).

Muscle Fiber Type Distribution and Capillarization

Muscle samples used for fiber type distribution were cut in five 10-μm-thick serial transverse sections at $-20°C$, preincubated at pH 4.3, 4.6, and 10.3 and afterward incubated for myofibrillar adenosine triphosphate reactions at

pH 9.4. The fiber type distribution included six of the seven subjects because one biopsy was too small to analyze (<75 fibers). The average number of fibers in the analysis was 189 ± 12 fibers (ranging from 102 to 211). Staining of the capillaries was performed on 8-μm-thick transverse sections of the muscle sample, cut on the same day as the muscle samples for fiber type distribution. The transverse sections were fixed for 2 min at room temperature in 2% formaldehyde and for 30 s in acetone at −20°C. Fiber type distribution and number of capillaries were determined under light microscopy, and individual fibers were classified under light microscopy as being Type I, Type IIA, or Type IIB fibers (4) based on the myofibrillar ATP staining. Photos of the images were analyzed on a computer using the software program Tema95 (version 1.04; CheckVision ApS, Hadsund, Denmark).

Blood Analysis

Blood samples were drawn in 2-mL heparinized syringes, and after sampling, a part of the blood ($\sim$1.5 mL) was rapidly centrifuged at 20,000g for 30 s and the rest (0.5–1.0 mL) was stored on ice. After centrifugation, the plasma was pipetted into Eppendorf tubes and placed in ice-cold water until they were stored at −20°C. Plasma samples were subsequently analyzed for K^+ by an ion-selective electrode using a Hitachi 912 (Hitachi 912 Automatic Analyzer; Roche Diagnostic, Indianapolis, IN). The whole blood was analyzed for lactate on an ABL 800 (ABL 800 Flex; Radiometer, Copenhagen, Denmark).

Statistical Analysis

Changes in performance (Yo-Yo IR2, AG, and SP tests) were evaluated using a Student's paired t-test (pre vs post). Furthermore, changes in muscle membrane proteins, enzyme activity, and capillarization were evaluated using a Student's paired t-test. Changes in blood lactate and plasma K^+ were evaluated using a two-way ANOVA on repeated measures, with blood sampling time and time of test (pre vs post) as the two factors.

RESULTS

Performance. After the SET intervention, Yo-Yo IR2 test performance was elevated ($P < 0.05$) by 11% (Table 1).

TABLE 1. Yo-Yo IR2 test level 2 ($n = 13$), 30-m and 10-m SP test ($n = 15$), as well as AG tests ($n = 13$) performance in trained soccer players before (Pre) and after (Post) a 5-wk SET intervention.

	Pre	Post
Yo-Yo IR2 (m)	778 ± 65	862 ± 63*
AG test (s)	7.16 ± 0.04	7.15 ± 0.04
30-m SP test (s)	4.32 ± 0.03	4.31 ± 0.03
10-m SP test (s)	1.78 ± 0.02	1.77 ± 0.02

Data are presented as means ± SEM.
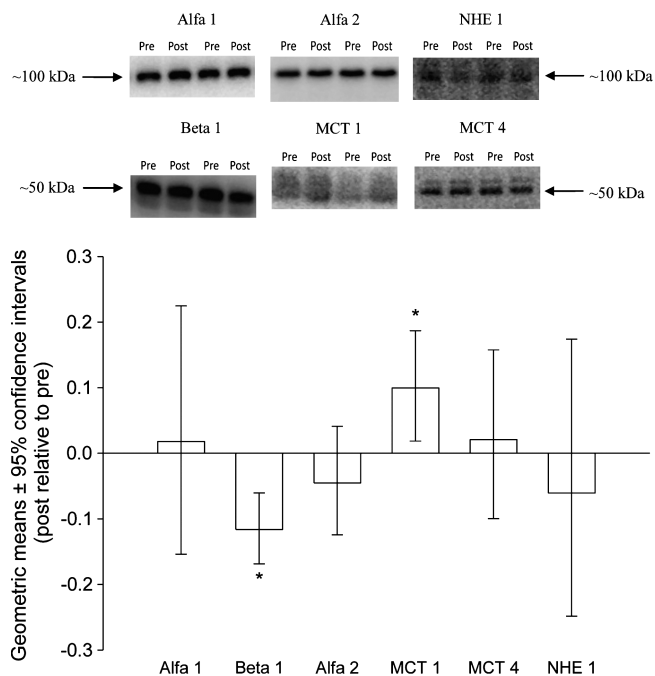* Different from Pre ($P < 0.05$).

FIGURE 1—Changes in muscle–ion transport protein expression in trained soccer players before (Pre) and after (Post) a 5-wk SET intervention. Representative blots are shown for each of the muscle–ion transport proteins. Values are geometric means ± 95% confidence intervals. *Different ($P < 0.05$) from Pre intensity.

Performance during the SP and AG tests, as well as performance during the incremental test to exhaustion (8.4 ± 0.3 vs 8.4 ± 0.3 min), was unchanged (Table 1).

Muscular adaptations. MCT1 expression ($n = 6$) was elevated ($P < 0.05$) by 9% after the SET intervention, with no change in the expression of MCT4 and NHE1 ($n = 6$; Fig. 1). The amount of the Na^+/K^+ pump subunit β_1 ($n = 6$) was lowered ($P < 0.05$) by 13%, whereas no change was observed for the α_1 ($n = 6$) and α_2 ($n = 6$) subunits. The relative number of Type I, Type IIa, and Type IIx ($n = 7$) before the SET intervention was 59% ± 6%, 29% ± 5%, and 12% ± 3%, respectively, and 58% ± 7%, 35% ± 7%, and 6% ± 1% after, with the number of Type IIx fibers being lower ($P < 0.05$) after the SET intervention. Capillary density ($n = 7$) after the SET intervention expressed as number per fiber (CFR), number around each fiber (CAF), and number per fiber area (μm^2) was nonsignificantly (<0.1) higher than before the SET intervention, with values being 3.0 ± 0.1 versus 2.7 ± 0.2, 5.7 ± 0.2 versus 5.3 ± 0.3, and 561 ± 34 versus 495 ± 45 before and after the SET intervention, respectively. Furthermore, PFK, CS, and HAD activity were not changed by the SET intervention ($n = 7$; Fig. 2).

Pulmonary $\dot{V}O_2$. $\dot{V}O_{2max}$ was unchanged by the SET intervention (60.5 ± 1.0 vs 61.2 ± 2.6 mL·kg^{-1}·min^{-1}). Oxygen uptake ($n = 7$) at 10 km·h^{-1} was lowered ($P < 0.05$) by 6% (35.9 ± 0.9 vs 33.8 ± 0.9 mL·kg^{-1}·min^{-1}), and it tended ($P < 0.1$) to be lower at 14 km·h^{-1} (47.5 ± 0.7 vs 46.1 ± 1.0 mL·kg^{-1}·min^{-1}).
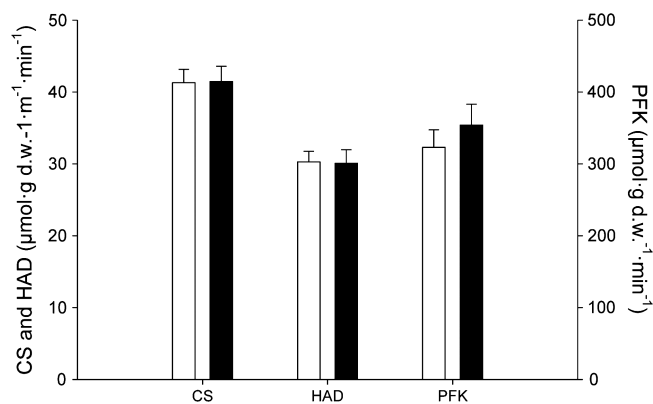
FIGURE 2—Changes in HAD, CS, and PFK enzyme activity in trained soccer players before (Pre) and after (Post) a 5-wk SET intervention. Values are means ± SEM.

Blood and plasma variables. Plasma K^+ ($n = 6$) levels during submaximal running (10 and 14 km·h^{-1}) and at exhaustion were not different after compared with before the SET intervention. Furthermore, no changes in blood lactate and pH were observed ($n = 7$; Table 2).

DISCUSSION

The major findings of the present study were that by adding ~3 min of SET a week for 5 wk during the season, trained soccer players improved their ability to perform repeated high-intensity exercise, had a lower oxygen uptake during submaximal running, and had an elevated expression of MCT1.

Yo-Yo IR2 test performance was 778 ± 65 m before the SET period, which is similar to values for other Danish Second Division players (771 ± 26 m), and it increased to 862 ± 63 m after the SET period. Thus, by just adding one weekly session of SET, i.e., ~3 min of effective working time, during the season, the ability to perform repeated high-intensity exercise was increased in trained soccer players. Accordingly, Thomassen et al. (26) found in trained soccer players that the sprint time in a repeated SP (10 × 20 m with 25 s of recovery) was lowered after only 2 wk of additional SET. The SET was similar to the one used in the present study, but the number of SET bouts performed per week in the study of Thomassen et al. (26) was higher (22.5 ± 1.2 vs 6.3 ± 0.7) than that in the present study. Moreover, in the study by Thomassen et al. (26), additional aerobic high-intensity training was performed, and the training volume was significantly reduced. In both recreationally active subjects (22) and endurance-trained runners (3,14), SET has been shown to improve the repeated short-term high-intensity exercise capacity as well as the short-term and long-term endurance capacity. Apparently, SET is effective in elevating the capacity to perform repeated intense exercises in moderately trained and even well-trained subjects who are used to perform high-intensity intermittent exercises.

Performances in the 10- and 30-m SP and the AG tests were unchanged, suggesting that the improvement in the Yo-Yo IR2 test performance was not due to changes in the ability to accelerate, perform high-speed turns, or sprint. The oxygen uptake during submaximal running was lowered after the SET period, which was also observed by Christensen et al. (6) after 2 wk of SET and aerobic high-intensity training in soccer players. Furthermore, a better running economy has been found in endurance-trained runners after a period of SET (3,12), and Iaia et al. (12) observed that the lowered oxygen uptake was associated with an increase in Yo-Yo IR2 test performance. Running economy is an important component of performance in an endurance event (7,23,25); it is, however, unclear whether the better running economy in the present study may have contributed to the improved performance during the repeated intense exercise carried out during the Yo-Yo IR2 test.

Krustrup et al. (18) have reported that HR at the end of the Yo-Yo IR2 test is not different from the peak HR obtained during exhaustive treadmill running, suggesting that the aerobic loading is almost maximal toward the end of the test. However, the maximum oxygen uptake was unaltered after the SET intervention period and cannot explain the improved performance. Muscle lactate accumulation has been shown to be high during the Yo-Yo IR2 test (18), and muscle pH was probably significantly lowered at the end of the test, which may have contributed to the development of fatigue. Therefore, the improved performance after the SET intervention may be related to changes in the transport of H^+ in the muscles. MCT1 expression was elevated by 9% after the SET intervention. A higher MCT1 expression has also been reported in untrained subjects after a period of endurance (8) and high-intensity (16,22) training. In addition, a study of trained soccer players found a nonsignificant increase (13%) in MCT1 expression after only 2 wk of SET and aerobic high-intensity training after the season (26). In contrast, no change in MCT1 expression was observed after a period of intensified training (4–9 wk) in endurance-trained runners (3,14). The difference may be related to the training history of the athletes because endurance training is known to elevate the amount of MCT1 and MCT4 (24), and it is likely that the endurance-trained runners already

TABLE 2. Plasma K^+ and blood lactate (mmol·L^{-1}) at rest, during running at 10 and 14 km·h^{-1}, as well as before (Pre) and immediately (0), 3, and 5 min after (Post) an exhaustive incremental test in trained soccer players before (Pre) and after (Post) a 5-wk SET intervention.

	Plasma K^+ (mmol·L^{-1}), $n = 6$		Blood Lactate (mmol·L^{-1}), $n = 7$	
	Pre	**Post**	**Pre**	**Post**
Rest	3.6 ± 0.1	3.8 ± 0.1	1.4 ± 0.1	1.2 ± 0.1
10 km·h^{-1}	4.3 ± 0.1	4.2 ± 0.1	1.7 ± 0.1	1.7 ± 0.1
14 km·h^{-1}	4.4 ± 0.1	4.5 ± 0.0	3.1 ± 0.5	3.1 ± 0.4
Pre	4.0 ± 0.1	4.0 ± 0.1	2.7 ± 0.4	2.8 ± 0.5
Post − 0	4.8 ± 0.2	4.9 ± 0.1	13.6 ± 1.1	13.1 ± 0.7
Post − 3	3.8 ± 0.3	3.6 ± 0.2	13.4 ± 0.9	12.6 ± 0.8
Post − 5	3.4 ± 0.1	3.4 ± 0.2	12.7 ± 1.0	12.0 ± 0.9

Data are presented as means ± SEM.

APPLIED SCIENCES

had a high expression of these proteins before the start of the training intervention. Nevertheless, SET seems to be a potent stimuli for increasing the expression of MCT1 in already trained soccer players, and the higher expression of MCT1 in the present study may have slowed the intracellular accumulation of H^+ and thereby delayed fatigue (2,16,24). It should be mentioned, however, that the importance of lowered muscle pH in fatigue development during high-intensity exercise has been questioned (1,2,5).

Cairns and Lindinger (5) suggest that a decrease of the sarcolemmal K^+ gradient is the dominant cellular process that contributes to fatigue during high-intensity exercise (5–20 min). In the present study, the expression of the Na^+/K^+ pump subunit β_1 decreased by $\sim13\%$, whereas the α_1 and α_2 subunits were unaltered. These findings are in contrast to other studies showing increases in the Na^+/K^+ pump subunits α_1, α_2, or β_1 when implementing high-intensity training for a duration of 4–10 wk (3,14,22) or intense submaximal training for 6 d (10). The difference may be related to various populations studied. As a consequence of the intense intermittent nature of soccer, the soccer players in the present study may have had a high expression of Na^+/K^+ pump subunits at the start of the intervention period, which may have reduced the effect of SET. However, the underlying mechanisms by which change in the expression of Na^+/K^+ pump subunits after high-intensity training occur are not well understood. Nevertheless, performance was enhanced after the SET intervention and it is not likely to have been caused by the changes in the expression of the Na^+/K^+ pump subunits, and the observations rather suggest that the lowered β_1 subunit expression is not of critical importance for the pump activity during exercise. Although it is a weak measure of muscle membrane transport of K^+, it is supported by the observation that plasma K^+ during the exhaustive treadmill running was not changed.

After the SET intervention, the amount of Type IIx fibers was reduced ($P < 0.05$) by 50% (from 12% to 6%). This observation is in contrast to previous findings of no change in trained soccer players (6) or increase in endurance-trained runners (12) after 2–4 wk of SET. The diverging results may be related to the reduced training volume in the latter studies, with a reduction in total training volume of $\sim64\%$ in Iaia et al. (12). Nevertheless, performance during high-intensity intermittent exercise increased in all three studies, suggesting that the distribution of Type IIx fibers is not of critical importance for the high-intensity intermittent exercise capacity. The capillarization tended ($P < 0.1$) to be higher ($\sim10\%$), which may have contributed to the increased performance during the Yo-Yo IR2 test, as suggested by Iaia et al. (13).

In the present study, it was not possible to include a proper control group because the coach would not allow a separation of the squad. It is, however, not likely that a control group would have changed performance level during the 5-wk intervention period. In support, Krustrup et al. (18) showed that Yo-Yo IR2 test performance in elite soccer players was unchanged during the season.

In summary, addition of one weekly SET session for trained soccer players during the season elevated Yo-Yo IR2 test performance, with a concomitant increase in MCT1 expression and a reduced oxygen uptake during submaximal running. In addition, the expression of the Na^+/K^+ pump subunit β_1 decreased by 13%, which apparently had no negative effect on the repeated high-intensity exercise capacity.

REFERENCES

1. Allen DG, Lamb GD, Westerblad H. Skeletal muscle fatigue: cellular mechanisms. *Physiol Rev*. 2008;88(1):287–332.
2. Bangsbo J, Juel C. Counterpoint: lactic acid accumulation is a disadvantage during muscle activity. *J Appl Physiol*. 2006;100(4):1412–3.
3. Bangsbo J, Gunnarsson TP, Wendell J, Nybo L, Thomassen M. Reduced volume and increased training intensity elevate muscle Na^+-K- pump alpha2-subunit expression as well as short- and long-term work capacity in humans. *J Appl Physiol*. 2009;107(6):1771–80.
4. Brooke MH, Kaiser KK. Three "myosin adenosine triphosphatase" systems: the nature of their pH lability and sulfhydryl dependence. *J Histochem Cytochem*. 1970;18(9):670–2.
5. Cairns SP, Lindinger MI. Do multiple ionic interactions contribute to skeletal muscle fatigue? *J Physiol*. 2008;586(Pt 17):4039–54.
6. Christensen PM, Krustrup P, Gunnarsson TP, Kiilerich K, Nybo L, Bangsbo J. $\dot{V}O_2$ kinetics and performance in soccer players after intense training and inactivity. *Med Sci Sports Exerc*. 2011;43(9):1716–24.
7. Conley DL, Krahenbuhl GS. Running economy and distance running performance of highly trained athletes. *Med Sci Sports Exerc*. 1980;12(5):357–60.
8. Dubouchaud H, Butterfield GE, Wolfel EE, Bergman BC, Brooks GA. Endurance training, expression, and physiology of LDH, MCT1, and MCT4 in human skeletal muscle. *Am J Physiol Endocrinol Metab*. 2000;278(4):E571–9.
9. Esfarjani F, Laursen PB. Manipulating high-intensity interval training: effects on $\dot{V}O_{2max}$, the lactate threshold and 3000 m running performance in moderately trained males. *J Sci Med Sport*. 2007;10(1):27–35.
10. Green HJ, Barr DJ, Fowles JR, Sandiford SD, Ouyang J. Malleability of human skeletal muscle Na(+)–K(+)-ATPase pump with short-term training. *J Appl Physiol*. 2004;97(1):143–8.
11. Iaia FM, Bangsbo J. Speed endurance training is a powerful stimulus for physiological adaptations and performance improvements of athletes. *Scand J Med Sci Sports*. 2010;20(2 suppl):11–23.
12. Iaia FM, Hellsten Y, Nielsen JJ, Fernstrom M, Sahlin K, Bangsbo J. Four weeks of speed endurance training reduces energy expenditure

during exercise and maintains muscle oxidative capacity despite a reduction in training volume. *J Appl Physiol*. 2009;106(1):73–80.

13. Iaia FM, Perez-Gomez J, Thomassen M, Nordsborg NB, Hellsten Y, Bangsbo J. Relationship between performance at different exercise intensities and skeletal muscle characteristics. *J Appl Physiol*. 2011; 110(6):1555–63.

14. Iaia FM, Thomassen M, Kolding H, et al. Reduced volume but increased training intensity elevates muscle Na$^+$–K$^+$ pump alpha1-subunit and NHE1 expression as well as short-term work capacity in humans. *Am J Physiol Regul Integr Comp Physiol*. 2008;294(3):R966–74.

15. Jensen JM, Randers MB, Krustrup P, Bangsbo J. Intermittent high-intensity drills improves in seasonal performance of elite football players. In: Reilly T, Korkuzus F, eds. *Science and Football VI: The Proceedings of the Sixth World Congress on Science and Football*. London (UK): Routledge; 2009. p. 296–301.

16. Juel C, Klarskov C, Nielsen JJ, Krustrup P, Mohr M, Bangsbo J. Effect of high-intensity intermittent training on lactate and H$^+$ release from human skeletal muscle. *Am J Physiol Endocrinol Metab*. 2004;286(2):E245–51.

17. Krustrup P, Mohr M, Amstrup T, et al. The Yo-Yo Intermittent Recovery Test: physiological response, reliability, and validity. *Med Sci Sports Exerc*. 2003;35(4):697–705.

18. Krustrup P, Mohr M, Nybo L, Jensen JM, Nielsen JJ, Bangsbo J. The Yo-Yo IR2 test: physiological response, reliability, and application to elite soccer. *Med Sci Sports Exerc*. 2006;38(9):1666–73.

19. Laursen PB. Training for intense exercise performance: high-intensity or high-volume training? *Scand J Med Sci Sports*. 2010; 20(2 suppl):1–10.

20. Laursen PB, Shing CM, Peake JM, Coombes JS, Jenkins DG. Interval training program optimization in highly trained endurance cyclists. *Med Sci Sports Exerc*. 2002;34(11):1801–7.

21. Lowry OH, Passonneau JV. *A Flexible System of Enzymatic Analysis*. New York (NY): Academic Press; 1972. p. 237–49.

22. Mohr M, Krustrup P, Nielsen JJ, et al. Effect of two different intense training regimens on skeletal muscle ion transport proteins and fatigue development. *Am J Physiol Regul Integr Comp Physiol*. 2007;292(4):R1594–602.

23. Morgan DW, Craib M. Physiological aspects of running economy. *Med Sci Sports Exerc*. 1992;24(4):456–61.

24. Pilegaard H, Terzis G, Halestrap A, Juel C. Distribution of the lactate/H$^+$ transporter isoforms MCT1 and MCT4 in human skeletal muscle. *Am J Physiol*. 1999;276(5 Pt 1):E843–8.

25. Saunders PU, Pyne DB, Telford RD, Hawley JA. Factors affecting running economy in trained distance runners. *Sports Med*. 2004; 34(7):465–85.

26. Thomassen M, Christensen PM, Gunnarsson TP, Nybo L, Bangsbo J. Effect of 2-wk intensified training and inactivity on muscle Na$^+$–K$^+$ pump expression, phospholemman (FXYD1) phosphorylation, and performance in soccer players. *J Appl Physiol*. 2010;108(4):898–905.

APPLIED SCIENCES