

lactate formation and acidifies more cytoplasm than VC leading to early fatigue (1,10,23,26). Because metabolic alterations are essential for muscle adaptation in endurance training (5,7), the additional application of EMS during endurance exercise might lead to greater adaptations and greater improvements of performance when compared with traditional endurance training. Based on previous studies, adding EMS to endurance training may lead to higher local stimuli on the skeletal muscle with reduced cardiovascular stress when compared with normal endurance exercise. To the best of our knowledge, there have been no studies to date that were designed to examine the cardiorespiratory, metabolic, and perceived effects of local superimposed EMS during cycling. However, before a new training stimulus is used, it should be described as clearly as possible (25). Therefore, the aim of this study was to quantify the systemic metabolic changes because of additional EMS during cycling at different intensities and the impact on skeletal muscle. According to the larger number of additionally recruited type 2 fibers and motor units (MUs) because of EMS, we hypothesized greater blood lactate levels, larger alterations in the acid-base balance, and a higher impact on skeletal muscle.

METHODS

Experimental Approach to the Problem

To test the hypothesis and the influence of additional EMS during cycling on metabolism and acid-base balance at different exercise intensities, the subjects performed 3 incremental step tests on a cycle ergometer (Schoberer Rad Meßtechnik SRM GmbH, Juelich, Germany): 1 without EMS and 2 tests with EMS (30 and 85 Hz) on 3 separate days in a randomly chosen order. All the tests were carried out at the same time of the day. Each test was separated by at least 5 days.

Subjects

Ten healthy, nonsmoking physical education students (mean \pm SD, age: 24.6 ± 3.2 years, weight: 77.1 ± 7.4 kg, height: 182.1 ± 6.4 cm, relative $\dot{V}O_{2\max}$: 54.1 ± 6.0 ml·min⁻¹·kg⁻¹) volunteered and gave written informed consent to participate in this study. The study protocol was performed in accordance with the declaration of Helsinki and the Ethical Committee of the university. The tested athletes were either soccer or handball players with at least 8 years of training experiences. At the time of testing, all the athletes were accustomed to approximately 3 endurance and 2 strength training sessions per week. All the subjects were tested during the preparation period. Before the tests, the subjects were fully familiarized with the laboratory exercise procedures and with EMS. All the participants were inexperienced in EMS training.

Procedures

On the test days, the subjects ingested 0.5 mL of water 1 hour before each test to assure a well-hydrated status and to provide a good conductance of the skin where the electrodes

were placed. A light meal was allowed 2 hours before the test. The food intake was partially standardized. That is, certain foods containing carbohydrates were recommended to the subjects, and the subjects were advised to ingest the same amount and composition of food before each test. Therefore, food intake was recorded before the first test and reproduced before the following. The subjects were not allowed to perform strenuous exercise 24 hours before testing.

In each session, the protocol consisted of cycling at 80 rpm with an initial workload of 100 W for 5 minutes and incremental 40-W increases every 5 minutes until volitional exhaustion was reached. For the 2 interventions with EMS (belt) electrodes were placed all around the thigh (44×4 cm) and the calf (27×4 cm) stimulating the major muscles of the thigh and the calf. Two electrodes (13×10 cm) were placed at the fundament. The settings of the EMS device (miha bodytec, Emersacker, Germany) are described in Table 1. The only difference between both trials with EMS was the frequency, set to 30 or 85 Hz, respectively. All other settings, including the intensity, were kept constant. The intensity was set individually for each subject at the maximum tolerated intensity according to each athlete's discomfort threshold and at an intensity at which proper pedaling was still possible.

Oxygen uptake and minute ventilation were measured with an open circuit breath-by-breath spiograph (nSpire, ZAN600USB, Oberthulba, Germany) throughout the testing, using standard algorithms with a dynamic account for the time delay between the gas consumption and volume signal. The spiograph was calibrated before each test using calibration gas (15.8% O₂, 5% O₂ in N; Praxair Deutschland GmbH, Duesseldorf, Germany) that comprised the range of anticipated fractional gas concentrations. A 1-L syringe (nSpire, Germany) was used for volume calibration. The heart rate was recorded in real time every 5 seconds during the tests using short-range telemetry (Polar S710, Polar Electro GmbH, Büttelborn, Germany). All respiratory and heart rate (HR) data were averaged every 30 seconds. A blood sample of 20 μ L from the ear lobe was collected at the end of 5-minute intervals into a capillary tube (Eppendorf,

TABLE 1. Settings of the EMS device.*

Training stimuli	Settings
Impulse frequency	30 Hz/85 Hz
Impulse type	Bipolar
Impulse width	400 μ s
Impulse rise	1 s
Off-time	5 s
On-time	10 s
Duty cycle	66.6%

*EMS = electromyostimulation.

Germany) and analyzed amperometric enzymatically for the blood lactate concentration using Ebio Plus (EBIOplus; EKF Diagnostic Sales, Magdeburg, Germany). At the same time points, another blood sample of 115 μL was collected and analyzed for blood gas parameters (pH, base excess [BE], H^+ , HCO_3^- , PO_2 , PCO_2) (AVL Omni 6; Roche Diagnostics GmbH, Mannheim, Germany). Additionally, the subjects were asked to rate their perceived exertion (Borg's scale 6–20) at the end of each step.

To assess the muscular demand of each trial, creatine kinase levels were measured before and 24 hours after each test. Therefore, 500 μL of blood was collected from the earlobe. After storage at 7°C for approximately 30 minutes for deactivation of coagulation factors, blood samples were centrifuged for 10 minutes at 1,861g and 4°C (Rotixa 50, Hettich Zentrifugen, Mühlheim, Germany). Afterward, the serum was stored at –80°C until analysis. Serum levels of CK (units per liter) were determined by using Autoanalyzer COBAS 400 (Roche).

Furthermore, saliva cortisol levels were measured before, directly after and 30 minutes after each test. Therefore, 1,000 μl of saliva were collected and stored at –80°C until analysis. Saliva levels of cortisol (nanograms per milliliter) were determined by using enzyme-linked immunosorbent assay kits (DRG SLV-2930, DRG Instruments GmbH, Marburg, Germany). The CK and cortisol samples were both analyzed in duplicate, and the mean was used for statistical analysis. The coefficient of variation for CK measurement is ≤ 1.9 and $\leq 2.65\%$ for cortisol, respectively.

Before and directly after exercise, a scale was used to assess a person's perceived physical state (PEPS) (16). The subjects had to rate their state of activity, training, flexibility, health, recovery, and their willingness to perform.

Statistical Analyses

Statistical analyses of the data were performed by using a statistics software package (Statistica for Windows, 7.0, Statsoft, Tulsa, OK, USA). Descriptive statistics of the data are presented as mean \pm SD. To test the hypothesis, if

additional EMS leads to stronger metabolic alterations, we used repeated-measures analysis of variance with Bonferroni post hoc test for the comparison of the 3 different interventions. For the comparison of CK values (pre vs. post and Δ pre-24 hours of the 3 interventions), a paired *t* test was used. Statistical differences were considered to be significant for $p < 0.05$. The effect size Cohen's *d* (defined as [difference between the means]/SD) was calculated for the comparison of CK pre vs. 24 hours values of each intervention (6). The thresholds for small, moderate, and large effects were defined as 0.20, 0.50, and 0.80, respectively (6), and the effect sizes are documented in Table 2. Measurement of the power output on 2 different days before the study revealed a technical error (%TEM) of 1.2%. The TEM for oxygen uptake, ratings of perceived exertion, and time to exhaustion was 2.8, 4.2, and 4.8%, respectively. Under our laboratory conditions, the coefficient of variation for repeated measurements of blood lactate concentration is routinely 1.2% at 12 mmol·L⁻¹. For PCO_2 , pH, BE, HCO_3^- , the corresponding coefficient of variation is 3.2%.

RESULTS

Because not all the subjects performed the same maximal intensity during the step test, data are presented in dependence of the percentage peak power output (PPO).

Rate of perceived exertion was significantly higher at 100% PPO, and total exercise time was significantly reduced when EMS was applied (without: 29.2 \pm 4.2 minutes; 30 Hz: 27.2 \pm 4.4 minutes; 85 Hz: 26.7 \pm 4.3 minutes).

Lactate concentrations were significantly higher at 75 and 100% PPO, respiratory exchange ratio (RER) at 100% PPO, respectively, with additional EMS (Figure 1A, E). Significantly lower BE values and bicarbonate concentrations (HCO_3^-) were found at 75 and 100% PPO, whereas pH showed no significant differences between the 3 trials (Figure 1B–D). PCO_2 was significantly lower at 75 and 100% PPO (Figure 1F), whereas PO_2 showed no significant differences.

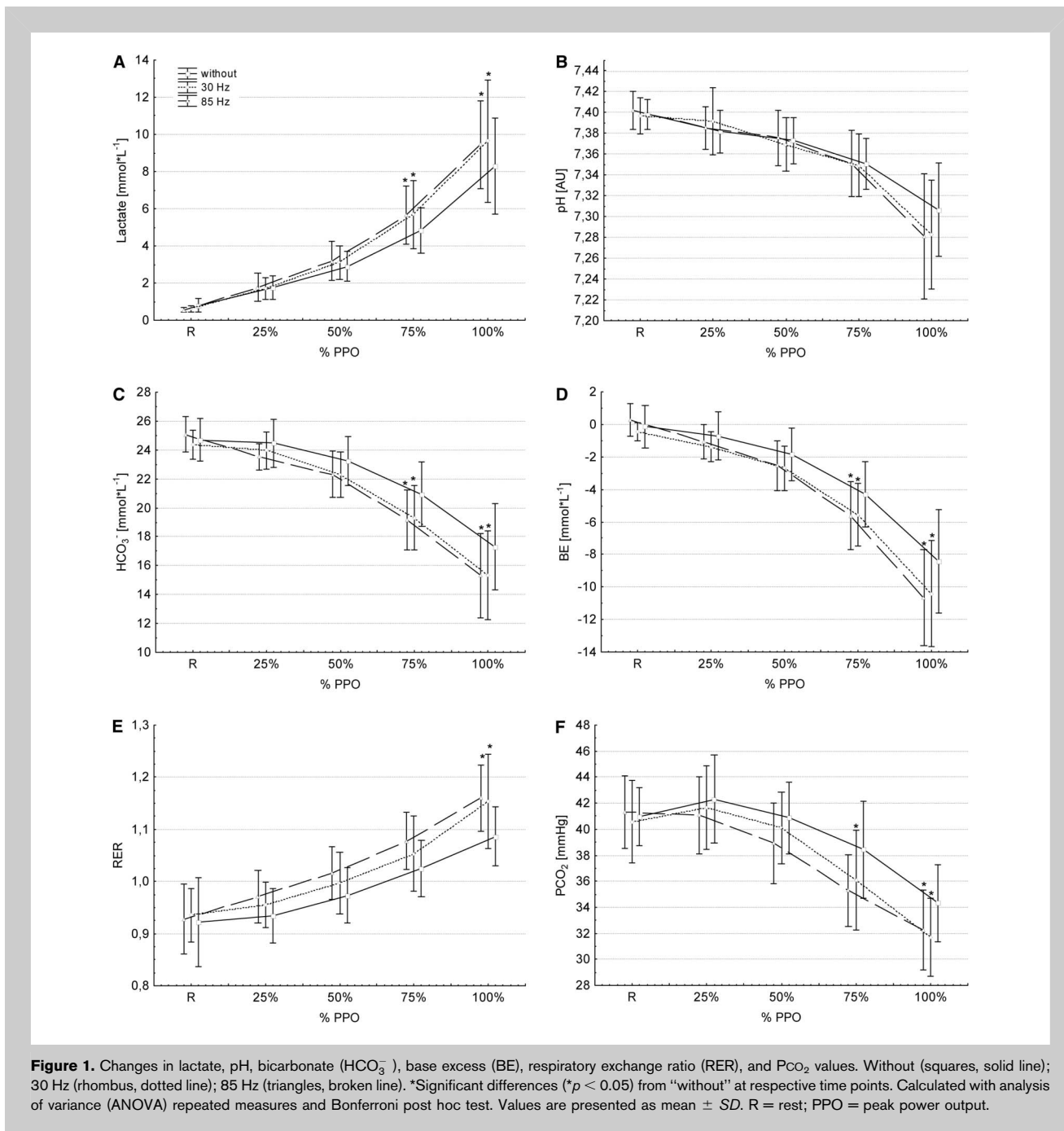
TABLE 2. Saliva cortisol and creatine kinase levels.

	Cortisol (ng·mL ⁻¹)			Creatine kinase (U·L ⁻¹)			
	Pre	0'	30'	Pre	24 h	Δ (Pre-24 h)	<i>d</i>
Without	5.2 \pm 1.4	5.8 \pm 1.9	12.7 \pm 9.3*	364 \pm 429	291 \pm 266	-73 \pm 171	0.2
30 Hz	5.3 \pm 3.5	6.3 \pm 2.1	8.5 \pm 3.0	672 \pm 793	1,213 \pm 1,405	542 \pm 1492	0.47
85 Hz	5.9 \pm 4.2	5.9 \pm 2.7	8.9 \pm 3.2*	308 \pm 317	623 \pm 490†	297 \pm 379‡	0.76

*Significantly different from prevalue and 0' values of the same intervention.

†Significantly different from "pre" of the same intervention.

‡Significantly different from "without." Effect size Cohen's *d* was calculated for the comparison of "pre" and "24 hours" post-creatine kinase values of the same intervention.



The HR at all levels was unaffected by the application of EMS as compared with that without EMS.

Saliva cortisol levels increased because of the exercise and were significantly higher 30 minutes postexercise compared with pre and 0-minute postexercise values. No significant differences were found between the trials (Table 2).

The CK levels increased significantly after the intervention with 85 Hz only. No significant changes were found for the

2 other trials. However, some subjects showed major increases as well when 30 Hz was superimposed, with increments of up to $+3,489 \text{ U}\cdot\text{L}^{-1}$. The calculation of Cohen’s effect size showed small effects for “without” (0.2) and 30 Hz (0.47) and moderate effects (0.76) for 85 Hz of EMS on CK levels (Table 2).

The comparison of absolute increases (Δ pre – 24 hour post) in CK levels showed significant differences between “without” and 85 Hz only (Table 2).

None of the attributes determined by the scale (PEPS) showed significant differences between the 3 interventions (data not shown).

DISCUSSION

The main findings of this study are greater metabolic changes (lactate, RER, BE, HCO_3^- , PCO_2) during cycling with superimposed EMS compared with normal cycling independent of frequency of EMS, mainly visible at higher workloads. Furthermore, EMS showed greater effects on CK levels 24 hours postexercise than did normal cycling, which reveals a higher demand and load on recruited skeletal muscles. No statistically significant differences in saliva cortisol levels were found between the interventions.

During voluntary muscle action in healthy subjects, MUs are recruited in an orderly fashion from small to large, according to the size principle of MU recruitment (11,12,21) in relation to the intensity of the stimulation (19). Under physiological conditions, low-level contractions such as those during low-intensity cycling are assigned to the smallest MUs, which are of the slow, fatigue-resistant type. With increasing force, MUs of larger size, which have a fast, fatigue-susceptible character, become involved (21). The fixed recruitment order ensures that MUs that are active for much of the time are composed of the type of fiber that is best suited for sustaining tension without fatigue for long periods (21).

In contrast to the normal recruitment order, electrical stimulation overrides the physiological recruitment order and can activate all the fibers in the muscle more or less simultaneously (21). Electrical stimulation recruits MUs in a nonselective, spatially fixed, and temporally synchronous pattern. Both slow and fast fibers are nonselectively activated with EMS at low or high force levels (9). This recruitment pattern may contribute to the increased metabolic changes and the increased muscle fatigue, which was shown by the alterations of the acid base balance, lactate values, and the shorter total exercise time in this study, when compared with normal cycling. Previous studies have shown that, for a given intensity and duration of stimulation, muscle fatigue appears earlier with isolated EMS than with VC (2,20), which is also apparent when EMS and VC are combined.

According to our results, previous studies have also shown that isolated EMS is highly demanding on muscle metabolism and can enhance energy consumption more than VC (13). According to the preferential recruitment of larger fibers, EMS strongly activates anaerobic glycolysis for energy production with lactate formation and induces a stronger acidification leading to early fatigue (1,10,23,26), which is accordance with our results. Kim et al. (13) showed that during an incremental 1-legged dynamic knee extension exercise test, lactate and ammonia efflux from the leg were higher with EMS than that during VCs, and the difference became larger with increasing exercise intensity (14). This might be the reason why significantly higher lactate values were only observed at higher intensities ($>75\%$ PPO) in this

study. Furthermore, the large number of additionally recruited type 2 fibers and MUs may lead to greater muscle lactate production especially during higher workload. The additional lactate produced because of the EMS might be oxidized at lower exercise intensities according to the cell-to-cell lactate shuttle theory and is therefore not visible in blood (3,4,8). At higher exercise intensities, the oxidative capacity might not be sufficient anymore, leading to increased blood lactate levels.

Because metabolic changes are important for the induction of cellular signaling cascades and adaptations (5,7), these results indicate that adding EMS to cycling may be an enhancing stimulus for aerobic training in athletes, and for patients who cannot perform high workloads. Even at lower exercise intensities, additional EMS may allow one to induce a high local stimulus on skeletal muscle. The nonselective recruitment may provide (clinical) advantages in that all fibers, regardless of type, have the potential to be activated at relatively low exercise intensities, which might lead to greater skeletal muscle adaptations and improvements of endurance performance, especially in fast twitch fibers (9). However, it should be taken into account that EMS leads to a higher demand and load on skeletal muscles mass involved and causes more muscle soreness and damage than VC as has been shown in the present and in previous studies (18).

The EMS and VC can be considered as complementary stimuli of a different nature, inducing different acute physiological effects. Therefore, in a context of chronic application (training program), the combination of EMS and VC could theoretically per induce greater physiological adaptations (19) not only in strength training but also in endurance training. In his review, Paillard (19) stated that the superimposition of the 2 types of contraction induced greater neuromuscular adaptations than EMS practiced alone (19). Previous studies have shown that long-term applications of isolated EMS can improve endurance (15). Because EMS and voluntary muscle contraction (VC) constitute different modes of muscle activation and induce different acute physiological effects on the neuromuscular system, the superimposed method might be even more promising. However long-term application of each mode of muscle activation can produce different muscle adaptations, which need to be further investigated. Thereby, the long-term influence of stimulation on muscle phenotype seems to depend primarily on the aggregate number of impulses delivered and not on the frequency (21).

PRACTICAL APPLICATIONS

The study demonstrates significant changes in metabolism at intensities $\geq 75\%$ PPO when EMS is added to cycling. Overall, these findings are in accordance with findings from EMS studies in strength training and reveal potential for metabolic changes when EMS is added to cycling. Because fast twitch fibers are substantially recruited only at exercise intensities $\geq 90\text{--}100\% \dot{V}O_{2\text{max}}$, $90\text{--}100\% \dot{V}O_{2\text{max}}$ should set the lower

training limit to enhance their oxidative capacity (17), which might not be applicable and practicable for athletes and patients, who cannot perform high workloads. According to the larger number of additionally recruited fast twitch fibers and MUs because of EMS even at low workloads (shown by the greater metabolic alterations in this study), adding EMS to cycling may have a positive benefit on the development of aerobic capacity. Even at lower exercise intensities, additional EMS may allow one to induce a high local stimulus on skeletal muscle and especially on fast twitch fibers, which may lead to higher improvements of aerobic capacity. Further research should focus on the chronic effects of cycling with additional EMS, on aspects such as muscle adaptations, muscle damage and changes in cardiorespiratory performance.

The authors state that they have no funding or conflict of interest to disclose. No funding was received for this work from any of the following organizations: National Institutes of Health (NIH); Wellcome Trust; Howard Hughes Medical Institute (HHMI).

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