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Original Article

Acute metabolic, hormonal, and psychological responses to strength training with superimposed EMS at the beginning and the end of a 6 week training period

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Abstract

Objectives: The aim was to determine metabolic and hormonal responses to strength training with or without superimposed electromyostimulation (EMS) at the beginning and the end of a 6 week training period. **Methods:** 20 strength trained subjects were randomly assigned to two groups. The first group (S) performed 4 sets of back squats with a constantly adjusted additional load of their individual 10 repetition maximum (10 RM) twice a week over 6 weeks. The second group (S+E) did the same training program with superimposed EMS on leg and trunk muscles. Physiological responses were determined before and after the first (TS 1) and the last training session (TS 12). **Results:** No significant differences of hormonal responses could be observed between groups and TSs. However, small to large effects on metabolism occurred between groups and TSs. Delayed onset muscle soreness (DOMS) was significantly higher 48h after TS 1 for S+E. **Conclusions:** Despite a higher DOMS after S+E, there is no acute effect of superimposed EMS on hormonal response to exhaustive resistance exercise. We suggest that, because of the high resistance during 10 RM bouts, most of the muscle fibers are already activated and superimposed EMS only activates few additional muscle fibers.

Keywords: Resistance Exercise, Squat Exercise, Hgh, Testosterone, Cortisol

Introduction

In general, exercise-induced hormonal responses are important for mediating signal pathways for both short-term homeostatic control and long-term cellular adaptations to any type of stress[1,2]. The endocrine system plays an important role in adaptations of strength training such as maximal strength and power by enhancing protein synthesis in muscle-cells[3-6] and functions in the nervous system[7,8]. However, it is still questionable if long term adaptations are affected by acute changes during and post-exercise or by chronic changes in resting concentrations[9]. Anyhow, testosterone and cortisol have been defined as important mediators in the response and adaptation to exercise training stimuli[2] functioning as biomarkers for anabolic and catabolic hormonal control, respectively[10,11]. Human growth hormone (hGH), which affects muscle hypertrophy, furthermore is known to affect substrate utilization while exercising[12]. In the past, the effects of different resistance exercise schemes on hormonal responses have been investigated[6]. In summary, strength training with moderate to high intensity constellations (70-80% 1RM) multiple sets (3-5), short rest intervals (60-120s) and 8-12 repetitions lead to great hormonal responses[13]. Besides mechanical stimuli, metabolic perturbations during strength training are discussed to have a great influence on the acute hormonal response and on gains in muscle strength and hypertrophy[14-16].

Electromyostimulation (EMS) is an alternative training method and can be applied for an intensification of resistance training. This intensification is due to the specific pattern of motor-unit recruitment imposed by EMS, which is a nonselective, spatially fixed and temporally synchronous pattern[17]. Previous studies showed that EMS is highly demanding on muscle metabolism, and can enhance energy expenditure and carbohydrate oxidation more than voluntary contraction[18,19]. EMS strongly activates anaerobic glycolysis for energy production with lactate formation and acidifies more cytoplasm than voluntary contraction[20,21].

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Therefore, the superimposed application of EMS might cause greater metabolic stress, and hence, greater hormonal responses and adaptations. In previous studies we were already able to show, that cycling with superimposed EMS leads to higher increases in circulating hormones post exercise[22]. However, according to strength training, data on superimposed EMS are rare. Most investigations of physiological responses to EMS mainly focused on passive and locally applied EMS at individual pain threshold[23-26]. Only a few studies combined EMS with superimposed movement[27] or investigated the effects of superimposed EMS on single muscle groups[19,28]. Whole body EMS devices are able to stimulate several muscle groups simultaneously, e.g. muscle chains or agonist/antagonist during multi joint movement like squat exercise. A superimposed stimulation additionally increases the activation especially in the eccentric phase[29], may be also leading to higher mechanical stimuli. These high mechanical stimuli might induce muscle damage, which is characterized by increases in circulating creatine kinase (CK) activity and delayed onset muscle soreness (DOMS)[30]. Although familiarization is documented for parameters of muscle damage like CK and DOMS, also known as the repeated bout effect[31], the occurrence of familiarization effects of the endocrine system to strength training or EMS are still unclear.

Although the impact of strength training has been widely investigated in the past, no investigation focused on the response of metabolism, hormones, and muscle damage to strength training with superimposed EMS. Therefore, the purpose of this study was to compare the acute metabolic and hormonal responses to additional loaded back squats with and without superimposed EMS at the beginning and at the end of a 6 week training period, in order to quantify the additional load of EMS and to identify possible familiarization effects.

Methods

Twenty male subjects were recruited to participate in this investigation. Inclusion criteria were as follows: 1) good health status without cardio-vascular or pulmonary disease, 2) strength training experience for at least 2 years, and 3) back squat exercise in a proper technique with an additional load equivalent to the 10 repetition maximum (10RM).

At the beginning, subjects were examined medically. Furthermore, study details and participation requirements were explained, and written informed consent was obtained. The study received approval from the Ethics Committee of German Sport University in Cologne and was conducted in accordance with the Declaration of Helsinki.

The experimental procedure of the study corresponded to a randomized trial with two different training groups: the 1st group performed 10RM back squat exercise (S) and the 2nd group performed 10RM back squat exercise with superimposed EMS (S+E). The general study design consisted of spirometric analysis and venous blood sampling during and after the first and the last training session of a 6 week training period. The subjects refrained from strenuous exercise for at least 48h before each testing session.

Training protocol

Two weeks before the start of the 6-week training period, two familiarization sessions took place. 10RM was determined as described by Baechle & Earle[32] and EMS was introduced to the S+E group.

Back squats were standardized for range of motion in the knee joints (180°-90°) and temporal and fractional distribution of contraction modes during the repetition (2s eccentric – 1s isometric – 2s concentric – 1s isometric) using biofeedback (Biofeedback 2.3.1, digimax, Hamm, Germany; distance sensor typ S501D, megaTron; Munich, Germany). The EMS surface electrodes (miha bodytec; Augsburg, Germany) were applied around the muscle belly of the lower legs (27 cm length x 4 cm height), the thighs (44 x 4 cm) and the buttocks (13 x 10 cm). Additionally, the upper body was stimulated at the lower back (14 x 11 cm) and at the abdominal muscles (23 x 10 cm) with two bilaterally paired electrodes which are integrated in a vest. Accordingly, the predominately stimulated muscle groups were rectus abdominis, erector spinae, gluteus maximus, quadriceps femoris, hamstrings, adductors, triceps surae and tibialis anterior.

The standardization of EMS intensity is difficult due to the different electrical conductivity of the skin and tissues. Furthermore, during dynamic exercise modes with superimposed EMS, the impulse intensity has to be down-regulated to ensure movement. Therefore, as standardization, the intensity of EMS was set at 70% of individual pain threshold. The maximum tolerated amperage was verified separately for each pair of electrodes before each session. Therefore, participants stayed in the starting position of the squat (170° inner knee angle) while applying EMS to their lower limbs muscles. The verification of individual pain threshold began with the electrodes at the buttock, followed by the thigh, the lower leg, the abdominal and the lower back electrodes. A bipolar rectangular pulse waveform with an impulse width of 350 µs was applied with 85 Hz. On/off-time was set at 5/1 s and was synchronized with the back squat movement (off-time during standing position (1s) and on-time during eccentric (2s), isometric (1s) and concentric (2s) contraction mode).

During the 6 week training period, all participants performed 12 TSs (two sessions per week), each with 2 and 3 days of recovery. In each TS 4 sets of squat exercise were performed in the same way as during 10RM testing. Between each set, 120s of recovery were completed passively in sitting position. Set 1 was performed with 50% 10RM and sets 2-4 with 100% 10RM. In the course of the training period, additional load was matched to 10RM for both groups. For S+E group EMS intensity was adjusted to maximum intensity that allows dynamic movements based on pre-verified values during familiarization.

Measurements

Respiratory measurements, blood sampling for determination of lactate concentration, serum hormone concentrations and the assessment of DOMS took place at TS 1 and TS 12. Both TSs were performed at the same time of the day (morning, between 8:00-10:00). Subjects were supposed to arrive 1 hour after rising

	Age (years)	Height (cm)	Body Mass (kg)		VO_2 at rest ($mL \cdot min^{-1}$)	
Group			**TS 1**	**TS 12**	**TS 1**	**TS 12**
S+E	22.1±1.9	183.9±6.2	83.7±8.9	83.7±8.2	391±54	388±48
S	21.9±1.6	183.5±6.6	78.3±4.2	78.5±3.7	387±83	379±66

Table 1. Anthropometric data and oxygen consumption (VO_2) at rest. Groups: "Strength" (S) and "Strength + EMS" (S+E) at training session 1 (TS 1) and training session 12 (TS 12). Data are given in means ± SD.

Parameter	**Group**	**TU 1**	**TU 12**	**% Delta (TU 1 vs. 12)**	**Cohen's d (TU 1 vs. 12)**	**Cohen's d TU 1 (S vs. S+E)**	**Cohen's d TU 12 (S vs. S+E)**
Additional load (kg)	**S+E**	91.5±12.5	106.5±15.7	+16	1.06	0.45	0.55
	S	85.0±11.4	97.8±15.9	+15	0.92		
	P	88.3±12.4	102.1±16.4 *	+16	0.95	-	-
EMS intensity (arbitrary units)	**S+E**	28.3±5.0	33.8±5.8 *	+20	1.02	-	-
	S	-	-	-	-		
Oxygen uptake during exercise [L]	**S+E**	14.0±2.7	15.7±1.7	+12	0.75	0.15	0.64
	S	13.6±2.5	14.4±2.3	+6	0.33		
	P	13.8±2.6	15.0±2.1	+9	0.51	-	-
EPOC 15 min [L]	**S+E**	4.2±1.1	5.3±1.1	+27	1.00	0.43	0.84
	S	3.7±1.2	4.2±1.5	+12	0.37		
	P	4.0±1.2	4.8±1.4	+20	0.61	-	-
Maximum lactate [$mmol \cdot L^{-1}$]	**S+E**	8.0±2.2	9.1±1.6	+13	0.57	0.71	0.99
	S	6.4±2.3	7.3±2.0	+15	0.42		
	P	7.2±2.3	8.2±1.9 *	+14	0.47	-	-
Delta CK 24h-pre [$U \cdot L^{-1}$]	**S+E**	+376±471	+48±196	-87	0.91	0.40	0.18
	S	+204±391	+19±104	-91	0.64		
	P	+290±442	+34±158 *	-89	0.78	-	-

Table 2. Training intensity, metabolism and delta CK 24h-pre. Groups: "Strength" (S) and "Strength + EMS" (S+E) at training session 1 (TS 1) and training session 12 (TS 12). Pooled data (P) only presented if ANOVA showed effects between TSs, but no group or interaction (group x TS) effects. Data are given in means ± SD. * = significant different to TS 1 ($p<0.05$).

up and they had a standardized caloric uptake consisting of 0.5 L low fat chocolate milk (1170 kJ; 13.5g protein; 41.5 g carbohydrate; 6.5g fat).

Assessment of oxygen consumption (VO_2) and lactate concentration. Respiratory gas exchange measures were assessed breath-by-breath via an open air spirometry system (ZAN 680 CPX, ZAN GmbH, Oberthulba, Germany) throughout the testing, using standard algorithms with dynamic account for the time delay between the gas consumption and volume signal. The respiratory gas exchange instrumentation was calibrated according to the manufacturer's guidelines with calibration gas. Volume of consumed O_2 was calculated during exercise and for 15 minutes pre- and post-exercise. Excess post-exercise oxygen consumption (EPOC) was determined by subtracting consumed O_2 15 min pre-exercise from consumed O_2 15 min post-exercise. Blood samples were analyzed for LA with an enzymatic-amperometric analyzer (Ebio plus, Eppendorf AG, Hamburg, Germany). The highest lactate was defined as post-exercise maximum lactate concentration. Blood samples were taken from the earlobe 2, 4 and 6 minutes after the last set.

Assessment of hormone concentrations. Venous blood samples were collected before (pre), immediately after (0´), 30 minutes (30´), 120 minutes (120´) and 24 hours (24h) after TS 1 and 12 to determine cortisol, testosterone, hGH and CK concentrations. The time of training for TS 1 and 12 was the same for all participants in an effort to limit the influence of circadian rhythms on hormonal concentrations. Venous blood (9.5 mL) was collected using the Vacutainer® blood withdrawl system (Becton Dickinson, Franklin Lakes, NJ, USA). After being stored at 7°C for approximately 30 minutes to allow for the deactivation of coagulation factors, blood samples were centrifuged for 10 minutes at 1.861 g and 4°C (Rotixa 50, Hettich Zentrifugen, Mühlheim, Germany). The serum was stored at -80°C until analysis. Serum concentrations of cortisol ($ng \bullet mL^{-1}$), testosterone ($ng \bullet mL^{-1}$) and hGH ($mLU \bullet mL^{-1}$) were determined by using human ELISA kits (Cortisol ELISA EIA-1887, Testosterone ELISA EIA-1559, and

			pre	0´	30´	120´	24h
Testosterone [ng*mL^{-1}]	**TS 1**	**S+E**	5.2±1.2	5.9±1.5	5.7±1.4	5.0±1.6	5.5±1.2
		S	7.1±1.9	7.8±2.3	6.8±1.9	6.1±1.6	6.5±2.7
	TS 12	**S+E**	5.0±1.4	6.5±2.2	5.5±1.8	5.1±1.5	5.2±1.6
		S	6.5±1.7	8.0±2.4	6.7±1.9	6.4±1.8	6.6±2.2
	Pooled data		6.0±1.8	7.1±2.3*	6.2±1.9	5.6±1.7	5.9±2.1
Cortisol [ng*mL^{-1}]	**TS 1**	**S+E**	146.5±29.5	150.5±36.2	135.5±32.5	90.7±32.6	112.6±35.2
		S	179.5±32.9	151.3±40.1	117.0±31.2	100.2±23.9	140.1±53.0
	TS 12	**S+E**	143.1±28.0	129.9±40.0	118.3±39.5	87.4±40.6	105.5±45.0
		S	162.1±48.9	137.2±46.7	113.0±47.5	101.6±21.6	135.4±50.7
	Pooled data		157.8±38.6	142.2±41.9*	121.0±39.2*	95.0±31.2*	123.4±48.7*
hGH [mlU*mL^{-1}]	**TS 1**	**S+E**	0.2±0.2	6.2±10.2	9.4±10.8	0.3±0.5	0.9±1.0
		S	0.7±1.4	8.1±9.5	10.2±8.4	0.5±0.7	0.9±1.1
	TS 12	**S+E**	0.1±0.2	7.2±8.1	9.2±8.3	0.4±0.4	0.5±0.7
		S	0.6±1.1	6.6±8.1	9.2±8.0	0.6±0.9	0.6±0.9
	Pooled data		0.4±0.9	7.0±9.0*	9.5±9.0*	0.5±0.7	0.7±1.0

Table 3. Hormonal responses. Testosterone, Cortisol and human growth hormone (hGH). Groups "Strength" (S) and "Strength + EMS" (S+E) at training session (TS) 1 and TS 12. Data are given in means ± SD. * = significant different to pre ($p<0.05$).

hGH ELISA EIA-3552; DRG Instruments GmbH, Marburg, Germany) and have been assayed in duplicates. CK concentrations were determined only at pre and 24h and are presented as increase after training (delta CK: 24h-pre).

Assessment of delayed onset muscle soreness (DOMS). The rating of muscle soreness was assessed by sitting down on a chair from an upright posture and standing up again from this position without using the arms. The subjects were then asked to rate their perceived physical pain using a 0-10 visual analog scale (VAS) pre, directly after, 24 h after and 48 h after each intervention. Visual analogue scales (VAS) have been used in research settings since the 1920's and is described as a reliable method[33,34].

Statistical analysis

All data were analyzed using Statistica for Windows (v.7.0; Statsoft, Tulsa, OK, USA). Descriptive statistics of the data are presented as mean ± standard deviation (SD). Data of anthropometry, training intensity and metabolism were compared using ANOVA repeated measures with regard to group (S and S+E) and TS (TS 1 and 12). As no significant differences between both groups were observed, all subjects were pooled in a single group, just analyzing differences between TS 1 and 12 using a paired t-test.

The acute hormonal responses to TS 1 and 12 were compared using ANOVA repeated measures [group (S and S+E) x time (pre, 0´, 30´, 120´, 24h) x TS (TS 1 and 12)] with Fisher post-hoc test. As no significant differences between both groups and TSs were observed, all subjects and TSs were pooled, just analyzing changes over time (pre, 0´, 30´, 120´, 24h) using ANOVA repeated measures with Fisher post-hoc test. $P<0.05$ was considered significant.

Cohen´s d defined as difference in means/standard deviation[24] was calculated within each group (S and S+E) over time (TS 1 to 12) and between groups S and S+E at TS 1 and 12 for parameter of training intensity, metabolism and delta CK (Table 2). Thresholds for small, medium, and large effects were 0.20, 0.50, and 0.80, respectively[24].

Results

Testing and training. For both training groups, age, height, as well as body weight and VO_2 at rest before and after the 6 week training period showed no significant differences between groups or TSs ($P>0.05$; Table 1). 10 RM improved significantly for both groups after 12 TSs in 6 weeks of squat exercise ($P<0.01$; Table 2). No significant differences occurred between the groups ($P=0.25$). Electrical stimulus intensity increased for S+E group ($P<0.01$).

Oxygen consumption. Over-all ANOVA showed a significant effect between TSs ($P=0.04$), but no group ($P=0.37$) or interaction ($P=0.41$) effects for oxygen uptake during training. For EPOC a significant effect was shown between TSs ($P=0.02$), but no group ($P=0.13$) or interaction effects ($P=0.26$). For S+E, Cohen´s d revealed large effects for oxygen consumption during training ($d=0.8$) and EPOC ($d=1.0$) between TS 1 and 12. For S, Cohen´s d revealed small effects for oxygen consumption during training (0.3) and EPOC (0.4) between TS 1 and 12. Medium ($d=0.6$) to large ($d=0.8$) effects were observed for oxygen consumption and EPOC between groups in TS 12 respectively (Table 2).

After pooling the groups, no significant differences from TS 1 to 12 for oxygen uptake during training ($P=0.06$) or for EPOC ($P=0.13$) were observed anymore. Cohen´s d revealed medium effects for oxygen consumption (0.5) and EPOC (0.6) respectively.

Lactate. Over-all ANOVA of maximum blood lactate concen-

			pre	post	24h	48h
VAS	TS 1	S+E	0.1±0.3	0.8±1.0	2.1±1.5*	2.5±2.0*#
		S	0.1±0.2	1.0±1.0*	0.6±0.8	0.7±1.0
	TS 12	S+E	0.3±0.3	1.0±0.6	1.5±1.2*	1.6±1.5*
		S	0.3±0.3	1.2±0.9*	0.5±0.5	0.4±0.5

Table 4. Changes in perceived physical pain. Groups: "Strength" (S) and "Strength + EMS" (S+E); pre, directly after (0´), 24 h and 48 h after training session (TS) 1 and TS 12. Data are given in means ± SD. * = significant different to pre ($p<0.05$). # = significant different to the other group at the same TS ($p<0.05$).

tration showed significant differences between TSs (P=0.03), but not between groups (P=0.05) or in interaction (P=0.90). Cohen´s d revealed medium effect sizes between TSs for S (0.5) and S+E (0.6) and medium (0.7) and large (1.0) effect sizes between groups at TS 1 and 12 (Table 2).

After pooling the groups, significant difference from TS 1 to 12 for maximum lactate concentration were observed (P=0.02). Cohen´s d revealed medium effect sizes (0.5).

CK. Increases of CK from pre to 24h post are presented as delta CK 24h-pre (Table 2). Over-all ANOVA showed significant differences between TS 1 and 12 (P=0.04), but no significant group (P=0.35) or interaction effect (P=0.53). Cohen´s d revealed large effects from TS 1 to 12 for S+E (-0.9) and medium effects for S (-0.6). Medium effects (0.4) are also shown between groups at TS 1only.

After pooling the groups, significant difference and large effects (0.8) for delta CK were observed (P=0.03) between TS 1 and TS 12.

Testosterone. Over-all ANOVA showed no significant differences in serum testosterone concentrations between groups (P=0.09) and between TS 1 and 12 (P=0.99), but over time within each session (P<0.01).

After pooling the groups and TSs, ANOVA revealed significant effects over time. Post-hoc analysis showed significantly higher values at 0´ in comparison to all other time points (P<0.05; Table 3).

Cortisol. Over-all ANOVA showed no significant differences in serum cortisol concentrations between groups (P=0.26) and between TS 1 and 12 (P=0.21), but over time within each session (P<0.01).

ANOVA revealed significant effects over time after pooling the groups and TSs. Post-hoc analysis showed significant differences between pre and all other time points (P<0.05; Table 3).

Human growth hormone. Over-all ANOVA showed no significant differences in serum hGH concentrations between groups (P=0.80) and between TS 1 and 12 (P=0.52), but over time within each session (P<0.01).

ANOVA revealed significant effects over time after pooling the groups and TSs. Post-hoc analysis showed significant higher values at 0´and 30´ in comparison to all other time points (P<0.05; Table 3).

DOMS. Over-all ANOVA showed significant differences in DOMS-rating between groups (P=0.02) and over time (P<0.01). Furthermore, an interaction effect for group x time (P<0.01) was observed. No significant differences were shown between TS 1 and 12 (P=0.42) and for group x TS (P=0.51). Post-hoc analysis showed a significant increase 0´ after exercise for S and 24h and 48h after exercise for S+E group at TS 1 and 12, respectively. Significant differences between groups occurred 48h after TS 1 (Table 4).

Discussion

The aim of the present study was to compare acute physiological responses to 4 sets of 10 RM squat exercise with or without superimposed EMS at the begin and the end of a 6-week training block. EMS was superimposed with submaximal intensity to agonistic and antagonistic muscles of legs and trunk which are predominately activated during squat exercise. We hypothesized a higher activation of stimulated muscles of S+E, and therefore a higher metabolic stress and a higher hormonal response. In order to quantify the training stimulus as accurate as possible, we used the resistance exercise determinants according to Toigo and Boutellier[35] and furthermore, measured the metabolic stress of the exercise. The superimposed EMS was set according to individual pain threshold. The major findings of the present study were, that no significant differences between groups could be observed in all measured metabolic and endocrine parameters during and after TS 1 and TS 12, except that superimposed EMS induced significantly higher DOMS 48h after TS 1. Furthermore, the elevated load and electrical stimulus intensity in TS 12 led to similar hormonal responses compared to TS 1, thus training stimulus was still great enough to stress the participants in both groups.

In the present study, neither additional load, nor metabolic parameters were significantly different between groups in TS 1 and 12. A higher activation of dynamic working muscles (agonistic and antagonistic) and stabilizing trunk muscles was suggested due to superimposed EMS. However, differences between groups for lactate were close to statistical significance (p=0.05), with medium to large effects between groups at TS 1 (cohen´s d=0.7) and 12 (1.0). Additionally, effects between groups are medium to large at TS 12 for oxygen consumption (0.6) and EPOC (0.8). In accordance, Kemmler et al. found impacts of superimposed EMS on energy expenditure during a low-intensity resistance exercise protocol[28]. However, in the study of Kemmler et al. more muscle groups of the upper body were stimulated and a consider-

ably lower intensity (no additional load) was applied. Therefore, the additional impact of EMS might be even greater compared to the high loads performed in the present study. Also for cycling with superimposed EMS, we could show significantly higher lactate levels compared to normal cycling at the same intensity[22]. It can be speculated, that because of the high resistance during 10 RM bouts of the present study, most of the muscle fibers are already activated, so less additional muscle fibers are activated by superimposed EMS, leading to similar metabolic reactions as without EMS. Another explanation might be the limited spatial recruitment of muscle fibers to EMS, which is quite superficial and largely incomplete. Differences between TS 1 and 12 might be explained by the higher load and higher electrical stimulus at TS 12. In fact, for pooled groups significant difference could only be observed for lactate (P=0.02) and narrow significant differences could be observed between TSs for oxygen uptake (P=0.06) and EPOC (P=0.13). Furthermore, medium effects for oxygen consumption (0.5), EPOC (0.6) and lactate (0.5) indicate a tendency to higher metabolism.

CK is usually measured as a damage marker and is associated with muscle soreness[30]. Especially eccentric accentuation is known to induce CK elevations[36]. Thus, the simultaneous activation of agonist and antagonist by EMS during con- and eccentric contractions would have suggested higher responses in CK levels for S+E. In the present study, increases in CK levels 24 h after TS 1 did not differ significantly between both groups. It should be noted that the CK-concentration showed high inter-individual responses to the load in both groups. Indeed, there was a small effect between groups at TS 1 (cohen's d=0.4). Furthermore, significantly higher perceived muscle soreness 48h following TS 1 was observed in the S+E group compared to S. Despite the small effect of S+E on CK levels, it seems that mainly high resistance induced CK increases rather than superimposed EMS. It is supposable, that peak-CK concentrations occur later 48-96 h following exercise[37]. However, according to the already large effort for the subjects, no further blood sampling was possible in the present study. For training at lower resistance during cycling, higher CK-activity was induced by superimposed EMS already 24 h following exercise[22]. EMS-induced isometric contractions at maximum pain threshold intensity and isometric contractions at the same force output lead to significantly higher CK-levels 72 h after exercise for stimulated contractions only[24]. In conclusion, we can only speculate about possible peak values and about differences between groups, due to data of DOMS. Higher DOMS with superimposed EMS could be attributed to microtraumata because of perpetual activation of the same pool of muscle fibers by EMS. The blunted CK levels at TS 12 compared to TS 1 are in accordance to the repeated bout effect after repeated mechanical and electrical stimulation of the muscles[31]. The repeated bout effect still seems to be present, despite progressive training loads and progressive electrical stimulation.

hGH is an anabolic hormone with further effects on lipid, carbohydrate, and protein metabolism[2]. Although it has not been established whether acute increases in hGH lead to local skeletal muscle hypertrophy during prolonged strength training, it has been suggested that a transient increase of hGH can produce an interaction with muscle cell receptors, aiding recovery and stimulating hypertrophy[38]. Since metabolic stress (lactate) has been suggested to influence hGH secretion[39,40], the absence of significant differences in metabolic parameters might be one explanation for no significant differences in hGH concentration in both groups. However, previous studies showed that intensity-dependent differences in lactate accumulation during strength training can lead to similar hGH concentrations[41]. The amount and duration of hGH responses of both groups were comparable to those being reported following hypertrophic resistance exercise protocols[42]. Studies that investigated sole EMS also showed a congruent increase in hGH[24,25,37]. Jubeau et al. compared 40 isometric contractions in a leg press machine induced by EMS at a maximal tolerable level or without EMS at the same force output. Although there were obvious differences in rest- and contraction modes in this study compared to the present intervention, total amount of time under tension (250 s vs. 240 s) and the involved muscle groups (in particular leg - and hip extensors) were approximately the same. Resulting hGH concentrations are of similar amount and duration like the hGH responses of the present study[24]. However, in the study of Jubeau et al., sole EMS induced significantly higher hGH levels than the (quite low) voluntary contractions of the same force output.

In the present investigation EMS is applied to several muscle groups, with an intensity, that is adjusted to enable squat exercise. Load and movement dominate the exercise to maintain voluntary aspects of coordination. To this method the results show, that mechanical stimulus of additional load is great enough to induce elevations in hGH concentration.

Testosterone as an anabolic hormone is known to influence muscle hypertrophy and strength[2]. Present results show that 4 sets of 10 RM squat exercise increased testosterone levels immediately after training for both groups. Immediate increases and the amount of testosterone concentration are in accordance with the literature dealing with hypertrophic training programs[13,42,43] or EMS[37]. Furthermore, there were no changes in the resting levels of testosterone for both groups (S, S+E) after the 6 week training period. This is in accordance to other studies, which investigate the influence of high training intensities and low training volume over multiple weeks on testosterone levels[44,45]. However, studies of combined strength and endurance training[46], or high-intensity endurance training[47] were able to show moderate changes in resting testosterone levels after training periods.

Disturbances in homeostasis due to exercise are known to increase the stress hormone cortisol. Thereby, the cortisol response seems to depend on the intensity and the volume of the exercise, which might be referred to it influences on lipid, protein and glucose metabolism[48]. More specific, cortisol was already shown to increase significantly in response to high-volume strength exercise (10 RM; multiple sets)[13,42]. Therefore, increases in cortisol would have been expected in the present study, too. However, immediate reduction in cortisol concentration for S probably reflects normal circadian variation. Throughout the diurnal rhythm cortisol concentration has a peak in the early morning with a following decline[49,50]. Despite the direct decrease, reductions seemed to be delayed by superimposed EMS especially at TS 1 (Table 3), although there were no significant differences between groups. In a previous study we were already able to show, that

superimposed EMS has an influence on cortisol levels compared to voluntary contractions[22]. Also other studies observed a direct decrease in cortisol levels after 45 stimulated isometric contractions of the quadriceps of one leg[37].

Although, all trials were carried out at the same time of the day, large inter-individual differences were observed even under resting conditions (<120 ng•ml^{-1} to >200 ng•ml^{-1}). Possible reasons could be different events in the morning, although subjects were supposed to arrive 1 hour after rising up and all subjects had a standardized resting phase of 30 min and a standardized caloric intake. Although, such inter-individual differences were already shown by previous studies[22,42] a "no exercise control group" would have taken diurnal decreases/increases into account. If there is any effect of superimposed EMS on cortisol levels, it can only be speculated based on the present data.

Conclusion

The results demonstrate that EMS superimposed to additional loaded squat exercise does not induce significantly higher endocrine responses, but small to large effects on metabolism, on CK and a higher individual pain-sensation 1-2 days after exercise. We suggest, that because of the high resistance during 10 RM bouts, most of the muscle fibers are already activated, so less additional muscle fibers are activated by superimposed EMS. It can be considered, that the increased pain sensation could be a disadvantage in the training process. However, it is our subjective evaluation that weak trunk muscles during back squat exercise might be supported by superimposed EMS, which could be beneficial at least for novices in complex strength training movements.

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