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ORIGINAL ARTICLE

Neuromuscular, hormonal and cardiovascular adaptations to eight-week HIIT and continuous aerobic training combined with neuromuscular electrical stimulation

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ABSTRACT

BACKGROUND: Whether high-or-low intensity exercise coupled with neuromuscular electrostimulation (NMES) affect IGF-1 and IGFBP-1 is unknown. The scope of this study was to test whether 8-week high-intensity interval training (HIIT) and continuous aerobic training (CA) combined with/without NMES performed at 65% and 120% of VO_{2max} on a cycle ergometer induce different metabolic adaptations.

METHODS: A randomized controlled trial with a parallel groups study design was used. Thirty healthy untrained male participants (age: 21.33±1.24 years, height: 177.80±5.97 cm, weight: 73.74±7.90 kg, lean body mass: 64.29±5.11 kg, percent body fat: 12.43±5.34%) voluntarily participated in this study. Six participants were allocated to Control, six to HIIT, six to HIIT+NMES, six to CA, and six to CA+NMES.

RESULTS: Pre- to post-test $_1VO_{2max}$, blood lactate concentrations, O_2 kinetics, peak torques at 60°/s and 180°/s were found statistically significant (P<0.05, P<0.001). IGF-1 pre 15 min in CA and IGF-1 post 30 min in HIIT group was found significantly higher compared to control group (16.93±8.40 *vs.* 6.05±4.25, P=0.024; 10.80±3.94 *vs.* 6.15±2.56, P=0.037), respectively. Additionally, IGFBP-1 were found significantly higher in CA+NMES group than HIIT group (0.95±0.67 *vs*. 1.23±0.56). Eight week post IGF-1/IGFBP-1 ratios were found higher in pre 15 min, post 30 min and post 24 h compared to baseline pre 15 min, post 30 min and post 24 h measurements in all groups (8.92±4.72 *vs.* 3.93±3.14; 9.41±3.72 *vs.* 3.99±1.76; 8.63±3.01 *vs.* 5.89±3.01, respectively). Also, IGFBP-1 post 30 min was significantly lower in HIIT+NMES while CA group showed significantly lower baseline and 24 h post IGFBP-1 compared to pre-test measurements (Z=-3.20, P=0.001; Z=-3.72, P=0.000; Z=-2.93, P=0.000).

CONCLUSIONS: HIIT and CA training induce different stimuli on IGF-1 and IGFBP-1 and NMES application combined with high-and-low intensity exercise is highly effective in improving athletic performance.

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KEY WORDS: Lactates; Cardiovascular system; Exercise test; Physiological adaptation; High-intensity interval training; Oxygen consumption.

The musculoskeletal system has a unique structure and rapidly responding to different physiological stimuli. Neuromuscular electrical stimulation (NMES) is the application of electrical stimulation to the peripheral nervous system through high-and-low-intensity electrical currents to provoke muscle contractions at equivalent rates to voluntary exercise such as aerobic and anaerobic exercises.[1] Because of the substantial performance improvements in maximal aerobic capacity, muscle strength and capacity for physical activity in both sedentary adults[2] and patients with chronic heart failure[3] NMES interventions has gained great interest in recent years. The recent studies revealed that by means of NMES exercise it is possible to induce cardiovascular responses to exercise and enhance energy expenditure.[4] Studies have shown that NMES training provides a significant increase in muscle strength and that dynamic voluntary activity with NMES can also induce neuromuscular and cardiovascular adaptations in the human organism. It is noted that the increase in muscle strength obtained by electrical stimulation in healthy individuals is

higher than obtained by exercise alone.[5, 6] It was also reported that fast twitch (FT) muscle fibers are contracted with voluntary contractions during physical activity, but these muscle fibers are also activated even under low stimulation threshold during NMES. High-frequency NMES applications also have a significant influence on oxidative capacity of muscles and endurance performance[7] by generating fast-slow muscle fiber-type transitions.[8]

NMES of skeletal muscle can produce changes in metabolic enzyme but little is known of the direct effect of NMES on the IGF system.[9] Anabolic hormones such as human growth hormone (hgH) have a vital role in regulating protein metabolism and hypertrophy in muscle mass, and shows its effect by stimulating insulin-like growth factor-I (IGF-1) synthesis.[10] IGF-1 plays an important role in regulating the proliferation, differentiation, regeneration of skeletal muscles, and specific functions of many cell types.[11] IGF-1 levels in the organism vary considerably depending on the muscle mass, the general protection of the musculoskeletal system, the metabolic rate and muscle strength.[12] IGF-1 has six binding proteins (IGFBP 1-6) which are effective in binding 95% of IGF-1 to the circulation.[13] Among all IGF binding proteins, IGFBP-1 is the only protein that shows the most rapid fluctuations in human plasma. Studies have shown that long-term endurance exercises significantly increase IGFBP-1 concentrations in humans.[14] It was also proposed that it is important to determine the ratio of IGF-1/IGFBP-1 in terms of measuring the active form and free bioavailability of IGF-1.[15, 16] However, the mechanism of these high concentrations involved in the circulation and their physiological significance has not been fully elucidated.

During physical exercise, the fundamental role of the cardiorespiratory system is to provide nutrients and sustained oxygen flow to the skeletal muscles and to remove the metabolic by-products of cellular respiration.[17] In terms of delayed onset of increased local muscular fatigue, an improved bioenergetic capacity has shown to be an important component to maintain high mechanical propulsive efficiency during the time to exhaustion at VO_{2max} (T_{Lim}-$_I VO_{2max}$)[18] which refers to the minimum intensity at which VO_{2max} elicits ($_I VO_{2max}$) to the point of exhaustion (T_{Lim}) allows more detailed insight in the evaluation of endurance capacity[19] because of variability of T_{Lim} even for elite athletes with similar VO_{2max} capacity. There are different type of training regimens with different intensities that require metabolic interaction of aerobic and anaerobic pathways such as high-intensity interval training (HIIT) and CA (Continuous Aerobic) which may promote specific responses to T_{Lim} at $_I VO_{2max}$. Since T_{Lim}-$_I VO_{2max}$ consists of the integration of neuromuscular, aerobic and anaerobic components and different type, intensity, or duration of exercise may promote different adaptations in human organism due to varying metabolic stress the scope of this study was to test whether 8-week HIIT and CA combined with/without NMES performed at 65% and 120% of $_I VO_{2max}$ on a cycle ergometer induce different adaptations on oxygen kinetics, time to exhaustion, isokinetic peak torque at different angular velocities, IGF-1, and IGFBP-1 parameters.

Materials and methods

Procedures

The training interventions were conducted three times per week throughout 8 weeks with a one-day interval. All participants gave written informed consent prior to participating with the study approved by the Institutional Review Board in accordance with the ethical standards of the Helsinki Declaration. This study was approved by the Research Ethics Committee. The anthropometric parameters were assessed using Bioelectrical impedance analysis (Tanita 418-MA Japan) prior to Cybex isokinetic sessions. Height was measured through stadiometer in the standing position (Holtain Ltd., Crymych, UK). All trainings were started 1-2 days after pre-tests. The training intensity was set individually for all participants and all were instructed to maintain their usual food intake, hydration, and physical activity. No additional strength training was allowed during the study period.

Study participants

Thirty healthy male college students with at least 3 years of sports history voluntarily participated in this study (age: 21.33±1.24 years, height: 177.80±5.97 cm, weight: 73.99±7.66 kg, lean mass: 64.64±7.34 kg, percent body fat: 13.17±5.00%), respectively. A randomized controlled trial with a parallel groups study design was used including HIIT+NMES and CA+NMES training groups with simultaneous NMES, HIIT and CA training groups without NMES and a control group in order to investigate the effects of the NMES stimulus combined with HIIT and continuous aerobic trainings on oxygen kinetics, time to exhaustion, isokinetic peak torque, IGF-1, and IGFBP-1 parameters.

Isokinetic strength measurements

Prior to the isokinetic measurements, the participants were seated in the upright position with the hips flexed at an

angle of 90° and performed dynamometer trials over a series of 10 submaximal repetitions both during knee flexion and extension at 180°/s followed by 5 maximal bilateral knee extension repetitions, from 90° of flexion to full extension (0°), at an angular velocity of 60°/s and 180°/s at a maximal effort. Gravity correction was made prior to all test sessions.

Assessment of VO_{2max}, $_{I}VO_{2max,}$ and time to exhaustion

VO_{2max}, $_{I}VO_{2max,}$ and T_{Lim} were measured in preliminary test session using Ergoline Ergoselect 100/200 cycle ergometer over a one-week interval. In the first visit, a progressive cycle ergometer test was used to determine VO_{2max} and $_{I}VO_{2max}$. Participants started to pedal at 50 W and were asked to pedal between 95-100 rpm. Each stage consisted of 2 min and load was increased 50 W with the completion of every stage until a plateau in VO_2 despite an increase in cycling intensity, a respiratory exchange ratio (RER) above 1.1, 90% of the predicted maximal HR. If the stage of 2 min could not be completed, the load of the previous stage was recorded as $_{I}VO_{2max}$. The load that VO_{2max} elicit was recorded as $_{I}VO_{2max}$ and was used to determine T_{Lim} and individual training intensity used during HIIT and CA trainings. Throughout the test, the Borg scale was used in assessment of perceived exertion during exercise.

In the following session, T_{Lim} at $_{I}VO_{2max}$ test was carried out at a constant load until volitional exhaustion. Following 10-min warm-up period at 60% of $_{I}VO_{2max}$, the load was immediately increased (in less than 20 s) up to $_{I}VO_{2max}$ and the participants were encouraged to pedal at a constant speed of 100 rpm to their volitional exhaustion. O_2 was measured breath by breath (CareFusion MasterScreen CPX, Carefusion, Hoechberg, Germany) and subsequently averaged over 15-second intervals. Before each test, the automated gas analyzer was calibrated according to the manufacturer's recommendations. Heart rate was also monitored and recorded throughout VO_{2max} and T_{Lim} test sessions using 12-lead ECG. Individual training intensity was determined using baseline $_{I}VO_{2max}$ parameters.

Collection of blood samples

In each testing session (pre and post $_{I}VO_{2max}$ testing sessions) 5 mL of fasting blood sample was withdrawn from brachial vein 15 min before (pre), 15-30 min after (post) and 24 h after the interventions (24 h-post) between 08:00 a.m. and 12:00 p.m. The participants were seated after the interventions for 15min until the first blood samples were collected. Blood samples were stored at -80 °C and analyzed after completing the study. Serum IGF-1 and IGFBP-1 samples were collected using Elabscience kits (IGF-1: detection range: 1.56~100 ng/mL; sensitivity: 0.94 ng/mL; IGFBP-1: detection range: 0.16~10 ng/mL; sensitivity: 0.10 ng/mL) and analyzed using enzyme-linked immunosorbent assay (ELISA)-method according to the manufacturer instructions (R&D Systems, Minneapolis, MN, USA). No nutrition supplements were allowed during the study period and all participants' nutrition on the test days was recorded. All participants were also instructed to maintain the usual nutrition throughout the study period. The enzyme-linked immunosorbent assay kits (Elabscience Biotechnology Co. Ltd, Wuhan, China) were used for the determination of serum IGF-1 and IGFBP-1 levels. All the measurements were taken based on the instructions of manufacturer.

In each $_{I}VO_{2max}$ testing session (pre-post) and at the end of every 2 min interval during $_{I}VO_{2max}$ test blood samples were collected from earlobe using Lactate Pro 2 handheld analyzer (LT-1730, Arkray Inc, Kyoto, Japan). Blood samples were also taken prior to T_{Lim} and 2 min after the intervention to determine blood lactate concentrations.

Neuromuscular electro stimulation protocol

NMES protocol was conducted using a COMPEX SP4.0 (Medicompex SA, Ecublens, Switzerland) four channel electric muscle stimulator. Biphasic symmetric rectangular pulsed currents (150 Hz) lasting 400 μs were used. COMPEX self-adhesive electrodes were used during muscle stimulation with COMPEX device. Positive snap electrodes (5×5 cm) that stimulate a 25cm^2 area of the muscle surface which also has membrane depolarization feature were placed on the proximal insertion of vastus medialis and vastus lateralis. The other negative electrode (10×5 cm), measuring 50 cm^2 was placed over the femoral triangle, 1-3cm below the inguinal ligament.

Each HIIT session consisted of a 5-min warm-up (65% $_{I}VO_{2max}$) followed by 1-min exercise at 120% of the $_{I}VO_{2max}$ followed by 1-min "loadless" cycling. This interval was repeated 8 times on training days 1 and 2 and progressed to 14 repeated intervals by the eighth session. Participants were given strong verbal encouragement and asked to maintain pedal cadence at 100 rpm throughout the test session. Participants assigned to HIIT+NMES training group continued the same training protocol using an additional NMES protocol (duration: 12 seconds ''On'' 8 seconds ''Off'', intensity: 45-60 Hz, current: 300 μs, wave: square wave form) throughout 8 weeks.

CA training was performed with the work rate set individually based on participant's pre-training $_{I}VO_{2max}$ for

30-48 min. The duration of training was determined 30 min for the first 2 weeks of training, 36 min for weeks 3-4, 42min for weeks 5-6, and 48 min for weeks 7-8. Participants were asked to maintain the cadence rate at 80 rpm throughout the test. Participants assigned to CA+NMES training group continued the same training protocol using an additional NMES protocol (duration: 20 seconds ''On'' 20 seconds ''Off'', warm-up frequency: 3 Hz, training intensity: 20 Hz, current: 300 μs, wave: square wave form) throughout 8 weeks.

Statistical analysis

G Power (3.1.9.2) program was used in sample size calculation. The effect size was determined as d=1.9811. Type I error level (α - error level) was set at 0.05 and Type II error (β) set at 0.20. The sample size was calculated as at least 5 participants for each group. The Shapiro Wilk-W test analysis of normality of distribution was followed by a two-way mixed ANOVA with repeated measures to analyze the results obtained for the subgroups before and after treatment (groups vs. pre/post-treatment). To analyze the results obtained for all groups before and after treatment; the Wilcoxon test for paired, Mann-Whitney U-test for non-paired and, to compare the results overtime a Kruskal–Wallis test with Bonferroni correction were performed for non-paired data. Correlations were assessed using Pearson product moment correlation coefficient. All results were presented as the mean±SD. The level of statistical significance was set at $P<0.05$ and $P<0.001$ for all comparisons. GraphPad Software GraphPad Prism 6 was used for graphical expression.

Results

Table I summarizes anthropometric, cardiorespiratory, serum IGF-1 and IGFBP-1 parameters within groups obtained at baseline and follow-up. Before the intervention period, body mass, $_{I}VO_{2max}$, $T_{Lim}VO_{2max}$, isokinetic peak torque at both angular velocities, blood lactate concentration, serum IGF-1 or IGFBP-1 parameters were not statistically significant among groups.

The results, displayed in Figure 1, show mean blood lactate concentrations for a given stage during $_{I}VO_{2max}$ test at baseline and the 8-week follow-up.

IGF-1 significantly elevated at post 30min and decreased at post 24 h and IGFBP-1 post 30 min was also found significantly decreased in HIIT+NMES compared to baseline measurements. However, IGFBP-1 reduced

TABLE I.—*Comparison of pre-test and post-test anthropometric, cardio respiratory, serum IGF-1 and IGFBP-1 parameters among the groups.*

Variable	Timepoint	Control (N.=6)	HIIT (N.=6)	HIIT+NMES (N.=6)	CA (N.=6)	CA+NMES (N.=6)
Body mass, kg	Before	75.27±7.25	77.13±7.22	74.50±3.72	68.12±9.64	75.22±7.79
	After	75.17±7.12	77.02±8.82	73.22±3.57	67.52±10.51	74.42±6.60
Lean weight, kg	Before	64.00±3.87	65.33±3.60	64.67±3.77	60.97±8.28	66.50±4.47
	After	63.90±4.09	66.75±3.36	64.63±3.30	60.07±9.40	66.25±5.00
Percent body fat, %	Before	14.60±5.54	14.62±6.68	13.16±4.01	10.37±4.19	11.58±5.45
	After	14.65±5.55	13.05±6.43	11.68±4.13	9.42±3.51	10.35±5.77
$_{I}VO_{2max}$, mL/kg/min)	Before	40.53±5.49	41.21±5.15	42.50±5.05	37.91±4.93	38.77±2.37
	After	38.93±1.77	46.92±4.49	51.34±3.55*	49.90±3.82*	47.70±4.49*
$T_{LIM}VO_{2max}$, mL/kg/min	Before	39.53±3.25	40.96±4.48	43.65±3.13	42.09±4.08	40.87±3.54
	After	39.43±2.06	44.72±3.85	50.82±2.97*	47.04±3.06	46.04±3.86*
IGF-1 $^{pre\ 15\ min}$, ng/mL	Before	5.84±6.80	8.74±11.81	12.80±12.97	14.10±16.04	11.53±8.26
	After	6.05±4.25	8.73±4.23	9.83±12.01	16.93±8.40	16.91±10.96
IGF-1 $^{post\ 30\ min}$, ng/mL	Before	8.24±6.62	8.27±8.16	12.69±9.97	13.68±12.61	11.74±9.52
	After	6.15±2.56	10.80±3.94	13.97±14.10*	13.17±7.75	16.05±9.80
IGF-1 $^{post\ 24h}$, ng/mL	Before	6.03±3.70	5.37±4.54	15.18±11.79	13.41±5.89	14.06±8.85
	After	6.91±2.73	5.59±1.61	7.72±7.23 *	12.28±8.17	11.76±4.85
IGFBP-1$^{pre\ 15\ min}$, ng/mL	Before	1.37±1.18	2.64±2.60	3.35±3.66	3.51±3.27	3.39±2.75
	After	1.15±1.27	2.02±2.74	1.19±1.46	1.88±0.96 *	1.79±1.26
IGFBP-1$^{post\ 30\ min}$, ng/mL	Before	1.97±1.14	2.40±1.48	3.20±2.88	2.90±1.98	2.96±1.49
	After	1.06±1.08	1.69±1.76	1.56±1.32*	1.70±1.21	1.46±0.85
IGFBP-1 $^{post\ 24h}$, ng/mL	Before	1.02±0.50	1.62±1.60	3.42±2.87	2.65±2.49	2.00±0.95
	After	0.66±0.25	0.95±0.67	2.38±3.60	1.55±1.08 *	1.23±0.56

Values are mean±SD.
*Significant change from pre-to post-training within same group ($P<0.05$).

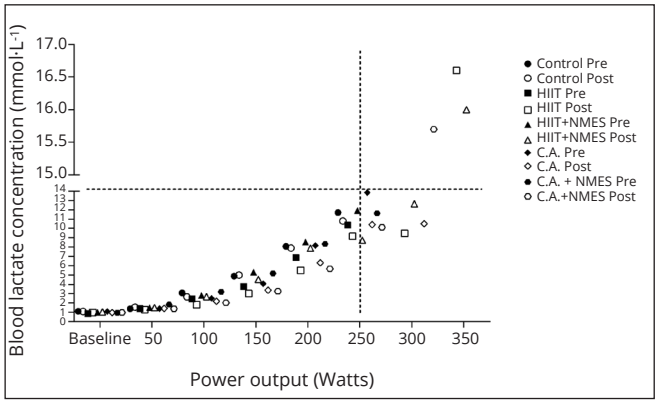

Figure 1.—Pre-and post-blood lactate values displayed as group means for a given stage during $_{I}VO_{2max}$ test. Notice the intersection point of dashed lines indicates the highest blood lactate concentration in baseline measurements.

significantly at post 30 min in HIIT+NMES (P=0.000) following the 8-week intervention period (Table II). Intragroup comparisons of post-test serum IGF-1 and IGFBP-1 was reported in Table II.

Eight week post IGF-1/IGFBP-1 ratios were found higher in pre 15 min, post 30 min and post 24 h than baseline measurements in all training groups (8.92±4.72 *vs*. 3.93±3.14, P=0.000; 9.41±3.72 *vs*. 3.99±1.76, P=0.000; 8.63±3.01 *vs*. 5.89±3.01, P=0.000, respectively). Changes in IGF-1/IGFBP-1 was not statistically significant in Control group.

There was not any statistically significant difference in $_{I}VO_{2max}$ among groups in baseline measurements (P=0.42). However, there was statistically significant improvements in $_{I}VO_{2max}$ in HIIT+NMES (43.65±3.13 *vs*. 50.82±2.97 mL/kg/min, P=0.028) and CA+NMES (40.87±3.54 *vs*. 46.04±3.86 mL/kg/min P=0.028) groups (Figure 2A). $T_{Lim}VO_{2max}$ increased by 20.8% in HIIT+NMES and 23.03% in CA+NMES group (Figure 2C). Significant difference (P=0.004) was observed in $T_{Lim}VO_{2max}$ between HIIT+NMES (51.34±3.55 mL/kg/min) and CA+NMES (47.70±4.49 mL/kg/min) group in follow-up measurements. Peak blood lactate concentration were significantly different subsequent to $_{I}VO_{2max}$ (Figure 2B) and $T_{Lim}VO_{2max}$ (Figure 2D) in CA+NMES and HIIT+NMES groups compared to control group in follow-up measurements.

Significant increases in right leg (303.17±46.14 *vs*. 304.17±31.98 Nm, P=0.014) and left leg (288.67±34.60 *vs*. 295.67±32.58 Nm, P=0.01) peak isokinetic quadriceps femoris torque at 60°/s angular velocity were observed from baseline to follow-up in HIIT+NMES group. A significant increase in peak isokinetic quadriceps femoris torque was also measured at 180°/s angular velocity in right leg (157.17±24.67 *vs*. 176.83±17.61 Nm, P=0.014) and left leg (157.17±15.63 *vs*. 170.83±24.11 Nm, P=0.042) following the HIIT+NMES intervention. Intragroup post-test comparisons of peak isokinetic torque was reported in Table III.

There was a positive correlation between $T_{Lim}VO_{2max}$ and knee extension (r=0.42, P<0.05), and $T_{Lim}VO_{2max}$ and knee flexion parameters (r=0.37, P<0.05), respectively. Both $_{I}VO_{2max}$ (r=-0.46, P<0.001) and $T_{Lim}VO_{2max}$ (r=-0.50,

TABLE II.—*Intragroup comparisons of post-test serum IGF-1 and IGFBP-1 parameters following 8 weeks of training.*

Variable	Groups	Mean±SD	Z	P value
IGF-1 pre 15 min, ng/mL	Control *vs*. CA	6.05±4.25 *vs*. 16.93±8.40	-2.25	0.024*
IGF-1 post 30 min, ng/mL	Control *vs*. HIIT	6.15±2.56 *vs*. 10.80±3.94	-2.09	0.037*
IGFBP-1 post 24 h, ng/mL	HIIT *vs*. CA+NMES	0.95±0.67 *vs*. 1.23±0.56	-2.09	0.037*

*Statistically significant difference between two groups at a significance level of P<0.05.

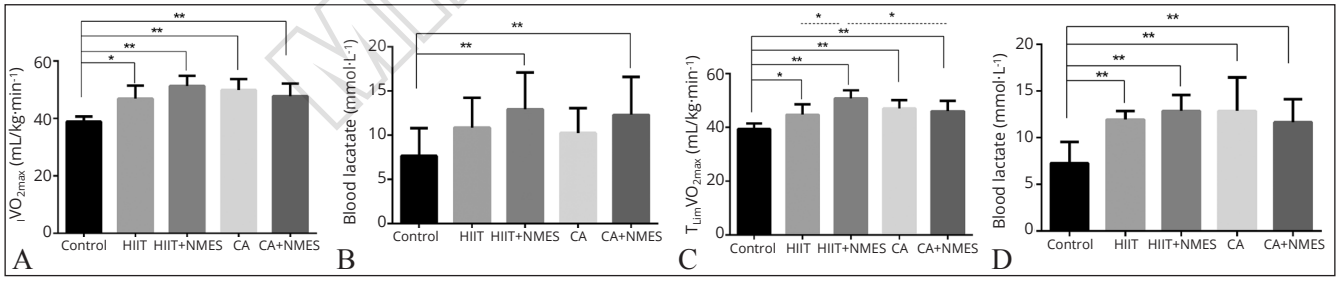

Figure 2.—A) Intragroup comparisons of post-test $_{I}VO_{2max}$; B) blood lactate concentration following $_{I}VO_{2max}$; C) $T_{Lim}VO_{2max}$; D) blood lactate concentration following $_{I}VO_{2max}$ following 8 weeks of training.

TABLE III.—*Intragroup comparisons of post-test 60 °/s and 180 °/s isokinetic strength parameters following 8 weeks of training.*

Variable	Groups	Mean±SD	Z	P value
60 °/s extension right, Nm	Control *vs.* HIIT+NMES	257.83±23.16 *vs.* 304.17±31.98	-1.94	0.053*
	HIIT+NMES *vs.* CA+NMES	304.17±31.98 *vs.* 249.00±26.59	-2.73	0.006**
	HIIT+NMES *vs.* CA	304.17±31.98 *vs.* 230.67±29.79	-2.41	0.016*
60 °/s extension left, Nm	HIIT+NMES *vs.* CA+NMES	295.67±32.58 *vs.* 247.33±27	-2.56	0.010*
	HIIT+NMES *vs.* CA	295.67±32.58 *vs.* 225.50±39.85	-2.24	0.025*
180 °/s extension right, Nm	HIIT+NMES *vs.* CA	176.83±17.62 *vs.* 151.00±17.74	-2.65	0.008**
	HIIT+NMES *vs.* CA+NMES	176.83±17.62 *vs.* 172.17±16.77	-2.08	0.037*
180 °/s extension left, Nm	Control *vs.* HIIT+NMES	134.50±18.99 *vs.* 170.83±24.11	-2.41	0.016*
	HIIT+NMES *vs.* CA	170.83±24.11 *vs.* 152.33±32.51	-2.89	0.004**
	HIIT+NMES *vs.* CA+NMES	170.83±24.11 *vs.* 155.83±23.91	-2.24	0.025*
180 °/s flexion right, Nm	Control *vs.* HIIT+NMES	93.33±7.94 *vs.* 117.00±8.65	-2.33	0.020*
	HIIT *vs.* HIIT+NMES	95.83±19.70 *vs.* 117.00±8.65	-2.08	0.037*
	HIIT+NMES *vs.* CA	117.00±8.65 *vs.* 98.33±11.36	-2.33	0.020*
	HIIT+NMES *vs.* CA +NMES	117.00±8.65 *vs.* 98.17±7.39	-2.56	0.010*
180 °/s flexion left, Nm	Control *vs.* HIIT+NMES	76.00±9.27 *vs.* 110.67±10.13	-2.57	0.010*
	HIIT+NMES *vs.* CA	110.67±10.13 *vs.* 86.33±10.86	-2.81	0.005**
	HIIT+NMES *vs.* CA+NMES	110.67±10.13 *vs.* 87.83±8.70	-2.56	0.010*

Statistically significant difference between two groups: *P<0.05; **P<0.001.

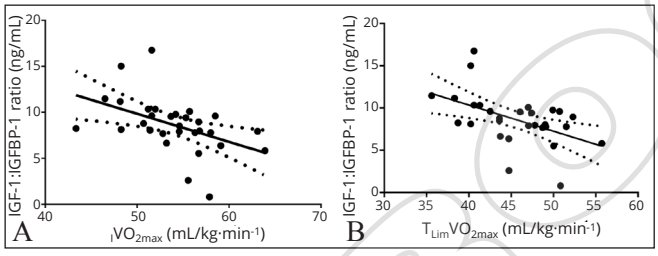

Figure 3.—A) Correlations between IGF-1/IGFBP-1 ratio and $_{I}VO_{2max}$; B) IGF-1/IGFBP-1 ratio and $T_{Lim}VO_{2max}$.

P<0.001) were found to be negatively correlated with the ratio of IGF-1/IGFBP-1 (Figure 3A, B).

The changes occurred in baseline (Figure 4A) and post 24 h IGF-1 (Figure 4C) parameters were negatively correlated with IGF-1/IGFBP-1 ratio while IGF-1 post 30 min (Figure 4B) was not correlated with IGF-1/IGFBP-1 ratio. Additionally, there was a positive significant correlation between IGFBP-1 and VO_{2max} parameters (Figure 4D) and a negative correlation between IGF-1 and HR (r=-0.492, P<0.05).

Discussion

To our knowledge, this is the first study that examines the effects of HIIT and CA training coupled with high-and-low-frequency neuromuscular electrostimulation on IGF-1, IGFBP-1, oxygen kinetics, and neuromuscular performance.

Studies to date indicated varying acute adaptations to HIIT training such as changes in the hormone levels, heart rate, venous blood glucose level and lactate levels, and metabolic adaptations. On the other hand, in the context of CA and NMES, biomarkers of particular interest suggested to be responsive to physical activity include such as insulin-like growth factor-1 (IGF-1) promoting growth and maintenance of muscle.[20] The results of the current study revealed statistically significant differences among groups in Serum IGF-1 and IGFBP-1 parameters from baseline to follow-up measurements (Table I). The data demonstrate the existence of a significant acute decrease in circulating IGF-I of 49% after HIIT+NMES exercise.

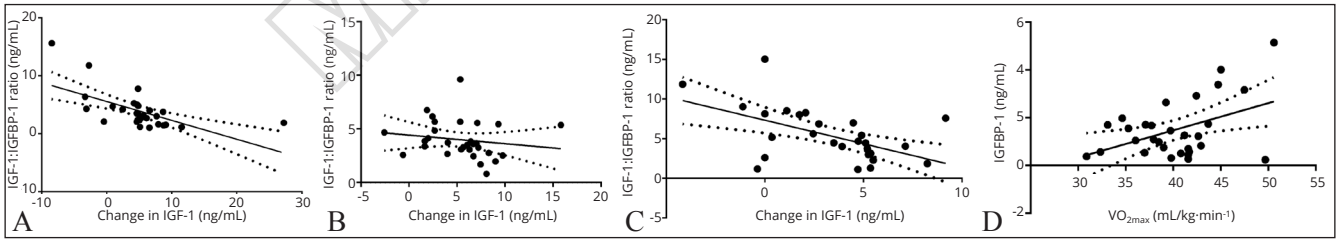

Figure 4.—A) Correlations between IGF-1:IGFBP-1 ratio and change in IGF-1 pre 15 min parameters; B) IGF-1:IGFBP-1 ratio and change in IGF-1 post 30 min parameters; C) IGF-1: IGFBP-1 ratio and change in IGF-1 post 24 hour parameters; D) IGFBP-1 and VO_{2max} parameters.

Similarly, Nemet *et al.*[21] investigated the effect of high-intensity exercises at the anaerobic threshold on IGF-1 and body mass index parameters and reported a decrease in IGF-1 levels even in weight-stable individuals. Nindl *et al.*[22] reported decreased IGF-1 levels following moderate to severe aerobic and HIIT exercises but the results were not found statistically significant. However, we found in this study that IGF-1 was significantly elevated after 30 min in HIIT+NMES group following 8 weeks of training. This suggests that, the use of NMES during high intensity exercises with shorts periods helps to protect the muscle mass and prolong the availability of IGF-1 which is necessary for the maintenance and growth of the cells. It was reported that ES of muscle cells *in vitro* not only directly modulates the gene expression of contractile proteins but also modulates proteins that are part of the IGF regulatory system.[9] However, compared to baseline and post 30 min measurements the ratio of IGF-1/IGFBP-1 determined 24 h later following $_{I}VO_{2max}$ test was found to be significantly elevated after 8 weeks of intervention among groups. It was noted in a study that the decrease of the ratio of IGF-1/IGFBP-1 indicates less free IGF-1 while the increased IGF-1 bioavailability suggests an anticatabolic effect.[23] It could be resulted from impaired protein synthesis, protein catabolism and amino acid release from muscle during exercise but stimulated during the recovery from exercise.

Furthermore, it was also asserted that the metabolic, structural, and functional changes occurring in the heart as a response to exercise are influenced by receptor signaling triggered by IGF-1 pathway which promote physiologic cardiac growth. Moreover, IGF-1 may also augment the adaptation of the heart to overload which in turn suppressed myosin heavy chain expression resulted from this adaptive mechanism enhances a balance between the economy of contraction and contractility.[24-26] As a result of different stimuli induced by different exercise modalities the negative correlation occurred between IGF-1 and HR in this study suggests that these hormones may provide additional regulation to acute or chronic metabolic changes induced by exercise.

Despite no significant reduction in fat mass between pre and post test, percent body fat values decreased in HIIT (10.74%), HIIT+NMES (11.25%), CA (9.16%) and CA+NMES (10.62%) training groups but increased in Control (0.34%) group in our study. Both HIIT+NMES and CA+NMES showed a greater decline in percent body fat compared to HIIT and CA groups without NMES following 8-weeks of training which suggests that NMES exercise resulted in greater calorie consumption and therefore yielded greater reductions in percentage body fat. It could be speculated that NMES accompanied training regimens may have been resulted in a greater decrease in percent body fat as a result of increased fatty acid oxidation in skeletal muscles during CA+NMES and increased insulin sensitivity and glycolytic enzyme activity during HIIT+NMES training. Gibala *et al.*[27] collected muscle biopsy samples in order to examine metabolic adaptations of the skeletal muscles to HIIT training and reported that the use of net glycogen stores decreased while β hydroxy acyl coenzyme dehydrogenase activity, catalyzing fat oxidation, increased significantly after HIIT training.

It has been stated that VO_{2max} capacity may increase up to 4% to 46% depending on the age, physical fitness level, duration and intensity of the activity.[28] There was no statistically significant difference between the pre and post VO_{2max} levels in Control (-3.95%) and HIIT (13.86%) groups while HIIT+NMES (20.8%), CA (31.63%) and CA+NMES (23.03%) groups showed statistically significant improvements in VO_{2max} in this study. These results revealed that NMES can be successfully used to elicit a physiological response in healthy individuals during high-intensity exercises, but recent evidence appears to suggest that there was no positive effect of low-frequency neuromuscular electrostimulation coupled with CA training on VO_{2max} performance. Based on the results of another study it was reported that NMES only induced approximately such as 76 kcal/h on total calories and 0.26 L/min on oxygen consumption without producing movement or voluntary exercise such as ergometer or treadmill training.[29] These findings supported the hypothesis that NMES is a useful tool for improving cardiopulmonary function and are in accordance with the results of our study.

During high intensity cycling exercises such as $_{I}VO_{2max}$ the energy required to maintain the activity after a few min will be fully met by anaerobic energy metabolism. As a result of the exposure of the organism to this level of stress, the lactate level in the muscles reaches 8-10 mmol/L during the performance.[30] Peak blood lactate concentrations determined using resting and 2 min after T_{Lim} was found statistically significant in favor of HIIT+NMES compared to other groups in our study (Figure 2D). Consistent with our results high lactate levels, up to 15.3 mmol/L, were recorded[31] even with traditional NMES techniques.[32] This indicates that NMES can induce large metabolic demand and exercising with this system appears to preferentially use carbohydrate as a substrate, that is, exercise at a very high respiratory quotient. Owing to use of NMES Vivodtzev *et al.* noted a significant improvement in maximal

voluntary contraction of the quadriceps muscle.[33] It was asserted that contractions of leg muscles stimulates leg muscle venous pump activity, increasing venous return, stroke volume, and cardiac output during high intensity exercises. Intriguingly, NMES results in increased strength and muscle endurance capacity as a results of hypertrophy and histochemical changes in muscles, by increasing capillary density, mitochondrial concentrations, and oxidative enzyme levels, associated with the transformation of muscle fiber. These changes proportionally increase aero-metabolic capacity, prevent of muscle atrophy, and increase resistance to fatigue during intense exercises.[34-36]

We found in this study that (Figure 1) the characteristics of HIIT+NMES training enabled the participants to cope with the fatigue during the high intensities and the improvement in the blood lactate concentration caused less fatigue and oxidative stress on the organism resulted from the exercise intensity. Although direct blood flow was not measured in the study, NMES has been previously shown to increase blood flow.[37] Therefore, NMES may have an effect on localized blood flow and increased metabolite removal from the fatigued muscles which possibly increase the efflux of contraction induced metabolites from the stimulated muscle mass owing to its role on muscle-pump, analgesic effects on muscle soreness and lymphatic drainage to the stimulated area.[38]

In contrast, the effect of lowering blood lactate may not necessarily enhance subsequent performance, especially given that lactate is no longer seen as a causative effect of muscle fatigue[39] but "a regulatory molecule that modulates the integration of metabolism" including glucose. Furthermore, traditional NMES techniques are known to induce different metabolic adaptations compared with voluntary exercise. The results of a study reported glycolytic-to-oxidative shift in the metabolic profile resulted from NMES and showed a significant shift in muscle fiber type from myosin heavy chain (MHC) MHC-2X towards MHC-2A and MHC-1 which indicates a muscle fiber shift towards oxidative metabolism.[8] Also, it could be speculated that as a result of the neural activations in quadriceps and rectus femoris muscles, which have a high level of growth hormone secretion, HIIT+NMES group showed the highest improvement in oxygen consumption during $T_{Lim}VO_{2max}$ compared to other groups by generating a possible fast-slow muscle fiber-type transitions. According to the literature, it was suggested that maximum strength training used in addition to the training could be very effective in improving T_{Lim} values in runners and cyclists.[40, 41] In our study, we used a 45-60 Hz and a current of 300 μs NMES on quadriceps and rectus femoris muscles coupled with HIIT training at which a training intensity 120% of individual $_{I}VO_{2max}$ rather than supplemental strength training. We designed a HIIT and CA testing protocol coupled with NMES application to test whether an increased neural activity as a result of the neuromuscular activity would favor an improvement in $T_{Lim}VO_{2max}$ performance. In this regard, the result of the current study revealed that NMES may have been an effective means of achieving the greatest improvements in $T_{Lim}VO_{2max}$ performance occurring with HIIT+NMES exercise. However; no statistically significant differences occurred among groups in time to exhaustion duration despite the highest T_{Lim} values were observed in HIIT+NMES group (Control: 184.35±113.81 s; HIIT:222.05±69.48 s; HIIT+NMES: 276.85±139.54 s; CA:203.09±81.41 s; CA+NMES:180.56±91.71 s) which also showed significant improvements in strength parameters at both angular velocities compared to other groups (Table III). The increase in both $T_{Lim}VO_{2max}$ and strength parameters reflect the intensity of NMES training. Additionally, the results of Pearson product moment correlation coefficient indicated positive correlations between $T_{Lim}VO_{2max}$ and knee extension, and $T_{Lim}VO_{2max}$ and knee flexion parameters, respectively. Maffiuletti *et al.*[42] reported that significant differences occurred in strength parameters and participants increased their vertical jump height by 17% following 8 weeks of NMES on knee extensor muscles. This shows that the resistance occurred in quadriceps and hamstring group muscles resulted from the high-intensity during pedaling coupled with NMES induce neuromuscular adaptations required for the optimal pedaling cycle but does not enable participants to tolerate the intensity during the time to exhaustion tests unless performing additional strength training for lower extremity muscles.

Conclusions

The results of the current study suggest that NMES can be used to elicit a physiological response and may have particular advantages of this exercise modality. Another significant finding of this study was that when used in addition to voluntary exercise NMES increases strength, muscle endurance and also VO_{2max} capacity by inducing histochemical changes in muscles which may possibly due to an increase in capillary density, mitochondrial concentrations, and oxidative enzyme levels. In terms of biomarkers in this study, exercise-induced alterations of IGF-I seems to depend on several factors such as exercise modality and intensity of the exercise. In particular:

• short-term cycle ergometer HIIT at 120% $_{I}VO_{2max}$ level increases circulating IGF-I concentrations after training while HIIT+NMES at the same intensity significantly decreases circulating IGF-I concentrations after 24 hours following the exercise, and IGFBP-1 levels after 30 min of exercise, without changing body weight;

• VO_{2max} positively correlates with changes in IGFBP-1 level both during pre- and post-trainings which shows the importance of exercise alone, rather than changes in body composition, on the regulation of IGF-I and IGFBP-1 before, during and after the exercise.

Further studies are needed to examine whether increased IGF-I levels subsequent to HIIT+NMES resulted from an exercise-GH-IGF-I interaction or a GH-independent direct effect of NMES on IGF-I and also longer periods of exercise using different type of interventions induce sustained and greater impacts on the changes in IGF-I and IGFBP-1 levels.

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