Neuromuscular performance after rapid weight loss in Olympic-style boxers

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Abstract

The present study investigated the effect of a 3% rapid weight loss (RWL) procedure on neuromuscular performance in elite, Olympic-style boxers. Nine boxers were randomly assigned to two experimental procedures (RWL and control, in a randomized counter-balance order) to perform 5-s maximum isometric voluntary contractions (MVC) of the dominant leg knee extensors prior to (MVC1), and following (MVC2), a sustained, isometric contraction at 70% MVC until exhaustion. The voluntary activation (VA) was determined using percutaneous muscle stimulation and interpolated twitch technique. High (at 80 Hz) and low (at 20 Hz) frequency tetanic impulses were also delivered before and after the sustained 70% MVC to assess peripheral fatigue. Hydration status, hemodynamic parameters, and lactate concentration were assessed throughout the study. Body-mass was reduced by ~3% (during RWL) compared to control ($p = .001$). As a result of the RWL protocol, MVC1 force output was 12% lower and VA deficits of 7% were observed after the fatigue protocol compared to control ($p = .001$). Following RWL, time to exhaustion for the sustained 70% MVC was 69 ± 20 s compared to 86 ± 34 s for control ($p = .020$). Peak lactate production was 53% lower in RWL compared to control ($p = .001$). In conclusion, the 3% RWL procedure translated into significant decline in neuromuscular performance for both brief and sustained contractions in competitive boxers.

Keywords: Dehydration, combat sports, caloric restriction, fatigue

Highlights

- The 3% RWL reduces maximal force production of the knee extensor muscles by ~12% in Olympic-style boxers.
- The force output and voluntary activation were each significantly reduced after a fatiguing contraction following 3% RWL protocols.
- Current findings suggest that neuromuscular fatigue development, as observed from the voluntary activation deficits, has a centrally-mediated component following a 3% RWL procedure.

Introduction

Muscle strength and power play an important role in the competitive success of Olympic-style boxing (Chaabène et al., 2015). Pre-competition rapid weight-loss (RWL) is a typical practice among many weight-class athletes that involves the "rapid" reduction of body-mass (B_M), primarily due to a loss of both intracellular and extracellular fluid, which in itself can lead to the development of serious, health and performance-related issues (Reale, Slater, & Burke, 2017). To achieve RWL and meet optimal competition B_M that (supposedly) translates into competitive success, Olympic combat athletes use different strategic approaches, including voluntary weight-loss (food and fluid restrictions) combined with passive heating (sauna exposures). Recently, Barley, Chapman, Blazevich, and Abbiss (2018) showed that 3% dehydration does indeed contribute to detrimental effects of the knee extensors (KE) after sustained contractions (at 85% maximal voluntary contraction – MVC, until exhaustion) in Australian combat athletes. In that study, athletes achieved the 3% B_M loss by using a 3 h passive

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heating protocol in an environmental chamber set at 40°C and 63% relative humidity. However, it has been well-established that passive hyperthermia alone attenuates neuromuscular performance in recreational and well-trained athletes (Morrison, Sleivert, & Cheung, 2006; Nybo & Nielsen, 2001; Périard, Caillaud, & Thompson, 2011). Unfortunately, this also means that a protocol which uses passive heating as its primary means to create RWL reductions cannot discriminate between the separate and combined effects of subsequent dehydration and hyperthermia on neuromuscular performance. Aside from these typical RWL procedures, athletes competing in Olympic combat sports undergo periods of intentional food and fluid restriction to manipulate B_M to achieve weight-class requirements.

Often, Olympic-style boxers will restrict their overall energy intake by ~40%, which tends to include a ~45% reduction in carbohydrate intake and ~35% reduction in water consumption over a 3–5 days period (Reljic et al., 2015). It has been demonstrated that 3–5% RWL via nutritional restriction impairs haematological parameters, but does not affect maximal oxygen uptake in combat athletes (Reljic, Feist, Jost, Kieser, & Friedmann-Bette, 2016). However, RWL procedure by up to 5% result in reduced skeletal muscle glycogen concentrations (by ~50%), concomitant with whole-body fluid deficits (Tarnopolsky et al., 1996). Yet, subsequent rehydration attempts fail to counter the deleterious effects of RWL during a limited recovery period (2–6 h) between their official weigh-in and competition start times (Artioli, Saunders, Iglesias, & Franchini, 2016; Pettersson & Berg, 2014). This is evident from biopsy findings that have determined a minimum of 17 h is required to completely replenish skeletal muscle glycogen concentrations in fighters who performed RWL via dietary restriction (Tarnopolsky et al., 1996). Consequently, a boxer's ability to repeatedly generate striking force throughout a match may be compromised by the incomplete restoration of skeletal muscle glycogen concentrations. Surprisingly, no study has quantified whether pre-competition RWL (food/fluid intake restrictions) may affect one's ability to efficiently activate and sustain voluntary muscle contractions, although it is clear that the excessive metabolic demands of Olympic-style boxing do impose certain muscle fatigue developments (Hanon, Savarino, & Thomas, 2015), Thus, the purpose of this study was to investigate the effects of RWL on neuromuscular performance in elite, Olympic-style boxers. We hypothesized that RWL would decrease neuromuscular performance and diminish force production capacity compared to the control condition and that the magnitude of decreases would be greater following an exhaustive, self-regulated contraction at a pre-defined 70% of MVC.

Material and methods

This study was approved by the National Committee for Medical Ethics at the Ministry of Health of the Republic of Slovenia (Ethics No. 0120-532/2015-2) and conducted in conformation with the guidelines of the Declaration of Helsinki. Following a detailed presentation of the study design, written informed consent was obtained from each boxer prior to further participation.

Study design and participants

Thirteen elite, internationally-competing male boxers were recruited to participate in this randomized, counter-balance, repeated measures investigation. All boxers underwent one familiarization session, and were then randomized (counterbalanced order) to one of two experimental conditions: (i) control trial or (ii) RWL trial; thus all participants acted as their own control. The exclusion criteria were outlined as follows: previous musculoskeletal injuries of lower-limbs, supplement consumption, international competitive experience, and diuretics misuse. To remove possible confounding factors of previous weight-loss habits, a validated questionnaire (Artioli et al., 2010) on weight management and its associated protocols was given to all athletes. Based on the above-mentioned criteria and their previous weight management history, a homogenous group of 11 boxers were selected for inclusion in the study; two were later excluded for not meeting pre-testing guidelines (e.g. supplement consumption). Thus, a total of nine boxers (24.2 ± 2.5 years, 1.83 ± 0.05 m, 76.3 ± 7.7 kg, 11.2 ± 2.5% body-fat percentage) completed all trials and included for further data analysis. The boxers were advised to keep a dietary intake log and repeat training bouts at the same volume and intensity throughout the duration of the study. They did not ingest any diuretics or dietary supplements over the course of the study or engage in any physical activity at least 24 h prior to experimental testing. On average, boxers reported 4.9 ± 3.5 years of competitive experience, 10.8 ± 1.3 training hours per week, and a minimum of 3 years of previous weight-loss experience.

Familiarization procedures

All boxers completed a full familiarization trial one week prior to taking part in any of the experimental procedures. The familiarization procedures included an assessment of their resting heart rate (RS 300x, Polar, Kempele, Finland), body-height and mass (Seca 769, Hamburg, Germany) and percent body-fat via Jackson and Pollock 7-site skinfold measure (Jackson & Pollock, 1978). In addition, to determine their hydration status, the boxers were educated in the technique of urine collection (mid-stream from the first-morning void), as outlined by the American College of Sports Medicine's (ACSM) guidelines (Sawka et al., 2007). During all experimental procedures the boxers collected their first-morning void, which they presented to the laboratory for urine specific gravity (U_{SG}) determination, on the day of experimental testing. The neuromuscular function measurement included a 5-second MVC with interpolated twitch and train stimulations. During brief contractions (5-s MVC), the athletes were coached to reach maximal effort as quickly as possible and to sustain this level for the duration of the contraction. After familiarization with MVC testing, an actual test was performed from which we determined the 70% MVC effort that would be used in the experimental trials. The fatiguing trial involved sustaining an isometric contraction of the dominant leg KE muscle group at 70% MVC until voluntary exhaustion. The exhaustion threshold was fixed at the point when the boxer could not sustain the contraction required to maintain the target level of muscle force for 5 s. In order for participants to maintain the 70% MVC contraction, visual feedback of muscle force and target force production was displayed on a screen throughout the entire experimental protocol. The KE muscle groups were chosen because their power generating capacity has been identified as essential for contributing to peak punching force in elite boxers (Filimonov, Koptsev, Husyanov, & Nazarov, 1985), and the 70% MVC intensity was chosen after pilot testing to impose a high degree of stress to the neuromuscular system, as previously suggested in the literature (Bigard et al., 2001).

Experimental measures

Following an overnight fast, all boxers reported to the laboratory between 7:30 and 8:30 h. The temperature and relative humidity in the laboratory were ~18°C and ~40%, respectively. The boxers took part in two experimental procedures (RWL and control), each separated at least two weeks of washout period. Immediately upon arrival to the laboratory, boxers were asked to provide the urine sample that they had collected themselves at home from their first-morning void, which was used to determine U_{SG}

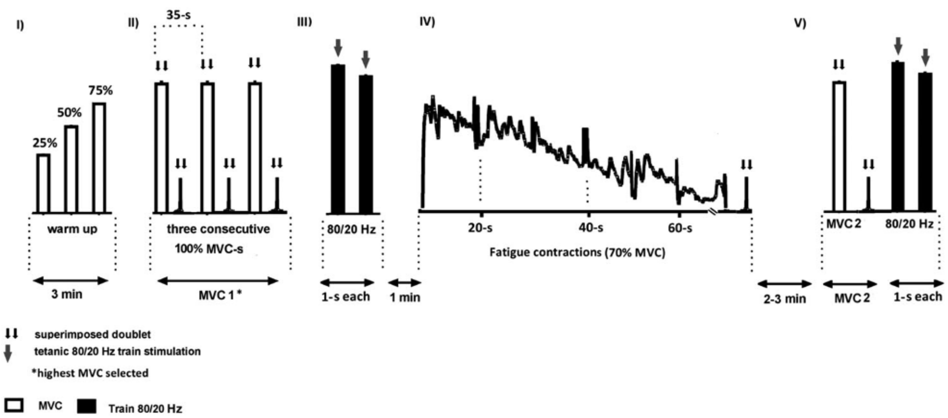

Figure 1. Schematic illustration of the neuromuscular assessment protocol example: Prior to muscle function testing, boxers completed a 10 min stepping warm up. (i) Brief MVC: Athletes completed a warm-up consisted of three 5-s contractions at 25%, 50% and 75% effort (predetermined during familiarization procedures), separated by at least 60-s; (ii) MVC, with interpolated twitch technique performed in one set of three 5-s MVCs, separated by at least 60-s, during which an electrical doublet was superimposed (100 Hz) and control doublet delivered within 5-s post contraction; The greatest MVC (MVC1) was selected as baseline for further analysis; (iii) high-frequency fatigue (HFF, at 80 Hz, with 41 pulses) and low frequency (LFF, at 20 Hz, with 11 pulses) tetanic stimulation: electrical bursts were delivered (separated by 30 sec) immediately after baseline MVC-s and before the fatigue protocol, as previously outlined by Martin et al. (2004); (iv) Fatigue protocol: boxers performed a self-regulated sustained contraction with 70% of their peak MVC (predetermined during familiarization) established as exhaustion threshold. The exhaustion threshold was fixed at the point when the boxer could not sustain the contraction required to maintain the target level of muscle force for 5 s. A post-activation control doublet delivered in the same manner as outlined above, following previously established criteria (Morrison et al., 2006; Nybo & Nielsen, 2001; Périard et al., 2011). Time-to-exhaustion was determined when the participants were unable to maintain the desired percentage of force production for five seconds; (v) Post-fatigue protocol; after completing the fatigue protocol, a final, 5-s MVC2 was superimposed and a control doublet was delivered within 5-s post contraction, followed by HFF (at 80 Hz) and LFF (at 20 Hz) tetanic stimulation bursts. More precisely, immediately after MVC2 electrical stimulation of the relaxed KE was performed, which consisted of delivering two consecutive, 0.5-s tetanic trains at 80 Hz (HFF) and 20 Hz (LFF), in identical fashion described above (ii and iii part of the experimental protocol outlined in Figure 1).

readings. Venous blood samples were taken after the athletes were rested, supine for 15 min. After blood sampling, semi-nude body-mass was measured. Following hydration status assessment, muscle force and voluntary activation level (VA) were measured. The experimental protocol consisted of five phases, which are depicted in Figure 1 with full details on total protocol duration explained in the legend of Figure 1 (supplementary file).

Neuromuscular assessment protocol

Boxers were seated on a commercially available dynamometer (S2P Ltd., Ljubljana, Slovenia), with the knee fixed at 60° flexion, secured by straps around the leg and chest to ensure movement restriction. For the purpose of the MVC assessment, the dominant leg was attached to a dynamometer lever with a calibrated force transducer (Z6FC3-200 kg, HBM, Darmstadt, Germany) at the lateral malleolus. Carbon rubber electrodes (5 × 10 cm, Medicompex, Switzerland) were placed proximally (anode) and distally (cathode) on the KE muscles. A custom-made constant current electrical stimulator (Wise Technologies Ltd., Ljubljana, Slovenia, max output voltage at 400-V) delivered square-shaped doublet pulses at 100 Hz (duration: 1 ms), to the relaxed KE-muscles (vastus lateralis, vastus medialis, rectus femoris). The optimal intensity of the superimposed doublet was determined at the start of the MVC assessment by increasing the current of the stimulus in 25 mA increments (beginning at 25 mA) until no further increase in doublet amplitude was observed (Morrison et al., 2006). The voluntary activation (VA) level was calculated as previously described by Merton (1954). The tetanic stimulation bursts at 80 Hz (41 pulses) and 20 Hz (11 pulses) were delivered on the KE muscles as described previously in the literature by Martin, Millet, Martin, Deley, and Lattier (2004), via two consecutive trains of squared wave pulses (duration: 0.5 s) to assess the excitation-contraction coupling efficiency, pre–post 70% sustained MVC fatigue protocol. Also, a ratio of the 20–80 Hz stimulations (20/80 index) was calculated since a decrease in this ratio is suggested to characterize low-frequency fatigue (LFF) development (Martin et al., 2004). The electrical bursts intensity ranged from 30 to 75 mA, and were gradually increased to reach one-third of pre-determined MVC output readings, recorded at baseline of 80 Hz tetanic stimulation, as suggested in the literature by Martin et al. (2010). All force signals were amplified, sent through an A/D conversion board and sampled at a frequency of 2 kHz, using in-house data-acquisition software (LabView, National Instruments, Austin, Texas, USA). Contractile characteristics of the KE muscles were calculated from the evoked resting doublet post MVC, as previously reported (Martin et al., 2004; Martin et al., 2010). The average value of force produced, during the MVC was sampled for 500 ms, prior to delivering percutaneous muscle stimulation. The contractile rate of force development (RFD) was calculated over a 50 ms time interval relative to the onset of evoked contraction (Maffiuletti et al., 2016).

Rapid weight loss protocol

During the familiarization testing, athletes were instructed to reach ~3% B_M reduction, via food and fluid restriction exclusively, within a 3-day time frame before returning to their next experimental session, following methods previously described for RWL (Reljic et al., 2015). The blood and urine analysis were performed immediately upon arrival to the laboratory by the same laboratory staff. According to self-reported diet logs, boxers used a combination of low carbohydrate diet and fluid restriction to accomplish the 3% B_M deficit.

Haematological measurements

Blood samples (~10 mL) were drawn from the antecubital fossa of the athlete's left arm and analysed using an automated haematology analyzer (AU680 Chemistry System, Beckman Coulter). The intra-assay reliability for estimated haematology concentration values was excellent (coefficient of variation <1%). Electrolytes concentration (Na^+, Ca^{2+}, K^+) were determined photometrically (AU-680 Chemistry System, Beckman Coulter). Change in plasma volume (Δ PV) was calculated in agreement with Dill and Costill (1974) while haematocrit values were multiplied by 0.96 and then 0.91 to correct for trapped plasma and the venous-to-whole blood haematocrit excess.

Urine sampling

The urine samples provided by the boxers were analysed (in duplicate) to determine U_{SG} values in agreement with the ACSM hydration testing guidelines (Sawka et al., 2007) (AtagoPal-10s refractometer, Tokyo, Japan). The refractometer was calibrated with distilled water before use and provides accurate readings to the 0.001 unit.

Lactate concentration

Finger-prick lactate concentrations (5 µL of capillary blood) were determined using a portable lactate analyzer (Accu-Chek, Accutrend Plus, Roche Diagnostics,

Table I. Haematological and biochemical indices of hydration status (data are given as mean ± SD).

Variables	Control	RWL	p-level
Erythrocytes (pL)	4.9 ± 0.5	4.8 ± 0.3	0.811
Leukocyte(10^9/L)	6.2 ± 1.4	6.0 ± 1.2	0.421
MCV(fL)	90.6 ± 9.4	93.6 ± 3.4	0.735
MCH (pg)	29.4 ± 3.4	30.4 ± 1.2	0.569
MCHC (g/L)	324.0 ± 9.7	324.4 ± 4.7	0.484
Hb (g/L)	144.9 ± 7.9	145.8 ± 2.5	0.595
Hct (%)	44.7 ± 2.0	45.0 ± 1.0	0.893
Δ PV (%)	—	1.2 ± 6.8	—
RDW (%[1])	13.6 ± 1.4	13.4 ± 0.6	0.972
Thrombocytes (10^9/L)	176.3 ± 48.3	177.8 ± 29.6	0.833
MPV (fl)	9.9 ± 2.03	9.7 ± 1.9	0.029*
Sodium (mmol/L)	135.0 ± 2.9	137.6 ± 2.5	0.091
Potassium (mmol/L)	4.1 ± 0.4	4.3 ± 0.2	0.364
Chlorides (mmol/L)	100.0 ± 2.7	101.3 ± 3.1	0.116

Notes: Abbreviations: RWL, rapid weight loss; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; Hb, hemoglobin concentration; Hct %, haematocrit; PV, plasma volume change; RDW, relative distribution of red blood cell; MPV, mean platelet volume. *Different from control ($p < 0.05$).

Switzerland), as suggested by Tanner, Fuller, and Ross (2010). Lactate readings were determined at three time-points during the experiment, once at the beginning (baseline), once immediately after completing the fatigue protocol, and 5-min after completing the fatigue protocol.

Statistics

Statistical software was used for all calculations (SPSS version 19.0, IBM Inc, Chicago, USA). Normality was determined using the Shapiro–Wilk test. Student's paired t-test was applied to establish differences between the hydration status indicators (body-mass, blood and urine data) over two experimental conditions. All primary outcome variables were entered into a two-way repeated measures GLM, taking into account the condition (control, RWL) and time (pre–post fatigue protocol assessment) as factors, with Greenhouse-Geisser correction applied if necessary. Where a significant F-test was identified, less conservative Fishers's post hoc was applied to determine multiple comparisons. Additionally, student t-tests were applied to establish pairwise comparisons between the fatigue data (time to exhaustion protocols). Homogeneity of variance was evaluated using Levine's test, while the Mauchly's test was used to confirmed compound symmetry. The degree of effect was determined for dependent variables using the partial eta-squared (η^2), while the partial eta squared readings of 0.02; 0.13 and 0.33 were rated differences as small, moderate and high (Pierce, Block, & Aguinis, 2004). Pearson's correlation coefficients were calculated between (i) percent change in brief MVCs and peak lactate concentration for both control and RWL conditions, and between (ii) percent change brief MVCs and percent change in B_M following RWL. Statistical significance was accepted at $p < .05$ for the main effects and $p < .10$ for interaction effects. All data are presented with mean ± SD.

Results

Preliminary findings

Normality, homogeneity of variance and sphericity assumptions were not violated in any of the dependent variables ($p > .05$). The intra-assay reliability among the three consecutive 5-s MVCs was satisfactory (coefficient of variation <6%).

Hydration status and haematology

Body-mass was significantly reduced after RWL (–2.9%, from 76.3 to 74.1 kg) compared to control trial ($p < .001$). Urine specific gravity exceeded hypohydration definitions in RWL 1.024 ± 0.005 g mL^{-1} ($p = .001$), when compared to control (1.018 ± 0.007 g mL^{-1}). Hemodynamic results indicated that only mean platelet volume decreased in response to RWL ($p = .029$, Table I).

Brief MVC-s

Regarding the MVC force output, a significant main effect of condition ($p = .001$, $\eta^2 = 0.71$) such that for the RWL condition, MVC1 started significantly lower than control (225 ± 37 vs. 257 ± 43 N, respectively). There was also a main effect of time ($p = .010$, $\eta^2 = .62$) in that MVC2 was lower than MVC1 by ~10% in both conditions ($p = .003$). A Fisher's post

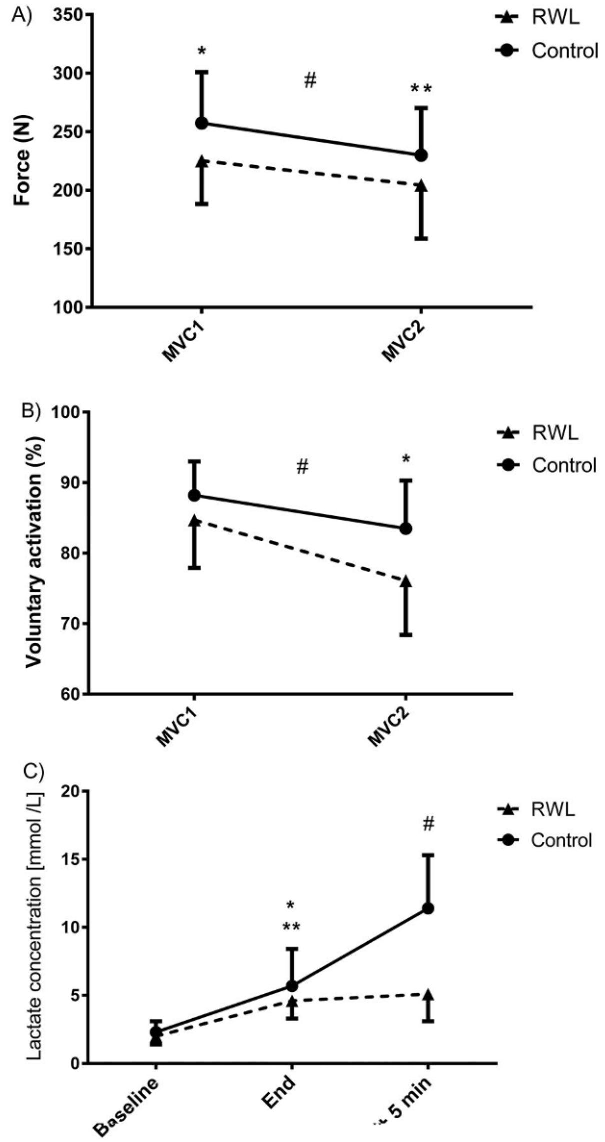

Figure 2. Force production (A) and voluntary activation (B) during brief maximal voluntary isometric contractions pre and post fatigue protocol; lactate production readings (C) determined at three time-points during the experiment, at the beginning (baseline), immediately after the fatigue protocol of the muscle function assessment, and a final sampling on the fifth minute post-fatigue protocol. *Fishers post hoc analysis of main effect of time ($p < 0.05$). **Fishers post hoc analysis of main effect of condition ($p < 0.05$). #Fishers post hoc analysis of interaction effect ($p < 0.10$).

hoc test also revealed a significant interaction effect between conditions, with greater MVC loss after RWL ($p = .001$, $\eta^2 = .31$; Figure 2A).

VA level was lower after the fatiguing protocol (from 86.5 ± 5.8 to 79.5 ± 8.0%; the main effect of time: $p = .011$, $\eta^2 = 0.60$, with 6/8 participants had a decrease), whereas the condition failed to meet statistical significance (from 85.8 ± 6.2 to 80.4 ± 8.2%, the main effect of condition $p = .058$, $\eta^2 = 0.23$), likely due to the small sample size. However, a Fishers' post hoc analysis of interaction effect revealed a reduction for RWL compared to control ($p = .001$, $\eta^2 = .19$; Figure 2(B)).

Contractile properties

The mechanical response of the KE muscles - peak doublet amplitude was reduced (main effect of time; $p = .001$, $\eta^2 = .72$), while the Fishers' post hoc of interaction effect revealed a greater decline in peak doublet amplitude in RWL when compared control after fatiguing contraction ($p = .050$). There were no interaction effects for half-relaxation time ($p = .887$) and time-to-end ($p = .989$), compared to the control respectively. Also, the contractile RFD was slower following fatigue protocol (main effect of time: $p = .020$; $\eta^2 = .71$), with no interaction effect observed for RFD ($p = .323$, Table II).

Resting train amplitude at 20 and 80 hz

The tetanic stimulation at 20 Hz applied on the resting KE-s did not reveal any effect of RWL or fatigue on resting force amplitude (interaction effect: $p = .331$, $\eta^2 = .03$). However, the half-relaxation time (main effect of time: $p = .001$, $\eta^2 = .53$) and time-to-end (main time effect: $p = .002$; $\eta^2 = .60$) were shorter, with Fishers' post hoc analysis revealing a significant decline after fatiguing contraction following RWL ($p = .023$; $p = .021$, respectively). A significant interaction effect was observed for RFD ($p = .090$; $\eta^2 = .33$), with no significant post hoc effects. In addition, there were no significant interaction effects in any outcome variables calculated from 80 Hz train stimulations. Lastly, there was no time ($p = .931$) or interaction ($p = .827$) effect observed for 20/80 Hz peripheral fatigue development index (Table II).

Fatigue protocol

Time to exhaustion was shorter by 16 s ($p = .020$) during the fatigue protocol (70% MVC) in RWL compared to control assessment. There were no significant differences observed in the resting post fatigue twitch properties, Table III. Peak lactate production was 53% lower in RWL compared to control (11.4 ± 3.9 vs. 5.1 ± 2.0, $p = .001$, $\eta^2 = .810$, Figure 2C).

Correlations

There was a significant correlation between the delta change in brief MVCs and peak lactate concentration for the control condition ($r = .65$, $p = .045$), but not after RWL ($r = -.29$, $p = .477$). Peak lactate

Table II. Doublet characteristics, 80 and 20 Hz train data before and after the fatigue protocol (data are presented as mean ± SD).

Variables	Control		RWL	
	pre	post	pre	post
Doublet				
Force (Nm)	79.3 ± 11.1	74.8 ± 9.9	74.4 ± 11.5	66.9 ± 16.5#
CT (ms)	70.8 ± 17.7	74.8 ± 16.5	66.2 ± 13.0	75.5 ± 23.2*
HRT (ms)	105.6 ± 38.9	85.4 ± 32.2	85.0 ± 33.5	64.5 ± 24.9*
TTE (ms)	211.2 ± 77.8	159.6 ± 59.9	168.6 ± 66.0	124.6 ± 57.2*
RFD (N m/s^{-1})	1218 ± 122	1169 ± 178	1175 ± 131	1086 ± 150*
20 Hz				
Force (Nm)	171.1 ± 46.6	162.9 ± 54.1	158.1 ± 33.5	149.2 ± 18.5
CT (ms)	558.9 ± 195.3	646.2 ± 132.1	570.1 ± 274.6	674.8 ± 387.1
HRT (ms)	223.9 ± 114.9	157.5 ± 82.4	177.0 ± 73.1	118.8 ± 69.2#
TTE (ms)	447.8 ± 229.8	315.4 ± 164.8	354.1 ± 146.2	237.4 ± 138.5#
RFD (N m/s^{-1})	716 ± 261	888 ± 207	923 ± 459	1099 ± 155#
80 Hz				
Force (Nm)	218.7 ± 35.8	215.6 ± 64.1	212.5 ± 29.2	199.3 ± 15.9
CT (ms)	769.2 ± 254.6	735.1 ± 278.6	758.8 ± 262.7	730.1 ± 292.9
HRT (ms)	272.6 ± 162.7	229.9 ± 155.8	250.4 ± 113.5	244.8 ± 136.7
TTE (ms)	550.2 ± 325.6	459.8 ± 311.7	497.6 ± 224.0	489.6 ± 273.3
RFD (N m/s^{-1})	1491 ± 261	1353 ± 441	1405 ± 241	1417 ± 277
20/80 Ratio	77 ± 15	76 ± 12	74 ± 8	75 ± 9

Notes: Abbreviations: RWL, rapid weight loss; Force (Nm), Resting peak twitch torque; CT, contraction time; HRT, Half-relaxation time; TTE, time to end, RFD, contractile rate of force development (N m/s^{-1}). *Fishers post-hoc analysis of main effect of time ($p < 0.05$). #Fishers post-hoc analysis of interaction effect ($p < 0.10$).

Table III. Fatigue protocol data – contractile characteristics and peak doublet twitch torque of the knee extensor muscles – (data are given as mean ± SD).

Variable	Control	RWL	*p*-level
Force (N m)	54.2 ± 11.8	55.6 ± 12.4	0.551
CT (ms)	76.6 ± 17.2	85.6 ± 24.4	0.479
HRT (ms)	188.2 ± 95.0	137.1 ± 57.1	0.279
TTE (ms)	376.3 ± 190.1	250.0 ± 96.8	0.140
Time to fatigue (sec)*	85.9 ± 34.8	68.8 ± 20.2*	0.020

Notes: Abbreviations: RWL, rapid weight loss; Force (Nm) – peak doublet twitch torque (post 70% MVC – fatigue protocol); CT, contraction time; HRT, half-relaxation time; TTE, time to end. *Different from control ($p < 0.05$).

concentrations were not available for one participant during the RWL condition. Correlations between MVC delta change and the 3% reduction in boxers' B_M was not significant ($r = .467$, $p = .205$).

Discussion

This is the first study to quantify the effects of RWL on the neuromuscular function of the KE muscles in Olympic-style boxers. It was hypothesized that neuromuscular function would be impaired following 3% RWL in part due to the combined fluid- and caloric- restrictions associated with such protocols. Indeed, after 3% RWL, MVC1 (or the pre-fatigue MVC) was ~12% lower, and VA deficits of ~7% were observed. Boxers' time to exhaustion during the fatiguing 70% MVC was 16 s shorter after RWL compared to control. Peak lactate production was ~53% lower after RWL compared to the control condition. Finally, the delta change in brief MVC force was positively correlated to peak lactate concentrations in the control condition exclusively.

According to data presented, the 3% RWL further accelerates neuromuscular fatigue development in Olympic style boxers, and especially when these athletes were not allowed to rehydrate. Indeed, a moderate RWL caused significant declines in brief MVC1 of the KE muscle groups immediately after 3% RWL. Previous research investigating neuromuscular fatigue in combat athletes after passive heating and subsequent 3% dehydration has reported 28% loss following sustained contractions, whereas there were no changes reported in brief isometric (5-s MVC) contractions (Barley et al., 2018). The former observations are not entirely consistent with data presented in the current study. In a recent meta-analysis, Savoie, Kenefick, Ely, Cheuvront, and Goulet (2015) had summarized findings from 34 studies and suggested that a moderate 2.9 ± 1.1% B_M loss is translated into 6.2 ± 1.1% decrease in maximal strength of the KE muscles, likely due to acute dehydration. However, findings complied by Savoie et al. (2015) were focused on the effects of acute dehydration on muscle endurance, strength, and jumping performance, while a separate analysis on the effects of acute dehydration on isometric force production was overlooked. This certainly influenced the overall magnitude of muscle power loss (–5.5 ± 1.0%), whereas the present study was looking at strictly isometric contractions following food and fluid deprivation. Force production loss in brief isometric contractions after 4% dehydration was reported by Hayes and Morse (2010) who found a –8.5% reduction in maximal force output. These data are more in line with the decreases found in the present study. However, Hayes & Mores were testing recreational athletes, and the participants underwent an active heating protocol (5 × 20 min jogging at ~80% heart rate max.) to achieve the 2.6% decreased in B_M, thus this research could not separate the combined effects of fluid loss and heat stress on decrements of neuromuscular function. Our boxers produced the RWL through food fluid restrictions exclusively to determine the sole contribution of RWL on neuromuscular function.

In the present study, force output and voluntary activation were each significantly reduced after a 70% MVC fatiguing contraction, notably more so after RWL (Figure 2). These findings illustrate an insufficiency of the central nervous system (CNS) to fully activate the KE-s after undergoing 3% RWL. Presumably, voluntary drive (i.e. central fatigue development) was influenced by substrate depletion and fluid loss. These data are in line with a seminal paper from Nybo (2003), who demonstrated that exercise-induced hypoglycaemia (induced via 3 h cycling exercise at constant power output) modulated force output and VA levels of the KEs in endurance-trained participants after completing 120 s isometric contractions. However, Nybo did not report contractile characteristics of the KEs in this work. The VA deficit observed in the present study ran in parallel to reductions in resting doublet amplitude (by ~9%), and shorter muscle half-relaxation times (by ~30%, Table II). A previous review on non-combat athlete populations had proposed that the crucial steps of the excitation-contraction coupling are compromised by reductions in skeletal muscle glycogen concentrations (Ørtenblad, Nielsen, Saltin, & Holmberg, 2011). Little data on humans is currently available in the existing literature, however, *in vitro* mice studies have demonstrated that excitation-contraction coupling efficiency is glycogen dependent in the flexor brevis muscle (Chin & Allen, 1997). Indeed, Chin and Allen (1997) observed a reduction in Ca^{2+} release (by ~43%) and depression in force production by ~24%, under conditions of reduced glycogen concentration (by ~27%), following intermitted 50 Hz tetanic stimulation, indicating that peripheral fatigue development was accelerated via substrate depletion and an inability to completely restore skeletal muscle glycogen concentrations (Ørtenblad, Westerblad, & Nielsen, 2013), especially following sustained contractions. Their findings indicated peripheral fatigue development. Conversely, data presented here suggest that the peripheral fatigue development, estimated via LFF index (Table II) t of the KE muscles, was non-significant and these changes in the LFF 20/80 ratio were consistent with data previously reported by Martin et al. (2010), who reported no notable changes in LFF in ultramarathon runners following a 24-h treadmill run.

The present data add to the sparse literature on the assessment of neuromuscular fatigue development in elite Olympic-style combat athletes utilizing RWL protocols. Indeed, current findings suggest that the neuromuscular fatigue development, as observed from the VA deficits, has a centrally-mediated component following 3% RWL procedure. Lactate production and clearance provide a gross indication of glycolytic energy pathway contributions, but do not inform on any relative degree of muscle fatigue development (Millet et al., 2002). However, lactate production seems to be predominantly affected via either glycogen depletion and/or oxygen availability. The current study was conducted at sea-level and did not include a hypoxic element to the investigation, however the participants were substrate depleted. In effect, the 3% RWL likely affected both intra- and extra-cellular osmolality due to variations in ion and metabolite concentrations, thus it is entirely plausible that lactate efflux from the muscle could have caused lower blood lactate concentrations seen in the RWL condition exclusively. Lactate clearance can also be affected by local ischaemia development following fatiguing isometric contraction at 70% MVC. It is important to note that even with such changes in lactate clearance, the magnitude of force reduction and contraction duration was different between our experimental procedures, with a two-fold lower lactate accumulation following RWL (Figure 2C). Correlations findings support the relationship between force decline and the lactate accumulation.

These findings suggest a few important practical applications that should be considered when testing or training elite combat athletes. First, neuromuscular function is impaired following RWL protocols even when they do not include a passive or active heating component to them. Secondly, for sports scientist who implement neuromuscular testing, it is critical to understand; routine muscle force testing scenarios should be extended beyond the classical muscle force/torque assessment, in order to get a better insight into the underlying mechanisms of force-generating capacity adjustments in combat athletes. Finally, for the athletes themselves, they should not overlook the deleterious effects a moderate, 3% RWL programme might have on their neuromuscular function, especially due to the limited recovery period between weigh-in and match start.

There are few limitations to the present study that must be acknowledged. The evoked response, such as the M-wave or H-reflex were not measured in the present study since significant changes were not anticipated (Ratel et al., 2015). The interplay between centrally-mediated fatigue in voluntary activation (and force development), and the peripherally-mediated lactate production is important to consider and fully quantify in further studies. Further studies research should also look into the effects of rehydration and weight re-gain on neuromuscular performance in Olympic combat athletes. Lastly, a relatively small number of participants ($n = 9$) represents a limitation to the present study. However, considering the above-mentioned exclusion criteria, this was a compromise we took to in attempt control for the internal and external validity of the study.

Conclusion

The 3% RWL reduces maximal force production in Olympic-style boxers. Boxers are not able to maintain force output after a fatiguing contraction following RWL protocols, and they are not able to produce the same peak lactate concentrations that were observed during baseline testing. Collectively, these data demonstrate that neuromuscular performance of boxers is impaired following RWL achieved via food and fluid restriction.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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