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Effect of tapering after a period of high-volume sprint interval training on running performance and muscular adaptations in moderately trained runners

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Running head: Tapering after high-volume sprint interval training

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Abstract

The effect of tapering following a period of high-volume sprint interval training (SIT) and a basic volume of aerobic training on performance and muscle adaptations in moderately trained runners was examined. Eleven (8 males, 3 females) runners (maximum oxygen uptake (VO₂-max): 56.8±2.9 mL·min⁻¹·kg⁻¹; mean±SD) conducted high-volume SIT (HV; 20 SIT sessions; 8-12 x 30-s all-out) for 40 days followed by 18 days of tapering (TAP; 4 SIT sessions; 4 x 30-s all-out). Before and after HV as well as midway through and at the end of TAP, the subjects completed a 10-km running test and a repeated running test at 90% of vVO₂-max to exhaustion (RRT). In addition, a biopsy from m. vastus lateralis was obtained at rest. Performance during RRT was better (P<0.01) at the end of TAP than before HV (6.8±0.5 vs 5.6±0.5 min; mean±SE), and 10-km performance was 2.7% better (P<0.05) midway through (40.7±0.7 min) and at the end of TAP (40.7±0.6 min) than after HV (41.8±0.9 min).

The expression of muscle Na⁺,K⁺-ATPase (NKA)α₁, NKAβ₁, phospholemman (FXYD1) and sarcoplasmic reticulum calcium transport ATPase (SERCA1) increased (P<0.05) during HV and remained higher during TAP. In addition, oxygen uptake at 60% of vVO₂-max was lower (P<0.05) at the end of TAP than before and after HV. Thus, short-duration exercise capacity and running economy was better than before the high-volume SIT period together with higher expression of muscle proteins related to Na⁺/K⁺ transport and Ca²⁺ re-uptake, while 10-km performance was not significantly improved by the combination of high-volume SIT and tapering.

Keywords: Running economy, Na⁺,K⁺-ATPase, sarcoplasmic reticulum calcium transport ATPase
New & Noteworthy

Short-duration performance became better after 18 days of tapering from ~6 weeks of high-volume sprint interval training (SIT), whereas 10-km performance was not significantly affected by the combination of high-volume SIT and tapering. Higher expression of muscle NKAα1, NKAβ1, FXYD1 and SERCA1 may reflect faster Na⁺/K⁺ transport, and Ca²⁺ re-uptake that could explain the better short-duration performance. These results suggest that the type of competition should determine the duration of tapering to optimize performance.
**Introduction**

Studies on trained subjects have shown performance improvements of ~3% when training volume is decreased and training intensity maintained for 7–21 days (22, 28, 39). The purpose of such interventions, termed tapering, is to reduce accumulated fatigue induced by a period of overload training while maximizing physiological adaptations and consequently performance (25). Tapering performed for an insufficient period may not overcome fatigue, while de-training and loss of athletic performance follows an extended period (27). Based on a meta-analysis of 27 studies, Bosquet et al. (2007), recommended that the best strategy to optimize performance is a 2-week tapering intervention where training intensity is maintained, training volume reduced by more than 20%, and training frequency withheld at a minimum of 80% pre-taper frequency (5). However, these general recommendations do not consider the type of competition as no distinction between short and long-duration performance was made. Furthermore, it is well established that the length of the taper needed is closely related to the training volume and/or intensity during the pre-tapering period (10, 37, 38).

Repeated bouts of exercise performed with near-maximal to maximal intensity for a duration up to 40-s per bout and recovery periods lasting more than 5 times the exercise duration provides the rationale for the term “sprint interval training” (SIT) (17). Studies investigating the effect of 2-3 weekly sessions of SIT and a basic volume of aerobic training reports 3-4% improved 10-km running and 40-km cycling performance in trained subjects (1, 20, 34), while maintained 10-km running performance is reported when SIT is performed ~4 times/week in runners accustomed to SIT (34). As the lack of improvement in the latter study might be explained by accumulated fatigue, it would be worthwhile exploring the
The effect of tapering after a period of high-volume SIT (~4 sessions per week) and a basic volume of aerobic training on short duration (~6 min) and 10-km running performance.

In between muscle contractions (i.e., the relaxation phase), the sarcoplasmic reticulum (SR) calcium transport ATPase (SERCA) transports calcium ions from the cytoplasm back into the SR thereby permitting muscle excitation-contraction coupling. SERCA1 is exclusively expressed in fast-twitch whereas SERCA2 is expressed in slow-twitch muscle fibers (30) and the SERCA pumps are reported to account for up to 50% of the energy used during muscle contractions (9, 36). Intense contractions can lead to marked muscle membrane depolarization, inexcitability and fatigue (12, 23, 31) with the action of the Na\(^+\),K\(^+\)-ATPase (NKA) pump being responsible for limiting the net fluxes of Na\(^+\) and K\(^+\) thereby preserving muscle membrane excitability and contractile function (8, 23). Increased muscle expression of NKA isoforms have been found in a number of SIT studies (1, 17, 18) and suggested to play an important role for improved short-duration performance after a period of SIT (14, 17). However, no study has examined the effect of a taper on the expression of proteins associated with Na\(^+\)/K\(^+\) transportation and Ca\(^{2+}\) re-uptake after a period of high-volume SIT.

Thus, the aims of the present study were to investigate the effect and time course of tapering after 40 days of high-volume SIT on short-duration exercise capacity (~6 min) and 10-km performance (~40 min) as well as on muscular adaptations related to Na\(^+\)/K\(^+\) transportation and Ca\(^{2+}\) re-uptake in moderately trained runners accustomed to SIT. It was hypothesized that the tapering period would lead to improved short and long-duration performance in association with higher expression of muscle Na\(^+\)/K\(^+\) transportation and Ca\(^{2+}\) re-uptake proteins.
Methods

Subjects

Eleven moderately trained male (n=8) and female (n=3) runners took part in the study. Subjects had an average age, stature, body mass, VO_{2-max} and HR_{max} of 29.2±4.5 (means±SD) yr., 176.6±10.1 cm, 72.4±9.8 kg, 56.8±2.9 mL·min^{-1}·kg^{-1}, and 189.1±8.4 bpm, respectively (males: 30.1±4.7 yr., 181.0±7.7 cm, 76.7±3.0 kg, 57.7±2.8 mL·min^{-1}·kg^{-1}, and 189.3±8.1 bpm; females: 26.7±3.5 yr., 165.0±4.4 cm, 58.2±8.3 kg, 54.1±1.4 mL·min^{-1}·kg^{-1} and 188.7±11.0 bpm). After receiving information about the study and the possible risks and discomforts associated with the experimental procedures, all subjects gave their written informed consent to participate. The study conformed to the Code of Ethics of the World Medical Association (Declaration of Helsinki) and was approved by the Ethics Committee of the capital region of Copenhagen (Region Hovedstaden; H-3-2014-048).

Training

Before the study period, 10 sessions of sprint interval training (SIT; 10 x 30-s all-out running interspersed by 3.5 min of rest) were performed during a 40-day familiarization period (FAM). In addition to SIT during FAM, 10 sessions of aerobic moderate speed running (AM; 30-60 min with an average heart rate (HR) of 60-85% of the maximal heart rate (HR_{max}) measured during an incremental running test to exhaustion before FAM) were performed (Fig. 1).

During the study period, subjects first completed a 40-day period with high-volume SIT (HV) consisting of four SIT sessions (8-12 x 30-s all-out running with 3.5 min of rest between bouts) with twelve repetitions day 1 and 5 and eight repetitions day 3 and 7 as well as two sessions of AM (30-60 min with an average HR_{max} of 60-85%) every eight days. Next, an 18-day tapering period (TAP) was
completed with SIT (4 x 30-s all-out running interspersed by 3.5 min of rest) being performed on day 3, 6, 11 and 14, and AM (20-40 min with an average HR\textsubscript{max} of 60-85%) performed on day 2 and 5 and 13 (Fig. 1). The SIT sessions were supervised and carried out after a thorough warm-up consisting of ~1.5 km aerobic moderate speed running, sprint-specific exercises (e.g. butt kicks, skips, skips for height) and dynamic stretches (e.g. leg cycling, leg drives, jump squats). Subjects performed the SIT sessions independently on the same day if they were unable to take part in the supervised training. The AM sessions were not supervised, but a training log was kept and analyzed to record intensity and duration. The weekly time spent on AM was lower (P<0.05) during TAP than HV (24±2 vs 57±4 min), while average heart rate was consistent (83±2 and 84±2 %HR\textsubscript{max}).

Before being included in the study, the subjects habitually ran 27±4 km/wk (122±32 min/wk at 84±3 %HR\textsubscript{max}) of which 0.9±0.4 km/wk was categorized as interval training. None of the subjects had experience with SIT. The weekly habitual running distance was reduced (P<0.05) by 37% to 17±1 km/wk during FAM, by 32% to 19±1 km/wk during HV, and by 49% to 14±1 km/wk during TAP, and included all running during SIT, AM, VO\textsubscript{2-max} and 10-km tests. The total running distance during TAP was 26% less (P<0.05) than during HV.

The present study is part of a larger study where the effects of 20 SIT sessions performed with either high (every second day) or low (every fourth day) volume on short-duration exercise capacity (~6 min) and 10-km running performance as well as on muscular adaptations related to Na\textsuperscript{+}/K\textsuperscript{+} transportation and Ca\textsuperscript{2+} re-uptake in moderately trained runners familiarized with 10 SIT sessions (every fourth day) was examined and compared (34).
Testing

On separate days, before and after HV and 8-10 and 16-18 days of TAP, subjects completed an incremental test to exhaustion (before and after HV only), a 10-km test, and a repeated running test at 90% vVO₂-max to exhaustion that was preceded by a muscle biopsy and a blood sample collected at rest after an overnight fast. Pulmonary oxygen uptake at the subject’s individual average 10-km running pace (v10-km; 13.8±0.3 km/h), determined at a screening 10-km running test before FAM, was measured before the incremental and repeated running tests. In addition, pulmonary oxygen uptake at the subject’s individual 60% maximal incremental speed (60% vVO₂-max; 11.7±0.2 km/h), determined at a screening incremental treadmill test to exhaustion before FAM, was measured before the 10-km tests. All measures of oxygen uptake were recorded by a breath-by-breath gas analyzing system (Oxycon Pro, Viasys Healthcare, Hoechberg, Germany).

Tests were separated from strenuous physical activity by a minimum of 48 hours and repeated at the same time of day throughout the study period, and subjects refrained from alcohol and caffeine 24 h prior to testing. Subjects were instructed to keep a diary journal two days prior to and during the first series of tests before the study period and replicate this diet before and during re-testing. One female runner did not perform the final performance tests during tapering, and one female runner did not perform the 10-km tests during tapering due to sickness and shin splints, respectively.

The incremental test to exhaustion (INC) consisted of walking for two min (at 5 km/h), six min at v10-km, two min at 14 or 15 km/h (dependent on v10-km), after which the speed increased by 1 km/h every min until exhaustion. During INC, maximum oxygen uptake (VO₂-max), the highest average value achieved over a 30-s period, was determined by a breath-by-breath gas analyzing system (Oxycon Pro), and HR was monitored by use of Polar Team² system (Polar Electro Oy, Kempele, Finland). Attaining
of maximal HR (judged against the screening INC before FAM, 189.0±2.6 bpm) together with an RER value of ≥1.15 were used as criteria (HR\textsubscript{max}: 188.3±2.8 vs 188.0±2.8 bpm; RER: 1.25±0.02 vs 1.24±0.02 before and after HV, respectively). During the last part of the test, the subject’s effort was encouraged by a researcher until voluntary termination. Based on the literature (16, 22, 32), no change in VO\textsubscript{2-max} was expected during tapering and to secure a reduction in volume (training + tests), the INC was therefore not performed during this period.

The 10-km test was performed on a running track (Østerbro Stadium, Copenhagen) in similar weather conditions (light winds, ~10-15°C). Tests were preceded by two bouts of six min of running on a treadmill at 60% vVO\textsubscript{2-max}.

Muscle biopsy and blood sampling were performed between 7 and 11 a.m. under standardized conditions after an overnight fast. First, the subject’s body mass was established and then a biopsy was collected at rest from the vastus lateralis muscle of the right leg under sterile conditions by use of local anesthetics (1 ml; 20 mg/l lidocaine without epinephrine) and the Bergström technique (4). The muscle sample (~100 mg wet weight) was immediately frozen in liquid N\textsubscript{2} and stored at −80°C until further analysis. Furthermore, a catheter was inserted into the antecubital vein and a ~7 ml blood sample was collected.

The repeated running test to exhaustion (RRT) followed the muscle biopsy and blood sampling and consisted of three bouts of running on a treadmill interspersed by rest periods in the following order: six min of running at v10-km (1\textsuperscript{st} bout); one h of recovery; two min of running at 90% vVO\textsubscript{2-max} (2\textsuperscript{nd} bout, 17.5±0.3 km/h) determined at a screening INC before FAM; five min of recovery; running to exhaustion at 90% vVO\textsubscript{2-max} (3\textsuperscript{rd} bout). All runs were preceded by two min of walking at 5 km/h and a
blood sample from the antecubital vein was collected immediately after running at v10-km, before and immediately after the 2\textsuperscript{nd} bout as well as before and immediately after and three min after exhaustion from the 3\textsuperscript{rd} bout.

**Muscle analysis**

*Western blotting* was performed to determine protein expression as described previously (33). In short, samples of ~2.5 mg dry weight (dw; freeze-dried) human muscle tissue was dissected free from blood, fat, and connective tissue. Samples were homogenized for 1 min at 28.5 Hz (Qiagen Tissuelyser II; Retsch, Germany) in a fresh batch of ice-cold buffer containing (in mM): 10% glycerol, 20 Na-pyrophosphate, 150 NaCl, 50 HEPES (pH 7.5), 1% NP-40, 20 β-glycerophosphate, 2 Na\textsubscript{3}VO\textsubscript{4}, 10 NaF, 2 PMSF, 1 EDTA (pH 8), 1 EGTA (pH 8), 10 μg/ml aprotinin, 10 μg/ml leupeptin, and 3 benzamidine, rotated for 1 h at 4°C and then centrifuged at 18,320 g for 20 min at 4°C to exclude nondissolved structures. The supernatant (lysate) was collected and used for further analysis. Total protein concentration in each sample was determined by a Bovine Serum Albumin (BSA) standard kit (Thermo Fischer Scientific, Waltham, USA), and samples were mixed with 6 x Laemmli buffer (7 ml 0.5 M Tris-base, 3 ml glycerol, 0.93 g DTT, 1 g SDS, and 1.2 mg bromophenol blue) and ddH\textsubscript{2}O to reach equal protein concentration before protein expression was determined by Western blot analysis.

Equal amounts of total protein were loaded in each well of precast gels (Millipore). All samples from each subject were loaded on the same gel. Proteins were separated according to their molecular weight by SDS-PAGE and semi-dry transferred to a PVDF membrane (Bio-Rad). The membranes were blocked in either 2% skimmed milk or 3% BSA in TBST including 0.1% Tween-20 before an overnight incubation in primary antibody at 4°C. The primary antibodies used were: (ab. cat number or name; company or donor; total amount of protein loaded, respectively): Na\textsuperscript{+}/H\textsuperscript{+} exchanger isoform 1
(NHE1; MAB3140; Millipore; 6-9 µg), Na⁺,K⁺-ATPase subunit alpha 1 (NKAα1; α6F; Development Studies Hybridoma; 9-12 µg), Na⁺,K⁺-ATPase subunit alpha 2 (NKAα2; 07-674; Millipore; 6 µg), Na⁺,K⁺-ATPase subunit beta 1 (NKAβ1; MA3-930; Thermo Scientific; 6 µg), phospholemman (FXYD1; 13721-1-AP; Proteintech; 6-9 µg) and FXYD1 phosphospecific protein expression (AB_FXYD1; AB_FXYD1–C2; Dr. D. Bers, Loyola University; 6-9 µg), sarcoplasmic reticulum Ca²⁺-ATPase 1 (SERCA1; MA3-912; Thermo Scientific; 6-12 µg), sarcoplasmic reticulum Ca²⁺-ATPase 2 (SERCA2; N-19 Sc-8095; Santa Cruz Technology; 7.5-12 µg), actin (A2066; Sigma Aldrich; 9-12 µg), calmodulin-dependent protein kinase II (CaMKII; 611293; BD Transduction Laboratories; 6-12 µg) and CaMKII phosphospecific site thr286 protein expression (CaMKIIphos; 3361; Cell Signaling Technology; 7.5-12 µg). The membranes were then incubated for 1 h in horseradish peroxidase-conjugated secondary antibody (rabbit anti-sheep (P-0163, DAKO), rabbit anti-goat (P-0449, DAKO), goat anti-mouse (P-0447, DAKO) and goat anti-rabbit IgM/IgG (4010-05; Southern Biotech) depending on the primary antibody source) at room temperature.

The bands were visualized with ECL (Millipore) and recorded with a digital camera (ChemiDoc MP Imaging System, Bio-Rad Laboratories, Hercules, USA). For each muscle sample, protein expression and phosphorylation were determined in duplicate and densitometry quantification of the Western blot band intensity was done using Image Lab version 4.0 (Bio-Rad Laboratories, Hercules, USA) and determined as the total band intensity adjusted for background intensity. Determination of the specific phosphorylation level and total protein expression was performed on separate membranes in separate analyses and phosphorylation level was calculated as the ratio of the total expression of the given protein (e.g. CaMKII phosphorylation/CaMKII expression).
Muscle enzyme activity was determined by use of ~2.5 mg dw muscle tissue dissected free from blood, fat, and connective tissue which was homogenized (1:400) in a 0.3 M phosphate buffer (pH 7.7) by 2 rounds of 30 s using a Qiagen TissueLyser II (Retch, Germany). Maximal activity of citrate synthase (CS), β-hydroxyacyl-CoA-dehydrogenase (HAD) and phosphofructokinase (PFK) was determined fluorometrically with NAD-NADH coupled reactions (21) on a Fluoroskan Ascent apparatus (Thermo Scientific, Waltham, USA) using Ascent Software version 2.6.

Muscle glycogen was determined as the glucose content of ~2 mg dw muscle tissue dissected free from blood, fat, and connective tissue that was extracted in 1 N HCl and hydrolyzed at 100°C for 3 h. Glucose content was determined by the hexokinase assay method (Roche Diagnostic, Mannheim, Germany) using a Hitachi 912 (Hitachi 912 Automatic Analyzer; Roche Diagnostic, Indianapolis, USA).

Blood analysis

For every blood sample collected at rest and during and after RRT, a total of ~7 mL blood was drawn in a heparinized 2-mL syringe and a 5-mL syringe. A part of the 2-mL blood sample (~1.5 mL) and the 5-mL sample (split into 2 x 2 mL tubes containing 30 μL EDTA) were centrifuged at 20,000g for ~2 min and the remaining whole blood from the 2-mL sample (~0.5 mL) was stored on ice for further analyses. After centrifugation, the plasma was transferred into tubes and placed in ice-cold water until they were stored at -20°C. Plasma samples were subsequently analyzed for testosterone and cortisol at rest; creatine kinase (CK) and immunoglobulin A (IgA) at rest and 3 min after exhaustion; epinephrine and norepinephrine at rest, immediately after the 2nd bout, and after exhaustion; and NH₃ at rest, before and after the 1st and 2nd bout, and immediately after as well as 3 min after exhaustion. Plasma CK activity and NH₃ content were analyzed by enzymatic kinetic assay methods (Roche Diagnostic,
Mannheim, Germany) using a Hitachi 912 (Hitachi 912 Automatic Analyzer; Roche Diagnostic, Indianapolis, USA). Plasma IgA was determined using an immunoturbidimetric assay method (Horiba, Montpellier, France) on an automatic analyzer (Pentra C400, Horiba, Montpellier, France). Plasma testosterone and cortisol were determined using ELISA kits (R&D Systems, Minneapolis, USA). Plasma epinephrine and norepinephrine were analyzed fluorometrically by the use of an enzymatic kit (2-CAT Plasma Elisa High Sensitive BA E-4500 WAKO Chemical, Neuss, Germany). Whole blood was analyzed for hemoglobin at rest; pH, K⁺, Na⁺, glucose, lactate and HCO₃⁻ at rest, before and after the 1st and 2nd bout and immediately after as well as 3 min after exhaustion on an automated blood gas analyzer apparatus (ABL800 Flex; Radiometer Medical, Copenhagen, Denmark).

Pulmonary oxygen uptake kinetics and running economy

Two transitions were used to determine VO₂ kinetics. Initially, errant breaths, defined as any value lying more than 4 standard deviations away from the local mean caused by swallowing and coughing, were removed. Then the VO₂ responses were linearly interpolated to give 1-s values and then averaged. The initial cardio dynamic component was ignored by eliminating the first 20 s of data after the onset of exercise. The data were fitted using a mono-exponential model:

\[ VO_2(t) \text{ at 60% } v \cdot VO_2\text{-max} = VO_2 \text{ (baseline)} + A \cdot (1-e^{-(t-TD)/\tau}) \]

With \( VO_2(t) \) being oxygen uptake to a given time while running at 60% \( v \cdot VO_2\text{-max} \). VO₂ baseline was calculated as the average \( VO_2 \) in the last min walking at 5 km/h. A, TD and \( \tau \) being the amplitude, time delay and time constant, respectively, for the response modeled with \( \tau \) describing the speed of the VO₂ response as the time to attain 63% of the amplitude.

Running economy (RE) was calculated with the following formula:
\[ \text{RE (ml O}_2\cdot \text{kg}^{-1}\cdot \text{km}^{-1}) = \text{VO}_2 (\text{mL} \cdot \text{min}^{-1}) \times 60 \text{ min} \cdot \text{h}^{-1} / \text{BM (kg)} / \text{km} \cdot \text{h}^{-1} \]

Where \( \text{VO}_2 \) is the average value during the last 2 min of running at 60% \( \text{vVO}_2\text{-max and v10-km} \), and BM is body mass.

**Statistics**

Performance, muscle expression and phosphorylation, muscle enzyme activity, muscle glycogen, body mass, pulmonary measurements and heart rate during submaximal exercise as well as blood and plasma variables at rest were evaluated by use of 1 way repeated measures analysis of variance (ANOVA). Blood variables at rest and during exercise were evaluated by use of 2 way repeated measures ANOVA. A Student Newman Keuls post hoc test was used for all pairwise comparisons of the mean responses if a significance level of \( P<0.05 \) was reached. Unless otherwise stated, data are presented as means±SE except data on muscle protein expression, which are presented as geometric means±95% confidence intervals.
Results

Running performance

The 10-km performance was 2.7% better (P<0.05) after both 8 (40.7±0.7 min) and 16 (40.7±0.6 min) days of TAP compared to the end of HV (41.8±0.9 min), but not different from before HV (41.2±0.7 min) (Fig. 2). Time to exhaustion during RRT did not change during HV, but was 22% longer (P<0.01) after 18 days of TAP than before HV (6.8±0.5 vs 5.6±0.5 min; Fig. 2).

Pulmonary measurements and heart rate during submaximal exercise

VO₂ and RE at 60% vVO₂-max were lower (P<0.05) after 16 days of TAP compared to before and at the end of HV (Table 2). RER, HR and oxygen kinetics (baseline, τ and TD) at 60% vVO₂-max were unchanged during the study period except for the amplitude, which was lower after HV and after 8 and 16 days of TAP compared to before HV (Table 2).

Neither VO₂, RE, RER nor HR at v10-km changed during the study period (Table 2).

Muscle protein expression

Expression of muscle NKAα₁, NKAβ₁, FXYD1 and SERCA1 was higher (P<0.05) after HV as well as 10 and 18 days of TAP than before HV (Fig. 3). Expression of muscle NKAα₂, SERCA2, CaMKII, actin, and NHE1, as well as phosphorylation state of FXYD1 and CaMKII, did not change during the study period.

Muscle enzyme activity and glycogen

Maximal activity of muscle CS, HAD and PFK as well as muscle glycogen were unchanged during the study period (Table 1).
Blood variables at rest and during the repeated running test

After 18 days of TAP, plasma epinephrine 3 min after RRT was higher (P<0.05) than before and at the end of HV as well as after 10 days of TAP (Table 3). After 18 days of TAP, plasma norepinephrine 3 min after RRT was higher (P<0.05) compared to before and at the end of HV (Table 3).

Plasma NH₃ was lower (P<0.05) at rest as well as after the 1st bout and before the 2nd bout of RRT after 10 and 18 days of TAP and lower (P<0.05) before the 3rd bout after 18 days of TAP than before HV (see Table, SDC 1).

After 18 days of TAP, blood lactate immediately and 3 min after RRT was higher (P<0.05) than before and at the end of HV, and higher (P<0.05) 3 min after RRT than after 10 days of TAP. After 10 and 18 days of TAP, blood pH immediately after RRT was lower (P<0.05) compared to before and at the end of HV. Blood HCO₃⁻ did not change during the study period (see Table, SDC 1).

After 18 days of TAP, blood glucose immediately and 3 min after RRT was higher (P<0.05) compared to before HV (see Table, SDC 1).

Plasma K⁺ did not change during the study period. Plasma Na⁺ at rest, after the 2nd bout, as well as immediately and 3 min after RRT was higher (P<0.05) after 10 and 18 days of TAP compared to before HV (see Table, SDC 1).

Blood hemoglobin as well as plasma testosterone (T), cortisol (C) and T:C ratio at rest did not change during the study period (see Table, SDC 2). Plasma IgA and CK did not change during the study period (see Table, SDC 3).
Discussion

The major findings of the present study were that after 10 and 18 days of tapering following a period of high-volume sprint interval training, short-duration exercise capacity was better than before the high-volume sprint interval training period, and that this was associated with higher expression of muscle NKAα₁, NKAβ₁, FXYD1, and SERCA1. In addition, following the tapering period, 10-km running performance returned to baseline, and running economy was improved compared to baseline.

Short-duration exercise capacity during the repeated running test did not change with the period of high-volume SIT, but was better after 18 days of tapering than before the high-volume SIT period. Thus, it appears that the tapering period was beneficial for short-duration exercise capacity (i.e., exercise lasting ~6 min), and that the subjects had not fully adapted to the high-volume SIT when the test was conducted ~48 h after the high-volume SIT period. The reason for the lack of improvement may have been residual fatigue at the end of the high-volume SIT period due to an imbalance between training load and recovery (35). However, there were no detectable changes in markers of muscle damage and overreaching, since the plasma level of CK, a marker of damaged muscle tissue (6), IgA, an immune antibody, and testosterone to cortisol ratio, an often used indicator of overtraining (24), were not changed compared to baseline levels.

In accordance with the present findings, Shepley et al. (32) reported that time to fatigue during treadmill running at a velocity equivalent to each subject’s best 1500-m time improved 22% after 7 days of tapering. The taper consisted of high-intensity/low volume training (1-5x 500-m intervals at 115-120% \( \text{vVO}_2\text{-max} \); 7.5 km equal to ~10% of pre-taper) in well-trained middle-distance runners (\( \text{VO}_2\text{-max}: 67 \text{ ml/kg/min} \)) and was performed after 4 weeks of 6 times training per week (80 km/wk). Likewise, Mujika et al. (26) found that 800-m performance improved ~2% after 6 days of daily high-
intensity interval training (HIIT; >100% of blood lactate steady state) with a progressive reduction in distance to 20% of the pre-taper HIIT volume after 3 weeks of overload training in well-trained middle-distance runners. The tapering duration needed appears to be related to the pre-tapering training intensity and volume/duration (10, 37, 38). Hence, the lower training intensity and smaller volume/duration during the pre-taper in the studies by Shepley and Mujika than in the present study (8-12x 30s at ~130% vVO2-max for ~6 weeks) could explain why short-duration performance improved after 6-7 days of tapering in the studies by Shepley and Mujika and not after 10 days of tapering in the present study. Thus, 18 days of tapering after ~6 weeks of frequent SIT seems to represent a time-point where short-duration exercise capacity (i.e. ~6 min) is improved.

The improved short-duration exercise capacity (i.e. performance during the repeated running test) was found together with higher expression of muscle NKAα1, NKAβ1, and FXYD1 which may have contributed to preserving muscle membrane excitability and thereby delayed the development of fatigue during the repeated running (8, 23). Accordingly, studies on the effects of SIT have shown improved short-duration exercise capacity concomitant with higher expression of NKA pump isoforms (1, 17, 18). In support, plasma Na+ concentrations were higher during and after the repeated running test to exhaustion after the period of high-volume SIT and in the tapering period compared to before the high-volume SIT period. Also lower plasma ammonia concentrations before and during the repeated running test to exhaustion were found indicating that the exercise stress was reduced during the taper compared to before high-volume SIT.

The SERCA pump transports calcium ions from the cytoplasm into the SR, and the higher expression of muscle SERCA1 after 18 days of tapering may have caused a faster calcium turnover and perhaps contributed to the improved short-duration exercise capacity during the repeated running test to
exhaustion. Similarly, a 5-wk period of sprint training (20 x 10-s all-out, 3 times per week) has been reported to increase the expression of muscle SERCA1 and SERCA2, which was associated with higher average power output during intermittent sprint cycling (10 x 8-s all-out) (29). SERCA1 is primarily distributed in type II muscle fibers (7) and as muscle expression of SERCA2, which are expressed in type I fibers (7), was unchanged during the study period, it may also suggest that adaptations occurred primarily in type II fibers and thus, that these fibers mainly contributed to the improved short-duration exercise capacity. This notion is supported by studies reporting that type II fibers are more affected than type I fibers by tapering (22, 28, 39).

Collectively, the possible increased muscle Na\(^+/\)K\(^+\) transport, and faster muscle Ca\(^{2+}\) re-uptake during exercise, due to higher muscle expression of NKA\(_{\alpha_1}\), NKA\(_{\beta_1}\), FXYD1 and SERCA1, may explain the improved short-duration exercise capacity found after 18 days of tapering. However, it may not be the only cause of the increased short-duration exercise capacity, since higher expression of muscle NKA\(_{\alpha_1}\), NKA\(_{\beta_1}\), FXYD1, and SERCA1 was found already after the high-volume SIT period and midway through the tapering period without significant improvements in short-duration exercise capacity. Blood lactate and pH was higher and lower, respectively, after the repeated running test after 18 days of tapering compared to before and after high-volume SIT as well as after 10 days of tapering. This suggests a greater anaerobic energy production from glycolysis possibly stimulated by the higher plasma levels of epinephrine. Thus, the higher anaerobic energy production may have contributed to the better short-duration exercise capacity after 18 days of tapering.

The 10-km performance was improved ~3% after 8 days of tapering. In accordance, Houmard et al. (1994) observed a 3% improved 5-km performance after 7 days of tapering that consisted of 400-m intervals at 5-km race pace and 85% reduced training volume in trained runners (16). Furthermore, a
1.5 and 3.6% improved 5-km performance was reported after a slow and fast exponential decay taper, respectively, in trained triathletes (2). 10-km performance was not improved further after 16 days of tapering and, thus, more than 8 days of tapering after ~6 weeks of high-intensity SIT does not appear to provide any additional benefit for 10-km running performance.

The observation of 10-km performance after 8 and 16 days of tapering not being significantly better than before the high-volume SIT, questions the beneficial effects of such programming (i.e., phases of overload and tapering) in subjects accustomed to SIT. While the lack of (statistical) improvement after the high-volume SIT period may be due to incomplete recovery or that the subjects were accustomed to the sprint interval training, it is worth highlighting that eight out of eleven subjects had a better 10-km time (2.9% on average) at some stage during the tapering period (i.e. after 8 or 16 days) than before the high-volume SIT period. Of these eleven, one subject did not perform any 10-km tests during tapering due to injury (see methods), which, in fact, makes it eight out of ten subjects that improved their 10-km time after the combination of high-volume SIT and 8 or 16 days of tapering.

Long-duration running performance is a function of VO2-max, running economy and relative work intensity (FVO2-max) (3) as well as anaerobic energy contribution. In the present study running economy at 60% vVO2-max was improved after 16 days of tapering, which is in agreement with other studies investigating the effect of tapering on running economy at a speed corresponding to 80% VO2-max after a 7-day taper (16), at 85% VO2-max after 2 and 3 weeks of taper, and at 65% VO2-max after 3 weeks of taper (15). The better running economy might have contributed to the improved 10-km performance after 16 days of tapering, but as 10-km performance was also improved after 8 days of tapering without any change in running economy, and no change in running economy was observed at v10-km at any point, other factors must have caused the improved 10-km performance during tapering.
Measures of FVO$_2$-max are complex, and impractical, and were not performed in the present study. FVO$_2$-max is related to the VO$_2$ at which lactate begins to accumulate in the blood (13), which in turn is affected by aerobic enzymatic activity, capillary density, muscle fiber type composition, distribution of power (amount of muscle mass and/or numbers of muscle fibers recruited) and movement technique (3, 11). The lactate and bicarbonate levels during v10-km running did not change during the study period indicating that FVO$_2$-max remained unchanged. Muscle oxidative enzyme (i.e. CS and HAD) activity was unchanged throughout the study period, which is in accordance with Luden et al. (22) who found no alteration in muscle CS activity after a 3-wk taper in competitive runners despite 3% improved cross-country race performance, but in contrast to the findings in the study by Shepley (32) where a 7-day taper led to ~18% increased CS activity in highly trained runners. Nevertheless, it does not appear like FVO$_2$-max was changed in the present study.

VO$_2$-max was not determined during the tapering phase, and it is therefore unknown whether VO$_2$-max changed during the tapering period. Other studies investigating the effect of tapering on VO$_2$-max have found unaltered VO$_2$-max (22, 32) after 1-3 weeks of taper. The findings of unchanged hemoglobin concentration throughout the study period could suggest that total body hemoglobin mass, a primary determinant of VO$_2$-max (19), was stable, which in turn supports that VO$_2$-max was not changed during the study period, and, thus, cannot explain the change in 10-km performance during the tapering period.

Blood pH was lower after the repeated running test with tapering suggesting that the anaerobic energy production capacity was improved, which may have contributed to the better 10-km performance midway through and at the end of the tapering period. The higher expression of NKA subunits and SERCA1 observed after the high-volume SIT period and throughout tapering might also have affected
10-km performance. Thus, possible faster Na⁺/K⁺ transport as well as faster Ca²⁺ re-uptake by higher
eexpression of NKA subunits and SERCA1 after high-volume SIT and throughout tapering may have
allowed for a faster pace during the 10-km test during and at the end of the tapering period.

In summary, short-duration exercise capacity was improved after 18 days of tapering following ~6
weeks of high-volume sprint interval training. 10-km running performance was not significantly
affected by the combination of high-volume sprint interval training and tapering, but performance did
improve after 8 days of tapering compared to after the high-volume sprint interval training period.
These changes were found together with higher expression of NKAα₁, NKAβ₁, FXYD1 and SERCA1
that may reflect faster Na⁺/K⁺ transport, and Ca²⁺ re-uptake that in turn could have contributed to be the
better performance. Furthermore, higher anaerobic capacity and improved running economy might
have contributed to the improved performance during tapering while residual fatigue, as a consequence
of the high exercise intensity and training volume, might account for the unchanged performance after
the high-volume SIT period.

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Disclosures

No conflicts of interest, financial or otherwise, are declared by the authors. The authors declare that the results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

Author contributions

CS: design of study, performed experiments, analyzed data, manuscript draft, edited and approved final version of manuscript
NA: performed experiments, analyzed data, edited and approved final version of manuscript
PMC: design of study, analyzed data, manuscript draft, edited and approved final version of manuscript
TK: manuscript draft, edited and approved final version of manuscript
JB: design of study, performed experiments, manuscript draft, edited and approved final version of manuscript
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Figure captions

**Figure 1.** Schematic presentation of the study design. The moderately trained runners started with a 40-day period of familiarization (FAM) to sprint interval training (SIT; x: number of repetitions per session), followed by a study period of 40-days high-volume SIT (HV) and 18 days of tapering (TAP). The training schedule of an 8-day block during FAM and HV and the 18-day TAP is presented. Before and after HV (arrows) as well as after 8-10 and 16-18 days of TAP, the subjects completed, on separate days, a 10-km running test (including pulmonary measurements at 60% \( \dot{V}O_2 \)-max) and a repeated running test at 90% of \( \dot{V}O_2 \)-max to exhaustion (RRT; including pulmonary measurements at \( v_{10\text{-km}} \)). In addition, a biopsy from m. vastus lateralis and a blood sample was obtained at rest (B&B).

**Figure 2.** Time to run 10 km (A) and time to exhaustion (TTE) during running at 90% \( \dot{V}O_2 \)-max in the repeated running test (B) before (Pre HV) and after (Post HV) a period of high-volume sprint interval training (SIT) as well as midway through (Mid Tap) and at the end of (Post Tap) 18 days of tapering in moderately trained runners accustomed to SIT. Data are presented as means±SE. **: different (P<0.01) from Pre HV. §: different (P<0.05) from Post HV.

**Figure 3.** Protein expression after (Post HV) a period of high-volume sprint interval training (SIT) as well as midway through (Mid Tap) and at the end of (Post Tap) 18 days of tapering in moderately trained runners accustomed to SIT. Data are related to before the period of high-volume SIT (Pre HV) and presented as means±95% CI. *: different (P<0.05) from Pre HV.
**SDC files**

536  Supplemental Digital Content 1.pptx

537  Supplemental Digital Content 2.pptx

538  Supplemental Digital Content 3.pptx
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Figure 3. Protein expression after (Post HV) a period of high-volume sprint interval training (SIT) as well as midway through (Mid Tap) and at the end of (Post Tap) 18 days of tapering in moderately trained runners accustomed to SIT. Data are related to before the period of high-volume SIT (Pre HV) and presented as means±95% CI. *: Different (P<0.05) from Pre HV.
Table 1. Maximal activity of muscle CS, HAD and PFK as well as muscle glycogen before (Pre HV) and after (Post HV) a period of high-volume sprint interval training (SIT) as well as midway through (Mid Tap) and at the end of (Post Tap) 18 days of tapering in moderately trained runners accustomed to SIT.

<table>
<thead>
<tr>
<th></th>
<th>Pre HV</th>
<th>Post HV</th>
<th>Mid Tap</th>
<th>Post Tap</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS (μmol·g·dw⁻¹·min⁻¹)</td>
<td>17.6 ± 2.8</td>
<td>22.0 ± 3.8</td>
<td>17.9 ± 2.3</td>
<td>18.6 ± 3.0</td>
</tr>
<tr>
<td>HAD (μmol·g·dw⁻¹·min⁻¹)</td>
<td>16.7 ± 0.8</td>
<td>16.7 ± 1.7</td>
<td>15.7 ± 1.2</td>
<td>17.1 ± 1.4</td>
</tr>
<tr>
<td>PFK (μmol·g·dw⁻¹·min⁻¹)</td>
<td>90.9 ± 20.4</td>
<td>131.2 ± 33.7</td>
<td>92.6 ± 23.1</td>
<td>95.7 ± 24.5</td>
</tr>
<tr>
<td>Glycogen (mmol·kg·dw⁻¹)</td>
<td>467 ± 30</td>
<td>473 ± 36</td>
<td>406 ± 23</td>
<td>449 ± 27</td>
</tr>
</tbody>
</table>

Data are presented as means±SE.
Table 2. Body mass as well as oxygen kinetics (baseline, amplitude, time delay and tau), $\text{VO}_2$, running economy (RE), respiratory exchange ratio (RER) and heart rate (HR) during running at 60% $\text{vVO}_2$-max and $\text{VO}_2$, RE and RER during running at v10-km before (Pre HV) and after (Post HV) a period of high-volume sprint interval training (SIT) as well as midway through (Mid Tap) and at the end of (Post Tap) 18 days of tapering in moderately trained runners accustomed to SIT.

<table>
<thead>
<tr>
<th></th>
<th>Pre HV</th>
<th>Post HV</th>
<th>Mid Tap</th>
<th>Post Tap</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body mass (kg)</strong></td>
<td>72.4 ± 3.0</td>
<td>72.2 ± 2.9</td>
<td>72.3 ± 3.0</td>
<td>72.3 ± 3.1</td>
</tr>
<tr>
<td><strong>60% vVO$_2$-max</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Baseline $\text{VO}_2$ (ml·min$^{-1}$)</strong></td>
<td>929 ± 67</td>
<td>1020 ± 84</td>
<td>987 ± 67</td>
<td>974 ± 77</td>
</tr>
<tr>
<td><strong>Amplitude $\text{VO}_2$ (ml·min$^{-1}$)</strong></td>
<td>2096 ± 140</td>
<td>*1949 ± 114</td>
<td>*1976 ± 126</td>
<td>*1987 ± 111</td>
</tr>
<tr>
<td><strong>Time delay (s)</strong></td>
<td>13.4 ± 0.8</td>
<td>12.4 ± 1.4</td>
<td>12.3 ± 1.8</td>
<td>11.3 ± 1.7</td>
</tr>
<tr>
<td><strong>$\text{VO}_2$ (ml·min$^{-1}$)</strong></td>
<td>2984 ± 174</td>
<td>2965 ± 163</td>
<td>2946 ± 182</td>
<td>*†2905 ± 155</td>
</tr>
<tr>
<td><strong>RE (ml·kg$^{-1}$·km$^{-1}$)</strong></td>
<td>209.5 ± 3.9</td>
<td>209.0 ± 2.8</td>
<td>207.0 ± 3.2</td>
<td>*†204.7 ± 2.6</td>
</tr>
<tr>
<td><strong>RER</strong></td>
<td>0.91 ± 0.01</td>
<td>0.90 ± 0.01</td>
<td>0.91 ± 0.01</td>
<td>0.92 ± 0.01</td>
</tr>
<tr>
<td><strong>HR (bpm)</strong></td>
<td>155 ± 3</td>
<td>154 ± 3</td>
<td>153 ± 3</td>
<td>152 ± 3</td>
</tr>
<tr>
<td><strong>v10-km</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td><strong>$\text{VO}_2$ (ml·min$^{-1}$)</strong></td>
<td>3378 ± 215</td>
<td>3391 ± 209</td>
<td>3375 ± 201</td>
<td>3396 ± 211</td>
</tr>
<tr>
<td><strong>RE (ml·kg$^{-1}$·km$^{-1}$)</strong></td>
<td>205.9 ± 2.6</td>
<td>206.7 ± 2.6</td>
<td>206.8 ± 4.4</td>
<td>207.2 ± 2.8</td>
</tr>
<tr>
<td><strong>RER</strong></td>
<td>0.97 ± 0.01</td>
<td>0.99 ± 0.01</td>
<td>0.97 ± 0.01</td>
<td>0.97 ± 0.02</td>
</tr>
<tr>
<td><strong>HR (bpm)</strong></td>
<td>161 ± 3</td>
<td>157 ± 4</td>
<td>157 ± 2</td>
<td>159 ± 4</td>
</tr>
</tbody>
</table>

Data are presented as means±SE. *: Different (P<0.05) from Pre HV. †: Different (P<0.05) from Post HV.
Table 3. Plasma epinephrine and norepinephrine before (Pre HV) and after (Post HV) a period of high-volume sprint interval training (SIT) as well as midway through (Mid Tap) and at the end of (Post Tap) 18 days of tapering in moderately trained runners accustomed to SIT at rest, after (Post 2\textsuperscript{nd} bout) 2 min running at 90\% v\text{VO}\textsubscript{2}-max and 3 min (REC3) after exhaustion in the repeated running test.

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>Post 2\textsuperscript{nd} bout</th>
<th>REC3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Epinephrine</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(nmol/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre HV</td>
<td>0.4 ± 0.1</td>
<td>1.5 ± 0.2</td>
<td>4.6 ± 0.6</td>
</tr>
<tr>
<td>Post HV</td>
<td>0.4 ± 0.2</td>
<td>1.4 ± 0.2</td>
<td>6.2 ± 1.1</td>
</tr>
<tr>
<td>Mid Tap</td>
<td>0.4 ± 0.1</td>
<td>1.2 ± 0.2</td>
<td>5.4 ± 1.0</td>
</tr>
<tr>
<td>Post Tap</td>
<td>0.3 ± 0.1</td>
<td>1.7 ± 0.2</td>
<td>*†#8.0 ± 1.7</td>
</tr>
<tr>
<td><strong>Norepinephrine</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(nmol/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre HV</td>
<td>3.2 ± 0.5</td>
<td>16.1 ± 2.5</td>
<td>45.6 ± 6.0</td>
</tr>
<tr>
<td>Post HV</td>
<td>3.8 ± 1.0</td>
<td>19.5 ± 2.9</td>
<td>53.8 ± 6.5</td>
</tr>
<tr>
<td>Mid Tap</td>
<td>3.4 ± 0.7</td>
<td>20.6 ± 2.6</td>
<td>52.1 ± 8.5</td>
</tr>
<tr>
<td>Post Tap</td>
<td>3.2 ± 0.4</td>
<td>22.9 ± 2.3</td>
<td>*57.9 ± 5.5</td>
</tr>
</tbody>
</table>

*Data are presented as means±SE. *: Different (P<0.05) to Pre HV. †: Different (P<0.05) to Post HV. #: Different (P<0.05) to Mid Tap.*