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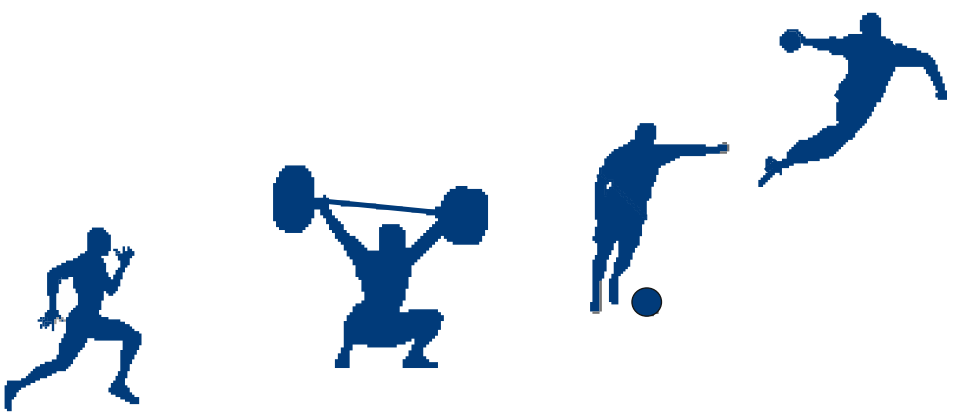

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# Reduced volume and increased training intensity elevate muscle $Na^+$-$K^+$ pump $\alpha_2$-subunit expression as well as short- and long-term work capacity in humans

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**Bangsbo J, Gunnarsson TP, Wendell J, Nybo L, Thomassen M.** Reduced volume and increased training intensity elevate muscle $Na^+$-$K^+$ pump $\alpha_2$-subunit expression as well as short- and long-term work capacity in humans. *J Appl Physiol* 107: 1771–1780, 2009. First published October 1, 2009; doi:10.1152/japplphysiol.00358.2009.— The present study examined muscle adaptations and alterations in work capacity in endurance-trained runners as a result of a reduced amount of training combined with speed endurance training. For a 6- to 9-wk period, 17 runners were assigned to either a speed endurance group with a 25% reduction in the amount of training but including speed endurance training consisting of six to twelve 30-s sprint runs 3–4 times/wk (SET group $n = 12$) or a control group ($n = 5$), which continued the endurance training (~55 km/wk). For the SET group, the expression of the muscle $Na^+$-$K^+$ pump $\alpha_2$-subunit was 68% higher ($P < 0.05$) and the plasma $K^+$ level was reduced ($P < 0.05$) during repeated intense running after 9 wk. Performance in a 30-s sprint test and the first of the supramaximal exhaustive runs was improved ($P < 0.05$) by 7% and 36%, respectively, after the speed endurance training period. In the SET group, maximal $O_2$ uptake was unaltered, but the 3-km (3,000-m) time was reduced ($P < 0.05$) from 10.4 ± 0.1 to 10.1 ± 0.1 min and the 10-km (10,000-m) time was improved from 37.3 ± 0.4 to 36.3 ± 0.4 min (means ± SE). Muscle protein expression and performance remained unaltered in the control group. The present data suggest that both short- and long-term exercise performances can be improved with a reduction in training volume if speed endurance training is performed and that the $Na^+$-$K^+$ pump plays a role in the control of $K^+$ homeostasis and in the development of fatigue during repeated high-intensity exercise.

fatigue; running economy; performance; potassium; $Na^+$-$K^+$-$Cl^-$ cotransporter isoform 1

WORK CAPACITY at different exercise intensities is determined by various factors. One way to study the importance of such factors is to change their regulatory systems by performing exercise training and then to examine the physiological response and work capacity during various types of exercise.

Muscle ion transport proteins involved in the exchange of $H^+$, $Na^+$, $K^+$, $Cl^-$, and lactate across the sarcolemma appear to be of importance in delaying fatigue during intense exercise (10, 47, 48). The $Na^+$-$K^+$ pump is pivotal in maintaining the muscle membrane potential during exercise (11), and $Na^+$-$K^+$-$Cl^-$ cotransporter isoform 1 (NKCC1) protein, primarily located in the sarcolemma, may also contribute to the maintenance of muscle function during intense exercise, possibly by adding to $K^+$ reuptake (62). In untrained human subjects, as first described in rat muscles (35), the $Na^+$-$K^+$ pump has been shown to be upregulated by different types of exercise training, detected either as content of functional pumps by [$^3$H]ouabain binding (25, 36, 41) or subunit specific by Western blot analysis (12, 45, 47). In addition, Nielsen et al. (47) observed that elevated levels of $Na^+$-$K^+$ pump $\alpha_1$- and $\alpha_2$-subunits after 8 wk of knee extensor training at supramaximal exercise intensities (speed endurance training) were associated with a reduced muscle interstitial $K^+$ concentration during exercise as well as better performance during intense exercise (47). Likewise, Iaia et al. (31) observed that well-trained subjects after a change from endurance to sprint training for 4 wk had a 29% higher expression of muscle $Na^+$-$K^+$ pump $\alpha_1$-subunits. This increase in pump content was associated with a reduced plasma $K^+$ level during exercise ($P < 0.05$) and improved performance during repeated intense running (31). In accordance, studies on well-trained subjects, who either performed strength training (42) or increased their training intensity (17, 40), have reported increased $Na^+$-$K^+$ pump concentrations as determined by the [$^3$H]ouabain-binding technique. In contrast, Aughey et al. (2) did not find changes in the abundance of any of the $Na^+$-$K^+$ pump α- and β-isoforms when already trained subjects performed a period of intensified training. The lack of effect in the latter study may have been a result of the exercise intensity being below the one corresponding to maximal $O_2$ uptake ($\dot{V}O_{2max}$). The effect of training on NKCC1 has been investigated in rats (22) and in one human study (31), where a tendency to a higher amount of NKCC1 (~14%) was observed for endurance-trained subjects after a period with sprint training. Thus, there is a further need to study the adaptations of these transport systems in trained subjects and a possible importance for work capacity during intense exercise.

Proteins controlling $H^+$ efflux from the muscle cell may be of importance for the work capacity of a contracting muscle (3, 18). $Na^+/H^+$ exchanger isoform 1 (NHE1) has been reported to increase as a result of high-intensity exercise training in rats and humans (33) and even when endurance-trained subjects changed to sprint training (31), which was associated with improved short-term performance. In human skeletal muscle, monocarboxylate transporters 1 and 4 (MCT1 and MCT4) facilitate lactate and $H^+$ exchange across the muscle membrane (32). A number of studies, including either endurance or high-intensity training programs of untrained subjects, have observed higher MCT1 protein density, and some have also observed higher MCT4 protein density (8, 33, 45). On the other hand, in a recent study (31) with endurance-trained subjects, neither the expression of MCT1 nor MCT4 increased after a sprint training period. This may be due to a reduced amount of

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training, since one study (5) has reported sprint training-induced changes in MCT1 transport proteins in endurance-trained subjects when the subjects maintained a high volume of training (~50 km/wk). Thus, the findings may suggest that, for trained athletes, a basic volume of training including frequent sessions of high-intensity exercise are necessary to change muscle MCT1 protein content and lactate transport capacity, but this needs to be studied.

Performance in distance running is a function of $\dot{V}O_{2max}$, relative work intensity, and running economy, which is defined as the energy used at a given submaximal running speed (13). In endurance-trained subjects, no or small changes in $\dot{V}O_{2max}$ are observed after a period with more intense training, whereas running economy has been shown to increase after a period of interval (6, 21, 27, 56), plyometric (50, 54, 57, 60), and strength (44) training. In a number of studies (50, 57, 58), better running economy has been associated with performance improvements. On the other hand, in a recent study (30), energy expenditure was diminished by 5–8% after a period with speed endurance training without a change in 10-km (10,000-m) performance, which may be due to a marked reduction in the total amount of training. Therefore, it would be of great interest to examine whether performance during long-term exercise and related variables can change by performing speed endurance training with a moderate reduction in the volume of training.

Therefore, the aim of the present study was to examine the effect of a reduced amount of training combined with speed endurance training on the adaptations of skeletal muscle ion transport proteins and their relation to the physiological response to exercise as well as short- and long-term work capacity. Seventeen endurance-trained subjects were studied before and after a 6- or 9-wk period where five subjects followed their normal training and twelve subjects reduced the volume of training and added sessions of speed endurance training.

## METHODS

### *Subjects*

Seventeen moderately trained male endurance runners took part in the study. All subjects were healthy nonsmokers, and none were on medication. Age, height, weight, and $\dot{V}O_{2max}$ were 34.8 ± 1.5 yr, 182.8 ± 1.5 cm, 74.0 ± 2.0 kg, and 63.0 ± 1.9 ml·kg$^{-1}$·min$^{-1}$, respectively (means ± SE). Subjects had been training and competing on a regular basis for a minimum of 5 yr, and before the study they were all running 4–5 days/wk with an average weekly distance of ~55 km. All participants were fully informed of any possible risks and discomforts associated with the experimental procedures before they gave their written informed consent to participate. This study conformed with the code of Ethics of the World Medical Association (Declaration of Helsinki) and was approved by the Ethics Committee of Copenhagen and Frederiksberg communities.

### *Intervention Period and Training*

An intervention (IT) period lasting for 6–9 wk was carried out in the last period of the competitive season. A parallel two-group, longitudinal (pre, post) design was used. Subjects were matched based on their seasonal best running performances (3,000 and 10,000 m) and randomly assigned to either an experimental speed endurance training group (SET group; $n = 12$) or a control group ($n = 5$). Four of the subjects in the SET group carried out a 6-wk IT period, whereas the remaining eight sujects conducted a 9-wk IT period. During the 9-wk IT period, the control group continued the training as during the period before the study (Fig. 1). During the IT period, the SET group carried out two to three sessions of speed endurance training a week. Each session consisted of 8–12 repeated 30-s running bouts at ~95% of maximal speed separated by 3 min of passive recovery. In addition, about once a week, the SET group did high-intensity aerobic training consisting of 4 × 4-min running at an intensity resulting in a heart rate (HR) >85% of maximal HR ($HR_{max}$; 1,100–1,250 m) separated by 2 min of passive recovery and one to two sessions of aerobic low-intensity (HR <75% of $HR_{max}$) or moderate-intensity (75–85% of $HR_{max}$) training. The total distance covered per week for the control and SET groups was 51.5 ± 3.6 and 33.2 ± 1.6 km, respectively (Fig. 1). The training speed was checked on a regular basis and modified

| | No. training sessions (·wk$^{-1}$) | Distance (km·wk$^{-1}$) | Duration (min·wk$^{-1}$) | Mean velocity (km·h$^{-1}$) | No. $A_{Hi}$ session (·wk$^{-1}$) | No. SE sessions (·wk$^{-1}$) |
|---|---|---|---|---|---|---|
| SET | 3.91±0.05$^{\#\#\#}$ | 33.2±1.6$^{\#\#\#}$ | 135.6±6.4$^{\#\#\#}$ | 14.7±0.1$^{\#}$ | 1.14±0.03$^{\#}$ | 2.22±0.03$^{\#\#\#}$ |
| CON | 4.55±0.20 | 51.5±3.6 | 226.3±15.0 | 13.7±0.1 | 1.75±0.36 | - |

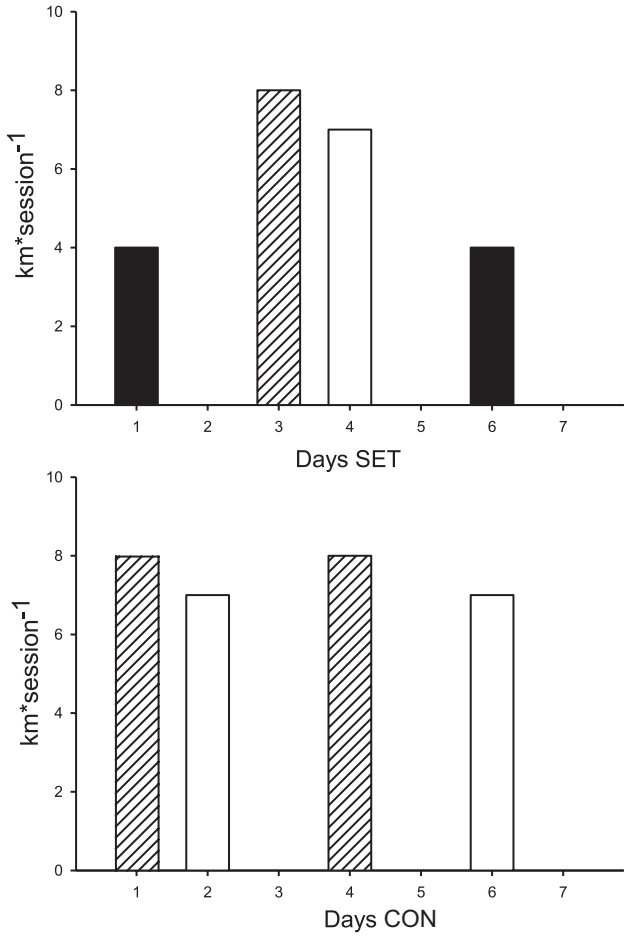


Fig. 1. Overview of the training performed during the intervention period for the control (CON) and speed endurance trained (SET) groups. *Top*: data for the total number of training sessions, distance covered, duration of the training session (expressed per week), mean velocity, number of high-intensity aerobic ($A_{HI}$) training sessions, and speed endurance (SE) training sessions are presented as means ± SE. #$P < 0.05$ and ###$P < 0.001$, significant difference between the control and SET groups. *Bottom*: 1 wk of training for the SET and control groups with speed endurance training (black bars), high-intensity aerobic training (open bars), and aerobic training (hatched bars) sessions.

every 14 days. In the SET group, all high-intensity aerobic and speed endurance training sessions were performed on a track and were carefully supervised. At the beginning of each training session, subjects were running 4.3 km with an average speed of ~13 km/h, and after the speed endurance training they ran ~1 km at a speed of ~11 km/h to recover. Subjects maintained their habitual life style and normal daily food intake. HR was recorded (Polar S610 HR monitor, Polar Electro, Kempele, Finland) in all training sessions in the IT period and immediately downloaded on portable personal computer after each training session. In addition, all training sessions for the control group were recorded and analyzed every week.

*Experimental Protocol*

Before and after the IT period, the participants completed the following tests: *1*) a 30-s sprint test (only the SET group); *2*) four 6-min runs at different submaximal running velocities (12, 14, 16, and 17 km/h) interspersed by 2 min of rest (SUB test) after a 10-min pause followed an incremental treadmill test (INC test) to exhaustion to determine $\dot{V}O_{2max}$; *3*) two exhaustive supramaximal treadmill runs (EX1 and EX2) at a speed of ~130% $\dot{V}O_{2max}$ separated by 2 min of passive rest [repeated supramaximal sprint (RS) test]; *4*) a 3,000-m race on a track (3-K); and *5*) a 10,000-m race on a track (10-K). Before the experiment, all subjects had high experience with treadmill running and 3- and 10-km racing on a track. The INC, EX1, EX2, and 30-s sprint tests were preceded by pretests to familiarize the subjects with the testing procedures. A muscle biopsy was taken before testing 48 h after the last training session.

All sets of tests were carried out at least 2 days apart, and the order of the tests was the same for the control and SET groups. The 3-km test was performed 48 h before the last speed endurance training session, and the 30-s sprint test was performed at the start of the last session. Forty-eight hours after the last training session, a muscle biopsy was obtained at rest, and the INC test was carried out after. The RS test was performed after another 48 h, and, after 24 h, subjects then underwent another speed endurance training session. After another 48 h of recovery, subjects completed the 10-km test. The control and SET groups performed the same number of training session between the tests.

*Testing Procedures*

On the day of testing, subjects reported to the laboratory 3 h after consuming a light meal. Subjects refrained from strenuous physical activity in the 48 h before testing and abstained from alcohol and caffeine consumption 24 h before testing. To minimize the effect of diet on muscle metabolism and performance, 2 days before any experimental testing the participants were also required to follow a nutritional strategy designed to ensure an adequate carbohydrate intake (~60% of total energy intake) and to record and replicate their individual dietary pattern during the 48 h before each testing day. All tests were preceded by 15 min of standardized warm-up.

A 30-s sprint test was carried out on a track, and the distance was recorded (only the SET group). Subjects completed the SUB, INC, and RS tests on a motorized treadmill under standard laboratory conditions. The calibration of the treadmill was checked before each testing session. Before the tests, subjects had a Polar S610 HR monitor (Polar Electro) fitted around their chests for continuous HR recordings, and a catheter (18 gauge, 32 mm) was inserted in an antecubital vein (not the subjects with a 6-wk IT period). The INC test started with a 3-min run at a preset speed (14 km/h), after which the speed was increased by 1 km/h every minute until volitional fatigue. Pulmonary $\dot{V}O_2$ was measured throughout the whole protocol by a breath-by-breath gas analyzing system (Jaeger MasterScreen, CPX, Viasys Heathcare, Hoechberg, Germany). The analyzer was automatically calibrated before each test with a gas of known $O_2$ and $CO_2$ concentration (53). $\dot{V}O_{2max}$ was determined as the highest value achieved over a 30-s period. A plateau in $\dot{V}O_2$ despite an increased power output and a respiratory exchange ratio (RER) of >1.15 were used as criteria for $\dot{V}O_{2max}$ achievement. Blood samples were taken at rest and at the end of each exercise bout as well as 3, 6, and 9 min after the exhaustive run. All blood samples were collected in 2-ml heparinized syringes.

In the RS test, subjects performed two supramaximal exhaustive runs (EX1 and EX2) at a speed corresponding to ~130% pretraining $\dot{V}O_{2max}$ separated by a 2-min rest period. The speed was determined by a linear extrapolation established from the individual relationship between exercise intensity and pulmonary $\dot{V}O_2$ obtained during the INC test. After a 10-min warm-up (at a running speed of 14 km/h), subjects rested for 5 min before starting the EX1 test. The exercises were terminated when the subject failed to maintain the speed. Subjects were not given any feedback. Pulmonary $\dot{V}O_2$ was measured as previously described. HR was collected at 5-s intervals. Blood samples were taken before and at the end of each exercise bout as well as 1 min after the EX1 test and 1.5 and 3 min after the EX2 test. All blood samples were collected in 2-ml heparinized syringes.

The 3- and 10-km trials were carried out on a 400-m track. To avoid racing tactics and strategies, the test was conducted on an individual basis with participants starting at 1-min intervals in random order. HR was measured before and during the tests with a Polar S610 HR monitor (Polar Electro) fitted around the chest.

Muscle biopsies were taken before and after the IT period (4). A small incision through the skin and fascia over the vastus lateralis muscle was made under local anesthesia (1 ml; 20 mg/l lidocain without adrenaline). The subject's left or right leg was randomly selected. The same leg was used for pre- and post-IT period biopsies. Tissue samples were immediately frozen in liquid nitrogen and subsequently stored at −80°C.

*Blood Analysis*

Immediately after being sampled, a part of the blood was rapidly centrifuged at 20,000 *g* for 30 s. Thereafter, the plasma was transferred to Eppendorf tubes and placed in ice-cold water until being stored at −20°C. Samples were subsequently analysed for $K^+$ by an ion-selective electrode using a Hitachi 912 Automatic Analyzer (Roche Diagnostic). Another part of the blood sample (100 μl) was hemolyzed using a 1:1 dilution with a buffer solution (Yellow Spring Instruments, Yellow Springs, OH) to which 20 g/l Triton X-100 was added (20) for the analysis of lactate (model 23, Yellow Spring Instruments).

*Muscle Analysis*

The frozen muscle biopsies were weighed before and after freeze drying to determine the water content. After the freeze drying, all connective tissue, visible fat, and blood were carefully dissected away under a stereo microscope in a room with a temperature of 18°C and a relative humidity below 30%.

*Muscle ion transport proteins.* Muscle tissue taken at rest (~4–5 mg dry wt) was homogenized on ice in a fresh batch of buffer (10% glycerol, 20 mM Na-pyrophosphate, 150 mM NaCl, 50 mM HEPES, 1% Nonidet P-40, 20 mM β-glycerophosphate, 10 mM NaF, 2 mM PMSF, 1 mM each EDTA and EGTA and 10 μg/ml each aprotinin and leupeptin and 3 mM benzamidine) with a Polytron 3100 (Kinematica) for not more than 30 s. After being rotated end over end for 1 h at 4°C, samples were centrifuged for 30 min at 17,500 *g* at 4°C, and lysates were collected as the supernatant. Protein concentrations were determined in the lysates using BSA standards (Pierce).

The lysates were diluted to appropriate protein concentrations in a ×6 sample buffer (0.5 M Tris base, DTT, SDS, glycerol, and bromophenol blue) and then boiled for 3 min at 96°C for protein denaturization. Equal amounts of total protein (5–15 μg in accordance to the antibody optimization) were loaded for each sample in different wells on either a 5% (NKCC1) or 10% precast Tris·HCl gel (Bio-Rad Laboratories). For comparisons, samples from the same subject were

always loaded on the same gel. The gel electrophoresis was done with 55 mA and maximum 150 V per gel in ~80–100 min, and, afterward, proteins were blotted to a polyvinylidene difluoride membrane using 70 mA and maximum 25 V per gel in 2 h. Membranes were incubated overnight with ~10 ml of primary antibody diluted in either 2% nonfat milk [monoclonal $Na^+$-$K^+$ pump α1-subunit, 1:500 dilution (α6F, Iowa Hybridoma Bank and C464.6, no. 05-369, Millipore); monoclonal $Na^+$-$K^+$ pump $\alpha_2$-subunit, 1:200 dilution (McB2, kindly donated by K. J. Sweadner to H. Bundgaard); polyclonal α2-subunit, 1:500 dilution (no. 07-674, Millipore); monoclonal $\beta_1$-subunit, 1:1,000 dilution (MA3-930, Affinity BioReagents); and polyclonal NKCC1, 1:200 dilution (Sc-21545, Santa Cruz Biotechnology)] or 3% BSA [monoclonal NHE1, 1:500 dilution; polyclonal MCT1, 1:1,000 dilution; and polyclonal MCT4, 1:1,000 dilution (MAB3140, AB3538P, and AB3316P, all from Millipore)]. After a brief wash in Tris-buffered saline-Tween, membranes were incubated with secondary antibody for 1 h at room temperature. The secondary horseradish peroxidase-conjugated antibodies used were diluted 1:5,000 in 2% nonfat milk or 3% BSA depending on the primary antibody (P-0447, P-0448, and P-0449, DakoCytomation). Membrane staining was visualized by a 5-min incubation with a chemiluminescent horseradish peroxidase substrate (Millipore) immediately before the membrane image was digitalized (KODAK Image Station 2000MM). The net band intensities were quantified as the total intensity minus the background intensity (Molecular Imaging Software, KODAK).

*Muscle enzymes.* Citrate synthase (CS), β-hydroxyacyl-CoA dehydrogenase (HAD), creatine kinase (CK), and phosphofructokinase (PFK) activity were determined fluorometrically on whole muscle (2 mg dry wt) homogenized (1:400) in 0.3 M phosphate-BSA buffer adjusted to pH 7.7 (39).

### *Statistics*

Before the IT period, Student's unpaired *t*-tests were used to compare subjects' characteristics between the two training groups (SET vs. control group). Two-way ANOVA for repeated measurements with control versus SET as a factor and pre versus post as a factor were used to determine the effects on muscle enzyme activity, relative HR, $\dot{V}O_{2max}$, and exercise performance (3-km run, 10-km run, and INC test). Separate for the SET and control groups, two-way ANOVA for repeated measurements with pre and post as a factor and the different sample times as a factor were used to determine the effects on blood variables during the SUB, INC, and RS tests, exercise performance in the RS test, running economy, and RER. To determine the effects on 30-s sprint test performance, we used one-way ANOVA for repeated measurements and a paired *t*-test. When an overall statistical difference was obtained, we used the Student-Newman-Keuls method as a multiple-comparison procedure to isolate which groups differed from the others.

Changes in muscle protein expression were examined on log-transformed data by applying a paired *t*-test for the SET and control groups. The individual signal intensity was related to the group pre mean before log transformation. From these log values, individual data points were left out in case of a >2-SD difference to the group mean value. Furthermore, even with continous analysis, the signal intensities from some of the samples were so weak that a quantitative analysis was impossible; these samples were then left out. Because of these criteria, the following numbers of subjects in the SET group were included in the analysis of the different proteins: $n = 12$, $Na^+$-$K^+$ pump $\beta_1$-subunit and NHE1; $n = 11$, NKCC1 and $Na^+$-$K^+$ pump $\alpha_1$- and $\alpha_2$-subunits; and $n = 8$, MCT1 and MCT4. For all proteins, all five subjects were included in the group. Data obtained with the two different antibodies against the $Na^+$-$K^+$ pump $\alpha_1$- and $\alpha_2$-subunits, respectively, were similar, and data were averaged and expressed only as $\alpha_1$- and $\alpha_2$-subunits. For all the analyses, the level of statistical significance was set at $P < 0.05$. Data are presented as means ± SE except for muscle protein data, which are presented as geometric mean ± 95% confidence intervals.

## RESULTS

### *Performance*

The SET group improved ($P < 0.001$) performance by 3.3% (pre: 10.4 ± 0.1 min vs. post: 10.1 ± 0.1 min) and mean speed at the 3-km run from 17.3 ± 0.2 to 17.9 ± 0.2 km/h. 10-km performance was improved by 3.1% (37.3 ± 0.4 vs. 36.3 ± 0.4 min) with mean velocity being 16.1 ± 0.2 km/h before and 16.6 ± 0.2 km/h after the IT period, whereas the performance of the control group was unaltered (Fig. 2). Six of the runners in the SET group conducted a new personal record on the 10-km after the IT period, with an improvement from 36.7 ± 0.3 to 35.5 ± 0.1 min. The time to exhaustion during the INC test was increased ($P < 0.001$) by 9.0% (570 ± 17 vs. 623 ± 16 s) for the SET group, resulting in a higher running speed for the SET group (20.5 ± 0.3 vs. 21.4 ± 0.3 km/h) compared with the control group (Fig. 3*A*). The SET group also had a better performance (36%, $P < 0.001$) in the EX1 test (108 ± 11 vs. 141 ± 9 s) but not in the EX2 test (70 ± 5

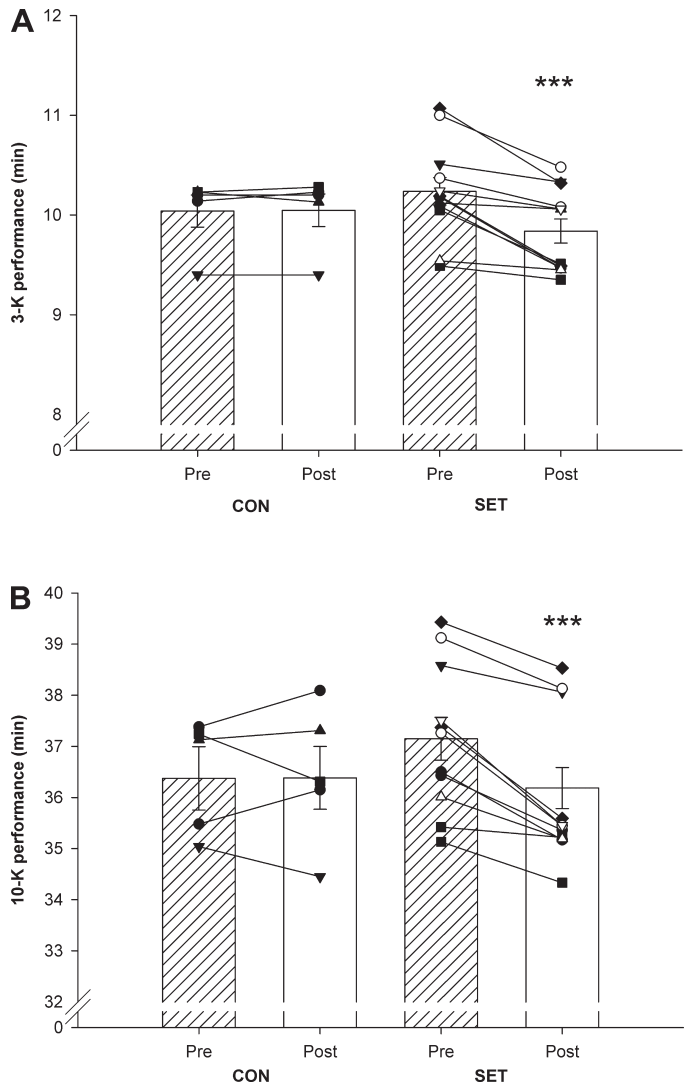


Fig. 2. Mean and individual values of 3-km (*A*) and 10-km (*B*) performances before (pre) and after (post) the intervention period in the control ($n = 5$) and SET ($n = 12$) groups divided into subjects covering the 6-wk (open symbols; $n = 4$) and 9-wk (solid symbols; $n = 8$) periods. ***$P < 0.001$, significant difference between pre and post values.

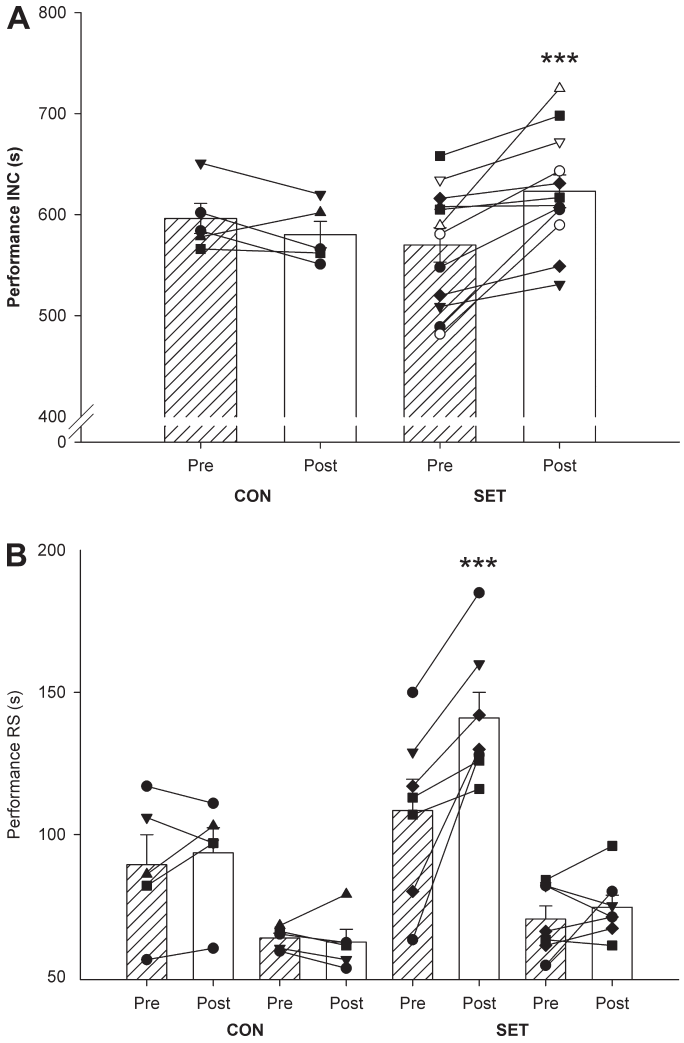


Fig. 3. Mean and individual values of performance during the incremental (INC) tests (*A*) and in the first (*left*) and second (*right*) runs of the repeated supramaximal sprint (RS) test (*B*) before (pre) and after (post) the intervention period in the control ($n = 5$) and SET ($n = 12$) groups divided into subjects covering the 6-wk (open symbols; $n = 4$) and 9-wk (closed symbols; $n = 8$) periods. ***$P < 0.001$, significant difference between pre and post values.

vs. 74 ± 4 s) and RS test (Fig. 3*B*). Performance of the control group in the INC and RS tests was the same before and after the IT period (Fig. 3). The runners in the SET group who had a 6-wk training period ($n = 4$) had a 11.0 ± 1.5-m (4.9%) improvement ($P < 0.05$) in the 30-s maximal running test, whereas the improvement ($P < 0.001$) in performance for the runners training for 9 wk ($n = 8$) was 10.8 ± 1.8 m (5.3%) and 14.0 ± 1.8 m (6.8%) after 6 and 9 wk, respectively. In the last 3 wk, the distance was increased ($P < 0.05$) by 3.3 ± 0.8 m (1.5%).

*HR During 3- and 10-km Runs*

In the SET group, the mean HR during the 3-km run was 175.5 ± 1.3 and 176.0 ± 2.6 beats/min, corresponding to 92.5 ± 1.1% and 92.7 ± 0.9% of $HR_{max}$, before and after the IT period, respectively. In the control group, the mean HR was 168.0 ± 4.4 (92.5 ± 1.1%) and 168.4 ± 3.8 (92.7 ± 0.9%) beats/min before and after the IT period, respectively. During 10-km run, the HR was 176.2 ± 2.6 (92.4 ± 0.5%) and 176.7 ± 1.5 (92.5 ± 0.8%) beats/min, respectively, in the SET group and 168.8 ± 2.5 (93.7 ± 0.8%) and 167.0 ± 2.9 (92.7 ± 0.7%) beats/min, respectively, in the control group.

*$\dot{V}O_{2max}$*

There were no changes in $\dot{V}O_{2max}$ for either the SET (4.44 ± 0.19 vs. 4.56 ± 0.19 l $O_2$/min ) or control (5.14 ± 0.16 vs. 5.04 ± 0.08 l $O_2$/min) groups. Correspondingly, $\dot{V}O_{2max}$ expressed per body weight was not changed for either the SET (61.0 ± 2.4 vs. 62.5 ± 1.9 ml $O_2 \cdot min^{-1} \cdot kg^{-1}$) or control (67.8 ± 2.4 vs. 67.0 ± 2.6 ml $O_2 \cdot min^{-1} \cdot kg^{-1}$) groups, and there were no differences between the groups.

*Muscle Ion Transport Proteins*

The expression of the $Na^+$-$K^+$ pump $\alpha_2$-subunit was 68% higher ($P < 0.05$) after the IT period (Fig. 4*B*), and the $Na^+$-$K^+$ pump $\beta_1$-subunit tended to be higher (10%, $P = 0.053$; Fig. 4*C*), whereas the expression of the $Na^+$-$K^+$ pump $\alpha_1$-subunit was unaltered (Fig. 4*A*). The expression of MCT1, MCT4, NHE1, and NKCC1 was not changed in the SET group (Table 1). The control group had no differences in the expression of any of the ion transport proteins (Fig. 4, *A–C*, and Table 1). The activity of HAD, CS, PFK, and CK was not changed during the IT period in either SET or control groups (Table 2).

*Physiological Response to Exercise*

*Running economy.* After the IT period, the SET group improved running economy at 12 km/h ($P < 0.05$) from 199 ± 7 to 193 ± 6 ml $O_2 \cdot kg^{-1} \cdot km^{-1}$, whereas at 14, 16, and 17 km/h, no significant differences were reached (Table 3). In the control group, no significant differences were observed (Table 3).

*RER values.* In the SET group, RER values at 12, 14, 16, and 17 km/h before and after the IT period were 0.933 ± 0.017 vs. 0.932 ± 0.015, 0.966 ± 0.019 vs. 0.948 ± 0.015, 1.003 ± 0.019 vs. 0.982 ± 0.015, and 1.056 ± 0.025 vs. 1.008 ± 0.012, respectively, with a significantly ($P < 0.01$) lower value at 17 km/h after the IT period (Table 3). In the control group, no significant differences in RER values were observed (Table 3).

*Plasma $K^+$ Concentration*

The $K^+$ concentration at rest, during the SUB test, and after the INC test was the same before and after the IT period in both the SET and CON groups (Table 4). In the SET group, the plasma $K^+$ concentration at the end of each of the two exhaustive runs (EX1 and EX2 tests) was lower ($P < 0.01$) after the IT period (Table 5), whereas no differences in $K^+$ concentration observed before and after the RS test. No differences in $K^+$ concentration during the RS test were observed in the control group (Table 5).

*Blood Lactate Concentration*

No differences in blood lactate concentration during the SUB test and at exhaustion in the INC test were observed before and after the training period in either the SET or control groups, but after the IT period, blood lactate concentration in the SET group was lower ($P < 0.01$) 3, 6, and 9 min into recovery (Table 4). Before, during, and after the RS test, no differences in blood lactate concentration were observed in either the SET or control groups (Table 5).

## DISCUSSION

The major findings of the present study were that inclusion of speed endurance training with a reduction in training volume not only resulted in improved short-term work capacity but also increased 3- and 10-km performance in endurance-trained runners. The improvements were associated with an ~70% higher expression of $Na^+$-$K^+$ pump $\alpha_2$-subunit and lower plasma $K^+$ concentrations during exhaustive running.

An amazing finding of the present study was that performance of these well-trained runners in the 3- and 10-km trials

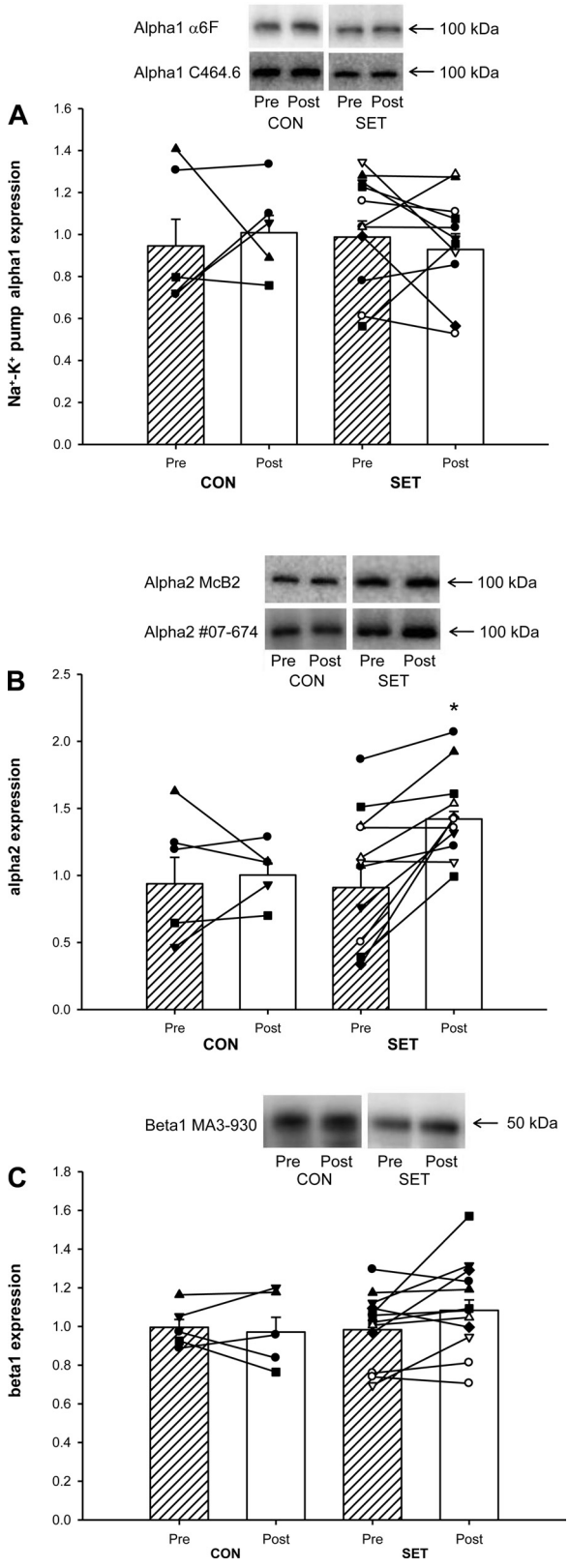


Fig. 4. *Bottom*: geometric mean values for the protein expression of the $Na^+$-$K^+$ pump $\alpha_1$-subunit (*A*), $\alpha_2$-subunit (*B*), and $\beta_1$-subunit (*C*) before (pre) and after (post) the intervention period in the control ($n = 5$) and SET ($n = 11$–12) groups divided into subjects covering the 6-wk (open symbols; $n = 4$) and 9-wk (closed symbols; $n = 7$–8) periods. *Top*: representative Western blots for the different antibodies used. $*P < 0.05$, significant difference between pre and post values.

Table 1. *Protein expression (post relative to pre) after the intervention period for the SET and control groups*

| | Control Group | | SET Group | |
|---|---|---|---|---|
| | Geometric means | 95% Confidence interval | Geometric means | 95% Confidence interval |
| Monocarboxylate transporter 1 | −5 | −42 to 58 | −2 | −8 to 4 |
| Monocarboxylate transporter 4 | −14 | −23 to −3 | −10 | −23 to 5 |
| $Na^+/H^+$ exchanger isoform 1 | −3 | −39 to 55 | 1 | −23 to 32 |
| $Na^+$-$K^+$-$Cl^-$ cotransporter isoform 1 | 17 | 3–33 | 24 | −7 to 67 |

Values are geometric means ± 95% CI in %; $n = 5$ subjects in the control group and 8–12 subjects in the speed endurance training (SET) group. Pre, before the intervention period; post, after the intervention period.

was significant elevated as a result of the speed endurance training and reduced amount of training. Furthermore, that six of the runners in the SET group at the end of the IT period made their best 10-km time ever with an improvement of >1 min, despite having been running for >5 yr and performing >20 10-km runs. These observations suggest that speed endurance training is a powerful stimulus to improve performance even in an event lasting >30 min despite that the duration of each exercise bout was 30 s. In our previous study (31), which also examined the effect of speed endurance training, no change in 10-km performance was observed, but in that study the amount of training was reduced by ~80%. Apparently, maintaining some aerobic sessions with high-intensity running, together with the speed endurance training, played a key role in causing the better performance in the present study. This is the first study to show increased long-term performance in endurance runners with a reduced volume of training and speed endurance training. Burgomaster et al. (9) found an increased cycle endurance capacity from 26 to 51 min in untrained subjects after a 2-wk period with six sessions of four to seven 30-s sprints, which was associated with elevated levels of the CS activity. In the present study, no changes in the activities of CS and HAD were observed, which may be due to the relative high levels before the IT period. On the other hand, a main effect of diminished RER was observed during submaximal running after the IT period in the SET group, with RER being significant lower at 17 km/h, which may suggest that the degree of fat oxidation was elevated at the velocity used during the 3-km test (17–18 km/h) and 10-km test (16–17 km/h). It may have allowed the same rate of muscle glycogen utilization at the higher running velocities after the IT period and may be part of the explanation of the better performance,

Table 2. *Enzyme activity before and after the intervention period in the control and SET groups*

| | Control Group | | SET Group | |
|---|---|---|---|---|
| | Pre | Post | Pre | Post |
| Citrate synthase | 41.7±7.4 | 42.3±3.3 | 45.3±4.7 | 43.7±4.2 |
| β-Hydroxyacyl-CoA-dehydrogenase | 22.1±4.3 | 21.5±2.9 | 29.9±3.2 | 26.6±2.1 |
| Creatine kinase | 5651±84 | 6166±360 | 5986±254 | 6606±234 |
| Phosphofructokinase | 58.1±8.1 | 71.9±4.5 | 95.6±11.0 | 105.6±13.5 |

Values are means ± SE (in $\mu mol \cdot g\ dry\ wt^{-1} \cdot min^{-1}$); $n = 3$ subjects in the control group and 8 subjects in the SET group.

Table 3. *Running economy ($\dot{V}O_2$) and RER during the submaximal test before and after the intervention period in the control and SET groups*

| | Running Speed | | | |
|---|---|---|---|---|
| | 12 km/h | 14 km/h | 16 km/h | 17 km/h |
| *Control group* | | | | |
| $\dot{V}O_2$, ml $O_2 \cdot kg^{-1} \cdot km^{-1}$ | | | | |
| Pre | 221.3±10.6 | 214.3±10.7 | 215.1±9.3 | 212.2±8.6 |
| Post | 209.5±6.7 | 205.0±8.5 | 205.4±8.6 | 207.1±9.3 |
| RER | | | | |
| Pre | 0.91±0.01 | 0.94±0.01 | 0.98±0.01 | 1.01±0.01 |
| Post | 0.89±0.02 | 0.93±0.02 | 0.96±0.02 | 0.98±0.02 |
| *SET group* | | | | |
| $\dot{V}O_2$, ml $O_2 \cdot kg^{-1} \cdot km^{-1}$ | | | | |
| Pre | 199.4±6.7 | 193.5±6.5 | 194.9±6.0 | 198.3±5.3 |
| Post | 193.0±5.7* | 189.7±5.2 | 189.7±5.3 | 193.3±4.7 |
| RER | | | | |
| Pre | 0.93±0.02 | 0.97±0.02 | 1.00±0.02 | 1.06±0.03 |
| Post | 0.93±0.02 | 0.95±0.02 | 0.98±0.02 | 1.01±0.01† |

Values are means ± SE; $n = 5$ subjects in the control group and 12 subjects in the SET group. $\dot{V}O_2$, $O_2$ uptake; RER, respiratory exchange ratio. *$P < 0.05$ and †$P < 0.01$, significant difference between pre and post values.

even though the role of muscle glycogen for performance during such events is not clear. $\dot{V}O_{2max}$ was not changed and cannot explain the improvements. A third important factor in long-term running performance is running economy (13). In the present study, a main effect of reduced running economy was observed after the IT period in the SET group, and the running economy was significant lower at 12 km/h. Similarly, Paavolainen et al. (50) found an improved running economy after a period where the subjects performed jump and sprint training, and the time allocated training was maintained. In addition, Iaia et al. (30) observed a better running economy when endurance-trained subjects only carried out speed endurance training. In contrast, Mikkola et al. (43) found an unaltered running economy when the volume was not changed but 19% of the training was performed as sprint, jump, and strength training once a week during an 8-wk period. Taken together, these studies suggest that sprint and frequent jump training with or without a significant reduction in training volume may lead to improvements in running economy for endurance-trained runners.

The amount of the $Na^+$-$K^+$ pump $\alpha_2$-subunit was higher after the reduced volume of training and speed endurance training, whereas there was no change in the $\alpha_1$-subunit and a tendency to elevated levels of the $\beta_1$-subunit. In contrast, Iaia et al. (31) observed that a change in training from regular endurance running to sprint training resulted in increased levels of muscle $Na^+$-$K^+$ pump $\alpha_1$-isoforms with no change in either $\alpha_2$- or $\beta_1$-isoform levels. The difference may be related to the difference in the amount of endurance training, since it has been shown to increase the expression of $Na^+$-$K^+$ pump $\alpha_2$- and $\beta_1$-isoform proteins (24), which probably are the most abundant subunits in muscle (26, 29, 49). In the study of Iaia et al. (31), the runners only performed speed endurance training, whereas the subjects in the present study carried out a significant amount of aerobic training in addition to speed endurance training. It appears that the combined high-intensity aerobic and speed endurance training in the present study provided a sufficient stimulus for increasing the level of the $\alpha_2$-subunit even though the amount of these isoforms may have been high before the study as the subjects were endurance trained. Likewise, in two studies (45, 47) of sedentary people performing repeated high-intensity training, elevated levels of $\alpha_2$-subunits were found. In only one of the studies (47), the level of the $\alpha_1$-subunit was higher after training. The lack of increase in the $\alpha_1$-subunit, which is in contrast to the finding of Iaia et al. (31), may suggest that a significant reduction in training volume is needed to cause a rise in the $\alpha_1$-subunit with speed endurance training in endurance-trained subjects. It may also have been an effect of the reduction in relative number of slow twitch fibers (data not shown), since $\alpha_1$-subunit expression has been shown to be higher in slow twitch compared with fast twitch fibers in rats (19). One of the explanations of the pronounced $Na^+$-$K^+$ pump upregulation in the present study may have been a higher intracellular $Na^+$ concentration during the training with high intensity compared with the normal submaximal

Table 4. *Plasma $K^+$ and lactate concentrations during submaximal running and after the incremental test before and after the intervention period in the control and SET groups*

| | | Running Speed | | | | | Recovery | | |
|---|---|---|---|---|---|---|---|---|---|
| | Rest | 12 km/h | 14 km/h | 16 km/h | 17 km/h | Exhaustion | 3 min | 6 min | 9 min |
| *Control group* | | | | | | | | | |
| $K^+$ concentration, mmol/l | | | | | | | | | |
| Pre | 3.68±0.16 | 4.26±0.12 | 4.28±0.21 | 4.66±0.28 | 4.92±0.17 | 5.40±0.12 | 3.44±0.15 | 3.18±0.22 | 3.38±0.15 |
| Post | 3.74±0.16 | 4.46±0.30 | 4.38±0.18 | 4.66±0.21 | 4.86±0.17 | 5.40±0.18 | 3.62±0.25 | 3.26±0.19 | 3.38±0.15 |
| Lactate concentration, mmol/l | | | | | | | | | |
| Pre | 0.66±0.05 | 0.94±0.13 | 1.09±0.15 | 1.66±0.34 | 2.78±0.62 | 7.07±0.95 | 7.25±1.05 | 6.55±1.00 | 5.93±1.07 |
| Post | 0.69±0.09 | 0.93±0.27 | 0.97±0.23 | 1.82±0.51 | 3.07±0.82 | 5.72±0.33 | 5.96±0.66 | 5.27±0.73 | 4.48±0.88 |
| *SET group* | | | | | | | | | |
| $K^+$ concentration, mmol/l | | | | | | | | | |
| Pre | 3.91±0.13 | 4.29±0.06 | 4.33±0.04 | 4.73±0.16 | 4.76±0.05 | 4.99±0.06 | 3.44±0.14 | 3.34±0.05 | 3.59±0.11 |
| Post | 3.70±0.13 | 4.26±0.10 | 4.35±0.13 | 4.49±0.12 | 4.61±0.08 | 4.97±0.27 | 3.66±0.18 | 3.31±0.09 | 3.31±0.14 |
| Lactate concentration, mmol/l | | | | | | | | | |
| Pre | 0.78±0.07 | 1.00±0.14 | 1.22±0.20 | 2.02±0.30 | 3.65±0.45 | 7.08±0.73 | 7.41±0.55 | 7.62±0.57 | 6.21±0.30 |
| Post | 0.81±0.07 | 1.06±0.08 | 1.12±0.09 | 1.92±0.11 | 3.24±0.18 | 8.31±0.71 | 8.72±0.59* | 8.47±0.64* | 7.87±0.77† |

Values are means ± SE; $n = 5$ subjects in the control group and 8 subjects in the SET group. *$P < 0.05$ and †$P < 0.01$, significant difference between pre and post values.

Table 5. *Plasma $K^+$ and lactate concentrations during and after the repeated supramaximal sprint test before and after the intervention period in the control and SET groups*

| | | | *Recovery Period 1* | | | *Recovery Period 2* | | |
|---|---|---|---|---|---|---|---|---|
| | Rest | EX1 | 1 min | 2 min | EX2 | 1.5 min | 3 min | 6 min |
| | | | | *Control group* | | | | |
| $K^+$ concentration, mmol/l | | | | | | | | |
| Pre | 3.94±0.10 | 5.93±0.30 | 5.06±0.57 | 4.24±0.21 | 5.33±0.11 | 4.34±0.09 | 3.46±0.11 | 3.24±0.09 |
| Post | 4.28±0.23 | 5.70±0.12 | 4.74±0.20 | 4.32±0.27 | 5.53±0.03 | 4.22±0.27 | 3.56±0.12 | 3.32±0.09 |
| Lactate concentration, mmol/l | | | | | | | | |
| Pre | 0.73±0.06 | 7.44±1.03 | 7.30±0.95 | 7.67±0.89 | 10.69±1.40 | 10.29±0.93 | 10.50±0.89 | 10.56±0.95 |
| Post | 0.77±0.11 | 7.06±1.22 | 6.14±0.70 | 6.62±0.53 | 9.89±1.30 | 8.86±1.11 | 8.95±0.89 | 8.59±0.89 |
| | | | | *SET group* | | | | |
| $K^+$ concentration, mmol/l | | | | | | | | |
| Pre | 3.89±0.03 | 5.63±0.21 | 4.86±0.16 | 4.44±0.21 | 5.46±0.22 | 4.24±0.07 | 3.45±0.06 | 3.31±0.06 |
| Post | 3.89±0.07 | 5.30±0.19* | 4.71±0.17 | 4.30±0.14 | 4.85±0.15† | 3.94±0.14 | 3.41±0.10 | 3.33±0.11 |
| Lactate concentration, mmol/l | | | | | | | | |
| Pre | 0.85±0.07 | 8.04±0.90 | 6.85±0.68 | 7.51±0.72 | 10.55±1.01 | 10.01±0.72 | 10.44±0.80 | 10.05±0.75 |
| Post | 0.92±0.08 | 9.41±0.75 | 7.30±0.95 | 7.67±0.89 | 10.69±1.40 | 10.29±0.93 | 10.50±0.89 | 10.56±0.95 |

Values are means ± SE; $n = 5$ subjects in the control group and 8 subjects in the SET group. EX1 and EX2, exhaustive supramaximal treadmill run *test 1* and *2*, respectively. *$P < 0.01$ and †$P < 0.001$, significant difference between pre and post values.

endurance training (55), since intracellular $Na^+$ is a possible transcription factor for the $Na^+$-$K^+$ pump (46). Thus, Ladka and Ng (37) used the $Na^+$ channel activator veratridine to modulate intracellular $Na^+$ and observed increased $\alpha_2$-subunit protein expression in $C_2C_{12}$ skeletal muscle cells. Furthermore, increased intracellular $Na^+$ was related to an upregulation of $Na^+$-$K^+$ ATPase molecules in cultured chick skeletal muscles (61).

The present study showed that performance during the INC test and during the first intense exercise bout to exhaustion (EX1; ~2 min) was elevated despite the reduction in the volume of training, which is in accordance with the study of Iaia et al. (31), where speed endurance training of the same type as in the present study was used and where the amount of training was lowered by 64%. A number of other studies (5, 15, 28, 38, 59) have demonstrated that performance during exercise lasting <5 min can be improved after a period including high-intensity exercise training. However, in contrast to these studies, the amount of training was significantly reduced in the present study and in our previous study (31), which suggests that the intensity, rather than the amount, of training is the main factor in performance improvement after a period of speed endurance training. It could be speculated that the elevated level of $\alpha_2$-isoforms in the SET group after the IT period may have increased the number of functional pumps, causing a lower accumulation of $K^+$ in the muscle interstitium during exercise and in the recovery from exercise (47, 48). In support of this, the plasma $K^+$ concentration was lower at the end of the repeated intense exercise bouts after the IT period. In agreement, in the study of Iaia et al. (31), the elevated level of $\alpha_1$-subunits after a period with sprint training was associated with a lower rate of increase in venous $K^+$ concentration during an intense exercise bout, and the lowering of plasma $K^+$ concentration 1 min after the intense exercise was correlated with the level of muscle $Na^+$-$K^+$ pump $\alpha_1$-subunits (31). It may be that the amount of $\alpha$-subunits are limiting for the formation of $Na^+$-$K^+$ pumps and that the elevated level of $\alpha_1$-subunits in the study of Iaia et al. (31) and $\alpha_2$-subunits in the present study after the IT period have lead to an increased content of active $Na^+$-$K^+$ pumps during exercise. Consequently, the reduction in muscle membrane potential may have been lowered and cell excitability preserved (10), and thereby the time to fatigue during supramaximal exercise is prolonged. Together, these findings support a role of muscle $Na^+$-$K^+$ pumps in the control of extracellular $K^+$ concentration and fatigue development during intense exercise. The expression of NKCC1 was not changed in the present study. Increases of 14–29% have been observed when training rats (22), and Iaia et al. (31) found in humans a nonsignificant 14% increase in NKCC1 with a reduced volume of training and sprint training. Apparently, a further reduction in training may have been needed for the amount of NKCC1 change, and alterations in NKCC1 expression cannot explain the slower development of muscle fatigue during intense exercise after the IT period.

The expression of muscle NHE1 was not changed in the present study, which is in contrast to the finding of Iaia et al. (31) and studies where untrained subjects performed a period of sprint training (34, 45). Apparently, the greater volume of training in the present study impaired the net synthesis of NHE1. Similarly, neither MCT1 nor MCT4 were changed with training, which is in accordance with the observations of Iaia et al. (31). On the other hand, most other studies (5, 8, 34, 45, 52) with untrained subjects using high-intensity intermittent training have shown a higher amount of MCT1, and one study (5) has reported sprint training-induced changes in MCT1 proteins in endurance-trained subjects. In that study, the subjects maintained a high volume of training (~50 km/wk). It may be that the subjects in the present study already had an elevated MCT1 protein content before the change in training since endurance training has been shown to increase MCT1 density (7, 14, 23) and lactate transport capacity has been observed to be higher in trained compared with untrained subjects (51). The finding of unaltered MCT4 levels is consistent with the majority of the other studies (5, 16, 34, 45). The unaltered NHE1 and MCT protein expression observed after the IT period is consistent with the finding of unchanged blood lactate concentrations during the SUB and RS tests. The finding of higher blood lactate levels in the recovery period

from the INC test is probably a reflection of the more work performed after the IT period. Apparently, improved short-term performance can occur without changes in some of the key $H^+$ transport proteins.

In summary, the present study showed that speed endurance training together with a reduction in total volume of training lead to both improved long- and short-term performance, which was associated with a significant elevated level of $Na^+$-$K^+$ pump $\alpha_2$-subunits and lower plasma $K^+$ concentrations during exhaustive running.

### *Perspectives and Significance*

The present study examined the muscular effects and performance aspects in relation to a change from regular endurance training to a reduced amount of training and additional speed endurance training. Significant changes in both long- and short-term performance were observed, which was associated with an increase in the $Na^+$-$K^+$ pump $\alpha_2$-subunit, whereas a number of metabolic enzymes and other key muscle ion transport proteins were unaltered. Future research should try to elucidate what are causing the changes and study the importance of muscle ion regulation for work capacity during exercise at various intensities. This study has important practical implications, as it suggests that in already trained subjects, further muscle adaptations can occur and performance can be improved by adding speed endurance training. Endurance runners may, therefore, in some periods, benefit from replacing overall volume of training with sessions of high-intensity exercise. This information is clearly of great interest not only for elite athletes but also for people participating in recreational activities. Specifically, in a health perspective, a prospective study (1) on United States male physicians has suggested that habitual vigorous exercise, as in the present study, diminishes the risk of sudden death during vigorous exertion.

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#### DISCLOSURES

No conflicts of interest are declared by the author(s).

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# HYPERACTIVATION OF SKELETAL MUSCLE STEM CELLS BY MEANS OF BLOOD FLOW RESTRICTED RESISTANCE EXERCISE: IMPLICATIONS FOR MUSCLE HYPERTROPHY IN SPORTS AND CLINIC SETTINGS

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## Blood flow restricted exercise

Blood flow restricted exercise at low-to-moderate loading intensity (20–50% 1RM) using concurrent blood flow restriction (BFRE) has gained increasing attention in both the scientific and applied fields (Manini & Clarck 2009, Wernbom et al. 2008). The growing popularity resides on observations that skeletal muscle mass and maximal muscle strength can be increased to a similar or greater extent with BFRE (Wernbom et al. 2008) compared to conventional heavy-resistance strength training (Aagaard et al. 2001). Further, BFRE seems to result in amplified hypertrophy responses and strength gains compared to exercise using identical loads and volume without vascular occlusion (Abe et al. 2006, Holm et al. 2008) although a potential hypertrophic role of low-intensity resistance training may also exist *per se* (Mitchell et al. 2012). Yet, the specific mechanisms responsible for the adaptive change in muscle morphology with BFRE remain largely unknown. Myofibrillar protein synthesis is increased following acute bouts of BFRE, along with an upregulated activity in the AKT/mTOR pathway (Fujita et al. 2007, Fry et al. 2010). In addition, a reduced expression of proteolysis-related genes (FOXO3a, Atrogin, MuRF-1) and myostatin, a negative regulator of muscle mass has been observed after acute BFRE (Manini et al. 2011, Laurentino et al. 2012).

## Myogenic satellite cells

Satellite cells (SCs) are undifferentiated myogenic precursor cells with the ability to re-enter the cell cycle to generate new muscle fibers and/or to provide new myonuclei to existing muscle fibers during postnatal growth (Kadi et al. 2005, Hawke & Garry 2001, Boldrin et al. 2010). In resting skeletal muscle quiescent SCs are located between the basal lamina and sarcolemma of the myofiber (Kadi et al. 2005, Pallafacchina et al. 2012). Resistance training appears to induce a renewal of SCs in human skeletal muscle of both old and young individuals (Zammit et al. 2006, Mackey et al. 2007, Kadi & Ponsot 2010) in a dose-dependant manner (Hanssen et al. 2012). Activation and proliferation of myogenic SCs are associated with accelerated and amplified hypertrophy responses following resistance training (Petrella et al. 2008, Olsen et al. 2006) and the amount of myonuclei in the myofiber has been proposed to impose a ceiling effect on the magnitude of myofiber hypertrophy (Kadi et al. 2004, Petrella et al. 2008). Exercise induced myonuclear addition occurs mainly in the presence of highly marked myofiber hypertrophy (Kadi & Thornell 2000, Kadi et al. 2004, Olsen et al. 2006, Petrella et al. 2008, Mackey et al. 2010). These findings suggest that SC activation play an essential role in conditions of amplified muscle protein synthesis by providing increased transcriptional capacity to the muscle cell, although recent data sugggest that overload-induced myofiber growth may well occur in the absence of myogenic SCs (McCarthy et al. 2011, Jackson et al. 2012). However, until very recently the effect of BFRE on myogenic SC activation and myonuclear addition has remained unknown.

## Effects of BFRE on contractile muscle function

Significant increases in maximal muscle strength (MVC) and power have been reported following BFRE using low-to-moderate training loads, despite relatively short periods of training (4-6 wks) (i.e. Takarada et al. 2002, Kubo et al. 2006; for review see Wernbom et al. 2008). Notably, the adaptive effect of BFRE on contractile muscle function (MVC, power) is comparable to that achieved by 12-16 wks of conventional high-volume heavy-resistance strength training (Wernbom et al. 2008). However, the effect of BFRE on rapid force capacity (rate of force development: RFD) has remained largely unexplored, while only very recently addressed (Nielsen et al, ICST 2012).

## Effects of BFRE on myofiber size

Substantial gains myofiber size and whole muscle CSA have been demonstrated following BFRE using low loading intensities (Abe et al. 2006, Ohta et al. 2003, Kubo et al. 2006, Takadara et al. 2002). In contrast, low-resistance training without blood flow occlusion typically results in no (Abe et al. 2006, Mackey et al.

2010) or only minor gains (<5%) (Holm et al. 2008) in myofiber size, although this notion recently has been disputed (Mitchell et al. 2012). Interestingly, the proliferation of myogenic SCs and formation of new myonuclei with BFRE seem to explain at least in part the very large gains in myofiber size that may be observed with this type of training (discussed below).

**Effects of BFRE on myogenic satellite cells and myonuclei number**

We recently investigated the involvement of myogenic SC proliferation and myonuclear addition in response to BFRE (Nielsen et al. 2012). Evidence of SC proliferation and myonuclear addition were observed following 3 weeks of BFRE, accompanied by substantial gains in myofiber size (Nielsen et al. 2012) (Fig.1). Density and number of Pax-$7^+$ SCs increased 1-2 fold (+100-200%) after 19 days of BFRE (Fig.2), thus markedly exceeding the 20-40% gain in SC number typically seen in response to months of conventional resistance training (Kadi et al. 2005, Olsen et al. 2006, Mackey et al. 2007). SC number and density increased to a similar extent in type I and II myofibers (Nielsen et al. 2012) (Fig.2) in contrast to the greater SC response in type II vs. type I fibers normally observed following prolonged heavy-load resistance training (Verdijk et al. 2009). In addition, myonuclei number increased significantly (+22-33%) with BFRE, while myonuclei domain (myofiber size/number of nuclei) remained constant (~1800-2100 $\mu m^2$) although demonstrating a slight albeit transient decrease at day 8 of training (Nielsen et al. 2012).

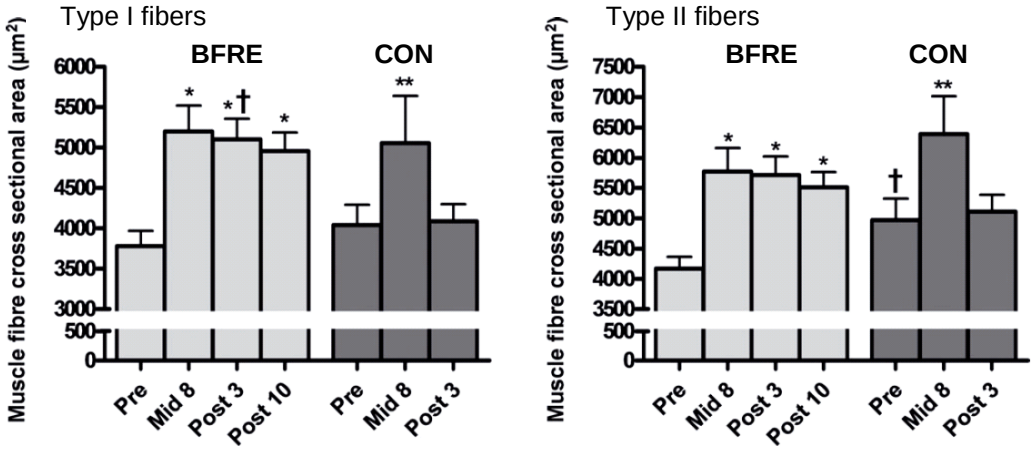


Fig.1

Myofiber cross-sectional area (CSA) measured pre and post 19 days of low-resistance (20%-1RM) blood flow restricted exercise (BFRE) or work/load matched exercise without blood flow occlusion (CON) in type I (left panel) and type II fibers (right panel). Changes relative to pre: * $p<0.001$, ** $p<0.01$, between group difference: † $p<0.05$. Adapted from Nielsen et al. 2012.

**Implications for myofiber growth**

The rise in SC activity induced by BFRE (cf. Fig.2) were accompanied by substantial myofiber hypertrophy (+30-40%) in type I and II myofibers from biopsies obtained at 3-10 days post training (Fig.1). In addition, BFRE led to significant gains in MVC (~10%) and RFD (16-21%) (Nielsen et al, ICST 2012).

Underlining the positive influence of SC proliferation on myofiber growth following BFRE, positive relationships were observed between the pre-to-post training change in mean myofiber area and gains in SC number and myonuclei number, respectively ($r = 0.51$-$0.58$, $p<0.01$).

No changes in any of the above parameters were observed in controls performing similar type of training without blood flow occlusion, except for a transient increase in type I+II myofiber size at 8 days of training.

**Potential adaptive mechanisms**

Myofiber CSA was found to increase for both fiber types after only 8 days of BFRE intervention (10 training sessions), and remained elevated 3 and 10 days post training (Nielsen et al. 2012). Unexpectedly, fiber CSA also increased transiently in control subjects performing non-occlusion exercise at day 8, but returned to baseline levels after 19 days of training. These observations suggest that the rapid initial change in myofiber CSA was influenced by factors other than myofibrillar protein accumulation, such as cell swelling.

Short lasting cellular swelling might arise from hypoxia-induced modification of selected membrane channels (Korthuis et al. 1985), stretch-induced opening of membrane channels (Singh & Dhalla 2010) or microfocal damage to the plasma membrane itself (Grembowicz et al. 1999). In contrast, the late-phase gain in myofiber CSA observed following 19 days of BFRE (cf. Fig.1) likely occurred as a result of myofibrillar protein accumulation, since myofiber CSA remained elevated 3–10 days post training along with a 7–11% sustained elevation in MVC and RFD.

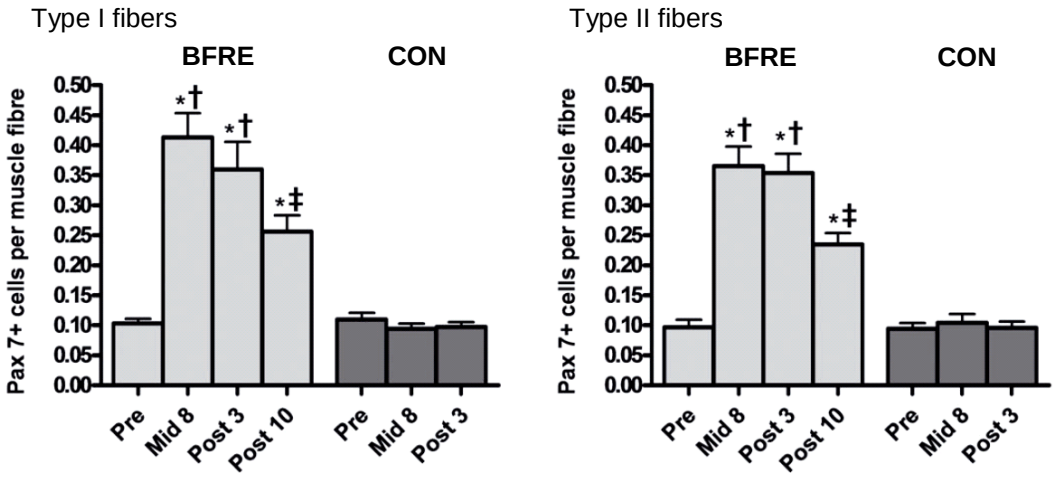


Fig.2
Myogenic satellite cell number pre and post 19 days of blood flow restricted exercise (BFRE) or work/load matched exercise without blood flow occlusion (CON) in type I (left panel) and type II fibers (right panel). Changes relative to pre: * $p<0.001$, between group difference: † $p<0.05$ Adapted from Nielsen et al. 2012.

The specific pathways of stimulatory action of BFRE on myogenic SCs remain largely unknown. Speculatively, down-regulated myostatin expression following BFRE (Manini et al. 2011, Laurentino et al. 2012) may play an important role, since myostatin is a potent inhibitor of myogenic SC activation (McCroskery et al. 2003, McKay et al. 2012) by suppressing Pax-7 signaling (McFarlane et al. 2008). Induction of IGF splice variants IGF-1Ea and IGF-1Eb (MGF) following BFRE may potentially also play an important role, since known to be strong stimulators of SC proliferation and differentiation (Hawke & Garry 2001, Boldrin et al. 2010). Mechanical stress on muscle fibers can trigger SC activation through the release of nitric oxide (NO) and HGF (Tatsumi et al. 2006, Punch et al. 2009). Consequently, NO may also be of importance for the hyper-activation of myogenic SCs observed with BFRE since transient rises in NO may likely occur in result of the ischemic conditions during BFRE.

**SUMMARY and FUNCTIONAL IMPLICATIONS**

Our recent data demonstrate that short-term low-load resistance exercise performed with partial blood flow restriction (BFRE) leads to marked proliferation of myogenic stem cells and results in myonuclear addition in human skeletal muscle, which contribute to the accelerated time course and marked degree of myofiber hypertrophy observed with this type of training. Tentative molecular signaling events involved in the hyper-activation of SCs by BFRE may involve increases in intramuscular IGF-1 production, local NO release and/or dimished myostatin activity, potentially with a contribution from yet other regulatory factors.

The accelerated and amplified hypertrophy response observed with low-intensity BFRE may be exploited to maximize muscle mass in strength/power athletes, and to prevent muscle loss in atrophic/sarcopenic patients and injured athletes alike. Further, the marked up-regulation in skeletal muscle SC content elicited by BFRE represents an effective non-pharmacological tool to increase the regenerative and adaptive capacity of the targeted muscles. In addition, the increase in myonuclear number resulting from exercise induced activation of myogenic SCs may contribute to the phenomenon of 'muscle memory' (Bruusgaard et al. 2010, 2012). Obviously, a number of safety aspects and potential exclusion criterions have to recognized with BFRE (Clarck et al. 2011, Loenneke et al. 2011), but when addressed adequately BFRE seem a safe and tolerable training modality in both young and old adults, including injured patients and athletes.

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Desde la década de los 70 se sabe que el músculo esquelético genera radicales libres (RL). Los primeros estudios desarrollados al respecto se centraron en el daño que un nivel excesivo de RL puede producir en la fibra muscular. Del mismo modo se demostró que el aumento de radicales libres influye en la fatiga muscular de ejercicios submáximos, ya que el uso de antioxidantes, que minimizan la acción de los radicales libres, produce un aumento de la fuerza submáxima. Sin embargo ciertos niveles de radicales libres son necesarios para la correcta generación de la fuerza. También estos, los radicales libres, son necesarios para la correcta señalización muscular en respuesta al ejercicio y por tanto para las adaptaciones que ésta regula. En relación a esto, la proteína quinasa activada por adenosin monofosfato (AMPK), es una enzima que activa varios procesos catabólicos y desactiva otra serie de procesos anabólicos. Entre los procesos que la AMPK activa (regulada por $pThr^{172}$-AMPKα) se encuentra el transporte de ácidos grasos al interior de la mitocondria para su oxidación o las adaptaciones celulares hacia un perfil más oxidativo. Recientemente se ha demostrado AMPK es activada tras ejercicio de sprint en cicloergometro, también se sabe que en cultivos celulares la actividad AMPK aumenta en hipoxia (por acción de los radicales libres). Para demostrar si el estrés oxidativo regula la actividad AMPK en ejercicio de sprint, reclutamos a 10 sujetos sanos (9 en el estudio II y III) que realizaron 4 test de Wingate combinando hipoxia, normoxia antioxidantes y placebo. En el **estudio I** demostramos que la combinación de ejercicio de sprint con hipoxia severa aguda ($FiO_2$: 0.1) no sólo no aumenta $pThr^{172}$-AMPKα sino que la inhibe. Los mecanismos que parecen regular dicha inhibición son dos: Primero en hipoxia la disminución de la cantidad de proteína total de SIRT1 (una proteína que regula positivamente la actividad AMPK) y su activación (vía $NAD^+/NADH^+$). Segundo el aumento en la fosforilación de las serinas alternativas de AMPK (un mecanismo inhibitorio de $pThr^{172}$-AMPKα) de manera dependiente de Akt. En dicha condición se produjo estrés oxidativo como pudo verse a través del aumento significativo de la carbonilación proteica en plasma y músculo esquelético. En el **estudio II** se demostró el aumento de la fosforilación de la $Thr^{172}$-AMPK producido por el ejercicio de sprint parece ser dependiente del nivel de radicales libres producidos por el propio ejercicio, pues al realizar el test de Wingate de 30 s tras la ingesta de antioxidantes se produjo

una inhibición de la fosforilación en la $Thr^{172}$-AMPKα. Dicha inhibición se explica por una disminución de la fosforilación en la $Thr^{286}$-CaMKII (una quinasa que se ha postulado como posible activadora de la proteína AMPK). Teniendo en cuenta que la fosforilación de $Thr^{172}$-AMPK fue bloqueada al realizar el test de Wingate en hipoxia severa aguda, en el **estudio III** comparamos un test de Wingate de 30 s realizado en hipoxia severa aguda ($FiO_2$: 0.1) con otro en similar condición pero tras la ingesta previa de antioxidantes. La disminución del estrés oxidativo por parte de los antioxidantes no solventó el bloqueo de la fosforilación de la $Thr^{172}$-AMPK. En ambas condiciones aumentó la fosforilación de de las serinas alternativas y de Akt, confirmando su efecto inhibitorio, los antioxidantes produjeron una disminución en la fosforilación de la fosforilación de la $Thr^{286}$-CaMKII como en el estudio II. Los resultados demostraron que la activación de AMPK por ejercicio de sprint es altamente dependiente del nivel de estrés oxidativo generado en el músculo y que los mecanismos que la regulan actúan a varios niveles.

# RESONANCIA MAGNÉTICA DE TEJIDO ADIPOSO Y MÚSCULO EN EL DEPORTE (Magnetic Resonance of adipose tissue and muscle in sport).

Fernando Idoate Saralegui

La prevalencia de la obesidad ha aumentado de forma constante en las últimas décadas. Disponemos de infinidad de estudios que evidencian los riesgos para la salud relacionados con la obesidad, acumulación grasa fundamentalmente en el compartimento visceral, o VAT (visceral adipose tissue) y en el compartimento intermuscular o IMAT (intermuscular adipose tissue). Por este motivo la evaluación y cuantificaciónde la grasa corporal con métodos no invasivos se han convertido en un componente importante en la investigación de la eficacia de las medidas propuestas contra la obesidad. Así mismo, existe una clara relación entre la calidad y cantidad de tejido muscular y resistencia a insulina (síndrome metabólico) así como con el síndrome de fragilidad en el anciano e incluso el pronóstico de pacientes oncológicos.

La aplicación de la tomografía computarizada (TC) y de la resonancia magnética (RM) en la investigación de la composición del cuerpo humano representa uno de los avances más importantes en la historia de la antropometría . Ambos métodos se han utilizado para avanzar en la compresnsión de las complejas relaciones entre la composición corporal humana y la enfermedad. La TC y la RM son los métodos de elección para la calibración de métodos de campo diseñados para medir el tejido adiposo y músculo esquelético in vivo, y son los únicos procedimientos disponibles para la cuantificación de los tejidos y órganos internos incluyendo el músculo esquelético y tejido adiposo. La TC permite obtener imágenes de cuerpo entero con tiempos de adquisición muy cortos, y su postproceso en el caso de cuantificación de tejido adiposo es más sencillo que la RM. Sin embargo la resonancia magnética es un método que no emite radiación ionizante, y es la técnica radiológica que proporciona mayor resolución tisular. Ambas técnicas de imagen seccional son las técnicas de elección en la valoración de estudios de intervención (por ejemplo dieta y/o ejercicio) en los que se desea comprobar cambios producidos global o regionalmente tanto en tejido adiposo como muscular . La RM es especialmente útil en la valoración la estructura muscular. Permite cuantificar in vivo volúmenes y áreas seccionales de músculos independientes, de modo que es posible monitorizar adaptaciones secundarias a ejercicio deportivo específico, rehabilitación, inmovilizacion o enfermedad. Los datos que obtenemos de la

segmentación con RM de los distintos músculos, tendones y estructuras óseas ofrecen datos que pueden ser utilizados para el desarrollo de modelos biomecánicos del sistéma musculoesquelético, aportando base científica al tratamiento de enfermedades relacionadas con el movimiento, desarrollo de nueva aproximaciones quirúrgicas o diseño de prótesis.

Pero la RM no sólo permite valorar los volúmenes musculares; existen técnicas de RM como el T2 mapping o RM-funcional que se han propuesto como método para la visualización de los cambios fisiológicos que los ocurrir en los músculos activados durante el ejercicio, permitiendo examinar la cantidad relativa de la actividad de los músculos, o partes de los músculos que participan en una tarea. El índice cuantitativo de la activación del músculo obtenido con estas técnicas de RM es el tiempo de relajación transversal T2. Los valores de T2 en músculos reclutados aumentan después de ejercicio excéntrico. El ejercicio intensivo se sabe que produce cambios en la cantidad y distribución de agua en los músculos esqueléticos. Se cree que el aumento de T2 inducido por el ejercicio es causado por la actividades metabólicas en el músculo, tales como el movimiento osmótico de agua muscular en los espacios miofibrillares y la dilatación vascular o aumento de flujo sanguíneo, asociados con la generación de la fuerza para la contracción muscular. Estas técnicas han permitido evaluar qué músculos y qué regiones musculares se reclutan durante programas de ejercicio eccéntrico diseñados para prevenir las roturas musculares producidas durante el ejercicio deportivo.

La evaluación de la estructura y función muscular, del depósito de grasa compartimental sí como la cuantificación de las adaptaciones y cambios producidos por intervenciones con distintos programas de ejercicio físico son posibles y accesibles gracias a las técnicas de imagen seccional.

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## Muscle biology in the –omics era

(Ola Hansson)

This talk will be describing how questions related to exercise physiology, in particular muscle biology, could be addressed by "omics" methodologies. Basic concepts of how genetic variation can be interpreted and translated into molecular mechanisms will be discussed. Data from two unpublished studies will be presented: (1) A skeletal muscle expression quantitative trail loci (eQTL) study, where we have integrated genetic variation, muscle gene expression and clinical phenotype data in order to investigate the genetic contribution to gene expression and insulin sensitivity. We identified 287 muscle eQTLs and 49 associations between gene expression and measures related to insulin sensitivity. These results were also extended with published genome wide association study (GWAS) data from the DIAGRAM and MAGIC consortia (T2D- and glycaemic-traits, respectively), with publicly available human muscle expression data, and with euglycemic hyperinsulinemic clamp phenotype data. Taken together, this highlighted phosphofructokinase (*PFKM)* as a regulator of skeletal muscle insulin sensitivity. (2) A compendium of gene expression microarray data for 2,852 human skeletal muscle biopsies, assayed across a wide variety of experimental and sample conditions will also be presented. The data was retrieved from public data archives, and manually re-annotated using a harmonized vocabulary. We analyse this compendium to characterise gene expression in skeletal muscle, and show that increasing age is associated with the activation of the PI3K/AKT/mTOR pathway and downstream targets regulating muscle growth, as well as the reduced expression of key enzymes for glucose metabolism, in particular glycolytic pyruvate processing. This is so far the largest gene expression based study of aging in human skeletal muscle, and the manually curated dataset of nearly 3,000 samples generated for this study will be publicly available.

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ORIGINAL ARTICLE

Øivind Foss · Jostein Hallén

# Cadence and performance in elite cyclists

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**Abstract** Many studies have attempted to describe the optimal cadence in cycling. However, the effect on performance has received little attention. The aim of the present study was therefore to examine the effect of cadence on performance during prolonged cycling (~30 min). Fourteen male elite cyclists performed two or five time trials at different cadences [60, 80, 100, 120 rpm or freely chosen cadence (FCC)]. The total work was the same between the time trials, and the subjects were instructed to complete each time trial as fast as possible by adjusting the workload with buttons mounted on the handlebar. Accumulated work and cadence was visualised on a monitor. Oxygen uptake was measured continuously and blood lactate concentration every fifth minute. Compared to 80 rpm, finishing times at 60, 100 and 120 rpm were 3.5, 1.7 and 10.2% slower ($P < 0.05$). Finishing time at FCC (mean 90 rpm) was indistinguishable from 80 and 100 rpm. Gross efficiency at 80 rpm was 2.9, 2.3, 3.4 and 12.3% larger than at 60, FCC, 100 and 120 rpm, respectively ($P < 0.05$). The maximal energy turnover rate was 1.7% higher at 100 than at 80 rpm ($P < 0.05$). This could not, however, compensate for the 3.4% lower efficiency at 100 rpm. This study demonstrated that elite cyclists perform best at their most efficient cadence despite the maximal energy turnover rate being larger at a higher cadence.

**Keywords** Cycling · Pedal frequency · Efficiency · Energy turnover rate · Performance

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## Introduction

For many decades, researchers have tried to find the optimal cadence in cycling. The major focus has been on the effect of cadence on efficiency (Gaesser and Brooks 1975; Hagberg et al. 1981; Faria et al. 1982; Böning et al. 1984; Coast and Welch 1985; Chavarren and Calbet 1999; Marsh et al. 2000; Hansen et al. 2002), but other measures have also been used (Patterson and Moreno 1990; Marsh and Martin 1995; Takaishi et al. 1996; Neptune and Hull 1999; MacIntosh et al. 2000). Patterson and Moreno (1990) stated that the term "optimal cadence" may differ depending on whether the term refers to most economical, maximum power producing, less fatiguing or most comfortable cadence. However, for a competitive cyclist, the optimal cadence is the cadence that produces the best performance. In cycling, performance is determined by the average speed (metres per second), and speed is determined by the energy turnover rate (joules per second) and the work economy (joules per metre) (speed = energy turnover rate/work economy) (di Prampero et al. 1986). Accordingly, both larger energy turnover rate and superior work economy will improve performance. It is well established that work economy is influenced by cadence, but to perform optimally one must choose a cadence that gives the optimal combination of both energy turnover rate and work economy. The effect of cadence on performance and maximal energy turnover rate has been rarely investigated. Nielsen et al. (2004) compared performance at a freely chosen cadence (FCC) against performance at FCC ±25% in moderately trained subjects during a constant load test to exhaustion (7–10 min duration). No difference was found between FCC (mean 78 rpm) and FCC −25% (mean 59 rpm), but time to exhaustion was longer in both compared with FCC +25% (mean 98 rpm). Foss and Hallén (2004a) compared performance between cadences of 60, 80, 100 and 120 rpm in elite cyclists during an incremental test to exhaustion (4–7 min duration). All subjects performed

best at 80 rpm and performance at different cadences correlated highly with efficiency, indicating that maximal energy turnover rate was relatively unaffected by cadence. However, it has been suggested that this is not the case during prolonged exercise. During short-term exercise (~3–8 min), maximal aerobic energy turnover rate is very much determined by maximal oxygen uptake, which is probably insignificantly affected by muscular fatigue. During prolonged exercise, muscle fatigue will affect maximal energy turnover rate and muscle fatigue may be dependent on cadence. Thus, it has been hypothesised that the reason why competitive cyclists do not choose the most efficient cadence is that fatigue develops faster at low cadences than at higher cadences; however, the evidence for this is lacking, even though some authors have claimed it based on indirect measurements (Patterson and Moreno 1990; Takaishi et al. 1996). To directly measure the development of fatigue during prolonged cycling at different cadences is difficult and has not been reported. An alternative and even more relevant measure is performance. Surprisingly, the effect of cadence on performance has been rarely studied during prolonged cycling (>10 min). Combined with continuous measurement of energy turnover, measurement of performance would enable calculation of both energy turnover rate and efficiency.

The aim of the present study was therefore to test the hypothesis that elite cyclists perform best at their most efficient cadence during prolonged cycling (~30 min).

## Methods

Fourteen healthy male road/terrain cyclists at national elite level from Sweden and Norway participated in this study. Physical characteristics of the subjects are presented in Table 1. Experimental procedures were described in detail to each subject and they gave their written informed consent. The study was approved by the Regional Ethics Committee and performed according to the Declaration of Helsinki. The experiments comply with the current laws of the country in which the experiments were performed.

**Table 1** Physical characteristics of the subjects. Values are means (SD), $n = 7$ in both experiments. $\dot{V}O_{2max}$ maximal oxygen uptake (mean of pre- and post-test), $\dot{V}_{Emax}$ maximal ventilation (mean of pre- and post-test), $HR_{max}$ maximal heart rate (the highest heart rate achieved in pre- or post-test)

| | Experiment I | Experiment II |
|---|---|---|
| Age (years) | 19.2 (0.9) | 24.1 (2.8) |
| Height (cm) | 185 (6.5) | 176 (5.2) |
| Mass (kg) | 73.7 (8.6) | 68.9 (4.8) |
| $\dot{V}O_{2max}$ (ml kg min$^{-1}$) | 71.2 (1.8) | 72.2 (3.0) |
| $\dot{V}O_{2max}$ (l min$^{-1}$) | 5.24 (0.54) | 4.97 (0.34) |
| $\dot{V}_{Emax}$ (l min$^{-1}$) | 179 (22) | 170 (20) |
| $HR_{max}$ (beats min$^{-1}$) | 189 (10) | 188 (7) |

### Experimental protocol

Two experiments were performed. Both experiments were preceded by a time-trial familiarisation test. In the first experiment (Exp. I), the effect of cadence on performance was investigated during five time trials at 60, 80, 100, 120 rpm and FCC ($n = 7$). The time trials were performed in a randomised order on an electromagnetically braked cycle ergometer with 2–3 days between trials. We found differences in performance between several of the cadences, but not between 80 and 100 rpm. The lack of statistical difference could be due to reduced reproducibility (low statistical power), because of the number of tests conducted within ~15 days. Therefore, a second experiment (Exp. II) was performed after Exp. I with a new group of elite cyclists ($n = 7$), where performance was tested at 80 and 100 rpm only.

### Pre- and post-experimental procedures

Prior to the experiments, maximal lactate steady state (MLSS) and maximal oxygen uptake ($\dot{V}O_{2max}$) were measured in each subject at 90 rpm. MLSS was found by a graded submaximal exercise test (Borch et al. 1993) and used as the start workload for each individual during the time trials. $\dot{V}O_{2max}$ was found by an incremental test to exhaustion of ~5 min and defined as the highest average oxygen uptake ($\dot{V}O_2$) attained during one consecutive minute (for details, see Foss and Hallén 2004a). Heart rate (HR) was measured continuously and time to exhaustion was registered. After the time trials were accomplished a second $\dot{V}O_{2max}$ test (post-test) was conducted in the exact same way as the pre-test. Three of the subjects in Exp. I did not perform the post-test.

### Time-trial test

Each time trial was preceded by three submaximal workloads of 5.5 min duration (unloaded pedalling, MLSS −100 and −50 W) (Fig. 1). At each workload, $\dot{V}O_2$ and HR were measured from 4 to 5 min, and a blood sample was taken after 5.5 min for determination of blood lactate concentration ($bLa^-$). The submaximal workloads were used for calculation of the $\dot{V}O_2$ slow component during the time trials (see later). To standardise energy consumption at the onset of each time trial, the time trials were preceded by a 3-min-long work bout at constant workload and cadence (150 W and 90 rpm).

During the first 5 min of the time trial, workload was fixed at an external power equivalent to each subject's MLSS [mean 314 (7) W; ~79% of $\dot{V}O_{2max}$] (Fig. 1). From the sixth minute, the subjects were instructed to complete the time trial as fast as possible by adjusting the workload in steps of 10 W up or down with buttons mounted at the handlebars. Total work (''distance'') of each time trial was calculated as an amount of work as large as their individual MLSS values (at 90 rpm)

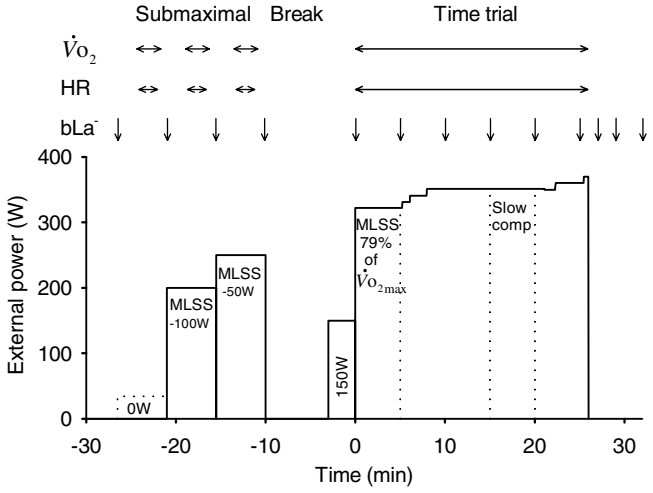


**Fig. 1** The protocol, including submaximal workloads and an example of the change in power output throughout the time trial. $\dot{V}O_2$ Oxygen uptake, *HR* heart rate, $bLa^-$ blood lactate concentration, *0W* "unloaded pedalling", *MLSS* maximal lactate steady state, $\dot{V}O_{2max}$ maximal oxygen uptake, *Slow comp* $\dot{V}O_2$-slow component

maintained for 30 min (Table 2). All changes in external power output and cadence were sampled at 100 Hz and processed by a computer. Accumulated work was visualised on a monitor, as a slide progressively increasing towards the "finish line", together with a cadence meter. This motivated the subjects to work with maximal effort and enabled them to maintain mean cadence within 0.3 rpm of actual cadence throughout the time trials (60–120 rpm). Finishing time was recorded and blinded for both the subject and test leader, and used as a measure of performance. The tests were conducted seated on the cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands) and the cycle ergometer was adjusted to each subject's normal riding position. The subjects used their own shoes and pedals with toe clips.

$\dot{V}O_2$ and HR were measured continuously throughout the time trials, except during the 17th minute. This period was used for oral water supplementation. The "missing" $\dot{V}O_2$ was estimated as the mean of the 16th and 18th minutes. Blood samples were taken from the fingertip immediately before the start of exercise, every 5 min, and at 1, 3 and 6 min post-exercise for determination of $bLa^-$ (Fig. 1). Subjects gave their rating of perceived exertion (RPE) immediately after cessation of the time trial (Borg 1970).

Room temperature varied by less than 3°C for each subject in all tests. All subjects refrained from eating during the last 2 h before reporting to the laboratory, and from vigorous activity on the previous day.

### *The $\dot{V}O_2$-slow component*

The $\dot{V}O_2$-slow component was calculated from $\dot{V}O_2$ measured in the 5th and 20th minutes of the time trial. The first 5 min of the time trial was conducted at a constant workload (MLSS). Thereafter, the subjects could change the workload to maximise their performance, except in the period between 15 and 20 min. At 14.5 min they had to decide which workload they should use for the next 5 min. In this way $\dot{V}O_2$ was at a "steady rate" in both the 5th and 20th minutes. The workload was, however, somewhat different at these two time points. Based on the individual linear relationship between the submaximal workloads (MLSS-100 W, MLSS-50 W and MLSS) (see Fig. 1) and $\dot{V}O_2$, $\dot{V}O_2$ was extrapolated to the actual workload in the 20th minute of each time trial for all cadences except for FCC (Fig. 2). The difference between the measured $\dot{V}O_2$ and the estimated oxygen demand was defined as the $\dot{V}O_2$-slow component.

### Metabolic measurements

An automatic system with a mixing chamber (Oxycon Pro, Erich Jaeger, Hoechberg, Germany) was used to measure respiratory variables (the breath-by-breath mode was used in one subject). The gas analysers were routinely calibrated against certified calibration gases of known concentrations. The flow turbine (Triple V, Erich Jaeger) was calibrated with a 3 l 5530 series calibration syringe (Hans Rudolph, Kansas City, USA). Gas and volume calibration was repeated until the difference between two consecutive calibrations was less than 1% (for details, see Foss and Hallén 2004b).

To examine the validity and reliability of the Oxycon Pro, a semiautomatic Douglas bag system was coupled in series with the Oxycon Pro during the time trials. Respiratory variables were measured every fifth minute with the Douglas bag system and compared with the Oxycon Pro in the same period. The drift in $\dot{V}O_2$ with the Oxycon Pro was negligible between 5–25 min, and

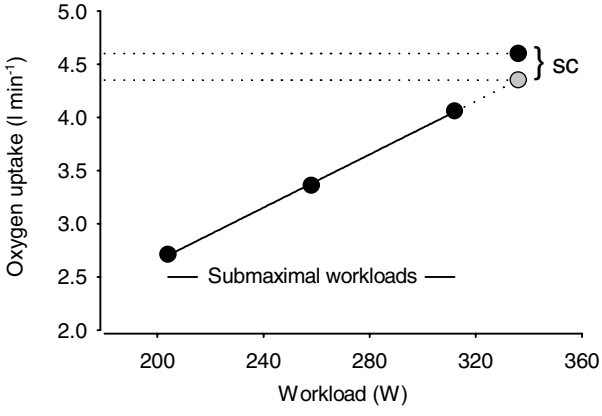


**Fig. 2** An example showing the calculation of $\dot{V}O_2$-slow component during the time trials. Based on the individual linear relationship between the submaximal workloads (MLSS-100 W, MLSS-50 W and MLSS) and $\dot{V}O_2$, $\dot{V}O_2$-slow component (*SC*) was defined as the difference between measured (*black circle*) and estimated $\dot{V}O_2$ (*grey circle*) in the 20th minute of the time trial. For details, see Methods

the between-system coefficient of variation was less than 1.1% (for details, see Foss and Hallén 2004b).

Blood samples for determination of $bLa^-$ were taken from a fingertip. The finger was rinsed with water and wiped before the skin was perforated by a small needle. About 50 µl of blood was taken in a heparinised capillary tube, from which 25 µl of non-haemolysed blood was injected with a pipette into the mixing chamber of the lactate analyser (YSI 1500 Sport, Yellow Springs Instruments, Ohio, USA). The lactate analyser was calibrated according to the instruction manual.

### Calculations

Aerobic energy turnover rate (Watts) was calculated using the $\dot{V}O_2$ and the corresponding respiratory exchange ratio ($R$) (Zuntz 1901). In 3.6% of the measurements $R$ was above 1, and 1.00 was used for the calculation. Anaerobic energy turnover rate (Watts) was calculated on the assumption that a 1 mmol $l^{-1}$ increase in $bLa^-$ is equivalent to the energy released by an oxygen consumption of 3.3 ml $kg^{-1}$ (di Prampero and Ferretti 1999). Net blood lactate accumulation was calculated as the difference between the peak post-exercise concentration and the $bLa^-$ at the onset of exercise. Blood lactate analysis was done on non-haemolysed blood. $bLa^-$ was therefore corrected assuming a fixed haematocrit (45%) and lactate concentration within the red blood cells to be 50% of that in plasma and independent of exercise intensity (Foxdal et al. 1990). Energy turnover rate (Watts) was calculated as the sum of the aerobic and the anaerobic energy turnover rate. Efficiency was calculated as gross efficiency (per cent) and defined as the ratio between external power output (Watts) and energy turnover rate (Watts).

### External power

The actual external power output of the cycle ergometer had previously been controlled at external powers from 100 to 300 W at 60, 80, 100 and 120 rpm, by an ergometer calibrator (Mod 17800, VacuMed, Calif. USA). The calibration revealed an almost perfect agreement between the cycle ergometer and the ergometer calibrator (see Foss and Hallén 2004a).

### Statistics

Repeated measures ANOVA was used to determine overall differences among test variables across 60, 80, 100, 120 rpm and FCC. Student's paired $t$-test was used to determine individual differences where overall differences had been found to be significant and in paired comparisons (Exp. II). The significance level was set at $P < 0.05$ (two-tailed). Results are presented as means (standard error, SE) if not otherwise stated.

## Results

### Time-trial tests

#### *Performance*

The time-trial performances in Exp. I were indistinguishable between 80, 100 rpm and FCC (mean 90 rpm, range 74–100 rpm), but approximately 3 and 10% superior at 80/100 rpm than at 60 and 120 rpm, respectively ($P < 0.05$) (Fig. 3A). However, in Exp. II, where only 80 and 100 rpm were tested, performance was 1.7% superior at 80 compared to 100 rpm ($P < 0.05$) (Fig. 3A).

#### *Gross efficiency*

Accumulated $\dot{V}O_2$ for both experiments was ~2.5% greater at 60, FCC and 100 rpm, and 13% greater at 120 compared to 80 rpm ($P < 0.05$) (Table 2). No difference was found in net blood lactate accumulation between cadences (Table 2). Consequently, accumulated energy turnover (kilojoules) during the time trials followed the same pattern as that for accumulated $\dot{V}O_2$. Because total work was the same among the various cadences, mean gross efficiency in Exp. I followed a reverse "U-shaped" curve with 80 rpm being 2.9, 2.3, 2.7 and 12.3% greater than at 60, FCC, 100 and 120 rpm, respectively ($P < 0.05$) (Fig. 3*B*). In Exp. II, gross efficiency was 3.4% greater at 80 than at 100 rpm ($P < 0.05$) (Fig. 3B).

#### *Energy turnover rate*

Mean $\dot{V}O_2$ (~4.4 l $min^{-1}$) was not different between the five cadences in Exp. I, but was 1.8% higher at 100 compared to 80 rpm in Exp. II ($P < 0.05$) (Table 3). Mean fractional utilisation of $\dot{V}O_{2max}$ ranged from 83 to 88% (Table 4). Mean $R$ was 0.94–0.96 for all cadences (Table 3), giving ~84% carbohydrate oxidation. Thus, aerobic energy turnover rate (1,498–1,559 W) was not different between cadences in Exp. I, but higher at 100 compared to 80 rpm in Exp. II (Table 2). Net increase in $bLa^-$ (~8 mmol $l^{-1}$) corresponded to an anaerobic energy turnover rate of ~25 W, and was similar between cadences in both experiments (Table 2). Accordingly, no difference was found in energy turnover rate between cadences in Exp. I, but in Exp. II, energy turnover rate was greater at 100 than at 80 rpm ($P < 0.05$) (Fig. 3C). This difference was due to differences in aerobic and not anaerobic energy turnover rate.

#### *$\dot{V}O_2$-slow component*

Pulmonary $\dot{V}O_2$ increased progressively (relative to workload) throughout the time trials at all cadences in both experiments. From the 5th to the 20th minutes of exercise, the overall increase was approximately 200 ml

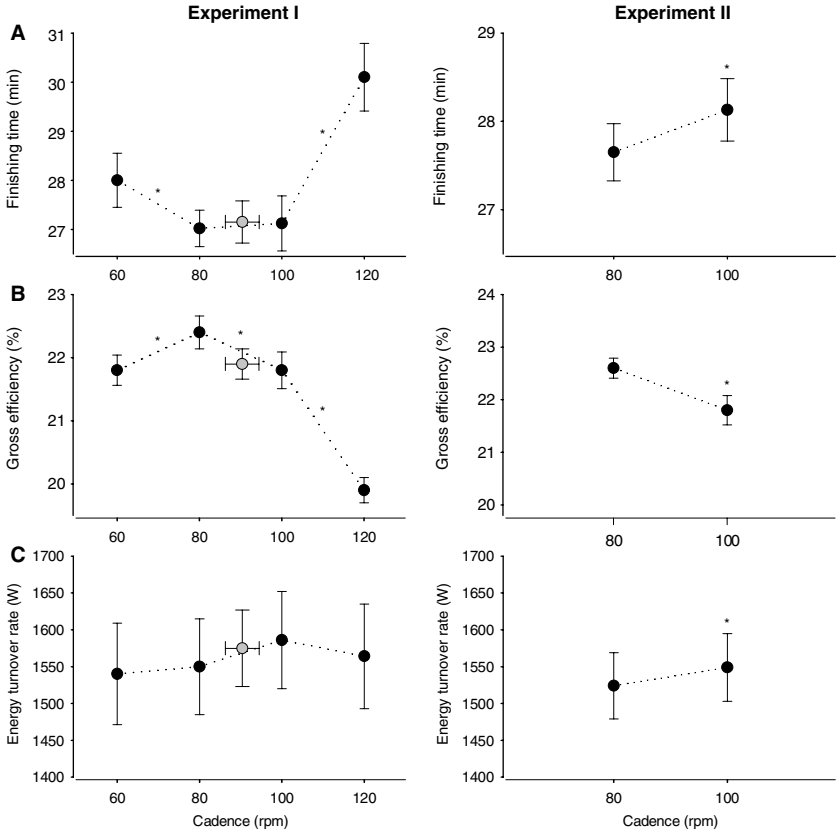


**Fig. 3** Finishing time (**A**), gross efficiency (**B**) and energy turnover rate (**C**) during the time trials at 60, 80, 100, 120 and freely chosen cadence (*grey circle*) (Experiment I) and at 80 and 100 rpm (Experiment II). Values are means (SE), $n = 7$ in both experiments. $^{*}P < 0.05$, significant difference between cadences

in Exp. I and II, but with no difference between cadences (Table 2).

*Ventilation, HR and RPE*

Mean ventilation ($\dot{V}_E$) during the time trials was higher at 120 than at 60 rpm in Exp. I, and higher at 100 than at 80 rpm in Exp. II ($P < 0.05$) (Table 3). Otherwise no differences existed between cadences. Mean HR was similar among cadences in Exp. I, while in Exp. II, HR was higher at 100 compared to 80 rpm ($P < 0.05$) (Table 3). Mean fractional utilisation of maximum ventilation and maximum HR ranged from 65 to 78% and from 87 to 93%, respectively (Table 4). No differences were found in RPE between cadences in Exp. I or II (Table 3).

Gross efficiency during submaximal exercise

During unloaded pedalling, energy turnover rate increased with increasing cadence from 208 W at 60 rpm to 578 W at 120 rpm in Exp. I. When external workload was added no differences were found in gross efficiency between 60 and 80 rpm at any workload (Exp. I), though the curve pattern indicated that the most efficient cadence gradually shifted from 60 to 80 rpm with increasing workload (Fig. 4). Gross efficiency decreased

**Table 2** Mean total work, accumulated oxygen uptake, $\dot{V}O_2$-slow component, aerobic energy turnover rate, net blood lactate ($bLa^-$) accumulation and anaerobic energy turnover rate during the time trials at different cadences in experiments I and II. Values are means (SE), $n = 7$ in both experiments. *FFC* Freely chosen cadence (mean 90 rpm, range 74–100 rpm)

| | Experiment I | | | | | Experiment II | |
|---|---|---|---|---|---|---|---|
| | 60 rpm | 80 rpm | 100 rpm | 120 rpm | FCC | 80 rpm | 100 rpm |
| Total work (kJ) | 562 (21) | 562 (21) | 562 (21) | 562 (21) | 562 (21) | 570 (15) | 570 (15) |
| Accumulated oxygen uptake (l) | 122 (5.1)* | 118 (4.1)* | 121 (4.4)* | 133 (4.4)* | 120 (4.3) | 118 (3.3)* | 122 (3.7) |
| $\dot{V}O_2$ slow component (ml $O_2$)[a] | 250 (41) | 190 (63) | 140 (56) | 200 (39) | – | 230 (28) | 190 (17) |
| Aerobic energy turnover rate (W) | 1,517 (66) | 1,524 (64) | 1,559 (64) | 1,544 (68) | 1,547 (52) | 1,498 (43)* | 1,524 (44) |
| Net $bLa^-$ accumulation (mmol $l^{-1}$) | 7.4 (0.7) | 8.0 (0.6) | 8.5 (0.9) | 6.7 (1.0) | 8.9 (0.4) | 8.8 (1.0) | 8.8 (1.3) |
| Anaerobic energy turnover rate (W)[b] | 23 (3) | 25 (3) | 27 (4) | 20 (4) | 28 (2) | 26 (4) | 25 (4) |

[a]See Methods for calculation
[b]Calculated according to di Prampero and Ferretti (1999)
$^{*}P < 0.05$, significant difference between cadences

**Table 3** Mean external power output, oxygen uptake ($\dot{V}O_2$), ventilation ($\dot{V}_E$), heart rate (*HR*), respiratory exchange ratio (R) and rating of perceived exertion (*RPE*) during the time trials at different cadences in experiments I and II. Values are means (SE), $n = 7$ in both experiments

| | Experiment I | | | | | Experiment II | |
|---|---|---|---|---|---|---|---|
| | 60 rpm | 80 rpm | 100 rpm | 120 rpm | FCC | 80 rpm | 100 rpm |
| External power output (W) | 335 (14)* | 347 (16) | 346 (16)* | 312 (15)* | 345 (13) | 351 (10)* | 344 (12) |
| $\dot{V}O_2$ (l min$^{-1}$) | 4.37 (0.19) | 4.38 (0.18) | 4.47 (0.18) | 4.44 (0.19) | 4.44 (0.15) | 4.27 (0.13)* | 4.35 (0.12) |
| $\dot{V}_E$ (l min$^{-1}$) | 116 (17) | 117 (17) | 123 (19) | 129 (20) | 122 (18) | 124 (5)* | 131 (5) |
| HR (beats min$^{-1}$) | 164 (4.3) | 164 (4.7) | 167 (4.9) | 170 (5.3) | 168 (3.8) | 171 (2.2)* | 174 (2.5) |
| *R* | 0.94 (0.01) | 0.95 (0.01) | 0.96 (0.01) | 0.95 (0.01) | 0.96 (0.01) | 0.95 (0.01) | 0.94 (0.00) |
| RPE[a] | 17.7 (0.6) | 18.0 (0.5) | 18.3 (0.7) | 18.7 (0.4) | 18.2 (0.7) | 18.1 (0.5) | 18.6 (0.4) |

[a]Rating of perceived exertion with a Borg scale (6–20)
*$P < 0.05$, significant difference between cadences

with increasing cadence from 80 to 120 rpm at an external workload of 204, 258 and 312 W in Exp. I, and from 80 to 100 rpm at 216, 266, 316 W in Exp. II (Fig. 4).

### $\dot{V}O_{2max}$ pre- and post-time trials

The time trials were carried out over 15 and 8 days in Exp. I and Exp. II, respectively. No difference was found in $\dot{V}O_{2max}$ tested before and after the time trials in either experiment. However, time to exhaustion during the $\dot{V}O_{2max}$ test tended to be longer in the post-test of Exp. I [321 (26) versus 351 (31) s; $P < 0.07$].

## Discussion

The present study shows that elite cyclists perform better at 80 rpm compared to 60, 100 and 120 rpm in a time trial lasting about 30 min. The highest gross efficiency was achieved at 80 rpm, while the average energy turnover rate was highest at 100 rpm, indicating less development of fatigue (better endurance) during 100 rpm.

### Efficiency

To study work economy in the field in terms of energy expenditure per metre is challenging, since many factors unrelated to the cyclist will affect the work. These factors will influence the power demanded by the rider at a certain speed, and they are in principle determined by five resistive elements: drive train friction, inertial forces associated with the acceleration of the bike, gravitational forces in climbing, tyre rolling resistance and aerodynamic drag. These factors may vary considerably during a road race and are only slightly influenced by the rider. Therefore, studies of work economy in cycling are very complex and more or less impossible to perform as an entirety. When using a cycle ergometer in the laboratory, the external factors can be eliminated or controlled and accurate measures of external power can be obtained. With metabolic measurements, accurate measures of rate of energy expenditure can also be obtained. In this way we can study the efficiency of the transformation of whole-body energy turnover to mechanical power output by the legs. It is from such studies that the conclusion has been reached that competitive cyclists use a cadence higher than the most efficient one. Since the experiments are conducted in the laboratory without the influence of external factors, it has been argued that these are not relevant for field cycling. This notion has been exaggerated by the fact that most laboratory experiments conclude that the most efficient cadence is as low as 50–60 rpm, far below the range that is preferred by competitive cyclists. However, we have recently shown that the most efficient cadence is higher than 60 rpm when exercising at high workloads (Foss and Hallén 2004a). At 125 W, direct measurements of $\dot{V}O_2$ showed that competitive cyclists were more efficient at 60 compared to 80 rpm, but at 350 W they were more efficient at 80 rpm compared to both 60 and 100 rpm. Importantly, at 350 W the workload is in the range that

**Table 4** Exercise intensity expressed as percentages of maximal oxygen uptake ($\%\dot{V}O_{2max}$), maximal ventilation ($\%\dot{V}_{Emax}$) and maximal heart rate ($\%HR_{max}$) during the time trials at different cadences in experiments I and II. Values are means (SE), $n = 7$ in both experiments. $\dot{V}O_2$ and $\dot{V}_E$ are expressed relative to mean of maximal pre- and post-tests at 90 rpm. HR is expressed relative to the highest heart rate achieved in pre- or post-tests. Statistics were run on the assumption that $\dot{V}O_{2max}$ is independent of cadence

| | Experiment I | | | | | Experiment II | |
|---|---|---|---|---|---|---|---|
| | 60 rpm | 80 rpm | 100 rpm | 120 rpm | FCC | 80 rpm | 100 rpm |
| $\%\dot{V}O_{2max}$ | 83.2 (1.1) | 83.6 (1.1) | 85.3 (0.7) | 84.7 (1.0) | 84.8 (1.1) | 86.0 (0.9)* | 87.6 (1.2) |
| $\%\dot{V}_{Emax}$ | 65.3 (2.2) | 65.4 (2.4) | 69.1 (2.8) | 72.5 (3.5) | 69.0 (3.7) | 73.5 (3.2)* | 77.6 (3.5) |
| $\%HR_{max}$ | 87.0 (0.7) | 87.4 (1.0) | 89.0 (1.1) | 90.3 (1.8) | 89.3 (1.0) | 91.3 (0.6)* | 92.7 (0.4) |

*$P < 0.05$, significant difference between cadences

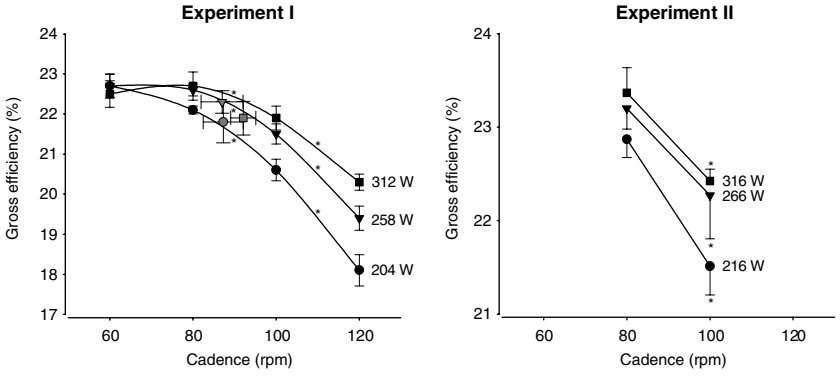


**Fig. 4** Gross efficiency during the submaximal workloads (MLSS-100 W, MLSS-50 W and MLSS) at 60, 80, 100, 120 and freely chosen cadence (*grey circle*) (Experiment I) and at 80 and 100 (Experiment II). One out of the subjects in experiment I was tested at MLSS-150 W, MLSS-75 W and MLSS. Values are means (SE), $n = 7$ in both experiments. *$P < 0.05$, significant difference between cadences

high-level road cyclists use competitively. The difference between the preferred and the most efficient cadence is therefore much less than previously assumed. The finding that the most efficient cadence is approximately 80 rpm at high workloads is based on measurements during steady-rate cycling at fixed workload and cadence. In the present study, the cyclists performed a time trial of about 28 min at self-selected and variable power with the goal to complete a given work as fast as possible. The average workloads ranged from 312 to 351 W (60–120 rpm, Table 3). Calculations of efficiency were based mainly (>98%) on total oxygen consumption and total work, which gave a very high accuracy with a very small coefficient of variance in the measurements (Foss and Hallén 2004b). Again, 80 rpm was the most efficient cadence (in 13 out of the 14 cyclists tested). Importantly, the measurements of efficiency in this study were not based on an arbitrary situation with constant workload, but in a competition-like profile with changing workload. The submaximal tests performed prior to the time trials revealed that gross efficiency at 80 rpm was not superior at lower workloads, confirming that the most efficient cadence is dependent on power. In summary, the present results show that the most efficient cadence for elite cyclists is approximately 80 rpm at workloads relevant for time trials of about 30 min duration.

One important phenomenon during prolonged high-intensity exercise is the time-dependent change in efficiency, commonly denoted the slow component of $\dot{V}O_2$. As expected based on exercise intensity, there was a significant $\dot{V}O_2$-slow component (~200 ml oxygen) in the period from 5 to 20 min in all time trials. This is close to the values found in professional cyclists (130 ml oxygen) at a somewhat lower exercise intensity (~80% of $\dot{V}O_{2max}$) (Lucia et al. 2000). From this it can be calculated that gross efficiency decreased by about 7% from the start to the end of the time trial, and therefore has a major impact on performance. However, as found by others (Billat et al. 1999), the $\dot{V}O_2$-slow component, and thus the decrement in efficiency, were independent of cadence.

The variations in gross efficiency between cadences (Figs. 3B, 4) may be explained by the differences in the internal work. The internal work is the power needed to overcome inertial and gravitational forces related to the movement of the legs (Ferguson et al. 2000; Sjøgaard et al. 2002). In cycling, the internal power increases in a curvilinear fashion, with cadence almost independent on the external workload applied (Hansen et al. 2004). During unloaded pedalling, energy expenditure increased in a similar way to that for internal power. However, as the most efficient cadence increased with increasing workload, the difference in internal power could not alone explain the differences in gross efficiency. Some of the variations seem, therefore, to be related to the true efficiency of leg muscles exercising at different contraction frequencies (Foss and Hallén 2004a).

It has been argued that gross efficiency is not the optimal measure of efficiency in cycling, since the energy expenditure used in calculation of gross efficiency includes energy for processes not directly contributing to the actual work accomplished. Instead it is suggested that delta efficiency, the ratio between the change in power and the change in energy expenditure is more applicable. Delta efficiency is held to be closer to true muscle efficiency and is shown to increase with increasing cadence, and, therefore, intuitively supports the fact that cyclists use a relatively high cadence (Chavarren and Calbet 1999). However, we argue that since maximal energy turnover rate, mostly determined by $\dot{V}O_{2max}$ is a major limiting factor, gross efficiency is the most relevant measure of efficiency. Regardless in which organ the oxygen is used, the delivery must be accomplished by the circulatory system, which is the main limiting factor for whole-body energy turnover rate (Andersen and Saltin 1985). The simple (but exact) model we are using states that speed (metres per second) is determined by the energy turnover rate (joules per second) and the work economy (joules per metre) (di Prampero et al. 1986). In the laboratory setting, speed is equivalent to external power, and work economy is equivalent to gross efficiency. This model could be more detailed, dividing the energy turnover rate into different organs, for instance the working skeletal muscles and non-working tissues. In this setting, other measures of efficiency than gross efficiency must be used. However, given the types of measurements we conducted in the present study, including pulmonary $\dot{V}O_2$ only, this division will not add to the understanding. For instance,

it is possible that energy turnover rate in non-contracting tissues changes as workload changes. Hence, gross efficiency is the relevant measure of efficiency in this kind of study.

## Energy turnover rate

The aerobic energy turnover comprised more than 98% of the total energy turnover during the time trials. Exp. II showed that the energy turnover rate was on average 1.7% higher at 100 than at 80 rpm. This difference was not evident in Exp. I ($P = 0.14$); however, time to exhaustion measured pre and post the experiments indicated that endurance had improved during Exp. I. Thus, it could be that the difference between 80 and 100 rpm in Exp. I was concealed due to a change in fitness level.

The most important factor determining the energy turnover rate during this type of prolonged exercise is $\dot{V}O_{2max}$. Several studies have investigated the effect of cadence on $\dot{V}O_{2max}$, but together they are inconclusive. In three studies it was found that $\dot{V}O_{2max}$ occurred within a range of cadences of 60–100 rpm (Hermansen and Saltin 1969; McKay and Banister 1976; Coast and Welch 1985), while in other studies no difference was found at cadences from 40 to 120 rpm (Beelen and Sargeant 1993; Zoladz et al. 2000, 2002; Foss and Hallén 2004a). Whether this is a true variation or a variation related to methodological issues is not known. Different protocols have been used and the fitness level of the participants has varied from sedentary to elite athletes. However, from these studies it can be concluded that the effect of cadence on $\dot{V}O_{2max}$ is limited and probably within the range ~0–1.5% at cadences between 60 and 120 rpm.

During the time trials, the subjects utilised about 85% of their $\dot{V}O_{2max}$. For different reasons, cadence may affect this percentage. Given no effect of cadence on $\dot{V}O_{2max}$ (i.e. the $\dot{V}O_{2max}$ measured at 90 rpm is valid for both 80 and 100 rpm), the fractional utilisation was higher at 100 compared to 80 rpm ($P < 0.05$) (Table 4). The 1.8% higher HR at 100 compared to 80 rpm supports an increased blood flow to the exercising muscles at 100 rpm. Moreover, the difference is identical to the relative difference in aerobic energy turnover rate between 80 and 100 rpm. This indicates that the ability to work at intensities close to the $\dot{V}O_{2max}$ is better preserved at a high cadence. This may be taken as reduced development of fatigue (better endurance). Reduced development of fatigue has been suggested in several articles, although none has directly measured fatigue during long term cycling. Takaishi et al. (1996) examined the effect of cadence on neuromuscular fatigue in moderately trained cyclists at ~85% of $\dot{V}O_{2max}$ with the use of EMG. They suggested that the lowest levels of neuromuscular fatigue are obtained at a cadence faster than the most efficient one. In support, Patterson and Moreno (1990) found a gradual decrease in the resultant force on the pedals with increasing cadence (up to 90–100 rpm), and suggested that this would minimise fatigue, even if the result would be a small increase in $\dot{V}O_2$. Further, Ahlquist et al. (1992) reported that glycogen depletion was greater in type II muscle fibres at 50 compared to 100 rpm after prolonged cycling (30 min) at ~85% of $\dot{V}O_{2max}$. This indicated a greater activation of the fatigue-sensitive type II muscle fibres at 50 rpm than at 100 rpm, thereby supporting the use of 100 rpm to avoid muscle fatigue. In summary, this study shows that maximal energy turnover rate is better preserved at 100 than at 80 rpm, indicating less development of fatigue at 100 rpm. However, this effect is small and cannot be extrapolated to higher cadences (Fig. 3C).

## Performance

In typical endurance sports such as cycling, performance is equivalent to speed. In the laboratory, speed is equivalent to external power. As previously described, the external power is determined by the gross efficiency and energy turnover rate. The present study shows that both efficiency and maximal energy turnover rate are influenced by cadence, but in different ways. Superior efficiency was achieved at 80 rpm, while superior energy turnover rate was achieved at 100 rpm. The relative change caused by a change in cadence will determine whether performance increases or decreases. In this study, efficiency was 3.4% greater at 80 rpm, while energy turnover rate was 1.7% greater at 100 rpm (Exp. II). This resulted in a 1.7% faster finishing time (29 s) at 80 rpm compared to 100 rpm (Exp. II). Energy turnover rate, which is mostly determined by $\dot{V}O_2$, was measured continuously during the whole exercise period. This is important for two reasons. It is valid, since temporal variation in both $\dot{V}O_2$ and $R$ is included, and it is accurate because of the long sampling time. This, together with a reliable ergometer, reliable oxygen measurements and experienced subjects enabled us to identify these differences, which are minor, but highly significant in sport. It is noteworthy that when the subjects cycled at 60 and 120 rpm, performance was approximately 1 and 3 min poorer than at 80 rpm, respectively. This demonstrates that there is a rather small window of preferable cadences and that cycling at a cadence that is too high is potentially worse than at a cadence that is too low. Further, the FCC did not seem to result in improved performance compared to 80 and 100 rpm (Fig. 3A). Freely chosen cadence (mean 90 rpm) followed the same pattern as expected if the cadence was fixed at 90 rpm (Fig. 3). Thus, cyclists do not seem to benefit from the ability to vary cadence throughout a race of this length.

## Optimal versus preferred cadence

The optimal cadence found in the present study (80 rpm) is lower than the cadence normally preferred by cyclists (90–105 rpm). Are cyclists using a cadence

that is too high? Professional cyclists generally have a higher $\dot{V}O_{2max}$ and are able to utilise a higher fraction of $\dot{V}O_{2max}$ than elite cyclists (Lucia et al. 1998, 2001; Padilla et al. 1999, 2000; Table 4) (Lucia et al. 1998, 2001; Padilla et al. 1999, 2000). This enables them to work with a higher power output (Watts per kilogram) than amateurs and to sustain an external workload in the range of 400–500 W throughout 1-h events (Padilla et al. 2000; Mujika and Padilla 2001). Based on the finding that the most efficient cadence increases with increasing workload, one cannot exclude the idea that professional cyclists are more efficient at cadences even higher than 80 rpm. Lucia et al. (2004) examined the effect of cadence on efficiency in professional cyclists. At a mean (SD) external workload of 366 (37) W, gross efficiency was higher at 100 compared to 60 rpm, but was not statistically different from 80 rpm. It is our experience that amateur cyclists often adopt their cadence from professionals in the belief that professionals have an optimal cadence. This could be wrong since the most efficient cadence changes with workload. Further, it is not known how cadence influences maximal energy turnover rate in professional cyclists. However, it could also be that professionals use a cadence that is too high. The selection of cadence in cycling is based more on experience than testing. Experience could be based on performance during races. The present study showed that the difference in performance between 80 and 100 rpm was about 30 s for a 28-min time trial. This is significant, but probably within the range of error for the self-experienced feeling of maximal performance. Their "experience" could also be based on the feeling of load. Again, these kinds of experience could fail to discover small differences. In addition, feeling is based on other variables than performance, such as muscle force, sensation of fatigue, etc. Further research is needed to investigate whether professionals also perform best at 80 rpm, and to relate the laboratory findings systematically to field conditions.

## Conclusion

The present study demonstrates that elite cyclists perform best at their most efficient cadence during time trials of ~28 min duration, despite maximal energy turnover rate being larger at a higher cadence.

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# Validity and Stability of a Computerized Metabolic System with Mixing Chamber



**Abstract**

The aim of this study was to validate a computerized metabolic system with mixing chamber (Oxycon Pro, Erich Jaeger GmbH, Hoechberg, Germany) against the Douglas bag method (1) over a large range of ventilations and (2) for drift during shorter (25-min time trial) and longer (~ three months) test periods. Eighteen well-trained/elite cyclists performed graded exercise tests, maximal oxygen uptake tests and time trial tests on an electromagnetic braked cycle ergometer. Respiratory variables were measured simultaneously, once or several times in every test by the Oxycon Pro and the Douglas bag method. (1) Overall, oxygen uptake was 0.8% (0.03 l·min$^{-1}$) lower with the Oxycon Pro than with the Douglas bag method with a coefficient of variation of 1.2% (n = 802) ($p < 0.05$). (2) During the time trials, oxygen uptake measured with the Oxycon Pro gradually decreased from 0.5% (0.02 l) lower than the Douglas bag method at 5 min to 1.0% (0.05 l) lower at 25 min ($p < 0.05$). Over the period of three months of testing, oxygen uptake measured with the Oxycon Pro gradually increased from 1.1% (0.04 l) lower than the Douglas bag method at the start to 0.5% (0.02 l) lower at the end ($p < 0.05$). This study demonstrates that a computerized metabolic system with mixing chamber is an accurate system for measuring oxygen uptake. This applies to testing over a large range of ventilations and for stability both during shorter and longer test periods.

**Key words**
Oxycon Pro · Douglas bag method · reproducibility · drift



## Introduction

The use of pulmonary oxygen uptake to estimate energy expenditure, cardiovascular function, and exercise capacity has been widely practiced for more than a century. Until about thirty years ago these measurements were primarily done by the Douglas bag method and this method still serves as the "gold standard". Today, computerized metabolic systems have taken over and these systems normally use breath-by-breath analysis. Expired gas concentrations and ventilation are measured continuously just outside the mouth and respiratory and metabolic data are calculated for each breath. This allows the study of rapid changes in oxygen uptake and is time-saving compared with the Douglas bag method. Breath-by-breath analysis requires very fast analyzers and a perfect temporal alignment between measurements of expired gases and ventilation. This is difficult to accomplish, especially at high breathing frequencies [6,8]. An alternative to breath-by-breath analysis is to use a computerized metabolic system fitted with a mixing chamber. The mixing chamber also offers the advantage of presenting data in real time and is just as time-saving as the breath-by-breath analysis, although the study of rapid changes in oxygen uptake is not possible. The expired gas from several breaths is mixed in a mixing chamber and a sample from this chamber gives an average expired gas concentration over those breaths. The gas concentrations together with ventilations measured anywhere in the gas flow give the respira-

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tory data, reducing the difficulties associated with rapid analyzers and alignment of gas concentrations and ventilation. Mixing chambers should therefore yield less error than breath-by-breath systems. Despite this, mixing chambers are rarely used and few studies have evaluated this method of measuring respiratory and metabolic variables. There may be several reasons for this. With the mixing chamber mode, it is not possible to measure respiratory variables breath-by-breath and therefore oxygen-uptake kinetics with high time resolution. Furthermore, systems with mixing chambers are more demanding to use, especially because of the required maintenance of the tube and the mixing chamber. Finally, purchasing a system with mixing chamber normally adds a significant extra cost. Given these arguments, it is not surprising that most laboratories choose the breath-by-breath systems. However, because of the technical challenges, the breath-by-breath systems are less reliable than the Douglas bag method and the coefficient of variance is typically larger than 5% [4]. We consider a coefficient of variance larger than 5% to be unacceptable. Based on the study of Bassett et al. [1] and our own experience, we believe that a proper system with a mixing chamber has a higher reproducibility than breath-by-breath systems and systems comparable to the Douglas bag method. Furthermore, many studies involving stable isotope technology (e.g., $^{13}CO_2$ breath measurements), have used mixing chambers [7]. Therefore the validation of such chambers is warranted.

Regardless of measurement technique, it is often necessary to measure oxygen uptake continuously during a longer period of time. This is done, for instance, to calculate total energy expenditure for a given work. Further, pulmonary oxygen uptake is usually a mandatory variable when cardiovascular function is examined pre and post intervention (a training period for instance). This requires that the computerized metabolic systems do not drift during continuous measurements and that the systems are stable for a longer time period. However, no studies have investigated the effect of short- and long-term drift in computerized metabolic systems.

Thus, the aim of the present study was to validate a computerized metabolic system equipped with a mixing chamber (Oxycon Pro, Erich Jaeger GmbH, Hoechberg, Germany) against the Douglas bag method at a large range of ventilations and to test the system for drift during shorter (25 min) and longer (~ three months) test periods.

## Materials and Methods

Seventeen male and one female well-trained/elite cyclists from Sweden and Norway participated. Their mean age was 21 ± 3 years (± SD), height 181 ± 8 cm, weight 73 ± 8 kg, and maximal oxygen uptake ($\dot{V}O_{2max}$) 70 ± 5 ml·kg$^{-1}$·min$^{-1}$. Experimental procedures were described in detail to each subject and they gave their written informed consent. The study was approved by the Regional Ethics Committee and performed according to the Declaration of Helsinki.

### Protocol

A computerized metabolic system with a mixing chamber (Oxycon Pro, Erich Jaeger GmbH, Hoechberg, Germany) was compared with the Douglas bag method during graded exercise tests, $\dot{V}O_{2max}$ tests and time trial tests at exercise intensities from 10 to > 100% of $\dot{V}O_{2max}$.

The tests were carried out on an electromagnetic braked cycle ergometer (Lode Excalibur Sport, Lode B.V., Groningen, The Netherlands) in a large (180 m$^2$) thermoneutral (18.5 ± 1.4 °C) and well-ventilated laboratory.

#### *Graded exercise test*

The graded exercise test started at a workload corresponding to ~ 50% of the subjects' $\dot{V}O_{2max}$. Workload was then increased 4 – 6 times by 25 W every 5.5 min at workloads from 175 – 350 W. Respiratory data were measured simultaneously by the Oxycon Pro and the Douglas bag method from 3 – 5 min at ventilations below ~ 50 – 60 l·min$^{-1}$ and otherwise from 4 – 5 min into each stage. The mouthpiece was put into the subject's mouth ~ 30 – 60 s before measurements started. This was done to assure complete flushing of "old air" in the tubes and mixing chamber before the measurements started.

#### *Maximal oxygen uptake*

The $\dot{V}O_{2max}$ test was conducted 15 min after the graded exercise test. Workload was initially set at 280 ± 24 W (± SD) and was thereafter increased with 25 W every 30 s until subjects reported having 1.5 – 2 min left until exhaustion. Workload was maintained at that level (460 ± 44 W) until exhaustion (4 – 7 min duration). Cadence was kept constant at 90 rpm during the test. Respiratory data were measured continuously by the Oxycon Pro and in the last 1.5 – 2 min also by the Douglas bag method (30-s bags). The highest oxygen uptake ($\dot{V}O_2$) attained by the Douglas bag method during one consecutive min was taken as $\dot{V}O_{2max}$ and compared with the Oxycon Pro in the same time period.

#### *Time trial test*

Each time trial was preceded by a warm-up conducted at three workloads (unloaded pedalling, 203 ± 25 W and 255 ± 25 W) of 5.5 min duration each. Respiratory variables were measured as described for the graded exercise test. The time trial test started at 306 ± 26 W. From then on, subjects were instructed to complete a predetermined amount of work (550 ± 48 kJ) as quickly as possible by adjusting the workload in steps of 10 W up or down with buttons mounted on the handlebar. All changes in external power output were registered by a computer. Mean workload for all time trials was 326 ± 34 W. Respiratory data were measured continuously with the Oxycon Pro (drinking was allowed in the 17th min) and every fifth minute by the Douglas bag method (time trial duration ~ 25 – 30 min). Mean exercise intensity for all time trials yielded ~ 85% of $\dot{V}O_{2max}$. The time trials were conducted three to six times for each subject, each time with a different cadence (60 – 120 rpm).

### Measuring procedures

During measurement, the subjects breathed through a rubber mouth piece connected to a Hans Rudolph 2700 series large two-way non-rebreathing valve (Hans Rudolph, Inc., Kansas City, USA). A nose clip was used to prevent nasal breathing. From the

two-way non-rebreathing valve, expired air was led through a 1.9 m long corrugated flexible plastic hose into the mixing chamber of the Oxycon Pro for subsequent measurements of oxygen ($O_2$) and carbon dioxide ($CO_2$) concentrations, temperature, and ventilation ($\dot{V}e$). From the flow turbine at the outlet of the mixing chamber a 1.0-m long corrugated flexible plastic hose was connected to a large 8600 series automatic three-way directional valve (Hans Rudolph, inc., Kansas City, USA) that directed the air from the mixing chamber into Douglas bags (Harvard apparatus, Kent, UK). The serial coupling enabled simultaneous measurements of the expired gases by the Oxycon Pro and Douglas bag method. A 220 $ml \cdot min^{-1}$ air sample was extracted from the mixing chamber during the analysis of gas concentrations and did not enter the Douglas bags. The volumes in the Douglas bags were corrected accordingly. Gas volumes used in metabolic calculations are expressed at standard conditions of temperature, pressure, and dry from water (STPD). Ventilation is expressed at body temperature, ambient pressure, and saturated with water (BTPS).

***Oxycon Pro***

The Oxycon Pro was calibrated according to the instruction manual before each test (Oxycon instruction manual ver. 4.5, Erich Jaeger GmbH, Hoechberg, Germany). Oxygen and $CO_2$ analyzers were calibrated with room air and certified calibration gases at 180 kPa (5.55 % $CO_2$ and 94.45 % $N_2$). The flow turbine (Triple V, Erich Jaeger GmbH, Hoechberg, Germany) was calibrated with a 3.00 liter 5530 series calibration syringe (Hans Rudolph, Inc., Kansas City, USA). The calibration syringe was calibrated before onset of the experiment with a motorized calibration syringe (Mod 17 800, VacuMed, California, USA). Both gas and volume calibration were repeated until the difference between consecutive calibrations was less than 1 %.

***Douglas bag method***

After the expired air had passed the mixing chamber it was led through a flow turbine (K 520, K L Engineering, California, USA) before it was directed by a large 8600 series automatic three-way directional valve (Hans Rudolph, Inc., Kansas City, USA) into the Douglas bags. The signals from this flow turbine were used to control the start and stop of air sampling in the Douglas bags exactly at the end of an expiration. This was controlled by a computer which also registered exact sampling time for each Douglas bag. The Douglas bags were thereafter emptied through a flow turbine (K 520, K L Engineering, California, USA) to a 0.4-bar intra bag pressure by a vacuum pump and analyzed for temperature (Pt 120), pressure (Wika, Klingenberg, Germany), $O_2$ concentration (Ametek S-3 A, Pittsburgh, USA), and $CO_2$ concentration (Ametek CD-3 A, Pittsburgh, USA). All bags were analyzed within 10 min after sampling. The Douglas bags were tested for leakage prior to and during the experiment. No difference was found in volume or gas concentrations measured immediately after compared with one hour after sampling. The accuracy of the flow turbine was verified with the same 3.00 liter calibration syringe that was used to calibrate the flow turbine of the Oxycon Pro. The flow turbine was stable during the test period. Temperature and pressure sensors were controlled against a mercury temperature and pressure meter (Leybold, Hürth, Germany). The $O_2$ analyzer was calibrated with room air (20.93 % $O_2$) and linearity was checked with a known $O_2$ concentration (12.00 %). The $CO_2$ analyzer was calibrated with room air (0.03 % $CO_2$) and with a known $CO_2$ concentration (5.00 %). Calibration was performed before each test. Good ventilation assured that the same gas concentrations were achieved in the laboratory as outdoors. This was verified by the stability of the difference between measured room air $O_2$ concentration (20.93 %) and a known oxygen concentration (12.00 %) after each test.

**Data handling and statistics**

Validation of the Oxycon Pro at a large range of ventilations included data from all three tests (n = 802). Bland Altman plots were used to show individual differences between the Oxycon Pro and the Douglas bag method (3). Validation of drift during continuous measuring was done with data from the time trial test. A total of 80 trials, each lasting just over 25 min, were examined. The stability of the Oxycon Pro during a longer test period (94 days) was evaluated with data from all three tests. Generally, at $\dot{V}e$ above ~ 50 – 60 $l \cdot min^{-1}$, $\dot{V}O_2$ measured for 1 min was used to compare the two systems. At $\dot{V}e$ below ~ 50 – 60 $l \cdot min^{-1}$ the mean of two minutes was used.

Student's paired *t*-test (two-tailed) was used to test differences between the Oxycon Pro and the Douglas bag method. Coefficient of variance (CV) of the differences between the Oxycon Pro and Douglas bag method was calculated as the standard deviation of the differences, divided by the means of both systems multiplied by $\sqrt{2}$. One-way repeated measures ANOVA was used to determine overall differences among test variables at 5, 10, 15, 20, and 25 min during the time trial test. The Person's r correlation coefficient was used to assess the degree of association between the differences of the two systems and the 94-day-long test period. A paired *t*-test was used to determine if the correlation coefficient was significantly different from zero. Significance level was set at $p < 0.05$. Results are presented individually or as mean ± standard deviation (SD).

## Results

**Validity and reproducibility at a large range of ventilations**

On average, $\dot{V}O_2$ was 0.8 % lower measured with the Oxycon compared with the Douglas bag method ($p < 0.05$) (Fig. **1 D** and Table **2**). The lower $\dot{V}O_2$ was a result of a 1.8 % lower $\dot{V}e$ and a 0.7 % higher Delta $O_2$ ($O_2$ concentration in the inspired air minus $O_2$ concentration in the expired air) ($p < 0.05$) (Fig. **1 B, C** and Table **1**). Delta $CO_2$ ($CO_2$ concentration in the expired air minus $CO_2$ concentration in the inspired air) was 0.6 % lower with the Oxycon Pro compared to the Douglas bag method ($p < 0.05$) (Fig. **1 A**). The average respiratory exchange ratio (RER) was 0.01 units lower with the Oxycon Pro than with the Douglas bag method ($p < 0.05$). The coefficient of variation (CV) was 1.4, 0.8, 1.0, and 1.2 for Delta $CO_2$, Delta $O_2$, $\dot{V}e$ (Table **1**), and $\dot{V}O_2$ (Table **2**), respectively. Differences between the Oxycon Pro and the Douglas bag method and the CV of the differences were independent of the absolute values of $\dot{V}e$ and $\dot{V}O_2$ except for the lowest range (Tables **1** and **2**). Both the Oxycon Pro and the Douglas bag method produced reasonable absolute $\dot{V}O_2$ measurements in relation to external power output (Fig. **4**).

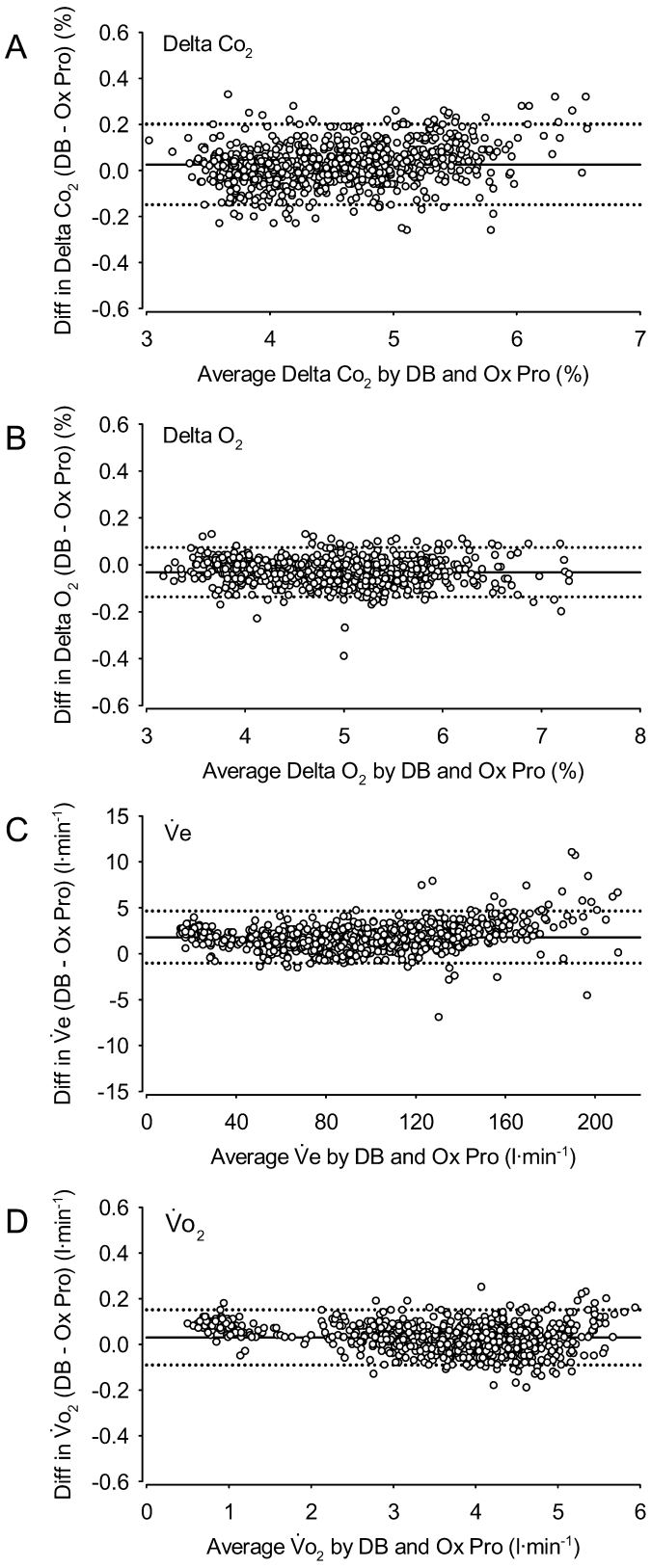


Fig. **1 A** to **D** Individual differences between the Oxycon Pro (Ox Pro) and the Douglas bag method (DB) at different levels of **A** Delta $CO_2$ ($CO_2$ concentration in the expired air minus $CO_2$ concentration in the inspired air), **B** Delta $O_2$ ($O_2$ concentration in the inspired air minus $O_2$ concentration in the expired air), **C** ventilation ($\dot{V}e$), and **D** oxygen uptake ($\dot{V}O_2$). Solid horizontal line represents mean difference (n = 802). Dashed horizontal lines represent ± 2 SD.

**Stability during continuous measuring**

During the time trial, the oxygen uptake measured with the Oxycon Pro gradually decreased from 0.5% (0.02 l) lower than the Douglas bag method at 5 min to 1.0% (0.05 l) lower at 25 min ($p < 0.05$) (Fig. **2 D**). In the same period the Delta $O_2$ measured with the Oxycon Pro drifted from being 1.1% higher to 0.4% higher than the Douglas bag method ($p < 0.05$) (Fig. **2 B**). Delta $CO_2$ and $\dot{V}e$ was constant over time (Fig. **2 A** and **C**).

**Stability during longer test periods**

The test of stability during 94 days of testing showed that $\dot{V}O_2$ measured with the Oxycon Pro gradually increased from 1.1% (0.04 l) lower than the Douglas bag method at the start of the test period to 0.5% (0.02 l) lower at the end of the test period ($p < 0.05$) (Fig. **3**).

## Discussion

The present study demonstrates that the Oxycon Pro with mixing chamber produces $\dot{V}e$, Delta $O_2$, and Delta $CO_2$ values that are very similar to the Douglas bag method. At the lowest exercise intensities ($\dot{V}O_2$ from 0.5 – 2.0 l·min$^{-1}$) $\dot{V}O_2$ was on the average 0.07 l·min$^{-1}$ lower with the Oxycon Pro than with the Douglas bag method. At moderate to very high exercise intensities ($\dot{V}O_2$ from 2.0 to 6.0 l·min$^{-1}$) $\dot{V}O_2$ was on the average 0.02 l·min$^{-1}$ lower with the Oxycon Pro than with the Douglas bag method. Altogether, $\dot{V}O_2$ was slightly underestimated with 0.8% (0.03 l·min$^{-1}$) by the Oxycon Pro. Average $\dot{V}e$ was 1.8% lower with the Oxycon Pro, thus lowering $\dot{V}O_2$ correspondingly (Table **1**). Conversely Delta $O_2$ was 0.7% higher with the Oxycon Pro, thus increasing $\dot{V}O_2$ compared to the Douglas bag method. The remaining difference could possibly be related to measurement of temperature in expired gases, as an error of only 1 °C could explain a difference of ~ 0.3%. The deviations in Delta $O_2$ and Delta $CO_2$ led to a 0.01-unit lower RER for the Oxycon Pro compared with the Douglas bag method. The measured $\dot{V}O_2$ was reasonable with respect to a wide range of external power outputs (Fig. **4**).

Just as important as the validity of a system is the reproducibility. Normally, when testing reproducibility, repeated measurements with the same system are performed when the same person is exercising at the same absolute exercise intensity [4]. The difference between two such measurements is the sum of the error in the measuring system in use and the biological variations. To be able to evaluate the reproducibility of the system only, a mechanical calibration system (metabolic simulator) can be used, or alternatively, as in this study, one can compare serial measurements of two systems. The disadvantage of the latter method is of course that one cannot distinguish the reproducibility of the two systems. Using this method we found an overall CV of less than 1.4% for Delta $CO_2$, Delta $O_2$, $\dot{V}e$ (Table **1**), and $\dot{V}O_2$ (Table **2**). This is considerably lower than the CV reported in a summary presented by Carter and Jeukendrup [4], but their CV also included biological variations. A standard deviation of a test/retest difference of 1.5% enables us to detect a difference between two situations of less than 1.5% with a power of 80% for 8 subjects, while a standard deviation of a test/retest difference of 3.0% will give a type II error in the same situation. For instance, with the Douglas bag method, with only 6 subjects we were able

Table **1** Oxycon Pro compared with the Douglas bag method at different absolute ventilations ($\dot{V}e$)

| *Measuring range $\dot{V}e$ ($l \cdot min^{-1}$)* | *15 – 39* | *40 – 79* | *80 – 119* | *120 – 159* | *160 – 210* | *15 – 210* |
|---|---|---|---|---|---|---|
| *Mean value ($l \cdot min^{-1}$)§* | 25.1 | 64.0 | 100.8 | 136.4 | 179.5 | 99.2 |
| *Mean difference (DB – Ox Pro) ($l \cdot min^{-1}$)* | 2.0 | 1.2 | 1.5 | 2.1 | 3.7 | 1.8 |
| *Coefficient of variation (CV)* | 2.4 | 1.1 | 0.8 | 0.8 | 1.0 | 1.0 |
| *Numbers compared (n)* | 78 | 192 | 265 | 217 | 50 | 802 |
| *Statistics (Ox Pro vs. DB)** | lower | lower | lower | lower | lower | lower |

* $p < 0.05$ between measurement systems; § mean of Oxycon Pro and the Douglas bag method

Table **2** Oxycon Pro compared with the Douglas bag method at different absolute oxygen uptakes ($\dot{V}O_2$)

| *Measuring range $\dot{V}O_2$ ($l \cdot min^{-1}$)* | *0.50 – 1.99* | *2.00 – 2.99* | *3.00 – 3.99* | *4.00 – 4.99* | *5.00 – 6.00* | *0.50 – 6.00* |
|---|---|---|---|---|---|---|
| *Mean value ($l \cdot min^{-1}$)§* | 1.02 | 2.67 | 3.56 | 4.43 | 5.29 | 3.69 |
| *Mean difference (DB – Ox Pro) ($l \cdot min^{-1}$)* | 0.07 | 0.04 | 0.02 | 0.02 | 0.06 | 0.03 |
| *Coefficient of variation (CV)* | 2.6 | 1.4 | 1.1 | 1.0 | 1.0 | 1.2 |
| *Numbers compared (n)* | 79 | 98 | 235 | 328 | 62 | 802 |
| *Statistics (Ox Pro vs. DB)** | lower | lower | lower | lower | lower | lower |

* $p < 0.05$ between measurement systems; § mean of Oxycon Pro and the Douglas bag method

to distinguish between the oxygen cost of cycling at 60 and 80 rpm at 350 W even if the difference was only 1.6% [5]. This would not be possible with poorer test/retest reproducibility.

In the present study, a drift in $\dot{V}O_2$ of 0.6% (0.03 l) was seen from 5 – 25 min with the Oxycon Pro compared with the Douglas bag method (Fig. **2D**). This drift was due to a 0.7% reduction of Delta $O_2$ by the Oxycon Pro in the same period, while no changes were seen in $\dot{V}e$ (Fig. **2B** and **C**). The Oxycon Pro measures ambient $O_2$ concentration continuously during testing, while the Douglas bag method uses $O_2$ concentration obtained immediately before each test to calculate respiratory variables. A slight decrease in ambient $O_2$ concentration would therefore lead to a gradual overestimation of the Delta $O_2$ for the Douglas bag method, but not for the Oxycon Pro. Ambient $O_2$ concentration changed at most with ± 0.02% units and Delta $CO_2$ was stable during the tests (Fig. **2A**). Any change in room air composition could therefore explain only minor parts of the 0.7% gradual decrease in Delta $O_2$. Mean $\dot{V}e$ increased gradually from 5 – 25 min (96 – 140 $l \cdot min^{-1}$) during the time trial. However, no relationship was seen between the increase in $\dot{V}e$ and the gradual drift in Delta $O_2$ ($p > 0.05$). Regardless of cause, the drift in $\dot{V}O_2$ was very small and without physiological significance.

The final purpose of this study was to examine the stability of the Oxycon Pro over many days of testing. Stability is important, especially in studies where submaximal and maximal oxygen uptake is evaluated before and after an intervention. During the 94 days of testing, a linear regression analysis revealed a 0.5% (0.02 l) drift in $\dot{V}O_2$ with the Oxycon Pro compared with the Douglas bag method. However, as for the drift found during continuous measurements, this drift was very small and without physiological significance.

The accuracy and reproducibility for the Oxycon Pro shown in the present study are higher than most other automatic metabolic systems reported [4,6,9,10]. However, most studies used breath-by-breath analysis. At moderate workloads (100 and 150 W) Carter and Jeukendrup [4] found a CV ranging from 4.4 to 6.5% with a breath-by-breath system (Oxycon Pro). Rietjens et al. [9] compared the Oxycon Pro with breath-by-breath system against the Douglas bag method at moderate to high workloads (95 – 375 W). They concluded that the Oxycon Pro is an accurate system for measurement of $\dot{V}O_2$ up to 5 $l \cdot min^{-1}$. At $\dot{V}O_2$ between 4 – 5 $l \cdot min^{-1}$, we estimated the CV from their data to be ~ 5%, similar to that found by Carter and Jeukendrup during moderate workloads [4]. Overall CV in the present study was 1.2% (Table **2**). Thus, reproducibility was much higher in the present study regardless of exercise intensity. This was not unexpected because Proctor and Beck [8] showed that breath-by-breath systems are vulnerable to even small deviations in the temporal alignment between ventilation and gas measurements. In a study by Bassett et al. [1], a similar precision level was achieved by mixing chamber as in the present study. It is therefore reasonable to draw the conclusion that automatic systems with mixing chambers produces more precise data than systems with the breath-by-breath technique. Since its introduction in 1973 [2], most laboratories have used breath-by-breath systems. This is probably because the breath-by-breath systems have a larger range of application and are cheaper. Based on the existing literature, however, it seems that mixing chambers should be preferred in studies where measurement of rapid changes in respiratory variables is not necessary and when $\dot{V}O_2$ is larger than ~ 4 $l \cdot min^{-1}$.

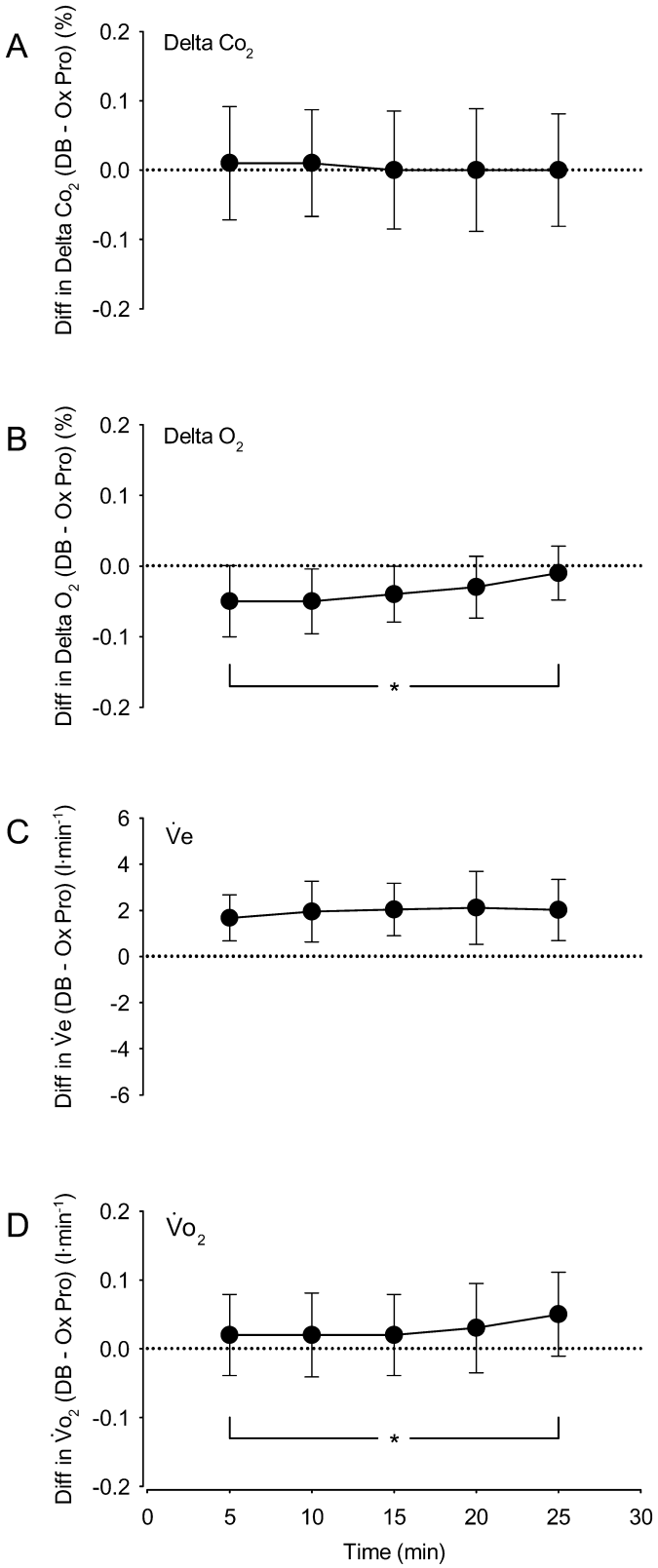


Fig. **2A** to **D** Mean difference in **A** Delta $CO_2$ ($CO_2$ concentration in the expired air minus $CO_2$ concentration in the inspired air), **B** Delta $O_2$ ($O_2$ concentration in the inspired air minus $O_2$ concentration in the expired air), **C** ventilation ($\dot{V}e$), and **D** oxygen uptake ($\dot{V}O_2$) between the Oxycon Pro (Ox Pro) and the Douglas bag method (DB) during continuous measuring for 25 min (time trial test). Mean values ranged between 4.90 – 3.82% (Delta $CO_2$), 5.14 – 3.98% (Delta $O_2$), 96 – 140 l · min$^{-1}$ ($\dot{V}e$) and 4.00 – 4.51 l · min$^{-1}$ ($\dot{V}O_2$) from 5 – 25 min of the time trial test. Dashed horizontal line is line of identity. Values are mean ± SD (n = 80). * $p < 0.05$.

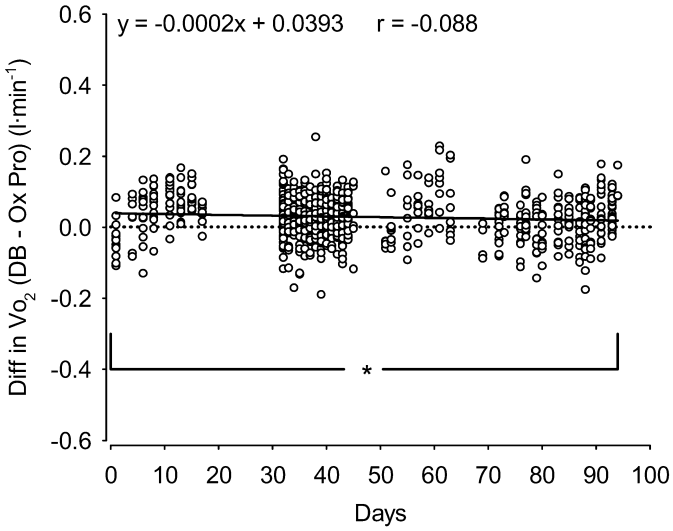


Fig. **3** Individual differences in oxygen uptake ($\dot{V}O_2$) between the Oxycon Pro (Ox Pro) and the Douglas bag method (DB) during 94 days of testing. Dashed horizontal line is line of identity. Solid horizontal line; linear regression (n = 802). * $p < 0.05$.

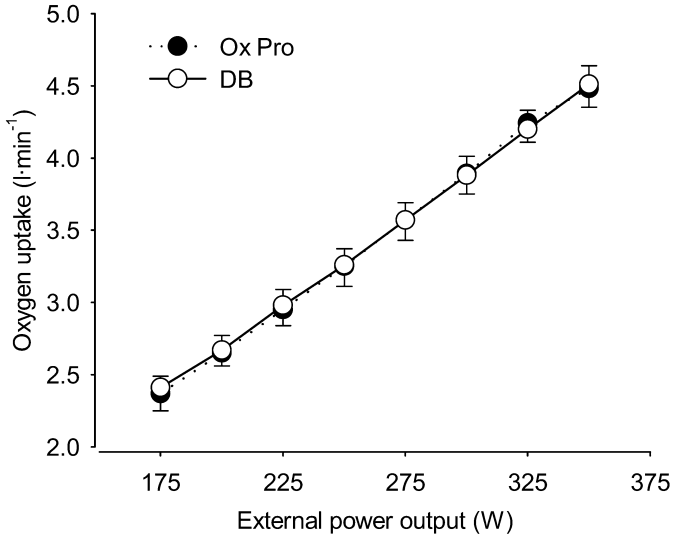


Fig. **4** Relationship between oxygen uptake and external power output for the Oxycon Pro (black symbols) and the Douglas bag method (white symbols) at a cadence of 90 rpm. Values are mean ± SD (n = 18).

## Conclusion

This study demonstrates that the Oxycon Pro with mixing chamber is a very accurate system for measuring oxygen uptake. This applies for testing over a large range of ventilations and for stability during both shorter and longer test periods.

## Acknowledgements

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**II Simposio Internacional en Ciencias del Deporte**

**Universidad Pablo de Olavide, de Sevilla**

# Avances en el estudio del sistema nervioso central en la producción de fuerza

***Miguel Fernández del Olmo***

La contracción muscular y el grado de tensión generada por la misma debe responder a los mismos procesos que constituyen el movimiento voluntario. Esto es, un sistema nervioso que influye sobre un sistema biomecánico (musculo-esquelético) y que interacciona físicamente con el entorno. Durante muchos años la contribución del sistema nervioso en la producción de fuerza y más concretamente las adaptaciones nerviosas al entrenamiento de la fuerza ha sido asumidas en base a evidencias indirectas.

En los últimos diez años ha aumentado notablemente la aplicación de técnicas neurofisiológicas en el estudio de las adaptaciones nerviosas al entrenamiento de fuerza. Técnicas tan diversas como el registro intramuscular de activación de unidades motoras, la combinación de estimulación eléctrica junto con registros electromiográficos para el estudio de reflejos espinales, la más reciente técnica de estimulación magnética transcraneal, técnicas de imagen como la resonancia magnética funcional, y el registro de potenciales corticales relacionados usando electroencefalografía, han sido aplicadas para el estudio de las adaptaciones nerviosas y su localización en el sistema nervioso central (SNC) en respuesta al entrenamiento de fuerza (revisión en Folland & Williams 2007).

El registro intramuscular de activación de unidades motoras ha permitido entender con mayor precisión el comportamiento de estas unidades motoras durante la contracción muscular y sus adaptaciones al entrenamiento de fuerza. Este registro permite explorar la frecuencia de descarga de estas unidades motoras, las cuales parece que se incrementan en respuesta al entrenamiento de fuerza. No obstante, este comportamiento está muy condicionado por las

características de la tarea con las que se evalúa la tensión muscular. Estudios recientes indican que en función del feedback que se proporciona durante la ejecución de la tarea la frecuencia de estimulación de las unidades motoras. Así, por ejemplo, si el feedback se refiere al nivel de fuerza a realizar o bien al grado articular a mantener (para una fuerza idéntica) las unidades motoras disminuirán su frecuencia de descarga y aumentarán su variabilidad mucho más en el último caso lo que irá acompañado en un menor tiempo de mantenimiento de la tensión muscular requerida (Maluf & Enoka 2005).

El estudio de las adaptaciones en relación al comportamiento reflejo se han centrado en la exploración del reflejo H y de la onda V (Aagaard et al., 2002). El primero como indicativo de la "excitabilidad espinal" y el segundo como indicativo de la "excitabilidad motorneuronal" de origen supraespinal. La mayoría de los estudios parecen coincidir en que el entrenamiento de fuerza va acompañado de un incremento en la onda V mientras cambios en el reflejo H parecen menos evidentes. No obstante la interpretación teórica mencionada anteriormente sobre ambas ondas es más que cuestionable por lo que no nos permite identificar de manera feaciente la localización de estas adaptaciones. De cualquier modo, estos estudios indican de manera indiscutible adaptaciones del SNC en respuesta al entrenamiento de fuerza.

Más recientemente se ha empezado a utilizar la técnica conocida como estimulación magnética transcraneal (TMS) para la localización de las adaptaciones nerviosas. Esta técnica permite estimular de manera no invasiva las neuronas motoras corticales, y registrar el potencial motor evocado (MEP) registrado mediante electromiografía sobre un músculo determinado. Aunque la amplitud del MEP podría reflejar una mediad en la "excitabilidad cortical", es bien sabido que la amplitud del MEP también se ve condicionado a nivel espinal. Por tanto, con la intención de localizar adaptaciones corticales y/o espinales es necesaria la combinación de registros corticales con medidas reflejas. Los estudios que utilizan esta metodología parecen indicar que las principales adaptaciones al entrenamiento de fuerza tiene lugar a nivel subcortical (Carroll et al. 2002).

Una estrategia alternativa al registro electromigráfico de potenciales evocados a nivel cortical (TMS) o a nivel espinal (ondas H y V), es el registro de las fuerzas que evocan la estimulación cortical y espinal. Si durante una contracción muscular un impulso eléctrico sobre el nervio motor o sobre la corteza motora provoca un incremento en la fuerza generada se puede asumir que no todas las neuronas motoras están siendo reclutadas o que las que están siendo estimuladas lo hacen a una frecuencia de descarga submáxima. Por tanto, con el objetivo de conocer la activación voluntaria, es decir, la capacidad de voluntariamente generar la máxima activación de un grupo muscular, se ha desarrollado la técnica conocida como "twitch interpolation", la cual consiste en medir la fuerza evocada por una estimulación externa (eléctrica o magnética) durante una contracción voluntaria máxima y compararla con la evocada en reposo. Los resultados de numerosos estudios empleado esta técnica nos ha permitido saber que lo humanos son capaces de desarrollar una activación voluntaria casi máxima (entre el 90-99% en función del músculo). Esto podría explicar el hecho de que la activación voluntaria no se vea influida por el entrenamiento de fuerza (o así lo reflejan la mayoría de los estudios), dado la poca "entrenabilidad" de este parámetro (revisión en Shield & Zhou 2004). Vale la pena mencionar, a modo de reflexión, que estudios utilizando resonancia magnética han indicado que durante una contracción voluntaria máxima el volumen muscular activado representa únicamente el 70% del volumen total (Kendall et al 2006). Estos resultados indicarían que o bien la twitch interpolation sobreestima la activación voluntaria o bien la resonancia magnética infraestima el volumen muscular. De cualquier modo, dicha discrepancia es una interesante cuestión que debería ser explorada en futuros estudios.

La "twitch interpolation" ha sido ampliamente utilizada para el estudio de la fatiga muscular, y más concretamente para diferenciar el origen periférico o central de la misma (ver revisión de Gandevia 2001). En este caso, la fuerza evocada por la estimulación en situación de reposo nos podría indica un origen periférico de la fatiga , mientras que la twitch interpolation un origen central. A este respecto, los estudios parecen indicar que el esfuerzo prolongado provoca una fatiga más central que periférica en contraste con una fatiga resultado de esfuerzos de breve

duración e intensos, de origen más periférico. De cualquier modo, la influencia periférica o central en la fatiga muscular, puede estar condicionada no únicamente por el tipo de esfuerzo pero también incluso por el movimiento que induce dicha fatiga.

Más recientemente se ha comenzado a utilizar otras técnicas, como la resonancia magnética funcional y la electroencefalografía para estudiar las adaptaciones a nivel cortical. Aunque su uso aún no está muy extendido, podrían aportar nuevos datos en relación a cambios plásticos que podrían acontecer en respuesta al entrenamiento de fuerza.

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# The Adaptations to Strength Training

## Morphological and Neurological Contributions to Increased Strength

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## Contents

## Abstract

High-resistance strength training (HRST) is one of the most widely practiced forms of physical activity, which is used to enhance athletic performance, augment musculo-skeletal health and alter body aesthetics. Chronic exposure to this type of activity produces marked increases in muscular strength, which are attributed to a range of neurological and morphological adaptations. This review assesses the evidence for these adaptations, their interplay and contribution to enhanced strength and the methodologies employed.

The primary morphological adaptations involve an increase in the cross-sectional area of the whole muscle and individual muscle fibres, which is due to an increase in myofibrillar size and number. Satellite cells are activated in the very early stages of training; their proliferation and later fusion with existing fibres appears to be intimately involved in the hypertrophy response. Other possible morphological adaptations include hyperplasia, changes in fibre type, muscle architecture, myofilament density and the structure of connective tissue and tendons.

Indirect evidence for neurological adaptations, which encompasses learning and coordination, comes from the specificity of the training adaptation, transfer of unilateral training to the contralateral limb and imagined contractions. The apparent rise in whole-muscle specific tension has been primarily used as evidence for neurological adaptations; however, morphological factors (e.g. preferential hypertrophy of type 2 fibres, increased angle of fibre pennation, increase in radiological density) are also likely to contribute to this phenomenon. Changes in inter-muscular coordination appear critical. Adaptations in agonist muscle activation, as assessed by electromyography, tetanic stimulation and the twitch interpolation technique, suggest small, but significant increases. Enhanced firing frequency and spinal reflexes most likely explain this improvement, although there is contrary evidence suggesting no change in cortical or corticospinal excitability.

The gains in strength with HRST are undoubtedly due to a wide combination of neurological and morphological factors. Whilst the neurological factors may make their greatest contribution during the early stages of a training programme, hypertrophic processes also commence at the onset of training.

High-resistance strength training (HRST) is one of the most widely practiced forms of physical activity. In the early weeks of a resistance training programme, voluntary muscle strength increases significantly and these gains continue for at least 12 months.[1] This type of exercise is used to enhance athletic performance, augment musculo-skeletal health and alter body aesthetics. The health benefits of HRST are primarily as a countermeasure to any circumstance where muscle weakness compromises function (e.g. sarcopenia, neuromusculo-skeletal disorders, or following immobilisation, injury or prolonged bed rest), but it also has a positive influence on metabolic and skeletal health. Whilst HRST is most readily associated with athletic events requiring strength and power, it has also been found to benefit endurance performance.[2] Thus, the adaptations to this type of activity are of considerable interest. This review addresses the morphological and neurological adaptations to HRST, assessing the evidence for these adaptations, their interplay and contribution to enhanced strength and the methodologies employed.

## 1. Morphological Adaptations

### 1.1 Changes in Whole-Muscle Size

It is a matter of common observation that regular high-resistance activity causes a substantial increase

in muscle size after a few months of training. This has been extensively documented in the scientific literature. Investigations employing a range of scanning techniques (e.g. magnetic resonance imaging [MRI]; computerised tomography [CT]; and ultrasound) have typically found significant increases in muscle anatomical cross-sectional area (ACSA) over relatively short training periods (8–12 weeks).[3-6] MRI is regarded as the superior method of determining muscle ACSA, because of its greater resolution,[7] and has been used increasingly in the last decade. In a careful, longer-duration study, Narici et al.[8] examined changes in muscle strength, ACSA (with MRI) and agonist muscle activation (with electromyography [EMG]) over 6 months of standard heavy-resistance training (figure 1). They demonstrated that whole-muscle growth (hypertrophy) evolved essentially in a linear manner from the onset of the training, with no indication of a plateau in this process after 6 months of training. Furthermore, after the first 2 months of training, quadriceps strength and ACSA appeared to increase in parallel. It is intuitive that the growth of skeletal muscle must slow or plateau eventually. Quantitative evidence comes from a training study by Alway et al.[9] with experienced bodybuilders (>5 years training experience). They found no change in biceps brachii ACSA or fibre area with 24 weeks of strength training.

Another common observation with HRST is the disproportionate increase in muscle strength compared with ACSA, indicating an increase in whole-muscle specific tension. Whilst of interest, there are numerous methodological problems with the direct comparison of these parameters, mainly involving the methodology of muscle-size measurement. The vast majority of investigations have measured ACSA at just one level as the index of muscle size. A recent reliability study of muscle-size measurement concluded that cross-sectional area (CSA) measured at just one level was less reliable than measurement of multiple sections and should only be used if a relatively large change in size is expected.[10] Theoretically, physiological CSA (PCSA), measured perpendicular to the line of pull of the fibres, would seem a more valid index of the muscle's contractile capability. However, the precise measurement of PCSA is problematic,[11] requiring the measurement of muscle volume and the angle of fibre pennation, as well as estimation of fibre length.[12] Alternatively, some studies have measured changes in whole muscle volume with MRI after resistance training (+14%, 12 weeks of elbow-flexor training;[13] +9.1%, 12 weeks of first dorsal interosseous training;[14] +12%, 9 weeks of quadriceps training;[5] +10%, 14 weeks of quadriceps training[15]). The question of which of these measures of muscle size is the most valid indicator of muscular strength is disputed. Bamman et al.[16] concluded that ACSA and PCSA were more strongly correlated with strength performance; however, Fukanaga et al.[17] reported higher correlations for PCSA and muscle volume with peak joint torque than for ACSA.

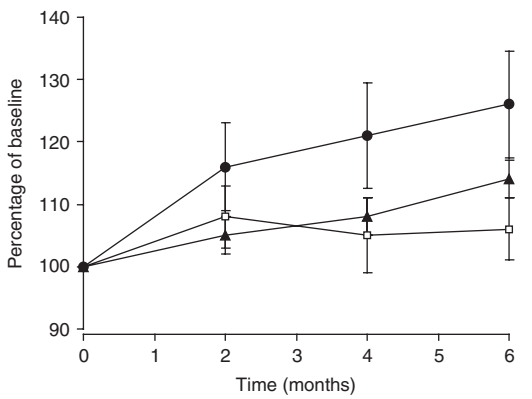


**Fig. 1.** Isometric maximal voluntary contraction (circles), integrated electromyography (squares) and quadriceps anatomical cross-sectional area (triangles) at mid-thigh during 6 months of strength training (data adapted from Narici et al.,[8] with permission).

A further confounding factor is that muscle-size measurements in relation to HRST have, to date, only been recorded in the passive state. Even during an isometric contraction, the contractile elements shorten and there can be considerable changes in muscle morphology and the mechanics of the musculo-skeletal system.[18,19] For example, as the medial gastrocnemius changes from rest to a maximum voluntary contraction at a fixed position (isometric), the angle of muscle fibre pennation doubles and the PCSA increases by 35%.[20]

Various indices of muscle size (ACSA, PCSA or muscle volume), as assessed by MRI, show significant changes after 8–12 weeks of regular training. This adaptation appears to proceed in a linear manner during the first 6 months of training. Unfortunately, the most valid muscle-size indicator of

strength is unclear and the confounding issue of size measurements taken at rest has not been addressed.

#### 1.1.1 Influence of Muscle Group

A greater hypertrophic response to resistance training has been observed in the upper body muscles compared with lower extremity muscles in previously untrained individuals.[21,22] When standard training was utilised, Welle et al.[23] found ACSA of the elbow flexors to increase by 22% and 9%, for young and old subjects, respectively; whereas, knee extensor ACSA increased by only 4% and 6%, respectively. A recent comparison of changes in muscle thickness (assessed by ultrasound) found a greater response to standard training for a range of upper body muscles compared with lower limb muscles.[6] A possible explanation for this is that lower limb muscles, particularly the anti-gravity quadriceps femoris and triceps surae, are habitually activated and loaded to a higher level during daily living activities than the upper body musculature,[22] and thus respond less to a given overload stimulus. An alternative explanation is the intermuscular differences in androgen receptor content, with some evidence for greater concentrations in the upper body muscles compared with lower limb muscles.[24]

#### 1.1.2 Influence of Sex

On average, the skeletal muscle of women typically has 60–80% of the strength, muscle fibre CSA and whole muscle ACSA of men.[25-28] Therefore, it is not surprising that the absolute changes in strength and muscle size after training are smaller in women[22] and in proportion to their smaller dimensions.[29] The lower blood androgen levels of women has also been hypothesised to cause less relative muscle hypertrophy in response to training when compared with men.[30-32] For lower body training, a number of studies have failed to find any difference between males and females with similar relative improvements both in terms of hypertrophic and strength adaptations after HRST.[6,22,33-37] For example, Tracy et al.[5] compared the hypertrophic response of the quadriceps of older men and women, finding an identical 12% increase in muscle volume after 9 weeks of training. In contrast, results for upper body training indicate there may be sex-mediated differences in the response to HRST.[38-40] A recent large-scale trial of 342 women and 243 men found greater increases in muscle ACSA in men (+2.5%, with MRI), but greater increases in strength in women (+25%, 1-repetition maximum; +6% isometric) after 12 weeks of identical training.[39] Potentially, the greater hypertrophy of males following upper body training might be due to the greater androgen receptor content of these muscles,[24] making them more responsive to higher blood androgen concentrations. The greater strength gains of females might reflect a greater capacity for neural adaptations,[41] perhaps due to less exposure and propensity towards upper body strength and power tasks that are not part of daily life in the untrained state.

#### 1.1.3 Influence of Age

There is no doubt that older adults, including nonagenarians, undergo skeletal muscle hypertrophy in response to HRST (mid-thigh ACSA: +9% after 8 weeks;[42] +9.8% after 12 weeks[43]). The absolute increase in muscle size is smaller in old adults compared with young adults, likely due to the smaller size of a typical older adult's muscles.[23] Some comparative studies suggest that the relative change in muscle volume or ACSA in response to HRST is not affected by age,[34,44] whilst others seem to suggest a smaller hypertrophy response in older individuals.[14,23,45] The variability in findings is most likely accounted for by the low subject numbers of these studies and the large inter-individual variation in response to HRST.[39]

#### 1.1.4 Selective Growth (Hypertrophy)

The extent of whole-muscle growth has been found to vary within the constituent muscles of a muscle group, as well as along the length of each constituent muscle.[4,8,46,47] For example, Housh et al.[4] reported an average hypertrophy of 23.2% for the rectus femoris, as opposed to 7.5% for the vastus lateralis (figure 2), and Narici et al.[8] found rectus femoris hypertrophy to vary from <10% to >50% at different lengths along the muscle. These authors went on to suggest that the hypertrophy of each component muscle may largely depend upon the extent of their loading and activation, which seems likely to be governed by the mechanics of each constituent muscle in relation to the training exercise(s). For example, the four constituents of the knee extensors (quadriceps) are each likely to have

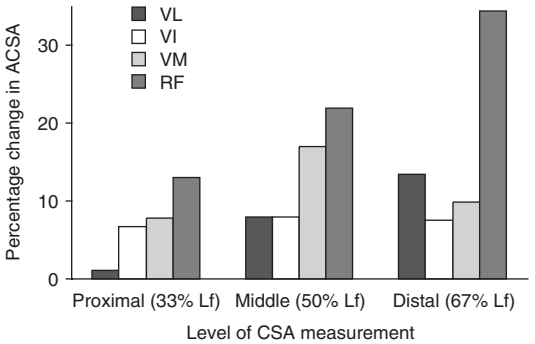


**Fig. 2.** Selective hypertrophy of the quadriceps femoris muscle after 8 weeks of isokinetic high-resistance strength training. The extent of hypertrophy varies according to the constituent muscle and level of cross-sectional area (CSA) assessment (adapted from the data of Housh et al.,[4] with permission). **ACSA** = anatomical cross-sectional area; **Lf** = length of the femur; **RF** = rectus femoris; **VI** = vastus intermedius; **VL** = vastus lateralis; **VM** = vastus medialis.

different length-tension relationships and thus different contributions to torque production at any given joint angle.

Some studies have found the greatest hypertrophic response of the whole quadriceps or biceps brachii muscles to be in the region of maximum girth/CSA (e.g. mid-thigh).[5,13,48] However, others have found this to occur in proximal[46] or proximal and distal[8] regions of the muscle, possibly due to the differences in the exercises prescribed. There is evidence that this phenomenon of selective growth can continue for an extended period of time. In experienced junior weightlifters (average age of 16.4 years), followed over 18 months of training, quadriceps ACSA increased by 31% at 30% femur length from the knee (Lf), but with no change at 50 or 70% Lf.[49] From a measurement perspective, selective growth suggests that multiple-slice MRI scanning may be required to accurately quantify the growth of muscle tissue. Theoretically, muscle growth can be achieved either by an increase in the CSA of muscle fibres (fibre hypertrophy), an increase in the number of fibres (fibre hyperplasia) or an increase in the length of fibres that do not initially run the length of the muscle.

## 1.2 Muscle Fibre Hypertrophy

An increase in the CSA of skeletal muscle fibres (fibre hypertrophy) is generally regarded as the primary adaptation to long-term strength training and has been widely documented (reviewed by McDonagh and Davies[50] and Jones et al.[51]). Fibre hypertrophy is thought to account for the increase in muscle CSA, facilitating the increase in the contractile material (number of cross-bridges) arranged in parallel and thus an increase in force production. Changes in fibre CSA in humans can only be evaluated by taking biopsy samples of skeletal muscle. Widely varying changes in mean fibre area in response to HRST have been reported. Training the triceps brachii for 6 months resulted in type 1 and type 2 fibre hypertrophy of 27 and 33%, respectively.[52] Aagaard et al.[11] found a mean increase of 16% in fibre area after 14 weeks of resistance training, which correlated significantly with the increase in muscle volume. Whilst the vast majority of studies have found significant increases in fibre CSA, Narici et al.[8] found no change in the mean fibre area despite muscle ACSA increasing by 19%. Such variability may be accounted for by a number of factors, including: (i) the poor reproducibility of the biopsy technique; (ii) the individual's responsiveness to training; and (iii) the precise nature of the training stimulus (e.g. muscle length, type and velocity of contraction, work intensity and duration). The poor repeatability of fibre area measurements with a single biopsy sample has been well documented (coefficient of variation = 10–24%).[53-57] This appears to be largely due to heterogeneity of fibre size within skeletal muscle, which may be partially influenced by the depth of the biopsy site,[58] as well as variability in perpendicular slicing of muscle tissue and tracing of cell borders.[56] Thus, while the weight of evidence strongly supports fibre hypertrophy, data from single biopsy samples must be treated with caution.[59]

### *1.2.1 Preferential Hypertrophy of Type 2 Fibres*

Preferential hypertrophy of type 2 fibres after strength training is another commonly reported finding.[60-63] The data presented by Hakkinen et al.[64] indicate a greater plasticity of type 2 fibres since they hypertrophy more rapidly during training and atrophy faster during detraining. Therefore, it is not surprising that many of the shorter studies (6–10 weeks) have only found significant hypertrophy of type 2 fibres,[11,63,65,66] whereas longer studies have more frequently found significant increases in the

fibre area of both type 1 and type 2 fibres.[52,64] The evidence from animal studies supports the greater hypertrophic response of type 2 fibres.[67] The proportion of type 2 fibres in human muscle has been significantly correlated with training-induced hypertrophy[45] and increases in strength.[65] However, strength gains have also been found to be unrelated to fibre composition[68] and positively related to the proportion of type 1 fibres.[63]

It has been suggested that type 2 fibres have a higher specific tension and their preferential hypertrophy contributes to the rise in the specific tension that is often observed for the whole muscle with training. However, there has been considerable debate about the specific tension of different fibre types. A review by Fitts et al.[69] concluded that there were no significant differences in specific tension between fibre types in rat or human muscle. In contrast, more recent work suggests greater specific tension of human fibres expressing the myosin heavy chain (MHC) IIX isoform, than in fibres expressing purely MHC I (+50%;[70] +20%;[71] +32%[72]). Studies that have related isometric specific tension to the fibre type composition of humans *in vivo* have found contradictory findings.[73-75] However, the proportion of type 2 fibres (or MHC II content) has been positively correlated with isokinetic strength at medium-to-high angular velocities[76] and relative force at high velocities.[73,77]

Recent evidence suggests that type 2 fibres have a significantly greater specific tension that, in combination with their greater hypertrophy response, likely contributes to increases in whole-muscle specific tension.

## 1.3 Myofibrillar Growth and Proliferation

MacDougall and colleagues[52] examined the myofibrillar structure of six subjects before and after 6 months of strength training. Despite wide variations in size, measurement of >3500 myofibrils in each condition revealed a significant increase in myofibrillar CSA (16%; $p < 0.01$), coincident with a 31% increase in mean fibre area. The methodology of this study was extremely thorough and their findings reinforced some earlier work of this group.[78] The packing density of the myosin filaments within the myofibril was also investigated at the centre and periphery of ≈500 myofibrils per subject. The packing density was extremely consistent within subjects, between conditions and within each myofibril, suggesting myofilament density was unchanged throughout myofibrils as well as being unresponsive to training. A three-fold increase in the number of myofibrils with 'splits' after training was also observed, which may indicate a longitudinal division of myofibrils post-training.

The uniformity of myosin-filament density throughout the myofibril indicated that myofibrillar growth was due to the addition of contractile proteins to the periphery of a myofibril. Furthermore, labelling studies have indicated that newly formed proteins tend to be found around the periphery of existing myofibrils.[79] The increase in myofibrillar CSA clearly contributes to the increase in muscle fibre area; however, the disproportionately greater increase in fibre CSA (two-fold more than myofibrillar area) suggests an additional adaptation. Given the consistency of the myosin filament packing and the increased number of myofibrils with 'splits' after training, the data of MacDougall et al.[52] is interpreted as evidence for an increase in myofibril number (i.e proliferation) after training.

### *1.3.1 A Possible Mechanism of Myofibrillar Proliferation*

The investigations by MacDougall and colleagues[52,78] indicate that myofibrillar growth and proliferation are the central morphological changes responsible for work-induced muscular growth in humans. During normal growth of mammalian muscle, myofibrillar number has been found to increase by as much as 15-fold.[80] In a series of investigations on the growth of post-natal mice, Goldspink[80,81] and Goldspink and Howells[82] proposed a mechanism for myofibrillar proliferation. Discrepancy in the arrays formed at the A and I bands causes the actin filaments to pull at a slightly oblique angle at the Z-disks. As myofibrillar size increases, the peripheral filaments will be subjected to a greater lateral displacement between the A band and Z-disk, and will pull with increasing obliquity (figure 3). Goldspink[80,81] proposed that if this were developed sufficiently in two half sarcomeres, it could cause the Z-disk to rupture.

Once one Z-disk has ruptured, the next Z-disk in the series may split in a similar manner until the entire myofibril has divided longitudinally. Evi-

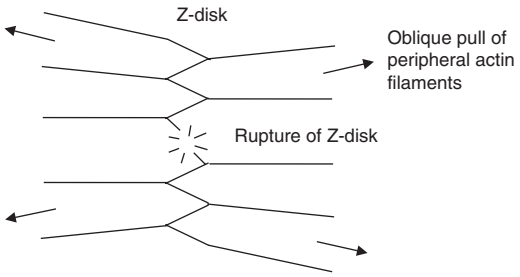


**Fig. 3.** Myofibrillar splitting due to the oblique pull of the peripheral actin filaments (redrawn from Goldspink,[83] with permission).

dence for myofibril splitting and Z-disk rupture leading to myofibrillar proliferation has also been found in growing avian and fish muscle.[84,85] Thus, in response to growth, and also likely HRST, myofibrillar proliferation takes place as a result of Z-disk rupture and longitudinal division, which limits myofibrillar size and facilitates their effective control and regulation.

#### *1.3.2 Satellite Cells*

Many investigators have found that the ratio of nuclear to cytoplasmic material remains fairly constant throughout a wide range of growth conditions (in animals;[86,87] and in humans[88,89]). In human muscle, Landing and colleagues[90] found a direct correlation between the number of myonuclei and fibre diameter. Hence, it seems that a single myonucleus may only be able to maintain a fixed volume of cytoplasmic material, and this ratio appears to be about twice as high for type 2 as for type 1 fibres.[89]

Animal work has shown that, during normal growth and maturation, the increase in muscle fibre size is due to the addition of new nuclei originating from satellite cell populations.[86,87] Unlike the myonuclei inside the fibre, satellite cells, situated beneath the basal lamina that surround each fibre, can undergo mitosis and typically one of the daughter cells then becomes a true myonucleus.[91] New myonuclei, derived from satellite cells, whilst no longer capable of dividing, begin to produce muscle-specific proteins that increase fibre size.[92,93] In overloaded adult cat muscle, Allen et al.[94] found that the increase in myonuclear number more than matched the increase in fibre volume. Rosenblatt and associates[95-97] studied changes in adult mammalian skeletal muscle in response to loading with an ablation model. These authors reported significantly less hypertrophy following prior irradiation of the muscle, which prevents the division of satellite cells. They concluded that satellite-cell proliferation is a prerequisite for hypertrophy following synergist ablation.

In humans, Kadi et al.[98,99] showed that both satellite cell numbers and myonuclei numbers were higher in elite powerlifters than in untrained controls (total nuclei +35% in type 1 and +31% in type 2 fibres).[98] These authors concluded that the extreme hypertrophy of the muscle fibres of these athletes was dependent upon the enhanced myonuclear content. Longitudinal studies of HRST have demonstrated increases in the satellite cell population after 9–14 weeks of training,[100-102] and recent research suggests rapid proliferation of satellite cells within 4 days of a single bout of largely eccentric high-load exercise.[103] However, the influence of HRST on myonuclear number and the nuclear to cytoplasm ratio has been more controversial. In response to 10 weeks resistance training, Kadi and Thornell[100] reported myonuclear and satellite cell numbers in the trapezius muscle to increase substantially, and by proportionally more than fibre CSA (figure 4). They concluded that additional myonuclei appeared to be required to support the enlargement of skeletal muscle fibres following even short-term resistance training. Hikida et al.[104] also found the nuclei to cytoplasm ratio to remain unchanged after 16 weeks of strength training that elicited a 30% increase in the size of the same fibres. However, Kadi et al.[102] reported no change in myonuclei number and an increase in the fibre area controlled by each my-

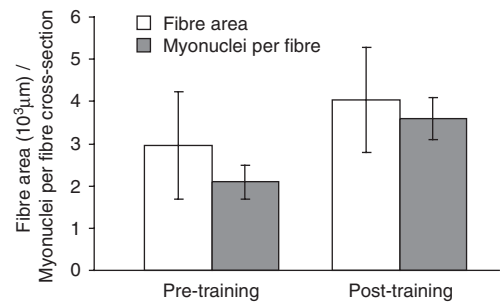


**Fig. 4.** The increase in fibre area during the early stages (10 weeks) of high-resistance strength training are matched by an increase in myonuclei number from proliferating satellite cells (data from Kadi and Thornell,[100] with kind permission of Springer and Business Media).

onucleus after 90 days of HRST. Taken together, these findings suggest that initial hypertrophy may involve a limited increase in the myonuclear domain and the quantity of cytosolic protein maintained by each nucleus, but thereafter, additional myonuclei derived from satellite cells are required.

In order for hypertrophy to occur, additional contractile proteins must be manufactured and functionally integrated into the existing fibres and myofibrils. This net accretion of muscle proteins clearly requires a sustained excess of synthesis over degradation. Increased protein synthesis is reliant upon up-regulation of either transcription or translation and is beyond the scope of this review. The regulation of protein synthesis is reviewed by Sartorelli and Fulco.[105]

## 1.4 Hyperplasia

Hyperplasia, an increase in the number of muscle fibres, could arise from fibre splitting/branching[106] with subsequent hypertrophy of daughter fibres and/or myogenesis.[107] Either of these processes could contribute to increased whole-muscle CSA and strength gains in response to HRST. However, the phenomenon of hyperplasia remains controversial.

### 1.4.1 Animal Studies

Work-induced splitting of muscle fibres has been observed and is thought to be responsible for hyperplasia in animal studies.[108-110] The methodology utilised of histologically counting the fibres in a cross-section at only one level in the muscle brings these results into question. Even in parallel fibred muscles, all the fibres may not run from origin to insertion. Consequently, a number of studies have used nitric acid digestion to dissociate and count the total number of fibres. Using total fibre counting Gollnick and colleagues[111] studied the response to compensatory hypertrophy (ablation) and chronic stretch models in the rat. They found no evidence for hyperplasia and attributed muscle enlargement entirely to hypertrophy of existing fibres. In contrast, Gonyea and et al.[112] carried out fibre counts after an average of 101 weeks of high-resistance training in cats. A significant increase in fibre numbers (9%; $p < 0.05$) was found and attributed to *de novo* formation from satellite cells, as no evidence for the longitudinal division of fibres was seen.

A review of 17 studies by Kelley[113] found less hyperplasia in mammalian muscle (8% vs 21% for avian muscle) and when the nitric acid digestion technique was used (11 %) compared with histological counting (21%). The degree of hyperplasia also seems to be dependent upon the experimental protocol that is used to induce the overload, with stretch causing more hyperplasia and small or no increase in fibre number with exercise or compensatory hypertrophy.[113,114]

### 1.4.2 Human Studies

The ethical and methodological problems of assessing the number of fibres in whole human muscles *in vivo*, make the investigation of hyperplasia in humans extremely difficult. Even in cadaver studies, there are large inter-individual differences that confound the observation of environmental adaptations.[115] The proliferative capacity of skeletal muscle tissue for regeneration is well documented.[116] Appell et al.[117] found evidence of new myotube formation from satellite cell activity after 6 weeks of endurance training. In response to HRST, Kadi and Thornell[100] discovered myotubes as well as small muscle fibres expressing embryonic and neonatal myosin heavy-chain isoforms. However, Appell[107] suggested that because of the slow rate of new fibre formation, hyperplasia could only have a small effect on muscle CSA and therefore strength improvements. A cadaver study by Sjostrom et al.[115] supported the idea of hyperplasia in adult humans, but again at a very slow rate in terms of functional changes.

The comparison of mean fibre size of resistance-trained subjects and controls has been used to infer or refute possible changes in muscle fibre number with HRST.[54,118-121] Given the previously discussed variability of fibre area measurements from biopsy specimens, often in combination with low subject numbers, this may produce erroneous conclusions. Somewhat more valid is the determination of fibre number by dividing the CSA, established with CT/MRI scanning, by the average fibre area measured in biopsy specimens. However, this relies upon extrapolating a constant fibre area and angle of pennation throughout the muscle, usually from a single biopsy sample, [111] which, as discussed in section 1.2, may not be that reliable for fibre area measurement.

Using this technique, Alway et al. [122] reported a significant correlation between fibre number and anatomical CSA in elite bodybuilders that could be attributed to either an adaptive response or a process of self selection. In response to 3 months of HRST, McCall et al.[123] found no change in the estimated fibre number, despite a 10% increase in CSA, and a comparison of muscle fibre number in bodybuilders and untrained subjects found no significant difference between the two.[124]

The quantitative contribution of hyperplasia to changes in human muscle CSA in response to exercise remains largely unknown. However, the study of human and mammalian muscle suggests hyperplasia accounts for, at most, a small proportion of the increase in muscle CSA in response to increased loading.

## 1.5 Other Morphological Adaptations

### 1.5.1 Changes in Fibre Type and Myosin Heavy-Chain Composition?

Most of the research on muscular adaptations to strength training provides evidence against substantial fibre type changes. In animals, a number of techniques used to manipulate muscle growth have revealed no change in gross fibre type with hypertrophy/atrophy,[67,125,126] although recent work indicates that more subtle changes can occur, specifically a transition of type 2B to type 2X.[127] In humans, resistance training also seems to produce subtle fibre-type changes. Several studies have found a significant increase in the number of type 2A fibres and a concomitant fall in type 2X fibres,[45,60,61,128] with one study reporting this change to occur after only 18 training sessions.[129]

The most recent classification system for identifying muscle composition is based on the expression of MHC isoforms. Schiaffino et al.[130] identified four separate MHC isoforms (I, IIA, IIB, IIX), with the majority of fibres expressing just one MHC isoform that is indicative of functional and metabolic properties, and generally corresponds to other fibre-type classification systems. In agreement with the findings on fibre type, measurements of muscle homogenate show the proportion of MHC IIX to fall by 5–11% with a similar rise in MHC IIA after 12–14 weeks of training.[131-133] Williamson et al.[132] examined single-fibre MHC expression before and after 12 weeks of HRST. These authors found increases in the proportion of fibres expressing purely MHC IIA (+24% for young women and +27% for young men) at the expense of a reduction of hybrid fibres (MHC I/IIA and IIA/IIX). In summary, subtle changes in fibre type and MHC composition appear to occur in the early phase (2–3 months) of training, but there is no evidence that this transformation continues over a prolonged period.

### 1.5.2 Density of Skeletal Muscle and Myofilaments

The gross muscle radiological density of skeletal muscle increases following strength training (+3%;[134] +5%[135,136]). Sipilä and Suominen[137] found an 11% increase in radiological density of the triceps surae after 18 weeks of strength training in elderly women. This measure of density involves much larger sections of muscle tissue than the packing density of myosin filaments examined by MacDougall et al.[52] and includes all of the constituents of whole muscle (e.g. fat and connective tissue). In rats, the discrepancy in fibre and whole-muscle size increases with overload has been taken to suggest that fibres develop at the expense of the extracellular compartment.[138] It is also interesting to note that many of the human studies employing the muscle biopsy technique have found greater hypertrophy than those using the measurement of anatomical CSA.[11,45,68,139]

Studies of the packing density of myofilaments have found this to be very consistent pre- to post-training.[52,134] More contemporary research has revealed that the specific tension of muscle fibre types, divided according to myosin heavy-chain expression, is unresponsive to 12 weeks of HRST[72,140,141] and similar for sedentary and long-term (>6 years) resistance-trained individuals.[142] Therefore, there is no evidence for an adaptation of cross-bridge density or the intrinsic contractile properties of skeletal muscle (specific tension) after HRST.

### 1.5.3 Tendon and Connective Tissue

Skeletal muscle is enveloped in a connective tissue matrix that may play a role in transmitting force to the tendons[143] and work-induced hypertrophy is known to elevate collagen synthesis in animal muscle.[144] However, there is evidence for a fixed

proportion of connective tissue in skeletal muscle throughout hypertrophy (≈13% in bodybuilders and untrained controls[124]), although this does not rule out the possibility of some plasticity in the connective tissue matrix. The arrangement of connective tissue, in relation to individual muscle fibres, could influence force production. For example, if connective tissue attachments were made between the tendons and intermediate parts of muscle fibres, then the effective CSA of a fibre would increase.[145] Essentially, a single longitudinal fibre with an extra tendinous attachment halfway along its length could, in effect, act with the force equivalent to two parallel fibres. Whether this occurs is unknown, but in theory, it could be tested, as it would cause substantial effects on the muscle mechanics.

Tendinous stiffness has been found to increase in animals in response to loading[146,147] and in humans after isometric[148] and isotonic HRST.[149,150] Reeves et al.[150] found 65% and 69% increases in patella tendon stiffness and Young's modulus, respectively, after 14 weeks of knee-extensor training. Tendon stiffness affects the time required to stretch the series elastic component and will therefore affect both the electromechanical delay and the rate of force development,[151] thus enhancing the rapid application of force. Increased stiffness also reduces tendon elongation and is likely to change the length-tension characteristics of a trained muscle, although this has not been formally investigated. A recent cross-sectional study found greater tendon thickness in athletes involved in high-force activity compared with controls.[152] In animals, high intensity running has been found to cause tendon hypertrophy.[153,154] However, longitudinal studies in humans up to 14 weeks of HRST have failed to find any evidence for this,[149,150] perhaps because this is too short a period. Alternatively, a biphasic response with an initial atrophy followed by hypertrophy has been observed in pig tendons in response to endurance exercise.[147,155] Intra-tendon structural changes in response to HRST in humans have not been investigated; however, animal studies suggest that increased diameter and packing density of collagen fibrils and changes in collagen crimp structure (waviness of fibrils)[156,157] are likely to influence tendon stiffness.

Whilst the proportion of connective tissue in skeletal muscle does not change with HRST, it is unknown if the arrangement of connective tissue changes. There is strong evidence for an increase in tendon stiffness, probably due to a range of structural changes, and tendon hypertrophy also seems probable given a sufficient training period.

#### 1.5.4 Muscle Architecture

The orientation of muscle fascicles (fibres), in relation to connective tissue/tendon and hence the relevant joint mechanics, influences muscular strength and may exhibit a degree of plasticity with HRST. As the angle of fibre pennation (AoP) increases, there is increased packing of muscle fibres within the same ACSA (essentially the effective PCSA increases), but less force from each fibre is resolved to the tendon due to their increasingly oblique angle of pull. Therefore, the effect of AoP on strength is a trade-off of these two factors (packing vs mechanical disadvantage). Alexander and Vernon[158] calculated that the force produced by a muscle of fixed external dimensions was proportional to the sine of twice the angle of pennation. According to this relationship, the optimum angle of pennation is 45°. Whilst most muscles have fibres that are pennate to the overall line of action, few muscles are pennate to this degree and therefore any increase in the angle of pennation would be expected to increase force, even if there were no increase in the anatomical CSA.

A number of studies have found a relationship between various muscle-size indicies and the angle of pennation, in a variety of strength-trained and control groups.[159-161] This may suggest that hypertrophy involves an increase in the angle of fibre pennation. An early report[162] found no change in the angle of pennation in the vastus lateralis (VL) after 12 weeks of training, although these authors conceded that the sensitivity of their ultrasound measurement technique may have been insufficient to detect changes in the angle of fibre pennation. Aagaard et al.[11] reported an increase in VL pennation angle from 8.0° to 10.7° (+36%) after 14 weeks of quadriceps HRST. The increase in pennation angle facilitated PCSA and thus isometric strength to increase significantly more (+16%) than ACSA or muscle volume (+10%). HRST of the triceps brachii

has been found to increase the angle of fibre pennation after 10 weeks (17.0–19.2°, +16%[163]) and 16 weeks (16.5–21.3°, +29%[164]). Reeves et al.[165] found the resting fibre pennation angle of the VL to increase by 28–35%, according to the knee-joint angle, after 14 weeks of HRST. More uniquely, these authors also measured pennation angle during maximal isometric contractions finding increases of 10–16% as a result of training.

These recent studies provide strong evidence that the AoP increases with HRST and, as most muscles have an AoP substantially below the optimum of 45°, this is expected to make a substantial contribution to increased strength.

## 2. Neurological Adaptations

Neurological adaptations to high-resistance training are of importance because of the specific nature of the adaptations in strength to the training task and also the apparent rise in specific tension after a period of strength training. In contrast with the morphological adaptations, considerable debate exists about the nature of the neurological changes that accompany strength training. Until recently, much of the evidence on neurological adaptation came from somewhat indirect evidence that could be questioned methodologically or neurophysiologically, and there remain extensive methodological considerations with many of the techniques used to evaluate neural adaptations. Recent work has more precisely delineated the specific neural mechanisms contributing to the training-induced increase in maximal-muscle strength.

Sale et al.[166] likened the expression of voluntary strength to a skilled act, where agonists must be maximally activated, while supported by appropriate synergist and stabiliser activation and opposed by minimal antagonist activation. Neural adaptations are essentially changes in coordination and learning that facilitate improved recruitment and activation of the involved muscles during a specific strength task.

### 2.1 Indirect Evidence of Neural Adaptations, Learning and Coordination

The disproportionately larger increase in muscle strength than size, particularly in the early stages of strength training, has been taken to indicate an increase in specific tension that is often largely ascribed to neurogenic factors. However, as discussed in section 1, numerous morphological changes could also account for this rise in specific tension (including changes in the architecture of muscle fibres, as well as the parallel and series elastic components, fibre type and preferential hypertrophy). Whilst some investigators, notably Aagaard et al.,[11] have attempted to include the contribution of some of these factors in order to calculate changes in muscle fibre specific tension *in vivo* after training, Gandevia[167] points out that it is difficult to estimate the cumulative effects of these necessary corrections.

#### *2.1.1 Specificity of Training Adaptations*

Other indirect, but more forceful, evidence for a substantial neurological adaptation comes from the observation in many strength-training investigations that the increase in dynamic lifting strength (1 repetition maximum) is disproportionately greater than the increase in isometric strength.[65,168] Undoubtedly, such findings point to a considerable facility for learning that is specific to the training task. Some proportion of this task specificity is attributable to postural activity associated with the task. As the human body is a linked mechanical system, it is necessary to orientate the body segments and set the base of support prior to forceful muscle activity.[169] Strength and power improvements after training are specific to the postures employed[170] and the role of fixator muscles and their sequence of contraction, which may be different for apparently similar exercises.[168] Recent work by Nozaki et al.[171] has highlighted the variability, between and within subjects on a trial-to-trial basis, of inter-muscle coordination and adjacent joint activity, during even seemingly straight-forward single-joint actions (e.g. knee extension). This evidence reinforces the fact that apparently simple actions undoubtedly require a degree of skill in order for optimal expression of strength.

#### *2.1.2 Cross-over Training Effect*

There is considerable evidence of a cross-over effect with training of one limb, causing strength increases in the contralateral untrained limb[172-174] (a review is presented by Zhou[175]). This supports

the hypothesis of a central adaptation in the response to training.[176] However, some studies have observed no cross-over effect.[3,136,177] It has been suggested that the cross-over training effect may be partially due to stabilising or bracing activity of the 'untrained limb' during exercise,[178] although the EMG activity of the contralateral muscle has been found to be only 15% of that recorded during a maximal voluntary contraction (MVC).[179] Certainly, the contribution of trained synergistic muscles, despite attempts to isolate a muscle group during strength measurements, might facilitate greater strength in the untrained limb.

The earliest phase of strength training may involve learning the right pattern of intermuscular coordination (i.e. stabilisers, synergists and antagonists),[168] and perhaps, once learned, this could be applied, for example, on the contralateral side.[167] Supporting evidence comes from the observation that cross-over training effects may also be muscle-action and velocity specific.[180,181] The magnitude of this type of preliminary learning seems likely to depend upon the prior level of physical activity and coordination/skill of the participants at the training task, and is a likely explanation for the diverse findings on cross-over effects. There is recent evidence that cross-over effects may extend beyond general learning and coordination and include changes in agonist activation. Using the interpolated twitch technique (ITT), Shima et al.[182] found significant increases in agonist activation of the trained and contralateral limb after 6 weeks of training.

#### 2.1.3 Imagined Contractions

In some muscles, imagined contractions appear to increase strength by inducing purely central nervous system adaptations.[183,184] Similar experiments on the abductor digiti minimi,[183] an intrinsic hand muscle, and the dorsiflexors[185] found equivalent strength increases for real and imagined training, which were greater than a control group. More recently, Zijdewind et al.[184] contrasted the influence of 7 weeks of imagined contractions, low intensity training or a control group on plantar flexor torque. These authors found substantially greater strength gains with imagined contractions (+36%) than for either controls (+14%) or low intensity training (+13%). In contrast, Herbert et al.[186] applied this idea to the elbow flexor muscles, finding imagined training produced strength gains only equivalent to a non-training control group and significantly less than real training. This could be because prior to training, the elbow flexors are closer to maximum activation than other muscle groups[187] and therefore have less capacity for central neurological adaptations. Whilst further research is clearly required, overall this evidence suggests that substantial increases in the strength of major ambulatory muscle groups can be made without physical activity and be independent of morphological adaptations. Mechanistically, it supports the role of central-cortical adaptations in response to regular HRST.

### 2.2 A Change in Agonist Activation?

The simple fact that, even during maximum contractions, recordings of force show substantial fluctuations has been taken to indicate that true maximum force is, at best, difficult to achieve.[167] Moreover, it has been widely suggested that healthy, but untrained individuals, cannot fully activate their muscles during maximum voluntary contractions, even when fully motivated.[188,189] With HRST, agonist muscle activation could increase through enhanced motor unit recruitment, or firing frequency, assuming these variables are sub-maximal prior to training.

#### 2.2.1 Electromyography

Surface electromyograph (SEMG) recordings have been used by many investigators in an attempt to measure the changes in agonist muscle activation. Numerous studies have reported agonist muscle SEMG to increase significantly with strength training, particularly during the first 3–4 weeks, and this has been taken as evidence for a change in the neural drive to a muscle.[33,46,48,172,173,190,191] Hakkinen and Komi[190] found the changes in SEMG to closely follow the changes in force over 16 weeks of training and 8 weeks of detraining (figure 5). In contrast, some studies have found no change in EMG after training.[3,8,192,193] In order to examine the factors responsible for the rapid increase in strength at the onset of a training programme, Holtermann et al.[194] observed changes in dorsiflexor strength and SEMG of the tibialis anterior with a large grid electrode, over 9 training sessions in a 5-day period. Whilst

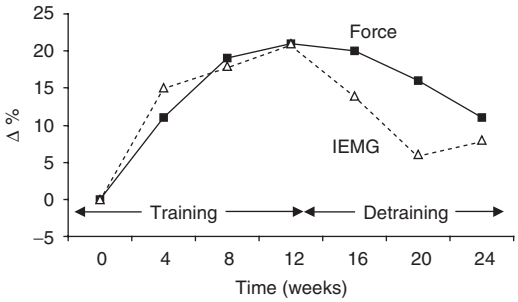


**Fig. 5.** Changes in the isometric force and surface electromyograph with 16 weeks of training and 8 weeks detraining (redrawn from Hakkinen & Komi,[190] with permission). **IEMG** = integrated electromyography.

strength increased by 16%, peak SEMG amplitude decreased by 11%. The controversy surrounding SEMG findings may be explained by a number of issues with SEMG measurement and interpretation. The technical difficulties of SEMG measurements are well recognised, and whilst electrode technology and signal processing of EMG recordings continues to improve, the reproducibility of EMG measurements remains questionable. Problems with relocating electrodes, variable impedance of the skin and subcutaneous tissue, as well as changes in muscle morphology, tend to confound the ability to reliably detect longitudinal changes in SEMG.

The interpretation of increased SEMG reflecting an increased neural drive is also considered a simplification. Firstly, SEMG is modified by changes in excitation-contraction coupling, specifically alteration of single-fibre action potential.[167] A number of factors change during a period of resistance training that are likely to alter single-fibre action potential and SEMG, including: fibre type; fibre size; membrane potential;[195] intramuscular ionic concentrations; and sodium-potassium pump content.[196,197] Secondly, the large, fast motor units tend to be more abundant towards the periphery of the muscle, close to the skin,[58,198] and any change in their activity may have an exaggerated effect upon SEMG recording. The confounding influence of these factors, and the variability in electrical impedance, can be controlled/normalised by measurement of the compound-muscle action potential (M-wave) produced by supramaximal nerve stimulation. Increased EMG, whilst the M-wave remained constant has been found,[199,200] whilst a parallel increase in EMG and M-wave has also been reported.[201]

Finally, whilst increased SEMG may reflect an increase in fibre recruitment or firing frequency, the summation pattern of EMG is also sensitive to changes in synchronisation. Out-of-phase summation can lead to cancellation of motor-unit action potentials that do not necessarily reflect any change in activation (possible changes in synchronisation are discussed in section 2.3.2).

#### 2.2.2 Tetanic Stimulation

The maximality of the neural drive to the agonist has been measured, by a variety of techniques, but typically only in relatively isolated circumstances (i.e. unilateral, single-joint isometric exercises). Supramaximal tetanic stimulation appears to be the most comprehensive method of evaluating the level of voluntary muscle activation, although a lack of activation of synergists and stabilisers does question the validity of this approach. As a result of the associated difficulties and discomfort, relatively few studies have been completed. The force from an isometric MVC has been found to match the force produced by tetanic stimulation in untrained subjects,[202-204] although the measurement sensitivity of these early investigations is dubious. After a period of training, comparison of changes in voluntary and electrically evoked force have also been used to elucidate the importance of the voluntary drive to strength gain. However, the evidence is equivocal, with reports that voluntary training increases[199,205] and has no effect[206,207] on the force of electrically evoked tetanic contractions. A third strategy in this regard has been to compare the effect of training with electrical muscle stimulation (EMS) to that of voluntary efforts. A number of studies have employed EMS training, reporting significant increases in strength,[208,209] similar strength increases as voluntary training[205,210,211] and greater strength and ACSA increases than voluntary training.[212] This evidence demonstrates that substantial improvements in strength are possible without central nervous system involvement.

#### 2.2.3 Interpolated Twitch Technique

The interpolated twitch technique has been extensively employed to measure the level of muscle activation.[213-215] In numerous studies, insensitive

forms of twitch interpolation have been used to conclude that untrained healthy subjects can achieve 'maximal' activation during isometric effort.[167] There is increasing acceptance of the importance of a number of technical and methodological issues in the use of this technique (see Folland and Williams[216] and Shield and Zhou[217]). The maximality of neurological activation appears to be muscle specific,[214] with, for example, the elbow flexors more completely activated than the quadriceps femoris.[187] Notably, more recent work provides evidence that activation of many muscle groups is rarely maximal, with, for example, considerable evidence that quadriceps femoris activation during isometric MVC is 85–95% in healthy, untrained subjects.[182,218-221] Whilst a number of older studies have found no increase in voluntary activation after resistance training,[136,177,222] again more recent investigations have found increased activation following training.[165,182,223,224] Another development in this field is the suggestion that the maximality of muscle activation during isometric effort may well be angle specific. Becker & Awiszus[225] found quadriceps activation at 40° knee-joint angle to be ≈20% lower than at 90° (figure 6a), and these findings have recently been replicated.[226]

#### 2.2.4 Dynamic Muscle Activity

Numerous authors have hypothesised that during slow concentric contractions, typical of maximum lifting tasks, there is a reduced neural drive.[189,228,229] Using EMG, Aagaard et al.[15] found evidence for inhibition of neural drive during maximal slow concentric movements, which was partially abolished after 14 weeks of HRST. Studies employing superimposed stimuli have tended to dismiss this suggestion.[230,231] However, using the ITT, Babault et al.[227] found activation to be significantly lower for slow concentric than for isometric contractions (89.7% vs 95.2%, respectively) [figure 6b].

During eccentric contractions, there is considerable evidence of a sub-maximal neural drive in untrained subjects. The eccentric portion of the *in vivo* force-velocity relationship for untrained individuals shows a marked difference in comparison with the *in vitro* relationship. Specifically, force is no greater during lengthening (eccentric) activity than isometric actions.[232] Notably, this discrepancy does not

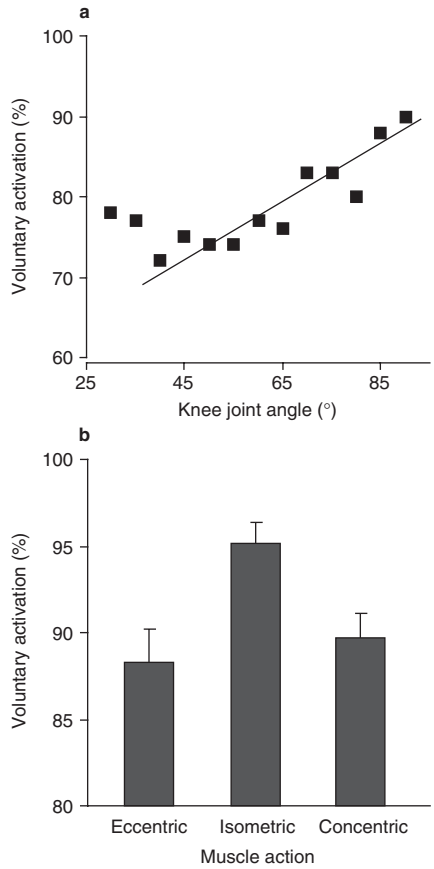


**Fig. 6.** Recent evidence using the interpolated twitch technique has suggested that the ability to maximally activate the agonist muscle varies with (**a**) joint position/muscle length (redrawn from Becker and Awiszus,[225] with permission of John Wiley & Sons, Inc.) and (**b**) type of muscle action (redrawn from Babault et al.,[227] with permission).

exist for voluntary contraction of elite power-trained individuals[232,233] and is removed with electrical stimulation of untrained subjects.[234] In addition, eccentric training of previously untrained individuals leads to considerably greater increases in eccentric-specific strength and EMG, than concentric training upon concentric strength and EMG.[235] Taken together, this evidence strongly indicates a failure in muscle activation during maximal eccentric efforts of untrained subjects either due to poor supraspinal activation or perhaps more likely spinal inhibition from a range of afferents (e.g. group Ib Golgi-organ afferents, group Ia, group II and group

III muscle-spindle afferents, and Renshaw cells), although the precise mechanism remains unknown.[15]

There is increasing evidence that previously untrained, yet healthy, subjects have scope for increasing the neural drive to agonist muscles. The magnitude of this central reserve, and hence the capacity for improvement with training is likely to depend upon the muscle group(s) under consideration, the type of muscle contraction, the muscle lengths and joint positions involved, as well as the complexity and familiarity of the movement task (i.e. bilateral or multi-joint activity).

## 2.3 Specific Mechanisms of Neurological Adaptation

Enhanced agonist muscle activation after HRST could be due to increased motor-unit recruitment or firing frequency. During a slow ramped contraction from rest, the contribution of these two factors to increased activation is highly dependent upon the muscle under consideration, with large muscles appearing to rely more on recruitment to achieve high levels of voluntary force.[236,237] Definitive evidence of an increase in motor-unit recruitment with training would require demonstration of a population of previously uninvolved motor units that can be recruited after training. Unfortunately, this is beyond the capability of current techniques. Clearly, both increased recruitment and/or firing frequency would involve some form of increased neurological drive either at the spinal or supraspinal level.

### 2.3.1 Firing Frequency

Using a large grid electrode, Holtermann and colleagues[194] evaluated changes in SEMG median frequency after 9 training session of the dorsiflexors. They found no change in median frequency, which is regarded as a measure of motor-unit recruitment,[238] despite a 16% increase in strength. Intra-muscular EMG recording techniques offer the potential to accurately investigate motor unit firing frequency (MUFF) of humans *in vivo*. The MUFF can be much higher for very brief periods (first three discharges) at the onset of a maximum voluntary effort (100–200Hz[200]), with much lower rates at the instant of maximum force generation (20–30Hz[236,237,239,240]). It is curious that with involuntary stimulation the force-frequency relationship observed for motor units in human muscle suggests that discharge rates of at least 50Hz are required to achieve maximum tetanic forces.[241,242] Taken in isolation, this might suggest considerable capacity for increases, perhaps up to 2-fold, in MUFF during maximum voluntary contractions, contributing to increased strength after training. However, it is thought that phenomena such as the catch-like properties of motor units[243] and twitch potentiation[244] may facilitate greater force production at lower frequencies than expected. An initial, brief, high-frequency burst of 2–4 pulses at the start of a contraction augments subsequent force production and is known as the catch-like property of skeletal muscle.[243] Twitch potentiation refers to the greater contractile response to a single pulse following muscle activity, may facilitate tetanic contractions at lower frequencies of innervation.

During maximum force generation, MUFF has been found to be higher in trained elderly weight lifters than age-matched controls (23.8Hz vs 19.1Hz, respectively).[245] Two longitudinal studies have found increased MUFF after HRST.[174,200] Van Cutsem et al.[200] trained subjects for 12 weeks (60 training sessions) with fast, ballistic contractions finding earlier motor-unit activation, extra doublets and enhanced MUFF at the onset of ballistic contractions after training. Whilst these adaptations are likely to contribute to gains in the rate of force development and acceleration during fast dynamic contractions, their effect on the rate of MUFF and strength at the instant of maximum force generation during slower, high force contractions is unknown. Patten et al.[174] reported no effect of two weeks of strength training on maximal MUFF. In this study, the largest changes (in strength and MUFF) appeared to occur between the two baseline tests, perhaps due to the unfamiliar nature of the movement (5th finger abduction), low subject numbers or the short duration of the training.

### 2.3.2 Synchronisation

Synchronisation quantifies the level of correlation between the timing of the action potentials discharged by concurrently active motor units. The motor units of strength athletes appear to exhibit greater synchronisation than untrained individuals

and HRST appears to increase synchronisation.[246,247] However, it is not clear how an increase in synchronisation could promote strength,[51,176] as at firing frequencies equivalent to MVC there is no effect of synchronisation upon force.[248,249]

#### 2.3.3 Cortical Adaptations

In humans, motor skill training with low force muscle activity has been demonstrated using neuroimaging techniques and transcranial magnetic stimulation to induce changes in the primary motor cortex, such as organisation of movement representations and increased cortical or corticospinal excitability for specific muscles and movements.[250-257] These adaptations might also offer an explanation for how imaginary training/mental practice could increase strength. However, more specific studies employing transcranial stimulation techniques in response to strength training found an unexpected decrease in corticospinal excitability after training of the first dorsal interosseous[258] and biceps brachii[259] muscles that would question any significant cortical adaptation.

#### 2.3.4 Spinal Reflexes

Afferent feedback in the form of spinal reflexes during contraction could enhance or dampen the supraspinal drive to the muscle. Evoked spinal reflexes have been investigated to examine any changes in spinal motoneurons after HRST, specifically their sensitivity to afferent feedback. The Hoffman reflex (or H-reflex) is an artificially elicited reflex that is used to test the efficacy of transmission of a stimulus as it passes from the afferent fibres through the motoneuron pool to the efferent fibres. It is thought to give an approximate measure of excitability of the motor neuron pool.[260] The V-wave is an electrophysiological variant of the H-reflex, but is delivered during an MVC, and may reflect efferent motor neuronal activity.[261] The H-reflex response has been measured at rest and found not to change after training,[223] although the relevance of this measurement has been questioned.[261] During maximum voluntary isometric contractions, Sale and colleagues measured the V1 and V2 wave responses after training, reporting both no potentiation[262] and a significant increase.[166] A recent study by Aagaard and co-workers[261] carefully assessed and controlled M-wave amplitude even during maximal contractions. These authors found a 20% increase in isometric strength was accompanied by increased V-wave and H-reflex amplitudes (55% and 19%, respectively) [figure 7] after 14 weeks of HRST. The increase in V-wave amplitude indicates enhanced neural drive from the spinal motoneurons, which these investigators concluded was most likely due to increased motoneuron firing frequency. The enhanced H-reflex after training further suggests that the increase in motoneuron output was caused, in part, by a rise in motoneuron excitability, although the greater increase in V-wave compared with H-reflex indicates enhanced supraspinal activation. Whilst these changes seem certain to contribute to enhanced strength, the quantitative functional significance of these effects remains unknown,[263] and this evidence is clearly contrary to the surprising decrease in corticospinal excitability that has been observed after training.[258,259]

#### 2.3.5 Antagonist Coactivation

The extent of antagonist activation during any given exercise depends on a wide range of factors, including the velocity and range of motion.[264] Any co-contraction of antagonists clearly reduces force output, but it also impairs, by reciprocal inhibition, the ability to fully activate the agonists. Cross-sectional studies have found lower coactivation in the strength/power of trained athletes than in untrained controls.[265,266] Carolan and Cafarelli[267] found a significant decrease in antagonistic activation that

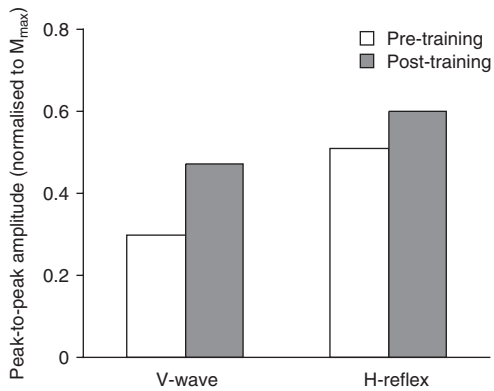


**Fig. 7.** V-wave and H-reflex amplitude (expressed relative to maximal compound muscle action potential [Mmax]) measured during isometric maximal voluntary contractions before and after 14 weeks of high-resistance strength training (data adapted from Aagaard et al.,[261] with permission).

mostly occurred in the first week of an isometric knee-extensor training programme. Hakkinen and colleagues[268] found reduced hamstring coactivation of older, but not middle-aged, participants after 6 months of knee-extensor HRST. However, other studies have found no change in antagonist activation after 9 dorsiflexor training sessions[194] or 14 weeks of knee-extension training with older adults.[165] During more complex multi-joint or whole-body movements, the level of antagonist activation may be greater, perhaps providing more opportunity for a reduction in coactivation with training.

## 3. Conclusion

A wide range of morphological and neurological factors are known to contribute to increased strength following HRST. An increase in the size of the exercised muscles is typically regarded as the major long-term adaptation, although this is highly variable between the muscles exposed to the training and along their length. Whole-muscle hypertrophy appears to proceed in a linear fashion during the first 6 months of training and is ascribed to hypertrophy of individual fibres by the processes of myofibrillar growth and proliferation, although hyperplasia may play a minor role. Whilst there may be an increase in the myonuclei to cytoplasm ratio by an upregulation of transcription or translation, satellite cells are activated in the very earliest stages of training. Their proliferation and fusion with existing myofibers enhances the number of myonuclei and appears to be intimately involved in the hypertrophy response. Muscle-fibre hypertrophy is typically greater in type 2 fibres and is accompanied by an increase in the angle of fibre pennation, which promotes a greater increase in PCSA and force production than is revealed by ACSA. These two factors are likely to contribute to increased strength and the apparent rise in whole-muscle specific tension, despite the fact that individual fibre-specific tension does not change.

The weight of indirect evidence (e.g. cross-over effects, task specificity, rapid gains in strength at the onset of a training programme), whilst not definitive, suggests a substantial neurological adaptation that may well be predominantly due to learning and changes in intermuscular coordination of agonists, antagonists and synergists. The rapid rise in strength at the start of a training programme, within the first 2 weeks, which is primarily due to neurological adaptations, significantly increases the loading and training stimulus to which the muscle is then exposed. This helps to maximise further strength gains, particularly morphological adaptations, which occur as training continues.

More sensitive use of the interpolated twitch technique suggests that untrained individuals may not be able to fully activate agonist muscles, and this central reserve appears to depend upon a range of task-specific factors. In addition, whilst controversial, the weight of SEMG measurements indicates an increase in agonist activation after training. Studies employing transcranial stimulation have found no evidence for cortical or corticospinal adaptation and are at odds with investigations of spinal reflexes that indicate an increased supraspinal drive, motoneuron excitability and a likely increase in MUFF after training.

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# Máximo lactato en estado estable: sí, ¿pero cuál?

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Máximo lactato en estado estable (en inglés "maximal lactate steady state", MLSS) se define como la velocidad o potencia más elevada de ejercicio a intensidad constante en la que la eliminación de lactato es igual a su producción, y se traduce por una concentración de lactato sanguíneo estable, tras un aumento en los primeros minutos de ejercicio[3,9]. Otros autores la definen como la mayor velocidad o potencia de ejercicio a intensidad constante a la que la concentración sanguínea de lactato permanece estable entre el minuto 10 y 30 de un ejercicio de intensidad constante, o sube menos de 1 mmol/l en ese período de tiempo, o también como la máxima intensidad de ejercicio constante que puede ser mantenida durante un largo periodo de tiempo sin que haya un aumento gradual y continuado de la concentración sanguínea de lactato[14]. Este concepto de estabilidad de lactato se conoce desde los años 20 y 30 del siglo pasado[2,8] y es muy relevante porque está más directamente relacionado con la marca deportiva en las disciplinas de larga duración que los valores de consumo máximo de oxígeno[4,10]. Los valores absolutos de concentración sanguínea de lactato durante el ejercicio a intensidad constante de MLSS pueden variar de un individuo a otro y oscilar entre 2 y 6 mmol/l[13]. En general, las personas poco entrenadas en resistencia suelen presentar unos valores de velocidad o potencia en MLSS bajos, con unas concentraciones sanguíneas altas (cerca de 4 a 6 mmol/l), mientras que los deportistas muy entrenados en resistencia presentan el caso contrario (altos valores de velocidad o potencia en MLSS y bajos valores de concentración sanguínea de lactato, cercanas a 2-3 mmol/l)[14]. La velocidad a la que se observa el MLSS suele estar comprendida entre los 10 y los 14 Km/hora en la mayoría de los deportistas masculinos de elite de los deportes de equipo, deportes de combate y disciplinas de velocidad en atletismo, mientras que son bastante mayores en los atletas mediofondistas o fondistas de elite (entre 17 y 20 Km/hora). Las deportistas de elite suelen tener valores un 10 a un 20% inferiores que los varones de su mismo deporte y nivel.

Si se realiza un ejercicio a velocidad o potencia constante ligeramente superior a la de MLSS, se observa un aumento gradual y continuo de la concentración sanguínea de lactato, superior a 1 mmol/l entre el minuto 10 y el minuto 30 de ejercicio[13]. Se considera que este aumento progresivo y continuo de la concentración sanguínea de lactato a lo largo del tiempo puede provocar disturbios

complejos del metabolismo que pueden llevar a una desestabilización del metabolismo e interferir con la capacidad para realizar ejercicio de larga duración[13]. Sin embargo, es menos conocido que es posible alcanzar situaciones de MLSS a velocidades superiores a la de MLSS de intensidad constante, siempre que se haga el ejercicio de manera fraccionada (realizando repeticiones de ejercicio intenso de corta duración, intercaladas por períodos de descanso o de ejercicio de intensidad moderada (menor de la velocidad de MLSS de intensidad constante)). De hecho, para cualquier intensidad de las repeticiones dada, superior a la velocidad o potencia de MLSS a intensidad constante, y una duración de períodos de descanso o de intensidad moderada dadas (inferior a la velocidad o potencia de MLSS a intensidad constante), existe una duración de la repetición que se acompaña de una situación de MLSS. Además, los valores de lactato sanguíneo en MLSS a estas intensidades superiores a la de MLSS de velocidad constante suelen ser tanto mayores, cuanto mayor sea la intensidad relativa a la que se realizan las repeticiones[7,12,15], pudiendo alcanzar valores cercanos a 10-12 mmol/l[5,12]. En estas condiciones de ejercicio fraccionado a MLSS también se observa, después de un cambio inicial brusco, una estabilidad de las concentraciones de lactato sanguíneo y muscular[12,15] y de otras variables musculares (fosfocreatina, ATP) y sanguíneas (amonio, ácido úrico)[1,5], que suele ser reflejo de una situación de homeostasis porque el aprovisionamiento de energía es capaz de satisfacer las necesidades de su utilización [11]. Por último, la situación de ejercicio en MLSS también se observa durante el ejercicio de fuerza muscular y también se caracteriza por una estabilidad de las variables sanguíneas y musculares indicadas con anterioridad[6]. El conocimiento y control de estos valores de intensidad a los que se produce la estabilidad del lactato sanguíneo debería ser de gran importancia para conocer las respuestas agudas de las diferentes sesiones de entrenamiento, conocer sus necesidades y tiempos requeridos de recuperación, programar el entrenamiento y analizar posteriormente las razones de la mejora o empeoramiento de la marca deportiva. En la presentación se presentan los resultados de algunos estudios que muestran el interés de conocer las situaciones de ejercicio de resistencia o de fuerza muscular de MLSS, para prescribir un entrenamiento más eficaz.

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**Central and peripheral limitations to $VO_2max$**

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The maximal oxygen uptake (VO2max) is a critical factor determining performance in sports events lasting more than 60 seconds. Although to increase endurance is not mandatory to improve VO2max, most studies have shown that in humans, improving VO2max (even without training) results in enhance endurance. In the $O_2$ cascade downstream steps can never achieve higher flows of $O_2$ than the preceding ones. This implies that VO2max cannot exceed systemic O2 delivery ($DO_2$) and is limited by the peripheral O2 extraction capacity ($O_2Ex$). Mathematically VO2max = $DO_2$ x $O_2Ex$. To determine what factor/s limit VO2max accurate measurements of DO2 and O2 must be obtained. The precise assessment of DO2 requires a simultaneous measurement of cardiac output (Q) and the concentration of O2 in arterial blood (CaO2). The most valid measures of Q and CaO2 are obtained by invasive procedures. Since Q depends also on central blood volume and preload a more useful assessment of Q can be obtained by obtained by measuring simultaneously central blood volume and preload. The latter can be achieved with transpulmonary thermodilution.

At the lung the transfer of $O_2$ is determined by the $O_2$ gradient between the alveolar space and the lung capillaries and the $O_2$ diffusing capacity (DLO2). While $DLO_2$ may be increased several times during exercise by recruiting more lung capillaries and by increasing the oxygen carrying capacity of blood due to higher peripheral extraction of $O_2$, the capacity to enhance the alveolocapillary $PO_2$ gradient is more limited. The transfer of oxygen from the alveolar space to the hemoglobin (Hb) has to overcome first the resistance offered by the alveolocapillary membrane (1/DM) and the capillary blood ($1/\theta Vc$). The fractional contribution of each of these two components to $DLO_2$ remains unknown. During exercise these resistances are reduced by the recruitment of lung capillaries. The factors that reduce the slope of the oxygen dissociation curve of the Hb (ODC) (i.e., lactic acidosis and hyperthermia) increase $1/\theta Vc$ contributing to limit $DLO_2$. These effects are accentuated in hypoxia. In fact a left-shift of the ODC may compensate for a reduction in [Hb] at altitude.

The greatest levels of muscle perfusion have been reported in the quadriceps muscle during knee extension exercise (250-450 $ml.min^{-1}.kg^{-1}$). A singularity of this exercise model is that the amount of muscle activated (about 2.5-3 kg) is small and, therefore, the pumping capacity of the heart is not taxed. Endurance training and high-intensity intermittent knee extension training increases maximal exercise vasodilatation by 20-30% mainly due to enhanced vasodilatory capacity, as maximal exercise perfusion pressure changes little with training. A great part of the increase in maximal exercise vascular conductance is explained by muscle hypertrophy and vascular remodelling.

The flow of oxygen from the muscle capillaries to the mitochondria is supposedly limited by muscle $O_2$ conductance ($DmcO_2$) (an estimation of muscle oxygen diffusing capacity). However, during maximal whole body exercise in normoxia higher flow of $O_2$ is achieved at the same pressure gradients after increasing blood [Hb], implying that in healthy humans exercising in normoxia there is a functional reserve in $DmcO_2$. Muscle O2 extraction can achieve maximal values between 95-97% in the legs and 85-87% in the arm muscles. Once the maximal values of O2Ex have been reached any improvement in VO2max will depend exclusively on the enhancement of O2 delivery.

The improvement of VO2max in middle-aged men appears to be achieved by enhancement of O2Ex, with no or little improvement of Q.

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TOPICAL REVIEW

# $\dot{V}_{O_2,max}$: what do we know, and what do we still need to know?

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**Maximal oxygen uptake ($\dot{V}_{O_2,max}$) is a physiological characteristic bounded by the parametric limits of the Fick equation: (left ventricular (LV) end-diastolic volume − LV end-systolic volume) × heart rate × arterio-venous oxygen difference. 'Classical' views of $\dot{V}_{O_2,max}$ emphasize its critical dependence on convective oxygen transport to working skeletal muscle, and recent data are dispositive, proving convincingly that such limits must and do exist. 'Contemporary' investigations into the mechanisms underlying peripheral muscle fatigue due to energetic supply/demand mismatch are clarifying the local mediators of fatigue at the skeletal muscle level, though the afferent signalling pathways that communicate these environmental conditions to the brain and the sites of central integration of cardiovascular and neuromotor control are still being worked out. Elite endurance athletes have a high $\dot{V}_{O_2,max}$ due primarily to a high cardiac output from a large compliant cardiac chamber (including the myocardium and pericardium) which relaxes quickly and fills to a large end-diastolic volume. This large capacity for LV filling and ejection allows preservation of blood pressure during extraordinary rates of muscle blood flow and oxygen transport which support high rates of sustained oxidative metabolism. The magnitude and mechanisms of cardiac phenotype plasticity remain uncertain and probably involve underlying genetic factors, as well as the length, duration, type, intensity and age of initiation of the training stimulus**

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*All that is gold does not glitter,*
*Not all those who wander are lost.*
*The old that is strong does not wither,*
*Deep roots are not reached by the frost.*

(J. R. R. Tolkein, 1955)

Maximal oxygen uptake ($\dot{V}_{O_2,max}$) is one of the most ubiquitous measurements in all of exercise science. The concept that there exists a finite rate of maximal oxygen transport from the environment to the mitochondria to support oxidative production of ATP to do physical work began with A.V. Hill (Hill & Lupton, 1923), and has been used diversely in clinical science as a measure of exercise performance (Mitchell *et al*. 1958; Levine & Stray-Gundersen, 1997; Hoppeler & Weibel, 2000; di Prampero, 2003), a marker of population-based fitness and cardiovascular disease (Blair *et al.* 1996; LaMonte *et al.* 2006), and even as a signal that patients with heart failure are on the verge of decompensation and should be referred for heart transplantation (Weber *et al*. 1987).

## $\dot{V}_{O_2,max}$: the classical view

The 'classical' view of maximal oxygen uptake is that maximal rates of oxygen utilization (and sustainable rates of oxidative ATP production) in skeletal muscle are limited under most circumstances by the ability of the heart to deliver oxygen to and be accommodated by the working muscle (Saltin & Strange, 1992; Bassett & Howley, 1997). In some sense, this construct must be true as $\dot{V}_{O_2,max}$ is easily altered by manipulations that increase ((Ekblom *et al.* 1972; Buick *et al.* 1980; Ekblom & Berglund, 1991) or decrease (Ekblom *et al.* 1972; Jilka *et al.* 1988; Pawelczyk *et al.* 1992; Levine *et al.* 1996) peripheral oxygen delivery without altering arterial $P_{O_2}$; and the maximal vasodilatory capacity of skeletal muscle clearly exceeds the ability of the heart to deliver blood and still maintain adequate arterial

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perfusion pressure (Secher *et al.* 1977; Richardson *et al.* 1999).

### Does a true $\dot{V}_{O_2,max}$ exist and can we measure it?

Some investigators have contended recently though that the absence of a clear and consistent plateau in $\dot{V}_{O_2}$ with increasing running speed in the early Hill experiments argues that the concept of $\dot{V}_{O_2,max}$ is not valid (Noakes, 1997). A number of scholarly reviews and rebuttals have been written about this issue (Saltin & Strange, 1992; Bassett & Howley, 1997, 2000; Bergh *et al.* 2000; Saltin & Calbet, 2006; Wagner, 2006) and these arguments will not be repeated here. To help answer this particular question and avoid the details of the 'what limits $\dot{V}_{O_2,max}$' debate for the moment, let's assume that there are 'upstream' and 'downstream' factors that provide oxygen to exercising skeletal muscle and then use it for physical work. The 'upstream' factors include all the physiological pathways that transfer $O_2$ from the environment to the blood, pump it to the periphery, and distribute it to and then inside the muscle cells. The 'downstream' factors include all the intracellular processes that occur once the $O_2$ molecule is transferred to the inside of the cell for oxidative production of ATP, and the neuromotor events that create calcium influx and muscle contraction.

A large part of the debate instigated by Noakes hinges on whether downstream factors, predominantly muscle motor recruitment, alone drive $\dot{V}_{O_2,max}$, or whether $\dot{V}_{O_2,max}$ has upstream limits independent of muscle motor recruitment. Indeed, Noakes has articulated what he considers a 'new model' of integrated performance physiology, which he has called The Central Governor Model (Noakes, 1997; Noakes *et al.* 2001, 2004*a*; Noakes & St Clair Gibson, 2004). In this formulation, a 'central governor' shuts down the body by putting a brake on muscle motor recruitment at very high work rates to avoid a 'disturbance of homeostasis'. So, during an incremental exercise test, the highest $\dot{V}_{O_2}$ that is achieved doesn't really reflect a true maximal ability to transport oxygen to the tissues and use it to make ATP to do physical work, because there remains lots of reserve that subjects don't 'choose' to evoke.

For the purposes of framing the debate, Dr Noakes frequently likes to place investigators into two camps: those who believe the brain plays a role in exercise performance, and those who do not (Noakes *et al.* 2004*b*). However this straw man is specious. No one disputes that 'the brain' is required to recruit motor units – for example, spinal cord-injured patients can't run. There is no doubt that motivation is necessary to achieve $\dot{V}_{O_2,max}$. A subject can elect to simply stop exercising on the treadmill while walking slowly because they don't want to continue; no mystical 'central governor' is required to hypothesize or predict a $\dot{V}_{O_2}$ below maximal achievable oxygen transport in this case.

For more than a century, cardiovascular scientists have appreciated that that 'central command' initiates the cardiovascular response to exercise and plays a critical role in the exercise pressor reflex (Mitchell *et al.* 1983; Mitchell & Victor, 1996; Williamson *et al.* 2006). This is especially true for the regulation of heart rate; for example, when voluntary effort ceases at the end of exercise, heart rate rapidly returns to normal, even if metabolic signals are trapped within skeletal muscle by vascular occlusion (Alam & Smirk, 1937). When skeletal muscle motor units are inhibited by curare, thus weakening the muscle contraction, the heart rate response to exercise is augmented (Leonard *et al.* 1985; Mitchell *et al.* 1989) as a function of increasing central command. Feedback to the brain from mechanically and metabolically sensitive skeletal muscle afferents also plays an essential role in increasing sympathetic nervous system outflow (Mitchell & Victor, 1996), as well as regulating the augmentation in cardiac output and the distribution of muscle blood flow – a response that is extremely tightly regulated with little effect of age, sex or fitness (McGuire *et al.* 2001; Fu & Levine, 2005). Indeed, when such signals are deranged, the cardiovascular response to exercise is dramatically altered, for example in patients with muscle metabolic disorders who may have 3–5 or, in extraordinary cases, more than 10 times the increase in cardiac output normally seen for a given increase in oxygen uptake (Lewis *et al.* 1984; Haller *et al.* 1991; Taivassalo *et al.* 2003). There are hundreds if not thousands of papers on animals and humans on the topic of cardiovascular regulation in healthy and patient populations, demonstrating the intimate connection between skeletal muscle and the CNS. These were reviewed thoroughly in a recent 'themed issue' of *Experimental Physiology* (Raven, 2006).

### So why the 'controversy'?

One obvious reason is that a clear and unequivocal 'plateau' in oxygen uptake with increasing work may be difficult to demonstrate during incremental tests to exhaustion in different populations. The classic strategy to overcome this limitation is to continue to repeat tests using increasing work rates, even if this requires discontinuous exercise, until the rise in $\dot{V}_{O_2}$ is smaller by some fraction than that expected from the change in external work (Taylor *et al.* 1955). The most comprehensive study in this regard was that by Taylor *et al.* (1955). They studied over 100 healthy young subjects and repeated exercise tests near maximal work capacity daily, increasing the treadmill grade until the increase in $\dot{V}_{O_2}$ was less than half to one-third that which was observed at submaximal workrates. Only 7 of 115 subjects failed to achieve this criterion, thus firmly establishing the concept of $\dot{V}_{O_2,max}$ as a measure of cardiorespiratory performance. However it must be acknowledged that this actual finite point can

be challenging to demonstrate with small increments in external work. This is especially true in subjects with relatively small anaerobic capacities, who are unable to sustain the high work rates long enough for oxygen uptake to stabilize, and for whom the difference between expected and measured changes in $\dot{V}_{O_2}$ is within the experimental noise of the technique.

Fortunately this issue was put to rest recently by a study which provides unequivocal experimental evidence validating the concept of $\dot{V}_{O_2,max}$ – that is, that there exists a finite rate of oxygen uptake of a given organism (at a particular fraction of inspired oxygen ($F_{IO_2}$) and fitness level), beyond which increasing the work rate does not lead to more oxygen uptake (Hawkins *et al.* 2007). In this study by Hawkins *et al.* (2007), a group of well-trained collegiate middle-distance runners performed an incremental exercise test, followed the next day by a 'supramaximal' test designed to determine anaerobic capacity from the accumulated oxygen deficit (Medbo *et al.* 1988). In this study, which included 156 pairs of incremental and supramaximal tests, the subjects were able to accomplish and sustain a *very large* amount of extra work – more than 30% greater than would be required to support such work rates oxidatively – using great motivation (and pain tolerance) and large amounts of muscle motor recruitment, and making energy for that work by high rates of glycolysis and substrate level phosphorylation; yet $\dot{V}_{O_2,max}$ was rarely higher, and *never* substantively so, than on the incremental test. Most importantly, it *never* even came close to the oxidative requirements of the higher workload, despite the clear documentation of a levelling off of oxygen uptake in response to the acute initiation of the very intense work rate. The central figure from this study is reproduced as Fig. 1. Thus it cannot be argued that during the incremental test to $\dot{V}_{O_2,max}$, a 'central governor' stopped the test before an actual $\dot{V}_{O_2,max}$ to avoid ischaemia or other disturbance, because the subjects were quite capable of exercising on a separate effort at a *much* higher external work rate yet no such ischaemia occurred. Others have reported similar data recently including reasonable estimates of myocardial work (Brink-Elfegoun *et al.* 2007). Finally, it is worth emphasizing that patients with coronary heart disease are regularly stressed beyond the point of myocardial ischaemia, which does not prevent them from continuing to exercise (Chaitman, 2005) – their 'central governors' must fail them all the time!

## If $\dot{V}_{O_2,max}$ exists, why is it so large in endurance athletes?

It should be emphasized that $\dot{V}_{O_2,max}$ is not equivalent to sport performance, by which I mean the time it takes to cover a specific distance under competitive circumstances, or scoring more points then an opponent in a team or individual game sport. Rather it is a physiological characteristic bounded by the parametric limits of the Fick equation:

> left ventricular (LV) end − diastolic volume − LV end−systolic volume) × heart rate × arteriovenous oxygen difference.

Some simple calculations help to highlight these boundaries. First, let's assume a maximum haemoglobin concentration for competitive athletes of 17 g dl$^{-1}$ for men, the upper limit established by the Federation Internationale de Ski (FIS) for allowing athletes to begin a race. If we assume 100% arterial oxygen saturation (i.e. no diffusion limitation or ventilation/perfusion ($\dot{V}/Q$) mismatch, certainly an overestimation in competitive athletes), and the lowest mixed venous oxygen saturations measured – 14% near the summit of Mount Everest (Sutton *et al.* 1988*b*), then the largest *possible* arteriovenous (a–v)

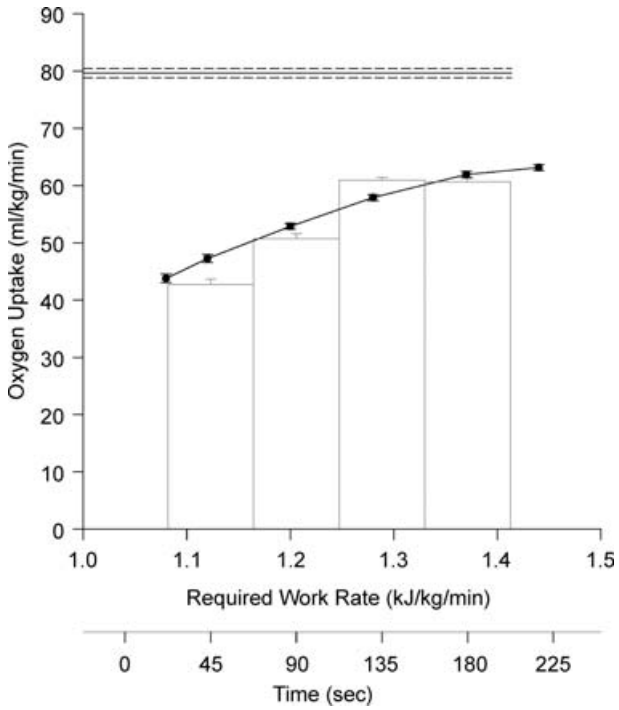


**Figure 1. Maximal $O_2$ uptake during incremental and supramaximal excercise (N = 156)**
Filled circles connected by lines represent Douglas bags obtained during the second minute of each 2 min stage run at a fixed speed with an increase in grade by 2% every 2 min. The last bag at the highest work rate was occasionally obtained earlier in the stage to accommodate subject exhaustion. Required work rate (dark abscissa) calculated directly from treadmill speed and grade. Open bars represent the last four 45 s Douglas bags collected continuously during the supramaximal test. The light abscissa reflects the time the bags were collected during the supramaximal test. Based on running economy determined individually at this grade (8%), the oxygen uptake required to perform this amount of work aerobically was calculated and shown as a solid line with 95% confidence limits (dashed line). From Hawkins *et al.* (2007) with permission from Lippincott Williams & Wilkins.

$O_2$ difference would be 200 ml $l^{-1}$ (20 vol percentage). This value is within 10–20% or so of the values that have been reported in elite athletes (Ekblom & Hermansen, 1968). Notably, peak a–v $O_2$ differences in non-athletes are not much below the values observed in elite athletes (Sutton *et al.* 1988*a*, 1992; Hagberg *et al.* 1985), arguing that the major factor underlying the large $\dot{V}_{O_2,max}$ of endurance athletes is a large cardiac output. This contention is buttressed by the observations made by Mitchell *et al.* (1958), now half a century ago, that the levelling off of $\dot{V}_{O_2}$ with increasing workrate at $\dot{V}_{O_2,max}$ was associated with a clear levelling off of cardiac output.

Since maximum heart rate of athletes is, if anything, lower than that of non-athletes (Rowell, 1986), it follows that the primary distinguishing feature of athletes is their large stroke volume. Since end-systolic volume has never been reported to be smaller in athletes than non-athletes, the single most important factor allowing this large stroke volume is a large end-diastolic volume.

Work in our laboratory more than 15 years ago demonstrated the mechanism for this unique characteristic (Levine *et al.* 1991), using direct invasive techniques. Endurance athletes have a markedly greater ability to use the Starling mechanism to increase stroke volume. Contractility was not different between athletes and non-athletes, so virtually all the difference in stroke volume was due to a large end-diastolic volume. Athletes were able to achieve such a large end-diastolic volume by virtue of markedly enhanced cardiac chamber compliance (Fig. 2). Both static compliance determined from pressure ($P$)–volume ($V$) curves, and operational compliance determined from $dV/dP$ of the $P$–$V$ curve were substantially larger in the endurance athletes (Levine *et al.* 1991). In these studies, the largest hearts for male athletes showed an end-diastolic volume in the supine position during volume infusion of around 250 ml, which generated a stroke volume of around 130–150 ml; for peak heart rate of 200 beats $min^{-1}$; this gives a peak cardiac output of about 30 l; Assuming maximal possible a–v $O_2$ difference, these characteristics would give a $\dot{V}_{O_2,max}$ of about 6–7 l $min^{-1}$ as seen in large, elite skiers or rowers (Ekblom *et al.* 1968; Jensen *et al.* 2001). To the best of my knowledge, the largest published cardiac output during exercise along with the largest stroke volume may be those reported in a world-class orienteer of 42.3 l $min^{-1}$ and world champion cyclist of 212 ml (Ekblom *et al.* 1968) respectively, though anecdotal unpublished comments have suggested the possibility of higher values (http://indurain.chez-alice.fr/). The highest reported $\dot{V}_{O_2,max}$ of which I am aware is 7.48 l $min^{-1}$ in a large, elite skier (Saltin, 1996). Studies in dogs (Stray-Gundersen *et al.* 1986) and pigs (Hammond *et al.* 1992) provide evidence that the pericardium provides a critical restraint to maximal LV filling. When the pericardium was removed in these studies, maximal LV end-diastolic volume was significantly increased, leading to increased cardiac output and $\dot{V}_{O_2,max}$. Thus the key distinguishing characteristic of elite endurance athletes is a large end-diastolic volume due to compliant heart and a distensible pericardium.

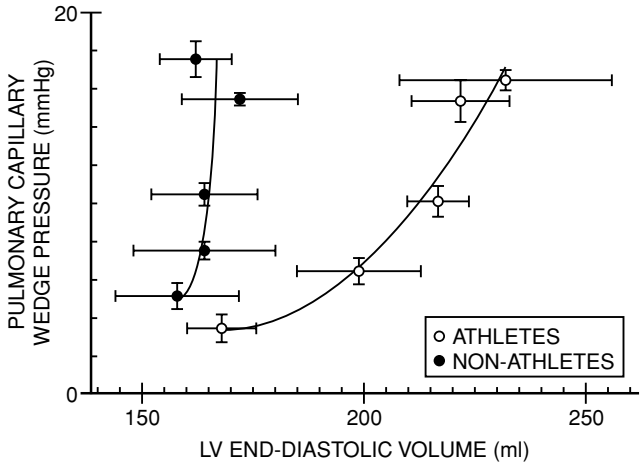


**Figure 2. Directly measured cardiac pressure–volume curves for athletes and non-athletic controls**
Note the marked improvement in both static and dynamic compliance in the endurance athletes. From Levine *et al.* (1991). Reproduced with permission.

Not only must the heart be compliant, but in order to fill to these large end-diastolic volumes during maximal exercise with very high heart rates, it must have very rapid diastolic relaxation with vigorous suction. Work by Ferguson *et al.* (2001) has shown that athletes do indeed have hearts that fill more rapidly at high levels of exercise intensity, which allows endurance athletes to continue to increase stroke volume at all levels of exercise (Gonzalez-Alonso *et al.* 2007). Diastolic suction develops because the remodelling of the athlete's heart (Pelliccia *et al.* 1999) increases the equilibrium volume of the left ventricle, which is the volume in the heart when transmural filling pressure is 0 mmHg (Nikolic *et al.* 1988; Yellin *et al.* 1990). When the heart contracts below the equilibrium volume in systole, it engages mechanical restorative forces which markedly augment the transmitral intraventricular pressure gradients that literally 'suck' blood from the left atrium across the mitral valve into the apex of the left ventricle (Yellin *et al.* 1990). This active process is particularly important during upright exercise when gravitational gradients must also be overcome to maximize venous return (Levine *et al.* 1997).

The point of going through these calculations is that there *must* be finite and fixed limitations to all the components of the Fick equation, thus providing absolute upper boundaries on maximum oxygen transport in humans (despite extraordinary rates of voluntary motor recruitment!). Assuming maximal possible a–v $O_2$ difference, and the highest reported exercise cardiac output, this absolute upper limit for $\dot{V}_{O_2,max}$ is about

8 l min$^{-1}$ (and almost certainly lower due to the low likelihood of matching maximal possible a–v $O_2$ difference with maximal cardiac output in the same athlete at the same time, due at least in part to ventilatory limitations which are prominent in endurance athletes (Dempsey & Wagner, 1999). Ultimately then, it is the broad concept of $\dot{V}_{O_2,max}$ that is important for this discussion, not the fine distinction of the specific limitations which may change under different circumstances. Moreover, it doesn't matter at all whether Hill did or did not actually 'prove' a plateau in $\dot{V}_{O_2,max}$ in 1923 – it is the concept that these parametric limits exist that is important for understanding exercise performance.

Of course the factors determining oxygen uptake are not independent, and where the 'limits' to $\dot{V}_{O_2,max}$ lie depends on the nuance of how the question is asked (Wagner, 2006). Thus, increasing cardiac output by itself may increase diffusion limitation at the lung or at the skeletal muscle and therefore not buy more oxygen transport; this problem is especially true at high altitude where the gradients driving diffusion of oxygen from the alveoli into the pulmonary capillary are greatly reduced (Johnson, 1967; Wagner, 1993, 1996*b*). Indeed maximal rates of diffusion in the periphery are so limited at high altitude that even increasing oxygen content of the blood by transfusion (Young *et al.* 1996) or erythropoietin (Lundby & Damsgaard, 2006) do not increase $\dot{V}_{O_2,max}$, even though increasing $O_2$ content by increasing $F_{IO_2}$ – emphasizing the importance of the pressure gradient from blood to muscle cell – obviously does (Savard *et al.* 1995; Boushel *et al.* 2001). Many detailed and scholarly discussions of the limitations to $\dot{V}_{O_2,max}$ have been reported by others (Saltin & Strange, 1992; Wagner, 1996*a*, 2006; di Prampero, 2003; Saltin & Calbet, 2006) to name only a few.

## So what? What is the relationship between $\dot{V}_{O_2,max}$ and performance?

Although $\dot{V}_{O_2,max}$ is not performance, it clearly is one of the major characteristics that determine performance in endurance sport (Peronnet *et al.* 1991; di Prampero, 2003). Sometimes this relationship may be obscure when only elite athletes with similar $\dot{V}_{O_2,max}$ values are considered (Noakes, 1997, 1998; Bergh *et al.* 2000). However, an elite athlete with a $\dot{V}_{O_2,max}$ of 80 ml kg$^{-1}$ min$^{-1}$ can surely run 5000 m faster than a recreational athlete with a $\dot{V}_{O_2,max}$ of 40 ml kg$^{-1}$ min$^{-1}$ can, so this characteristic is clearly closely tied to endurance performance (Bergh *et al.* 2000; di Prampero, 2003). Of course, it depends on the distance being covered, which determines the rate of optimal/possible energy utilization and the substrate used to produce ATP, how much $\dot{V}_{O_2,max}$ contributes to performance (Peronnet *et al.* 1991; di Prampero, 2003; Joyner & Coyle, 2007).

Moreover, it has been widely recognized for decades that $\dot{V}_{O_2,max}$ is not the only characteristic that determines how fast an athlete can travel, especially as the differences in outcome at a world-class level are measured in fractions of a second. Other traits such as sport-specific economy, anaerobic capacity and, for longer distances, fuel utilization and the speed or oxygen uptake at the maximal steady state will all contribute to the final count on the stop watch (Joyner, 1991; Peronnet *et al.* 1991; di Prampero, 2003; Joyner & Coyle, 2007). Since maximal oxygen delivery has little to do with most of these factors, it should not surprise anyone that athletes can perform at work rates higher than $\dot{V}_{O_2,max}$ for brief periods of time, even if $\dot{V}_{O_2,max}$ is indeed limited by cardiovascular performance. Every sprinter knows this fact – but no amount of voluntary motor recruitment can allow an athlete to run at a rate of 10 m s$^{-1}$ for the distance of a marathon. Why is this? Is it because humans do not have the motivation to sustain such high rates of muscle contraction for more than 10 s or so? Because a 'central governor' knows that it is dangerous to do so for more than 100 m? Or is it because human skeletal muscle cannot produce enough ATP at a high enough rate for a sustained period of time (or regenerate it on a sustained basis via oxidative metabolism) to support this kind of external work? It seems clear that the muscle (not the brain) is fatiguing over these brief bursts of extremely high levels of motor recruitment, though of course such local signals are communicated to the brain and influence the athlete's sense of how fast they can continue to run. Such sprint athletes are able to generate so much force from such intense motor recruitment that they rip their muscles apart (Thelen *et al.* 2006), yet they don't stop sprinting because of inadequate central drive or myocardial ischaemia. No one disputes the fact that motivation and voluntary motor recruitment influenced by sensations coming from skeletal muscle (not the vague action of a 'central governor') play a role in exercise performance, and in no way does this reality violate any tenet of the concept of $\dot{V}_{O_2,max}$ and cardiovascular performance.

It may be instructive to examine how $\dot{V}_{O_2,max}$ changes with hypoxia at altitude, to demonstrate the tight relationship between $\dot{V}_{O_2,max}$ and performance, even in athletes with relatively uniform $\dot{V}_{O_2,max}$. It has long been known that $\dot{V}_{O_2,max}$ decreases with high-altitude exposure or hypoxia (for review, see Fulco *et al.* 1998), and that in athletes, this decrease is evident at altitudes as low as a few hundred metres (Terrados *et al.* 1985; Lawler *et al.* 1988; Gore *et al.* 1996; Wehrlin & Hallen, 2006). The mechanism for this reduction is related to diffusion limitation in the lung, which is exaggerated in athletes with high pulmonary blood flows (Johnson, 1967; Torre-Bueno *et al.* 1985; Levine & Stray-Gundersen, 1999) and who may develop exercise-induced hypoxaemia even at sea level (Dempsey & Wagner, 1999) (*see* Fig. 3).

Not only does $\dot{V}_{O_2,max}$ decrease, but exercise performance at altitude clearly deteriorates at all running distances greater than 800 m (events lasting longer than 2 min (Peronnet *et al.* 1991). Noakes *et al.* (2001) have suggested that this decrease in $\dot{V}_{O_2,max}$ and performance is a function of reduced motor recruitment, and argue that it provides evidence in support of the central governor model. However, this speculation has recently been proved convincingly to be incorrect.

Some insight can be obtained from an early study by Medbo *et al.* (1988), who elaborated on the Krogh and Lindhard concept of the accumulated oxygen deficit as a measure of anaerobic capacity. In this study the investigators performed supramaximal exercise on a treadmill after careful assessment of individual running economy. During the uphill run, the total energy expended during the test was divided into aerobic and anaerobic components, and the anaerobic capacity was defined as the difference between the predicted cost of the total work if all energy had been derived from oxidative sources, and the directly measured accumulated oxygen uptake. Central to the proof that this measure is truly representative of anaerobic capacity is the demonstration that it is independent of oxygen uptake, and unaffected by hypoxia. Consistent with this hypothesis, the data showed that there was no difference in the anaerobic capacity measured under normoxic and hypoxic conditions, equivalent to an altitude of 3500 m; 100% of the reduction in performance (slower speed, lower grade) was due to a reduction in accumulated oxygen uptake. However, in order to keep the duration of the test constant in normoxia and hypoxia, the speed and grade of the treadmill had to be reduced.

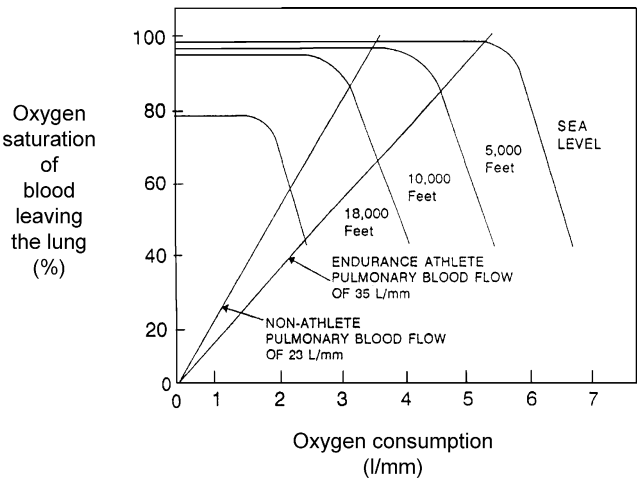


**Figure 3. Effect of different pulmonary blood flows (i.e. cardiac output) on oxygen saturation at different altitudes**
Note that a non-athlete with a peak pulmonary blood flow of 23 l min$^{-1}$ would be well saturated at sea level and low altitude up to 5000 feet. (1500 m); however, an endurance athlete with a peak pulmonary blood flow of 35 l min$^{-1}$ would be barely compensated at sea level and would have clear desaturation even at low altitude. From Levine & Stray Gundersen (1999) as modified from Johnson (1967). Reproduced with permission (© Elsevier 1999).

More recently, Wehrlin & Hallen, (2006) extended this work by performing repeated supramaximal running tests in a group of trained athletes at multiple low to moderate altitudes from 300 m to 2800 m. In order to ensure that motor recruitment and power output were the same in all tests, each supramaximal test was performed at exactly the same speed, at 107% normoxic velocity at $\dot{V}_{O_2,max}$. Despite keeping the speed absolutely constant at all altitudes, $\dot{V}_{O_2,max}$ was reduced progressively and linearly by 0.6% per 100 m altitude, in direct proportion to the reduction in oxygen saturation ($S_{pO_2}$) (Wehrlin & Hallen, 2006); performance was reduced by 1.4% per 100 m altitude in direct proportion to the decrease in $\dot{V}_{O_2,max}$. This study provides strong evidence that: (a) $\dot{V}_{O_2,max}$ is closely tied to oxygen transport, even when the differences are quite small, especially in well-trained endurance athletes; and (b) the reduction in $\dot{V}_{O_2,max}$ at altitude is not likely to be due to decreased motor unit recruitment since running speed was constant at all altitudes studied.

## What next?

So why do athletes stop exercising at $\dot{V}_{O_2,max}$? This is a complex question that is beyond the scope of this essay, and the answer unquestionably varies depending on the circumstances of the exercise effort. Certainly, oxygen-dependent pathways are involved in energetic failure (Kindig *et al.* 2005), though these may be more important in muscle fibres that are less rather than more oxidative (Howlett & Hogan, 2007). A very recent series of 'mini-reviews' summarizes the state of the art in this field (McKenna & Hargreaves, 2007) and emphasizes that there is a multiplicity of factors responsible for inducing local muscle fatigue, including failure of sarcoplasmic reticulum calcium release (Allen *et al.* 2007), impaired sodium/potassium pump activity (McKenna *et al.* 2007), and slowed cross-bridge cycling (Fitts, 2008) due to a variety of metabolic mediators including reactive oxygen species (Ferriera & Reid, 2008). It is also clear that these muscle factors stimulate a number of neural pathways (Todd *et al.* 2007) that ultimately lead to reduced central motor drive and neural activation (Amann & Calbet, 2007; Amann *et al.* 2006). Under certain conditions such as severe acute hypoxia, central fatigue may be quite prominent and cause exercise effort to be compromised even before peripheral fatigue develops (Amann *et al.* 2007). It is highly likely that many of these factors are redundant, and may be more or less prominent in leading to cessation of effort under different circumstances. Defining the link between metabolic demand, cardiovascular control (including the regulation of cardiac output and local

muscle blood flow), and fatiguing exercise including the afferent receptors, neural pathways and central integration will be an important direction for future research.

Lastly, it is intuitively obvious to anyone who has grown up on a playground that some individuals are more gifted athletically than others. Not only are there those that are bigger, stronger and faster, but also those with more endurance. Recent evidence suggests that at least some amount of $\dot{V}_{O_2,max}$ is heritable (Bouchard *et al.* 1998; Hagberg *et al.* 2001), though identification of specific genes has been less convincing. For example, despite the early enthusiasm for genes that govern the angiotensin converting enzyme (Myerson *et al.* 1999; Gayagay *et al.* 1998), it is quite clear that large numbers of successful endurance athletes do not have the 'endurance genotype' (Gayagay *et al.* 1998; Woods *et al.* 2001; Tsianos *et al.* 2004; Lucia *et al.* 2005; Scott *et al.* 2005). How much does this have to do with differences in underlying genotype or, more importantly, gene–environment interactions? What are the limits of phenotypic plasticity in response to training? For example, training studies in our lab show the ability to achieve the same LV mass as elite endurance athletes with 1 year of training (Morrow *et al.* 1997, 1998; Levine *et al.* 1998). However, LV end-diastolic volume and compliance remain much lower than we have previously reported in cross-sectional studies of endurance athletes (Levine *et al.* 1991). Is this a function of pericardial constraint which needs more than 1 year to dilate? Do we just need many more years of training? Or rather is it more important that training occurs during growth and development to achieve optimal cardiac size and compliance (Saltin *et al.* 1995)?

In conclusion, the key take home messages from this essay are: (1) $\dot{V}_{O_2,max}$ is an important determinant of endurance performance which represents a true parametric measure of cardiorespiratory capacity for an individual at a given degree of fitness and oxygen availability; (2) the primary distinguishing characteristic of elite endurance athletes that allows them to run fast over prolonged periods of time is a large, compliant heart with a compliant pericardium that can accommodate a lot of blood, very fast, to take maximal advantage of the Starling mechanism to generate a large stroke volume; (3) athletes stop exercising at $\dot{V}_{O_2,max}$ because of severe functional alterations at the local muscle level due to what is ultimately a limitation in convective oxygen transport, which activates muscle afferents leading to cessation of central motor drive and voluntary effort.

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# Movement Velocity as a Measure of Loading Intensity in Resistance Training

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Key words
- bench press
- muscle strength
- 1RM prediction
- isoinertial assessment
- exercise testing
- weight training

## Abstract

This study examined the possibility of using movement velocity as an indicator of relative load in the bench press (BP) exercise. One hundred and twenty strength-trained males performed a test (T1) with increasing loads for the individual determination of the one-repetition maximum (1RM) and full load-velocity profile. Fifty-six subjects performed the test on a second occasion (T2) following 6 weeks of training. A very close relationship between mean propulsive velocity (MPV) and load (%1RM) was observed ($R^2 = 0.98$). Mean velocity attained with 1RM was $0.16 \pm 0.04\,m \cdot s^{-1}$ and was found to influence the MPV attained with each %1RM. Despite a mean increase of 9.3% in 1RM from T1 to T2, MPV for each %1RM remained stable. Stability in the load-velocity relationship was also confirmed regardless of individual relative strength. These results confirm an inextricable relationship between relative load and MPV in the BP that makes it possible to: 1) evaluate maximal strength without the need to perform a 1RM test, or test of maximum number of repetitions to failure (XRM); 2) determine the %1RM that is being used as soon as the first repetition with any given load is performed; 3) prescribe and monitor training load according to velocity, instead of percentages of 1RM or XRM.

## Introduction

One of the main problems faced by strength and conditioning coaches is the issue of how to objectively quantify and monitor the actual training load undertaken by athletes in order to maximize performance. Several acute training variables have been identified for resistance training program design (exercise type and order, intensity or load, number of repetitions and sets, and rests between sets) [24]. Manipulation of these variables shapes the magnitude and type of physiological responses and, ultimately, the adaptations to strength training [2,32]. Exercise intensity is generally acknowledged as the most important stimulus related to changes in strength levels [23], and has been commonly identified with relative load (percentage of one-repetition maximum, 1RM) [12]. This approach requires coaches to individually assess the 1RM value for each athlete. Direct assessment of 1RM, however, has some potential disadvantages worth noting. It may be associated with injury when performed incorrectly or by novice subjects and it is time-consuming and impractical for large groups [3,4,25]. Furthermore, experience tells us that the actual RM can change quite rapidly after only a few training sessions and often the obtained value is not the subject's true maximum.

An alternative way to prescribe loading intensity is to determine, through trial and error, the maximum number of repetitions that can be performed with a given submaximal weight. For example, 10RM refers to a weight that can be lifted ten times, but no more. Several studies [16,19,30] have been conducted to identify the relationship between selected percentages of 1RM and the number of repetitions to failure, establishing a repetition maximum continuum [23]. It is believed that certain performance characteristics are best trained using specific RM load ranges [6,32]. This method certainly eliminates the need for a direct 1RM test, but it is not without drawbacks either. Although training using exhaustive efforts is common practice in strength training, increasing evidence shows that training to repetition failure does not necessarily improve the magnitude of strength gains and that it may even be counterproductive by inducing excessive fatigue, mechanical and metabolic strain for sub-

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sequent sessions [9,10,18,33,34] as well as undesirable transitions to slower fibre types [12]. Furthermore, after performing the first set to failure the number of repetitions in following sets is reduced, regardless of recovery [29]. Hence, by the second or third set it is likely that the athlete may not be training within the prescribed intensity range. Prediction models for 1RM derived from regression equations based on maximum number of repetitions to failure have also been proposed [3,4,25,28,35] as an alternative to direct 1RM assessment.

The aforementioned limitations suggest trying to find better ways to objectively monitor training load during resistance exercise. Movement velocity is another variable which could be of great interest for monitoring exercise intensity but surprisingly, as some authors have noticed [19,27], it has been vaguely mentioned in most studies. The lack of use of this variable is likely because until recently it was not possible to accurately measure velocity in typical isoinertial resistance training exercises. Thus, most of the research which has addressed velocity of movement in strength training has done so mainly in studies that used isokinetic dynamometry [1,5,7,22] which, unfortunately, is not an ideal or common training setting. The actual velocity performed in each repetition could perhaps be the best reference to gauge the real effort which is being incurred by the athlete, although, to the best of our knowledge, there are yet no studies that have specifically examined this issue. Therefore, the purpose of the present study was to analyze the relationship between movement velocity and relative load (%1RM) in the concentric bench press (BP) in order to assess the possibility of using velocity data to estimate relative load.

## Materials and Methods

### Subjects

One hundred and twenty young healthy men volunteered to take part in this study. The subjects' mean ± SD age, height, body mass, body fat percentage, and one-repetition maximum (1RM) bench press were: 24.3 ± 5.2 years, 1.80 ± 0.07 m, 78.3 ± 8.3 kg, 13.2 ± 4.1 %, 87.8 ± 15.9 kg, respectively. Subjects' weight training experience ranged from 1.5 to beyond 4 years (2–3 sessions per week). No physical limitations or musculoskeletal injuries that could affect testing were reported. The study met the ethical standards of this journal [14] and was approved by the Research Ethics Committee of Pablo de Olavide University. After being informed of the purpose and experimental procedures, subjects signed a written informed consent form prior to participation.

### Testing procedures

All 120 subjects performed an isoinertial strength test (T1) with increasing loads up to the 1RM for the individual determination of the full load-velocity relationship in the BP exercise. A subset of the total sample (56 subjects) performed the same test on a second occasion (T2), following 6-wk of resistance training. During this time each subject trained following his usual routine (2–3 sessions/wk) using free-weights, which included the BP (3–5 sets, 4–12 repetitions, 60–85% 1RM, 2–4 min inter-set rests). Subjects were instructed to avoid training to repetition failure and to perform concentric actions at maximal velocity. A detailed description of the strength testing procedures used in this study has recently been reported elsewhere [31].

### Measurement equipment and data acquisition

Height was measured to the nearest 0.5 cm during a maximal inhalation using a wall-mounted stadiometer (Seca 202, Seca Ltd., Hamburg, Germany). Body mass and fat percentage were determined using an 8-contact electrode segmental body composition analyzer (Tanita BC-418, Tanita Corp., Tokyo, Japan).

A Smith machine (Multipower Fitness Line, Peroga, Spain) was used for all tests. An isoinertial dynamometer (T-Force Dynamic Measurement System, Ergotech, Murcia, Spain) was used for mechanical measurements. This system consists of a cable-extension linear velocity transducer interfaced to a personal computer by means of a 14-bit resolution analog-to-digital data acquisition board, and custom software. Vertical instantaneous velocity was directly sampled by the device at a frequency of 1 000 Hz. The propulsive phase was defined as that portion of the concentric phase during which the measured acceleration ($a$) is greater than acceleration due to gravity (i.e. $a \geq -9.81\ m \cdot s^{-2}$). Mean test velocity was defined as the mean of the velocity values, calculated each 5% from 30–95%1RM, and derived from second-order polynomial fits to load-velocity data for each test.

### Statistical analyses

Standard statistical methods were used for the calculation of means, standard deviations (SD) and *Pearson* product-moment correlation coefficients (r). Intraclass correlation coefficients (ICC) were calculated with the one-way random effects model. Coefficients of variation (CV) were calculated as typical error of measurement expressed as a percentage of the subjects' mean score. One-way ANOVA was used to detect differences between subgroups of subjects. *Scheffé* post-hoc test was used to identify the source of any significant differences. The *Kolmogorov-Smirnov* test was applied to determine the nature of the data distribution for the velocity attained with the 1RM load. Relationship between relative load and velocity was studied by fitting second-order polynomials to data. Significance was accepted at the $P \leq 0.05$ level.

## Results

### Relationship between relative load and velocity

After plotting mean propulsive velocity (MPV) against %1RM and fitting a second-order polynomial to all data points, a very close relationship ($R^2 = 0.98$) between these two variables could be observed (**Fig. 1**). Individual curve fits for each test gave an $R^2$ of 0.996 ± 0.003 (range: 0.983–0.999; CV = 0.3%). The mean MPV attained with each percentage of 1RM was obtained from these polynomial fits, from 30% 1RM onwards, in 5% increments (**Table 1**).

### Influence of the velocity attained with 1RM

Mean velocity attained with the 1RM load ($V_{1RM}$) was $0.16 \pm 0.04\ m \cdot s^{-1}$, and followed a normal distribution, ranging from $0.06\ m \cdot s^{-1}$ to $0.24\ m \cdot s^{-1}$. A significant, although low, correlation ($r = 0.27$, $P < 0.01$; **Fig. 2**) was found between $V_{1RM}$ and mean test velocity.

### Stability in the load-velocity relationship after modifying the 1RM

A subset of 56 subjects performed a retest after 6-wk of training for the purpose of studying the differences that could exist in the MPV attained with each relative load after modifying their max-

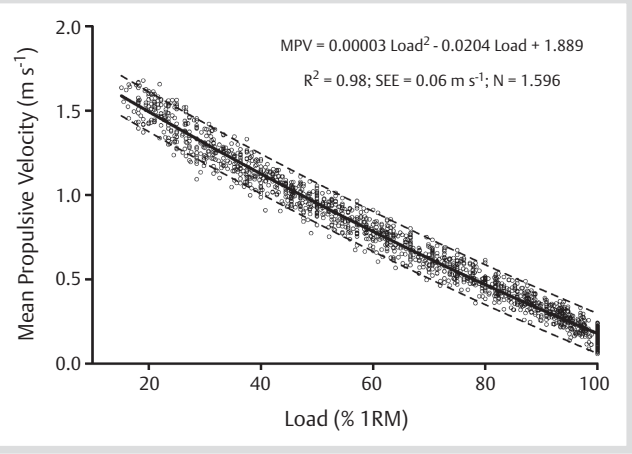


**Fig. 1** Relationship between relative load (% 1RM) and MPV directly obtained from 1 596 raw data derived from the 176 incremental tests performed in the BP exercise. Solid line shows the fitted curve to the data, and the dotted lines indicate limits within which 95% of predictions will fall.

**Table 1** Changes in mean propulsive velocity ($m \cdot s^{-1}$) attained with each relative load, from initial test (T1) to retest (T2), after 6-wk of training, in the bench press exercise.

| Load (%1RM) | T1 | T2 | Difference (T1–T2) |
|---|---|---|---|
| 30% | 1.33 ± 0.08 | 1.33 ± 0.08 | 0.00 |
| 35% | 1.24 ± 0.07 | 1.23 ± 0.07 | 0.01 |
| 40% | 1.15 ± 0.06 | 1.14 ± 0.06 | 0.01 |
| 45% | 1.06 ± 0.05 | 1.05 ± 0.05 | 0.01 |
| 50% | 0.97 ± 0.05 | 0.96 ± 0.05 | 0.01 |
| 55% | 0.89 ± 0.05 | 0.87 ± 0.05 | 0.01* |
| 60% | 0.80 ± 0.05 | 0.79 ± 0.05 | 0.01 |
| 65% | 0.72 ± 0.05 | 0.71 ± 0.05 | 0.01 |
| 70% | 0.64 ± 0.05 | 0.63 ± 0.05 | 0.01 |
| 75% | 0.56 ± 0.04 | 0.55 ± 0.04 | 0.01 |
| 80% | 0.48 ± 0.04 | 0.47 ± 0.04 | 0.01 |
| 85% | 0.41 ± 0.04 | 0.40 ± 0.04 | 0.01 |
| 90% | 0.33 ± 0.04 | 0.32 ± 0.04 | 0.01 |
| 95% | 0.26 ± 0.03 | 0.25 ± 0.03 | 0.01 |
| 100% | 0.19 ± 0.04 | 0.18 ± 0.04 | 0.00* |

* Does not exactly coincide with T1-T2 due to the shown values being the result of rounding to two decimal places. Values are mean ± SD (N = 56).

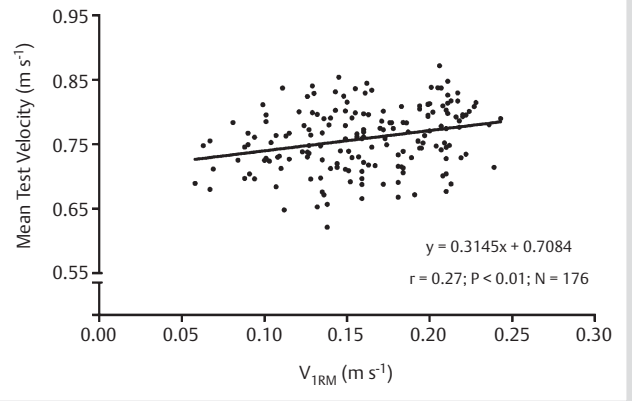


**Fig. 2** Correlation between mean velocities attained with the 1RM load ($V_{1RM}$) and mean test velocity.

imal strength. From T1 to T2, the mean 1RM value improved by 9.3 ± 6.7% (changing from 86.9 ± 15.2 kg to 94.5 ± 15.2 kg). Despite this fact, the difference in mean test velocity was only of $-0.01 \pm 0.05\ m \cdot s^{-1}$ or, when expressed as absolute values, of $0.02 \pm 0.02\ m \cdot s^{-1}$, changing from $0.78 \pm 0.05\ m \cdot s^{-1}$ to $0.76 \pm 0.05\ m \cdot s^{-1}$. **Table 1** shows the differences in MPV attained with each percentage of 1RM for the 56 subjects who performed twice the BP test. Despite the observed change in 1RM values from T1 to T2, after 6-wk of training, mean ICC for MPV attained with each load (%1RM) was 0.87 (range: 0.81–0.91; CV: 0.0–3.6%; 95% confidence interval: 0.68–0.95). When plotting percentage of change in the 1RM values against the differences between mean test velocity from T1 to T2, a negative and significant correlation could be identified ($r = -0.42$, $P < 0.01$). A positive, but non-significant, correlation ($r = 0.23$, $P = 0.091$) was found when comparing changes in $V_{1RM}$ from T1 to T2 and differences in mean test velocity.

**Fig. 3** provides examples of the load-velocity relationships for three representative subjects. **Fig. 3a** corresponds to one subject who improved his 1RM value by 11.8% (from 85–95 kg). $V_{1RM}$ in T1 ($0.16\ m \cdot s^{-1}$) was almost identical to that of T2 ($0.14\ m \cdot s^{-1}$), while MPV with each %1RM and mean test velocity remained stable. **Fig. 3b** shows an extreme case, the subject who showed the greatest change in the load-velocity curve from T1 to T2. He improved his 1RM (14.8%, from 115–132 kg), but $V_{1RM}$ in T2 ($0.06\ m \cdot s^{-1}$) and mean test velocity ($0.69\ m \cdot s^{-1}$) were both considerably lower to those of T1 ($0.17\ m \cdot s^{-1}$ and $0.75\ m \cdot s^{-1}$, respectively). MPV attained with each relative load were lower in T2 than in T1. Finally, the subject whose curves are shown in **Fig. 3c** did not improve his maximal strength (1RM value slightly decreased by 2.2%, from 112.5–110 kg). For this subject, $V_{1RM}$ in T1 ($0.10\ m \cdot s^{-1}$) and T2 ($0.12\ m \cdot s^{-1}$) were very similar, and mean test velocity was the same on both occasions ($0.73\ m \cdot s^{-1}$). MPV attained with each percentage of 1RM in T1 and T2 were almost identical.

## Stability in the load-velocity relationship regardless of individual relative strength

In order to study whether the velocity attained with each %1RM was dependent upon individual strength levels, subjects were ranked according to relative strength ratio (RSR) and the total sample of 176 tests was further divided into four subgroups: group 1 (G1), n = 45, RSR ≤ 0.97; group 2 (G2), n = 44, 0.97 < RSR ≤ 1.09; group 3 (G3), n = 44, 1.09 < RSR ≤ 1.22; and group 4 (G4), n = 43, RSR > 1.22. Mean test velocity for G4 was significantly lower ($P < 0.05$) than for all other groups. No significant differences in $V_{1RM}$ were found between groups, although certain tendency towards slightly lower values was detected for the strongest group (G4) (**Table 2**).

## Predicting load (% 1RM) from velocity data

A prediction equation to estimate relative load (Load, %1RM) from mean propulsive velocity data (MPV, in $m \cdot s^{-1}$) could be obtained: $Load = 8.4326\ MPV^2 - 73.501\ MPV + 112.33$ ($R^2 = 0.981$; SEE = 3.56% 1RM). In the case that mean concentric velocity (MV) is used, the resulting equation was: $Load = 7.5786\ MV^2 - 75.865\ MV + 113.02$ ($R^2 = 0.979$; SEE = 3.77% 1RM).

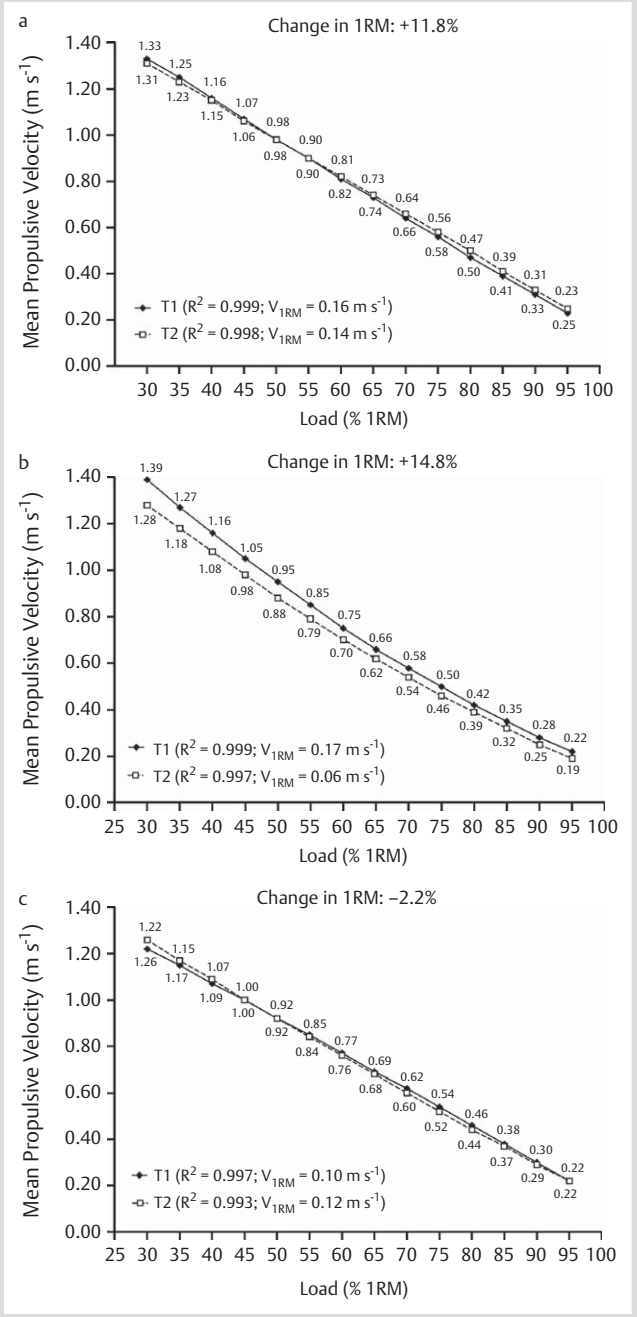


**Fig. 3** Load-velocity relationships for three representative subjects in the bench press exercise. Solid (T1) and dashed (T2) lines are second-order polynomial curve fits. (**a**) 1RM increased from 85 kg (T1) to 95 kg (T2); (**b**) 1RM increased from 115 kg (T1) to 132 kg (T2); (**c**) 1RM decreased from 112.5 kg (T1) to 110 kg (T2).

## Discussion

The main finding of this investigation was that the mean velocity attained with a given absolute load can be used as a very good estimate of the relative load (%1RM) that load represents. The force-velocity relationship of skeletal muscle is a well-known phenomenon since the work of Nobel laureate A.V. Hill [15] and has been extensively studied under in vitro and in vivo conditions [5,13]. Similarly, the load-velocity relationship has already been described for isoinertial resistance exercise performed with maximal voluntary effort [8,21]. However, the role played by movement velocity has often been overlooked in the everyday practice of strength training. To the best of our knowledge, the present study is the first to examine the relationship between relative load (% 1RM) and mean velocity. Our results highlight the practical importance of considering movement velocity for monitoring training load in resistance exercise.

**Table 2** Comparison of mean test velocity and mean velocity attained with the 1RM load ($V_{1RM}$) between subgroups of different relative strength.

| Subgroup | RSR* | Mean Test Velocity ($m \cdot s^{-1}$) | $V_{1RM}$ ($m \cdot s^{-1}$) |
|---|---|---|---|
| G1 (n = 45) | 0.90 ± 0.07† | 0.762 ± 0.050 | 0.164 ± 0.040 |
| G2 (n = 44) | 1.04 ± 0.03† | 0.768 ± 0.046 | 0.168 ± 0.037 |
| G3 (n = 44) | 1.16 ± 0.04† | 0.770 ± 0.034 | 0.163 ± 0.048 |
| G4 (n = 43) | 1.41 ± 0.18† | 0.735 ± 0.056# | 0.149 ± 0.044 |

* RSR: Relative Strength Ratio, defined as 1RM value divided by body mass. † All groups significantly different from each other ($P < 0.05$). # Significantly different from all other groups ($P < 0.05$). Values are mean ± SD

The extremely close relationship ($R^2 = 0.98$) observed between relative load and MPV (**Fig. 1**) makes it possible to determine with great precision which %1RM is being used as soon as the first repetition of a set is performed with maximal voluntary velocity. It also allows us to determine the real intensity of effort being incurred by an athlete when using any load from 30% to 95% of 1RM. Furthermore, if repetition velocity is habitually monitored it is possible to determine whether the proposed load (kg) for a given training session truly represents the real effort (%1RM) that was intended.

Since the observed differences in velocity between each 5% increment in relative load (from 30–100%1RM) vary between 0.07 and 0.09 $m \cdot s^{-1}$ (**Table 1**), it can be estimated that when a subject increases the MPV attained with a given absolute load by 7–9 hundredths of a metre per second, performance (1RM value) would have improved by 5%. The same reasoning would be applicable had the subject decreased the velocity attained against the same absolute load.

The observed values for $V_{1RM}$ are well in agreement with those reported by Izquierdo et al. [19], the only study to our knowledge that has paid attention to the velocity attained with the 1RM. As one could expect, $V_{1RM}$ influences the mean velocity attained with each %1RM. As shown in **Fig. 2**, as $V_{1RM}$ gets higher, there exists a tendency ($r = 0.27$, $P < 0.01$) towards higher velocities with each %1RM. This may further indicate that some maximum value for $V_{1RM}$ should be established in order for 1RM load to be considered true and valid. This also means that when $V_{1RM}$ is not actually measured, as frequently occurs, the values of mean velocity correspondent to each %1RM, as well as these percentages themselves, can easily differ from the true values. In our study, the observed mean $V_{1RM}$ was 0.16 ± 0.04 $m \cdot s^{-1}$; adding a standard deviation to the mean, the resulting velocity is 0.20 $m \cdot s^{-1}$. Since up to this velocity, the aforementioned tendency towards higher velocities with each %1RM is very low ($r = 0.17$, NS), a practical recommendation is made for only considering as true RM those repetitions whose mean concentric velocity is not greater than 0.20 $m \cdot s^{-1}$ in the BP exercise. As $V_{1RM}$ exceeds this figure, mean velocities attained with each %1RM and relative loads themselves deviate from their true values. Nonetheless, practice shows that sometimes it is not possible for a subject to get $V_{1RM}$ under 0.20 $m \cdot s^{-1}$, but being cognizant of this fact is important for coaches and sport scientists to be able to more precisely adjust the training load. Moreover, everyday

training and testing experience tells us that the $V_{1RM}$ value is different for each resistance training exercise.

Another interesting finding of the present study is that MPV attained with each %1RM is not modified when a subject's 1RM value changes after a period of strength training. When 56 athletes performed a retest after 6-wk of training having on average improved their 1RM by 9.3%, changes in MPV with each %1RM (30–95%) were minimal, from 0.00–0.01 $m \cdot s^{-1}$ (**Table 1**), despite such a notable change in maximal dynamic strength. These results clearly show that mean velocity attained with each %1RM is a very stable indicator of the actual percentage of 1RM that each load (weight) represents. However, there was a significant tendency ($r = 0.41$, $P < 0.01$) for mean test velocity to decrease with larger improvements in 1RM. This is explained by the fact that as individual performance (1RM) improves, $V_{1RM}$ tends to be slightly reduced (**Table 2**). Thus, the subjects with the highest relative strength (G4 group, RSR = 1.41) reached their 1RM with a slightly, but not significant, lower mean velocity than the rest of subgroups (0.164, 0.168 and 0.163 for G1, G2 and G3, respectively; and 0.149 for G4). By contrast, mean test velocity for G4 was significantly lower than that reached by the less strong groups (**Table 2**). Similar findings have been reported when examining BP and half-squat performance of athletes from sports with different strength demands [17, 19].

Further evidence of the importance of $V_{1RM}$ comes when examining the three representative cases presented in **Fig. 3**. We can conclude that it is precisely $V_{1RM}$ that determines the subtle changes in MPV attained with each %1RM between test and retest. In the first case (**Fig. 3a**), the subject clearly improves his performance (+12%, from 85–95 kg). Since $V_{1RM}$ in T1 and T2 are very similar (0.16 and 0.14 $m \cdot s^{-1}$, respectively), the differences in MPV attained with each %1RM range from 0.00–0.03 $m \cdot s^{-1}$, and mean test velocity only differs in 0.01 $m \cdot s^{-1}$ between T2 and T1 (0.78 and 0.77 $m \cdot s^{-1}$, respectively). However, in the second example (**Fig. 3b**), with a 1RM improvement similar to the previous case (+15%), but with very different $V_{1RM}$ in T1 (0.17 $m \cdot s^{-1}$) and T2 (0.06 $m \cdot s^{-1}$), all MPV are lower in T2 (ranging from −0.11 to −0.03 $m \cdot s^{-1}$) in a similar proportion (~0.07 $m \cdot s^{-1}$), and mean test velocity differs in 0.06 $m \cdot s^{-1}$ between T2 and T1 (0.69 and 0.75 $m \cdot s^{-1}$, respectively). Lastly, when the athlete's 1RM value slightly decreased (-2%) but maintained a very similar $V_{1RM}$ in T1 and T2, mean test velocities of T1 and T2 were almost identical (**Fig. 3c**).

Velocity seems to be the steadiest parameter in isoinertial strength assessment [20]. Despite the fact that our subjects significantly modified their maximal strength levels as a result of 6-wk of training, the ICC obtained for MPV attained with each %1RM, from 30–95%, when comparing test and retest were very high (0.81–0.91; CV: 0.0–3.6%), thus showing the high reliability of the velocity parameter and suggesting its use for strength assessment follow-up.

For testing and evaluation purposes, it is important to consider that the velocity attained with each percentage of 1RM can be slightly different according to the exact method of calculating mean velocities; that is, whether we use mean velocity of the whole concentric portion of the lift (MV) or mean velocity of only the propulsive phase (MPV). This is explained by the fact that as the lifted loads are lighter, the braking phase becomes larger. The larger the braking phase, the greater is the difference between MV and MPV. This means that when using velocity to monitor and/or prescribe loading intensity we must clearly specify the exact parameter that is being used, especially when light loads are used. We advocate the use of MPV because it better represents the true neuromuscular potential of a subject against a given absolute load [31].

Mean velocities attained with each %1RM are very similar to those reported in previous research on the BP exercise [11, 21], but differ from the surprisingly low velocities observed in some studies [8, 26]. Since the exercise protocols used do not seem to be much different, these divergent findings are difficult to explain but might perhaps be attributable to the type of Smith machine and hydraulic braking mechanism used in the latter studies.

## Conclusions

The main findings of the present study were that: 1) there exists an inextricable relationship between relative load and mean velocity that allows us to use one to estimate the other with great precision; and 2) mean velocities attained with each %1RM can differ very slightly due to differences in $V_{1RM}$.

## Practical Applications

These findings have important practical applications for the prescription and monitoring of training load in resistance training, making it possible to:

1) Evaluate an athlete's strength without the need to perform a 1RM test or a test of maximum number of repetitions to failure (XRM).
2) Determine what is the %1RM that is being used as soon as the first repetition with a given load is performed with maximal voluntary velocity. This allows us to determine the real effort being incurred when training with loads from 30–95% 1RM.
3) Prescribe and monitor training load according to velocity, instead of percentages of 1RM, that are highly modifiable on a daily basis; or XRM, which forces us to train to muscle failure to approximately know the %1RM we are working with.

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# Velocity Loss as an Indicator of Neuromuscular Fatigue during Resistance Training

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**ABSTRACT**

SÁNCHEZ-MEDINA, L., and J. J. GONZÁLEZ-BADILLO. Velocity Loss as an Indicator of Neuromuscular Fatigue during Resistance Training. *Med. Sci. Sports Exerc.*, Vol. 43, No. 9, pp. 1725–1734, 2011. **Purpose**: This study aimed to analyze the acute mechanical and metabolic response to resistance exercise protocols (REP) differing in the number of repetitions (R) performed in each set (S) with respect to the maximum predicted number (P). **Methods**: Over 21 exercise sessions separated by 48–72 h, 18 strength-trained males (10 in bench press (BP) and 8 in squat (SQ)) performed 1) a progressive test for one-repetition maximum (1RM) and load–velocity profile determination, 2) tests of maximal number of repetitions to failure (12RM, 10RM, 8RM, 6RM, and 4RM), and 3) 15 REP (S × R[P]: 3 × 6[12], 3 × 8[12], 3 × 10[12], 3 × 12[12], 3 × 6[10], 3 × 8[10], 3 × 10[10], 3 × 4[8], 3 × 6[8], 3 × 8[8], 3 × 3[6], 3 × 4[6], 3 × 6[6], 3 × 2[4], 3 × 4[4]), with 5-min interset rests. Kinematic data were registered by a linear velocity transducer. Blood lactate and ammonia were measured before and after exercise. **Results**: Mean repetition velocity loss after three sets, loss of velocity pre-post exercise against the 1-m·s$^{-1}$ load, and countermovement jump height loss (SQ group) were significant for all REP and were highly correlated to each other ($r$ = 0.91–0.97). Velocity loss was significantly greater for BP compared with SQ and strongly correlated to peak postexercise lactate ($r$ = 0.93–0.97) for both SQ and BP. Unlike lactate, ammonia showed a curvilinear response to loss of velocity, only increasing above resting levels when R was at least two repetitions higher than 50% of P. **Conclusions**: Velocity loss and metabolic stress clearly differs when manipulating the number of repetitions actually performed in each training set. The high correlations found between mechanical (velocity and countermovement jump height losses) and metabolic (lactate, ammonia) measures of fatigue support the validity of using velocity loss to objectively quantify neuromuscular fatigue during resistance training. **Key Words:** MUSCLE STRENGTH, WEIGHT TRAINING, BLOOD LACTATE, AMMONIA, BENCH PRESS, FULL SQUAT

Knowledge of the mechanical and physiological aspects underlying resistance training (RT) is essential to improve our understanding of the stimuli that affect adaptation (8). Configuration of the exercise stimulus in RT has been traditionally associated with a combination of the so-called *acute resistance exercise variables* (exercise type and order, loading, number of repetitions and sets, rest duration, and movement velocity) (25,35). Although most of these variables have received considerable research attention, a question that remains ignored in the literature is the possibility of manipulating the number of repetitions actually performed in each set with respect to the maximum number that can be completed. It seems reasonable that the degree or level of effort is substantially different when performing, e.g., 8 of 12 possible repetitions with a given load (8[12]) compared with performing all repetitions (12[12]). Lack of attention to this issue is likely due to an assumption that RT should always be performed to muscular failure. However, increasing evidence seems to suggest that reaching repetition failure may not necessarily improve the magnitude of strength gains (10,14,20,21). Furthermore, in the case of not exercising to failure, the optimal number of repetitions to perform under different loading conditions to achieve certain training goals has not been established.

Muscle fatigue is recognized as a complex, task-dependent and multifactorial phenomenon whose etiology is controversial and still a matter of much debate (12,13,29). Despite the many definitions of fatigue that have been proposed (2,4,12,13), a common element to most of them is the observation of an exercise-induced transient decline in muscle force–generating capacity. This decrease in force production is accompanied by an increase in the level of effort required to perform the exercise until eventually, if continued, task failure occurs (13,39). However, fatigue limits not only a fiber's capacity for maximal force generation but also the maximum velocity of shortening decreases and a slowing of relaxation occurs (2). Consequently, power output will be affected. In fact, an increased curvature of the force–velocity relationship is a major factor in the loss of muscle power (22). Therefore, all definitions of fatigue necessitate a decline in force, velocity, or power (39).

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During typical resistance exercise in isoinertial conditions, and assuming every repetition is performed with maximal voluntary effort, velocity unintentionally declines as fatigue develops (18). However, few studies analyzing the response to different RT schemes have described changes in repetition velocity or power (1,18,19,26). It thus seems necessary to conduct more research using models of fatigue that analyze the reduction in mechanical variables such as force, velocity, and power output over repeated dynamic contractions in actual training or competition settings (7,39).

Therefore, the purpose of the present study was to quantify the extent of neuromuscular fatigue while performing popular multijoint RT exercises for the upper (bench press) and lower body (squat) by analyzing the acute mechanical (velocity loss) and metabolic (blood lactate and ammonia) response to 15 types of resistance exercise protocols (REP) differing in the number of repetitions actually performed in each set with regard to the maximum predicted number. We hypothesized that both repetition velocity loss within a set and loss of velocity before versus immediately after exercise against a submaximal, individually determined, load would be highly correlated to indicators of metabolic stress and thus could be used to quantify the actual level of effort incurred during typical RT sessions.

## METHODS

### Subjects

Eighteen men (age = 25.6 ± 3.4 yr, body mass = 75.9 ± 9.1 kg, height = 176.6 ± 7.5 cm, body fat = 12.2% ± 3.7%) volunteered to take part in this study. Subjects were either professional firefighters or firefighter candidates with an RT experience ranging from 3 yr to beyond 5 yr. They were divided into two groups depending on the exercise to be performed: bench press (BP, $n$ = 10) or full squat (SQ, $n$ = 8). Initial one-repetition maximum (1RM) strength was 95.0 ± 14.9 kg for the BP and 97.1 ± 23.0 kg for the SQ group. In the 3 months preceding this study, subjects had been training two to three sessions per week and were capable of performing their respective exercise with proper technique. No physical limitations, health problems, or musculoskeletal injuries that could affect testing were found after a medical examination. None of the subjects were taking drugs, medications, or dietary supplements known to influence physical performance. The study was approved by the Research Ethics Committee of Pablo de Olavide University, and written informed consent was obtained from all subjects.

### Study Design

During a period of approximately 8 wk, 21 exercise sessions were conducted in the following order: 1) an initial test with increasing loads for the individual determination of 1RM strength and full load–velocity relationship, 2) five tests of maximal number of repetitions to failure (XRM: 12RM, 10RM, 8RM, 6RM, 4RM), 3) 15 REP differing in the number of repetitions (R) actually performed in each set (S) with regard to the maximum predicted number of repetitions (P) (S × R[P]: 3 × 6[12], 3 × 8[12], 3 × 10[12], 3 × 12[12], 3 × 6[10], 3 × 8[10], 3 × 10[10], 3 × 4[8], 3 × 6[8], 3 × 8[8], 3 × 3[6], 3 × 4[6], 3 × 6[6], 3 × 2[4], 3 × 4[4]). All these sessions were conducted on separate days, with 48 h of recovery time except the initial 1RM test, the XRM assessments, and the 3 × 12[12], 3 × 10[10], 3 × 8[8], and 3 × 6[6] REP (i.e., the most demanding protocols) after which 72 h of recovery was allowed. Sessions were performed in the evenings, at the same time of day for each participant and under similar environmental conditions (20°C–22°C and 55%–65% humidity). During the present study, subjects did not perform any other RT besides some abdominal and lower-back strengthening exercises, and their endurance conditioning only consisted of running (BP group) or swimming (SQ group) twice per week (30 min at an intensity corresponding to 70%–80% of HR reserve).

### Testing Procedures

**Initial session and 1RM determination.** An introductory session was used for body composition assessment, medical examination, and familiarization with testing protocols. Subjects arrived to the laboratory in the morning in a well-rested condition and fasted state. After being medically screened and their body composition determined, they carried out some practice sets with light and medium loads in their respective exercise (BP or SQ), while researches emphasized proper technique. On the evening of the following day, individual load–velocity relationships and 1RM strength were determined using a progressive loading test. A detailed description of the BP testing protocol has been recently provided elsewhere (31). The BP was performed imposing a momentary pause (~1.5 s) at the chest between the eccentric and concentric actions to minimize the contribution of the rebound effect and allow for more reproducible, consistent measurements. In the SQ group, subjects started from the upright position with the knees and hips fully extended, stance approximately shoulder-width apart and the barbell resting across the back at the level of the acromion. Each subject descended in a continuous motion until the top of the thighs got below the horizontal (ground) plane, the posterior thighs and shanks making contact with each other, then immediately reversed motion and ascended back to the upright position. Auditory feedback based on eccentric distance traveled was provided to help each subject reach his previously determined squat depth. Unlike the eccentric phase that was performed at a normal, controlled speed, subjects were required to always execute the concentric phase of either BP or SQ in an explosive manner, at maximal intended velocity. Warm-up consisted of 5 min of stationary cycling at a self-selected easy pace, 5 min of static stretches and joint mobilization exercises, followed by two sets of eight and six repetitions

(3-min rest) with loads of 20 and 30 kg, respectively. Initial load was set at 20 kg for all subjects and was gradually increased in 10-kg increments until the attained mean propulsive velocity (MPV) was <0.5 $m \cdot s^{-1}$ in the BP or <0.8 $m \cdot s^{-1}$ in the SQ group. Thereafter, load was individually adjusted with smaller increments (5 down to 1 kg) so that 1RM could be precisely determined. The heaviest load that each subject could properly lift while completing full range of motion was considered to be his 1RM. Trained spotters were present when high loads were lifted to ensure safety. Three attempts were executed for light (<50% RM), two for medium (50%–80% RM), and only one for the heaviest (>80% RM) loads. Interset rests ranged from 3 (light) to 6 min (heavy loads). Only the best repetition at each load, according to the criteria of fastest MPV (31), was considered for subsequent analysis.

**Maximum repetition number assessment.** For the XRM load assessments, subjects warmed up by performing four to five sets of five down to two repetitions (3-min rests), progressively increasing weight up to the load corresponding to ~70% (12RM), ~75% (10RM), ~80% (8RM), ~85% (6RM), or ~90% (4RM) of their previously determined 1RM. This was carefully controlled for each participant from his individual load–velocity profile because it has been recently shown that mean velocity can be used to precisely estimate loading intensity (15). After a 5-min rest, subjects completed one set to failure, whereas kinematic data from every repetition were registered.

**Acute REP.** The 15 types of REP were performed always using three sets and 5-min interset recoveries. Two measures were taken to ensure that the maximum predicted number of repetitions for each session was as accurate as possible. First, previous XRM assessments were used as a reference to individually determine absolute load for each REP. Second, because of the considerable number of exercise sessions undertaken in this study, strength levels were expected to change. Consequently, before starting each REP, adjustments in the proposed load (kg) were made when needed so that the velocity of the first repetition matched that expected from each subject's relative load–velocity relationship. In each session, subjects warmed up by performing three sets of six down to three repetitions (2-min rests) with increasing loads up to the individual load that elicited a ~1.00-$m \cdot s^{-1}$ (1.04 ± 0.01 for SQ and 1.03 ± 0.01 for BP) MPV ($V_{1\ m \cdot s^{-1}}$). This value was chosen because it is a sufficiently high velocity, which is attained against medium loads (~45% RM in BP and ~60% RM in SQ), and it allows a good expression of the effect of loading on velocity, besides being a relatively easy-to-move and well-tolerated load. The $V_{1\ m \cdot s^{-1}}$ load (kg) was thus taken as a preexercise reference measure against which to compare the velocity loss experienced after the three exercise sets. Subjects executed three maximal-effort consecutive repetitions against the $V_{1\ m \cdot s^{-1}}$ load right before starting the first set and again immediately after completing the last repetition of the third set (load was changed in 10–15 s with the help of the spotters). Furthermore, the participants from the SQ group performed five maximal countermovement jumps (CMJ), separated by 20-s rests, right after executing the three preexercise repetitions with the $V_{1\ m \cdot s^{-1}}$ load and again after the final three postexercise repetitions with that load. On each occasion, CMJ height was registered, the highest and lowest values were discarded, and the resulting average was kept for analysis. Strong verbal encouragement and velocity feedback in every repetition was provided throughout all sessions to motivate participants to give a maximal effort.

## Mechanical Measurements of Fatigue

Three different methods were used to quantify the extent of fatigue induced by each REP. The first method analyzed the decline in repetition velocity during the three consecutive exercise sets. It was calculated as the percent loss in MPV from the fastest (usually first) to the slowest (last) repetition of each set and averaged over the three sets. The second method examined the percent change in MPV pre–post exercise attained with the $V_{1\ m \cdot s^{-1}}$ load. The average MPV of the three repetitions before exercise was compared with the average MPV of the three repetitions after exercise, i.e., 100 (average $MPV_{post}$ − average $MPV_{pre}$)/average $MPV_{pre}$. Figure 1 shows an example of these velocity losses for a representative subject and protocol. The third method (only applied to the SQ group) involved the calculation of percent change in CMJ height pre–post exercise.

## Blood Lactate and Ammonia Analyses

Capillary whole blood samples were drawn from the fingertip before exercise and immediately after each REP. Postexercise samples for the analysis of lactate (5 $\mu$L) were taken at 1, 3, and 5 min, whereas samples for ammonia (20 $\mu$L) were obtained at 1, 4, and 7 min during recovery to determine peak concentration. The Lactate Pro LT-1710 (Arkray, Kyoto, Japan) portable lactate analyzer was used

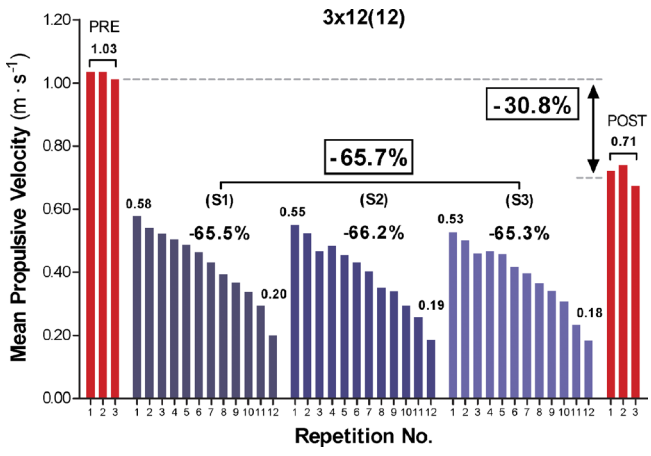


**FIGURE 1—Example of quantification of percent velocity losses after a 3 × 12[12] REP for a representative subject in the BP exercise. Both MPV loss over three sets (−65.7%) and MPV pre–post exercise against the $V_{1\ m \cdot s^{-1}}$ load (−30.8%) were calculated.**

for lactate measurements. Ammonia was measured using PocketChem BA PA-4130 (Menarini Diagnostics, Florence, Italy). Both devices were calibrated before each exercise session according to the manufacturer's specifications. Reliability was calculated by assessing twice 15 different samples over the physiological range (1.3–17.0 $mmol \cdot L^{-1}$ for lactate and 35–150 $\mu mol \cdot L^{-1}$ for ammonia). The coefficient of variation (CV) ranged from 2.6% to 4.1% for lactate and from 3.0% to 5.2% for ammonia.

### Measurement Equipment and Data Acquisition

Height was measured to the nearest 0.5 cm during a maximal inhalation using a wall-mounted stadiometer (Seca 202; Seca Ltd., Hamburg, Germany). Body weight was determined, and fat percentage was estimated using an eight-contact electrode segmental body composition analyzer (Tanita BC-418; Tanita Corp., Tokyo, Japan). Jump height was measured using an infrared timing system (Optojump; Microgate, Bolzano, Italy). A Smith machine (Multipower Fitness Line, Peroga, Spain) that ensures a smooth vertical displacement of the bar along a fixed pathway was used for all sessions. A dynamic measurement system (T-Force System; Ergotech, Murcia, Spain) automatically calculated the relevant kinematic parameters of every repetition, provided auditory velocity and displacement feedback, and stored data on disk for analysis. This system consists of a linear velocity transducer interfaced to a personal computer using a 14-bit resolution analog-to-digital data acquisition board and custom software. Instantaneous velocity was sampled at a frequency of 1000 Hz and subsequently smoothed with a fourth-order low-pass Butterworth filter with a cutoff frequency of 10 Hz. A digital filter with no phase shift was then applied to the data. Validity and reliability were established by comparing the displacement measurements obtained by this device with a high-precision digital height gauge (Mitutoyo HDS-H60C; Mitutoyo, Corp., Kawasaki, Japan) previously calibrated by the Spanish National Institute of Aerospace Technology. After performing the comparisons with 18 different T-Force units, the mean relative error in the velocity measurements was found to be <0.25%, whereas displacement was accurate to ±0.5 mm. In addition, when simultaneously performing 30 repetitions with two devices (range = 0.3–2.3 $m \cdot s^{-1}$ mean velocity), an intraclass correlation coefficient (ICC) of 1.00 (95% confidence interval = 1.00–1.00) and CV of 0.57% were obtained for MPV, whereas an ICC of 1.00 (95% confidence interval = 0.99–1.00) and CV of 1.75% were found for peak velocity. The velocities reported in the present study correspond to the mean velocity of the propulsive phase for each repetition. Mean propulsive values are preferable to mean concentric values because they avoid underestimating an individual's true neuromuscular potential when lifting light and medium loads, as well as being more stable and reliable than peak values (31). The propulsive phase was defined as that portion of the concentric phase during which barbell acceleration ($a$) is greater than acceleration due to gravity (i.e., $a > -9.81\ m \cdot s^{-2}$).

### Statistical Analysis

Correlations are reported using Pearson product–moment correlation coefficients ($r$). Relationships between mechanical losses and ammonia concentration were studied by fitting second-order polynomials to data. An independent-samples $t$-test was used to examine differences between exercises, whereas a related-samples $t$-test was used to analyze velocity and CMJ height pre–post changes as well as to compare preexercise and postexercise lactate and ammonia levels. Data are presented as mean ± SD. Significance was accepted at $P \leq 0.05$. Analyses were performed using SPSS software version 15.0 (SPSS, Chicago, IL).

TABLE 1. Mechanical and metabolic measurements of fatigue after each REP.

| | Loss of MPV over Three Sets (%) | | Loss of MPV with $V_{1\ m \cdot s^{-1}}$ Load (%) | | Loss of CMJ Height (%) | Lactate ($mmol \cdot L^{-1}$) | | Ammonia ($\mu mol \cdot L^{-1}$) | |
|---|---|---|---|---|---|---|---|---|---|
| REP | SQ | BP | SQ | BP | SQ | SQ | BP | SQ | BP |
| 3 × 12[12] | 46.5 ± 3.8 | ***63.3 ± 4.0 | 21.3 ± 9.1 | **32.8 ± 8.5 | 19.3 ± 4.4 | 12.5 ± 1.9 | ***8.2 ± 1.3 | †††125 ± 34 | †††111 ± 20 |
| 3 × 10[12] | 37.1 ± 7.7 | ***51.1 ± 5.5 | 14.6 ± 5.5 | **24.9 ± 6.9 | 16.6 ± 3.9 | 10.6 ± 1.2 | ***6.7 ± 1.0 | †62 ± 14 | †††71 ± 11 |
| 3 × 8[12] | 32.3 ± 7.6 | 36.5 ± 4.3 | 10.6 ± 1.9 | **15.3 ± 3.6 | 11.4 ± 1.9 | 8.0 ± 1.4 | **5.7 ± 1.4 | 49 ± 16 | 49 ± 18 |
| 3 × 6[12] | 20.2 ± 4.3 | *24.2 ± 2.3 | 9.7 ± 2.1 | 8.1 ± 1.4 | 9.6 ± 1.4 | 4.9 ± 0.3 | 4.2 ± 0.9 | 46 ± 14 | 45 ± 12 |
| 3 × 10[10] | 45.7 ± 7.0 | ***58.4 ± 4.5 | 21.0 ± 8.9 | *30.5 ± 8.3 | 17.0 ± 3.6 | 11.7 ± 2.2 | ***7.8 ± 1.2 | †††97 ± 19 | †††89 ± 16 |
| 3 × 8[10] | 32.3 ± 5.5 | ***46.1 ± 4.2 | 15.2 ± 4.3 | 17.7 ± 3.9 | 13.6 ± 1.9 | 8.6 ± 1.3 | ***6.0 ± 0.6 | 62 ± 20 | ††64 ± 17 |
| 3 × 6[10] | 22.0 ± 8.0 | *29.8 ± 4.5 | 11.0 ± 4.1 | 13.6 ± 2.6 | 10.3 ± 2.1 | 6.3 ± 1.6 | **4.6 ± 0.8 | 48 ± 10 | 47 ± 13 |
| 3 × 8[8] | 39.8 ± 4.0 | ***56.9 ± 3.7 | 18.2 ± 5.0 | *26.8 ± 7.9 | 15.8 ± 4.0 | 10.4 ± 2.1 | **7.5 ± 1.4 | †78 ± 28 | †††79 ± 20 |
| 3 × 6[8] | 29.4 ± 9.4 | *39.0 ± 4.5 | 10.5 ± 1.2 | *13.9 ± 3.2 | 11.3 ± 2.2 | 7.1 ± 2.1 | **4.8 ± 1.0 | 52 ± 22 | 54 ± 19 |
| 3 × 4[8] | 21.2 ± 8.6 | 24.8 ± 2.9 | 9.2 ± 1.7 | 9.6 ± 1.5 | 6.0 ± 2.0 | 4.5 ± 0.8 | *3.4 ± 0.9 | 42 ± 11 | 49 ± 19 |
| 3 × 6[6] | 41.9 ± 4.9 | ***56.8 ± 5.7 | 14.7 ± 5.4 | **24.7 ± 6.6 | 13.2 ± 2.6 | 10.0 ± 1.7 | ***6.9 ± 1.1 | 65 ± 23 | ††68 ± 14 |
| 3 × 4[6] | 28.1 ± 6.1 | *33.8 ± 3.6 | 10.3 ± 3.0 | 9.2 ± 2.8 | 9.9 ± 2.7 | 5.2 ± 0.9 | *4.0 ± 0.9 | 56 ± 21 | 52 ± 17 |
| 3 × 3[6] | 19.6 ± 7.1 | 23.7 ± 3.0 | 8.0 ± 2.6 | 7.2 ± 1.4 | 6.4 ± 2.2 | 3.5 ± 0.6 | 3.1 ± 0.7 | 47 ± 16 | 51 ± 21 |
| 3 × 4[4] | 32.0 ± 5.1 | ***49.8 ± 6.6 | 9.5 ± 1.9 | ***18.8 ± 3.8 | 10.6 ± 3.4 | 6.9 ± 1.7 | **4.9 ± 1.0 | 61 ± 28 | 53 ± 16 |
| 3 × 2[4] | 16.6 ± 4.5 | 18.9 ± 4.4 | 6.1 ± 1.8 | 5.2 ± 1.2 | 5.7 ± 1.3 | 3.0 ± 0.6 | 2.6 ± 0.7 | 41 ± 12 | 48 ± 14 |

Data are mean ± SD. Losses are reported as positive values.
BP, bench press ($n$ = 10); SQ, squat ($n$ = 8).
Significant differences between SQ and BP exercises: * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$.
Significant differences between preexercise and postexercise ammonia: † $P \leq 0.05$, †† $P \leq 0.01$, ††† $P \leq 0.001$.
Postexercise lactate significantly different ($P \leq 0.001$) from preexercise for all REP and both exercises except 3 × 2[4] in BP ($P \leq 0.01$).
Loss of MPV over three sets, loss of MPV with $V_{1\ m \cdot s^{-1}}$ load, and loss of CMJ height were significant ($P \leq 0.001$) for all REP and both exercises.

## RESULTS

**Velocity and CMJ height losses.** Both percent loss of MPV over three sets and loss of MPV pre–post exercise with the $V_{1\ m\cdot s^{-1}}$ load, gradually increased as the number of performed repetitions in each set approached the maximum predicted number of repetitions for each type of REP (Table 1). Velocity losses were significantly greater for BP compared with SQ for most types of REP. This difference in the magnitude of loss of velocity between exercises increased as the number of performed repetitions approached the maximum (Table 1). MPV losses, both over three sets and pre–post with $V_{1\ m\cdot s^{-1}}$ load, were statistically significant ($P \leq 0.001$) for all REP and both exercises. The decrease in CMJ height pre–post exercise was greater as the number of performed repetitions approached the maximum for each REP. Postexercise CMJ height was significantly different ($P \leq 0.001$) from preexercise after all REP.

**Relationships between mechanical measurements of fatigue.** A very high correlation was found between relative loss of MPV over three sets and loss of MPV pre–post exercise with the $V_{1\ m\cdot s^{-1}}$ load for both SQ ($r = 0.91$; Fig. 2A) and BP ($r = 0.97$; Fig. 2B) exercises. For the SQ group, similarly high correlations were found between percent loss of CMJ height pre–post exercise and (a) MPV loss over three sets ($r = 0.92$, $P \leq 0.001$; Fig. 3A) and (b) loss of MPV pre–post with the $V_{1\ m\cdot s^{-1}}$ load ($r = 0.93$, $P \leq 0.001$; Fig. 3B).

**Blood lactate and ammonia response.** Peak postexercise lactate concentration linearly increased as the number of performed repetitions in each set approached the maximum predicted number of repetitions, both in SQ and in BP (Table 1). For any REP, lactate levels were always higher after the SQ compared with the BP exercise, these differences being significant for most of the protocols analyzed (Table 1). Postexercise ammonia levels were significantly higher than preexercise resting values for the 3 × 12[12], 3 × 10[12], 3 × 10[10], 3 × 8[10], 3 × 8[8], and 3 × 6[6] REP in BP; and 3 × 12[12], 3 × 10[12], 3 × 10[10], and 3 × 8[8] REP in SQ (Table 1). Peak postexercise ammonia concentration did not increase above basal resting values (≤50 $\mu mol \cdot L^{-1}$) when the number of performed repetitions in each set was half the maximum predicted number. No statistically significant differences in postexercise ammonia were found between SQ and BP for any REP.

**Relationships between mechanical and metabolic measures of fatigue.** A nearly perfect correlation between MPV loss over three sets and postexercise peak lactate was found for both SQ ($r = 0.97$, $P < 0.001$) and BP ($r = 0.95$, $P < 0.001$) exercises (Fig. 4A). Very high correlations were also found between loss of MPV pre–post exercise with the $V_{1\ m\cdot s^{-1}}$ load and lactate for SQ ($r = 0.93$, $P < 0.001$) and BP ($r = 0.97$, $P < 0.001$) (Fig. 4C). Unlike lactate, which linearly increased with greater velocity loss (Figs. 4A, C), the response of ammonia to loss of velocity followed a curvilinear relationship and better fitted a quadratic regression (Figs. 4B, D). Thus, from a MPV loss

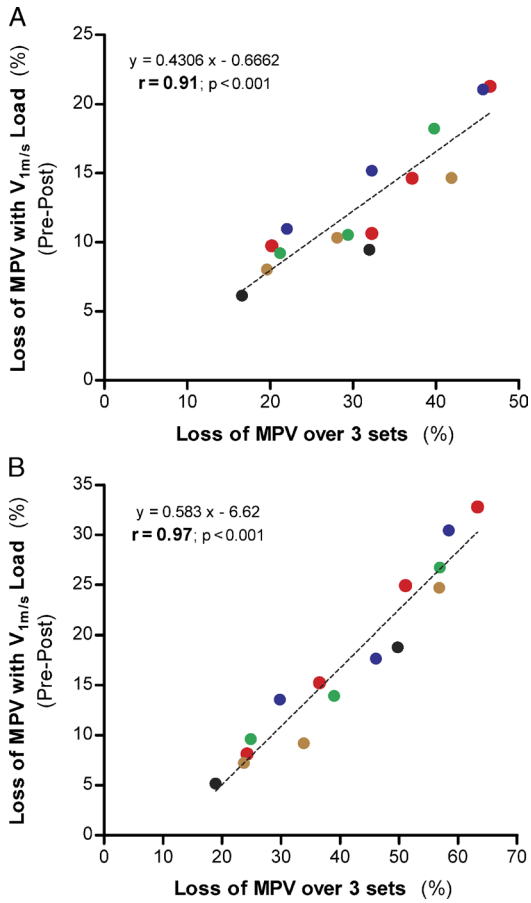


**FIGURE 2—Relationships between relative loss of MPV over three sets and loss of MPV pre–post exercise against the $V_{1\ m\cdot s^{-1}}$ load in SQ (A) and BP (B) exercises. Each data point corresponds to one of the 15 different REP analyzed. Different symbol colors are used to differentiate between the maximum predicted number of repetitions (P) for each REP: black (P = 4), brown (P = 6), green (P = 8), blue (P = 10), and red (P = 12).**

over three sets of ~30% (SQ) or ~35% (BP), blood ammonia levels started to increase steadily above resting values (Fig. 4B). When considering the loss of MPV pre–post with the $V_{1\ m\cdot s^{-1}}$ load, the magnitudes of velocity loss from which ammonia increased above resting values were ~15% (SQ) and ~20% (BP) (Fig. 4D). Percent loss of CMJ height pre–post exercise was highly correlated with lactate ($r = 0.97$, $P < 0.001$; Fig. 3C). Ammonia showed a curvilinear response to loss of CMJ height so that from ~12% loss of CMJ height, ammonia increased steadily above resting levels (Fig. 3D).

## DISCUSSION

To the best of our knowledge, this is the first study to analyze the acute response to manipulating the number of repetitions actually performed in each training set with regard to the maximum number of repetitions that can be completed. Although some research has compared the effect of failure versus nonfailure training approaches on strength gains (9,10,14,20,21,38), the mechanical and metabolic

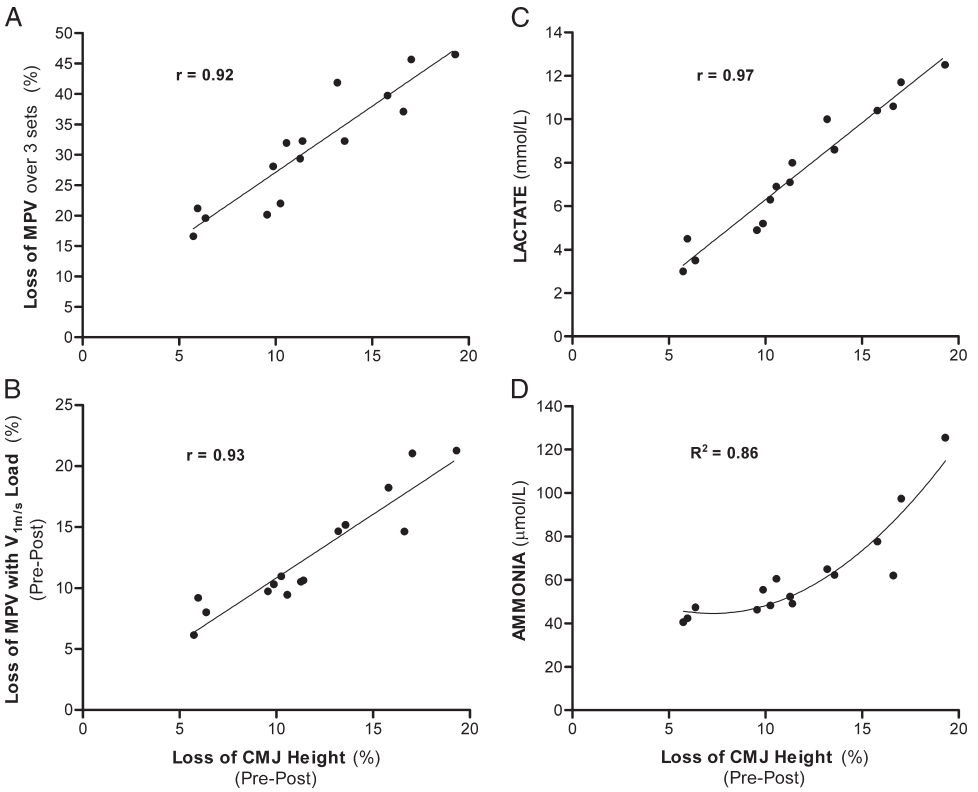


**FIGURE 3—Relationships between relative loss of CMJ height pre–post exercise and loss of MPV over three sets (A), loss of MPV pre–post exercise against the $V_{1\ m \cdot s^{-1}}$ load (B), lactate (C), and ammonia (D) for the SQ exercise group. Each data point corresponds to one of the 15 different REP analyzed.**

responses to different repetition schemes in which a set is ended before reaching muscular failure had not been previously analyzed. In the present study, a detailed examination of 15 different types of REP was conducted under controlled conditions to assess whether loss of repetition velocity could be used as an objective indicator of the extent of neuromuscular fatigue induced by typical RT sessions. Our results indicate that, by monitoring repetition velocity during training, it is possible to reasonably estimate the metabolic stress and neuromuscular fatigue induced by resistance exercise. A unique finding of this study is that ammonia, unlike lactate, shows a curvilinear response to loss of repetition velocity during RT. Some REP, especially those consisting of eight or more repetitions per set leading to failure (3 × 12[12], 3 × 10[10], and 3 × 8[8]), caused ammonia to significantly rise above resting values, which could indicate an accelerated purine nucleotide degradation, thereby suggesting that such protocols may require longer recovery times.

Most of the literature examining neuromuscular fatigue has traditionally used isolated muscle preparations, both *in vitro* and *in situ*, as well as electrically stimulated muscle fibers. Isometric or isokinetic contractions made before and immediately after the fatiguing task, as well as during the activity, have been commonly used to quantify fatigue (27,29). Although such laboratory experiments are certainly necessary to identify the physiological mechanisms underlying the onset of muscle fatigue, they bear little resemblance to the majority of muscle actions performed in actual sports training and competition settings. Hence, there is a need to use fatigue protocols and outcome measures closer to isoinertial *in vivo* training movements (7,27). Because fatigue is postulated to be a continuous rather than a failure-point phenomenon (7), the gradual decrease in repetition velocity that takes place during repeated dynamic contractions can be interpreted as evidence of impaired neuromuscular function and its measurement could provide a relatively simple yet objective means of quantifying the extent of fatigue.

The present study confirms that the magnitude of velocity loss experienced during RT gradually increases as the number of performed repetitions in a set approaches the maximum predicted number. This was an expected result because it is known that velocity naturally slows down during a training set as fatigue develops (11,18,26). However, to the authors' knowledge, the actual values of velocity loss (Table 1) after a wide range of REP performed within the most typical RT intensity range (~70%–90% 1RM) had not been previously described. A finding worth noting is that greater MPV losses were experienced for BP compared with SQ for all protocols analyzed (Table 1). This is in agreement with previous results from Izquierdo et al. (18) who compared the pattern of repetition velocity decline when performing sets to failure with loads corresponding to 60%,

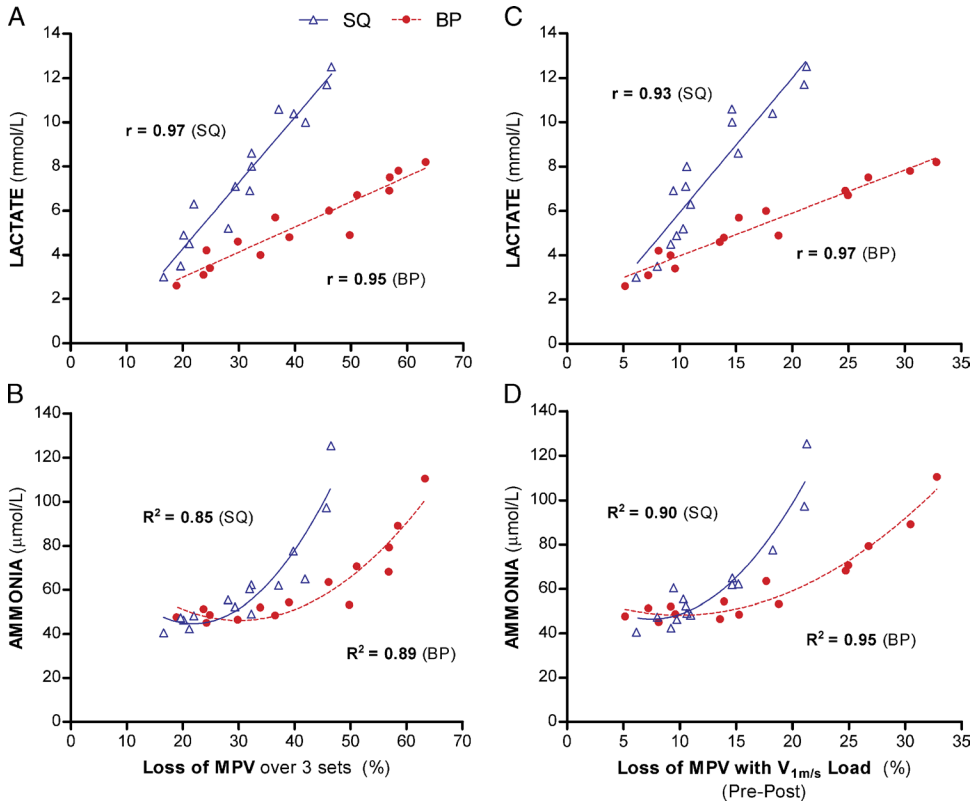


**FIGURE 4—Relationships between relative loss of MPV over three sets and peak postexercise: lactate (A) and ammonia (B); and between MPV pre–post exercise against the $V_{1\ m\cdot s^{-1}}$ load and lactate (C) and ammonia (D) for the BP and SQ exercises. Each data point corresponds to one of the 15 different REP analyzed.**

65%, 70%, and 75% 1RM in the BP and half-squat exercises. The greater velocity loss in the BP could be because 1RM for this exercise is attained at a considerably slower mean velocity (~0.16 $m\cdot s^{-1}$) than that for the SQ (~0.35 $m\cdot s^{-1}$) (15,18). The lower 1RM mean velocity in the BP could be related to the greater movement control and smaller muscle groups involved in this exercise (more localized fatigue) compared with the SQ (fatigue distributed among a greater amount of muscle mass). The relative position of the "sticking region" in these exercises may also explain these velocity differences, as the squat allows more time/distance for force production after such region. We must finally consider that the BP was performed in a concentric-only (no rebound) action, whereas the SQ exercise is influenced by the stretch–shortening cycle that takes place when transitioning from an eccentric to a concentric action.

In essence, all models of fatigue entail two components: fatigue induction and fatigue quantification (27). In the present study, fatigue was quantified using two different methods: 1) percent decline in MPV over the three consecutive exercise sets and 2) percent change in MPV pre–post exercise attained with the $V_{1\ m\cdot s^{-1}}$ load, as well as percent change in CMJ height pre–post (SQ group only). Because fatigue has been traditionally defined as a loss of force-generating capability with an eventual inability to sustain exercise at the required or expected level (4,13), the post-exercise decline in movement velocity experienced against a given submaximal load (in this case, the $V_{1\ m\cdot s^{-1}}$ load) can be considered as a good expression of neuromuscular fatigue. Indeed, in addition to force reduction, other aspects of neuromuscular performance that are affected by fatigue are muscle-shortening velocity (decreases) and relaxation time (increases) (2). Because of fatigue, the load that was lifted at ~1.00 $m\cdot s^{-1}$ in a rested, preexercise state, will be moved at a considerably slower velocity after the REP. The subject will undoubtedly perceive a greater effort when moving the same absolute load in the fatigued state, a situation that corresponds well with the definition of Enoka and Stuart (13). Besides being a relatively easy-to-move and well-tolerated load for most RT exercises, the $V_{1\ m\cdot s^{-1}}$ load is quick to determine as part of the warm-up and facilitates the calculation of percentage losses.

Similar to loss of MPV over three sets, the magnitude of loss of MPV pre–post with the $V_{1\ m\cdot s^{-1}}$ load gradually increased as the number of performed repetitions in each set approached the maximum predicted number for each type of REP (Table 1). Relative loss of velocity with the $V_{1\ m\cdot s^{-1}}$ load was of lesser magnitude than MPV loss over three sets and higher for BP compared with SQ, especially as the number of performed repetitions increased toward maximum (Table 1). The same pattern of decline was observed when analyzing loss of CMJ height pre–post exercise for

the SQ group, which seems to follow the same rationale. Loss of CMJ height is equivalent to loss of vertical velocity at take-off, so in essence we are quantifying fatigue by the loss of muscle-shortening velocity. Several studies have used measurements of vertical jump height pre–post exercise to quantify the extent of fatigue. Smilios (33) observed CMJ height losses of 33% and 23% after exercise to failure in the leg press with loads of 70% and 90% RM, respectively. These reductions are greater than those obtained in the present study (19% and 11%) in the equivalent REP of 12[12] (~70% RM) and 4[4] (~90% RM). However, in Smilios's study (33), participants were not required to perform each repetition with maximal voluntary effort, and the number of repetitions actually performed with each load was not reported, which makes it difficult to compare with our data. Rodacki et al. (30) induced fatigue by requesting subjects to extend and flex their knees to failure in a weight machine. The loads used corresponded to ~50% (extensors) and ~40% (flexors) of each subject's body mass. Mean losses in CMJ height of 14% (extensors) and 6% (flexors) were found. Data from Rodacki et al. (30) suggest that the incurred fatigue and degree of effort was highly variable between participants (~10–26 repetitions in extension; ~18–36 repetitions in flexion) and thus precludes direct comparison with our data. Gorostiaga et al. (16) examined CMJ height loss after typical sprint training workouts in 400-m elite runners. They found reductions of 5%–19% in CMJ height pre–post exercise, with no clear relationship to sprint distance. Comparing our findings with those of these investigations is difficult because the protocols used to induce fatigue, the samples, and even the type of actions and movement velocities greatly differed between studies. Nevertheless, it seems clear from this body of research that loss of CMJ height can be used as an indicator of neuromuscular fatigue.

In the present study, very high and significant correlations ($r$ = 0.91–0.97) were found between the three different types of mechanical measures used to assess neuromuscular fatigue (Figs. 2 and 3A, B). These relationships are an important finding for the quantification and monitoring of training load during RT. The fact that there exists such a close relationship between loss of MPV over three sets and loss of MPV with the $V_{1\ m\cdot s^{-1}}$ load in two exercises as different as SQ (Fig. 2A) and BP (Fig. 2B), as well as between both variables and loss of CMJ height in the SQ group (Figs. 3A, B), is a novel finding that emphasizes the validity of using percent loss of repetition velocity within a set as an indicator of neuromuscular fatigue. The relationships observed in Figure 2 also mean that, for a given percent loss of velocity within a set, the degree of fatigue incurred during RT is very similar irrespective of the number of repetitions the subject is able to perform (shown in different colors in Fig. 2), at least in a range from 4 (~90% RM) to 12 (~70% RM) repetitions.

The validity of using percent velocity loss to quantify neuromuscular fatigue during RT is further supported by the relationships observed between mechanical measures of fatigue and metabolic stress (acute lactate and ammonia responses) (Figs. 3C, D and 4). Lactate increased linearly as the number of performed repetitions approached the maximum predicted for each type of REP (Table 1) and showed extremely high correlations ($r$ = 0.93–0.97) with loss of MPV over three sets (Fig. 4A), loss of MPV pre–post exercise with the $V_{1\ m\cdot s^{-1}}$ load (Fig. 4C), and loss of CMJ height (Fig. 3C). The highest peak lactate values (~10.5–12.5 mmol·L$^{-1}$ in SQ and ~7.5–8.0 mmol·L$^{-1}$ in BP) were obtained when performing 8–12 repetitions per set. Lactate levels were significantly higher for SQ than BP after most REP analyzed (Table 1), which can be attributed to the greater muscle mass involved in the full squat. The REP that resulted in the highest lactate response were 3 × 12[12], 3 × 10[12], 3 × 10[10], and 3 × 8[8], i.e., the type of protocols commonly used to induce muscle hypertrophy, which is in line with previous research (23,24,34). Interestingly, peak lactate values after the 3 × 6[12] BP protocol (4.2 ± 0.9 mmol·L$^{-1}$) were very similar to those found by Abdessemed et al. (1) when performing 10 × 6[12] under different interset recovery conditions. They found that blood lactate did not significantly increase after the third set when using 3-min (4.7 ± 0.8 mmol·L$^{-1}$) or 5-min (3.6 ± 0.7 mmol·L$^{-1}$) rests. However, the 1-min rest condition resulted in a significantly greater lactate elevation in sets 4 to 10, concomitant with much greater reductions in mean repetition power output.

A unique and interesting finding of the present study is that ammonia response, unlike lactate, shows a curvilinear relationship to loss of velocity (Figs. 4B, D) and seems independent of the exercise (BP or SQ). Peak postexercise ammonia only increased above basal resting levels when the number of performed repetitions in each set was at least two higher than half the maximum predicted number (Table 1), thus suggesting the existence of a certain "level of effort threshold" to be exceeded for blood ammonia to respond. This nonlinear response of ammonia is similar to that found in some studies, which analyzed the physiological response to incremental exercise (3,6,32), but to our knowledge, it had not been previously documented for RT. An increase in blood ammonia levels during short-term high-intensity exercise is usually interpreted as indicative of an accelerated ammonia production by muscle resulting from the deamination of AMP to IMP. A loss of purines has been documented after high-intensity exercise sessions such as repeated sprints (17,36). Because *de novo* synthesis of nucleotides is a slow and energy-consuming process, muscle performance can remain significantly reduced up to 48–72 h after exercise (17). According to the results of this study, the REP that resulted in blood ammonia significantly higher than resting levels were 3 × 12[12], 3 × 10[12], 3 × 10[10], and 3 × 8[8] in SQ and 3 × 12[12], 3 × 10[12], 3 × 10[10], 3 × 8[10], 3 × 8[8], and 3 × 6[6] in BP, with considerably greater values for those leading to failure in each set (Table 1). It seems plausible to suggest that these types of protocols may cause an accelerated purine nucleotide

degradation that would increase the amount of time needed for recovery after training. The mean peak postexercise ammonia concentrations in this study (125 $\mu mol \cdot L^{-1}$ in SQ, 110 $\mu mol \cdot L^{-1}$ in BP for the 3 × 12[12] REP) are similar to those obtained by Izquierdo et al. (19) but lower than the extremely high values (>200 $\mu mol \cdot L^{-1}$) found after 3 × 10RM or 3 × 5RM in multiple exercises with only 1-min recovery (24).

Although some studies have reported the point within a set where a significant reduction in velocity (18) or power output (1,26) was observed, the optimal time to terminate a set before reaching failure has never been clearly established. Although the present study does not come up with a definitive answer to that question, it does, however, provide us with some valuable information that may indicate when it could be appropriate to end a set. According to our results (Table 1; Figs. 3 and 4), a maximum MPV loss of ~30% for SQ and ~35% for BP could be established to prevent blood ammonia to significantly rise above resting levels. These theoretical thresholds for velocity loss could be used as a preliminary reference to undertake a longitudinal study aimed to examine the effect of training with different repetition velocity losses (e.g., 15%, 30%, and 45%) on neuromuscular performance (1RM strength, rate of force development, maximal power production, etc.).

Monitoring repetition velocity during resistance exercise seems important because both the neuromuscular demands and the training effect itself largely depend on the velocity at which loads are lifted. A velocity- or power-based approach to RT is not entirely new, and authors such as Bosco (5) and Tidow (37) already provided some initial guidelines for putting it into practice. However, the role placed by movement velocity has not been sufficiently investigated (28). The findings obtained in the present study strongly support the use of velocity monitoring to control the degree of incurred fatigue. Because loads must be specific to ensure an optimal training stimulus, setting a certain velocity loss threshold during RT can serve to avoid performing unnecessary repetitions that may not be contributing to the desired training effect. Furthermore, the immediate velocity feedback the athlete receives during each session may increase the potential for adaptation. With this training approach, instead of a certain amount of weight to be lifted, strength and conditioning coaches should prescribe resistance exercise in terms of two variables: 1) first repetition's mean velocity, which is intrinsically related to loading intensity (15); and 2) a maximum percent velocity loss to be allowed in each set. When this percent loss limit is exceed the set must be terminated. The limit of repetition velocity loss should be set beforehand depending on the primary training goal being pursued, the particular exercise to be performed, as well as the training experience and performance level of the athlete. More studies are warranted to further explore this velocity-based approach to RT.

In conclusion, the present data show that the relationship between the number of repetitions actually performed in a set and the maximum predicted number that can be completed is an important aspect to take into account when prescribing resistance exercise because the velocity loss and metabolic stress clearly differ when manipulating these variables. The high correlations found between mechanical (velocity and CMJ height losses) and metabolic (lactate, ammonia) measures of fatigue support the validity of using velocity loss to objectively quantify neuromuscular fatigue during RT. The nonlinear response of blood ammonia to loss of repetition velocity could perhaps be used as a reference to indicate the point within a set where the exercise should be terminated when the main training objective is to improve movement velocity or maximal power production. Future experimental research should compare the effects of training with different magnitudes of velocity loss on neuromuscular performance. The present study is expected to contribute to the field of exercise science by allowing a more rational characterization of the RT stimulus.

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**II SIMPOSIO INTERNACIONAL EN CIENCIAS DEL DEPORTE**

**UNIVERSIDAD PABLO DE OLAVIDE, DE SEVILLA. Sevilla, mayo de 2013**

**"*La velocidad de ejecución como indicador de la carga, la fatiga y el efecto del entrenamiento* *(Speed of execution as indicator of the load, the fatigue and the training effect)*"**

**Juan José González Badillo**

## RESUMEN

***La velocidad como objetivo del entrenamiento***
Si pretendemos mejorar la condición física orientada a la mejora del rendimiento deportivo, el objetivo general y común a cualquier tipo de entrenamiento es mejorar la velocidad ante cualquier carga que se utilice para medir el efecto del entrenamiento. Y si se trata de mejorar el rendimiento específico (en cualquier especialidad deportiva) en acciones discretas (aisladas) o repetidas (tanto continuas como intermitentes), el objetivo permanente es mejorar la velocidad siempre ante la misma carga: peso corporal o peso corporal más algún instrumento. En este sentido, sólo existe una excepción, la halterofilia, cuyo objetivo consiste en mantener siempre la misma velocidad ante cargas cada vez mayores. Esto indica que la mejora de la *velocidad* está en la base de todo el entrenamiento que realiza cualquier deportista durante su vida deportiva.

***La intensidad y la carga del entrenamiento y su relación con la velocidad***
La solución tradicional a la dosificación de la intensidad en el entrenamiento de fuerza ha sido utilizar como referencia el porcentaje de 1RM o el XRM. Pero ambas opciones presentan inconvenientes importantes.

Los inconvenientes de utilizar 1RM como referencia son los siguientes:

- Modificación de la RM con el entrenamiento
- Inexactitud de la medida de la RM
- Riesgo de sobrecarga excesiva al medirla
- Influencia en el propio proceso de entrenamiento si se mide con frecuencia

De lo anterior se deduce que la RM no se debería, y no es necesario, medirla nunca a ningún deportista, y menos a los jóvenes o personas no deportistas

Los inconvenientes de utilizar como referencia un XRM son los siguientes:

- Necesidad de entrenar siempre con el máximo número posible de repeticiones por serie (máximo carácter del esfuerzo)
- Esto puede producir:
  - Excesiva fatiga, porque obliga a realizar un carácter del esfuerzo máximo en cada serie
  - Aumentar el riesgo de lesión, por la misma razón indicada en el punto anterior
  - Reducción de la velocidad de ejecución ante cualquier carga como efecto del entrenamiento
- Además, se ha comprobado que la utilización de un XRM no ofrece mejores resultados que el entrenamiento con un carácter del esfuerzo menor

La solución a estos problemas empieza por una interpretación adecuada de lo que es la *programación del entrenamiento*

La *programación* del entrenamiento no es más que la *expresión* de una sucesión o serie ordenada de *esfuerzos que guardan una relación de dependencia entre sí***.**

El *esfuerzo* es el *grado real de exigencia* en relación con las posibilidades actuales del sujeto: *carácter del* esfuerzo (CE). Por tanto, el CE será la relación entre lo realizado (lo que se hace) en una serie o en una sesión y lo realizable (lo que se podría hacer). Todos los entrenamientos tienen un (su) carácter del esfuerzo.

Por tanto, dado que *lo que se programa cada día es un determinado grado o carácter del esfuerzo*, la solución adecuada para la estimación y dosificación de la carga estaría en ser capaces de medir de manera precisa dicho grado o carácter del esfuerzo. Esto se consigue si conocemos dos indicadores de la carga:

1. El Grado de Esfuerzo que representa *la primera repetición de una serie*
2. El Grado de Esfuerzo que representa *la pérdida de velocidad dentro de la serie*

Para estimar el primero de estos indicadores es necesario conocer *la velocidad de cada porcentaje de la RM*

Para estimar el segundo es necesario conocer *el porcentaje de pérdida de velocidad dentro de la serie y su efecto mecánico* (pérdida de velocidad pre-post esfuerzo ante una misma carga) *y metabólico* (grado de estrés metabólico) con el fin de validar la fatiga, como expresión del grado de esfuerzo, generada por la serie o la sesión de entrenamiento

En el primer caso, hemos podido comprobar que *cada porcentaje tiene su propia velocidad* (González-Badillo, J.J. y Sánchez-Medina, L, 2010), y si esto es así, podemos conseguir las siguientes aplicaciones prácticas:

- Evaluar la fuerza de un sujeto sin necesidad de realizar en ningún momento un test de 1RM ni un test de XRM
- Determinar con alta precisión qué porcentaje de 1RM real (qué grado de esfuerzo) está utilizando el sujeto nada más realizar la primera repetición de una serie a la máxima velocidad posible con una carga dada
- Programar, dosificar y controlar el entrenamiento con alta precisión
  - Esto significa que si se mide la velocidad cada día, se puede determinar si la carga propuesta al sujeto (kg) representa fielmente el verdadero esfuerzo (% real de 1RM) que se ha programado para él, así como cuál es la evolución del efecto del entrenamiento a través del ciclo de entrenamiento
- Poder programar el entrenamiento de fuerza con todos los sujetos, desde los niños hasta los deportistas más avanzados o para personas no deportistas, sin necesidad de realizar esfuerzos inapropiados para dosificar las cargas y comprobar el efecto del entrenamiento
- Estimar la mejora en el rendimiento cada día sin realizar ningún test, simplemente midiendo la velocidad con la que se desplaza una misma carga absoluta

Con respecto al Grado de Esfuerzo que representa *la pérdida de velocidad dentro de la serie*, el objetivo es conocer la relación entre la pérdida de velocidad en la serie y la fatiga. Habiendo cuantificado la fatiga por la pérdida de velocidad pre-post esfuerzo ante una carga dada y por la pérdida de altura del salto (CMJ), nuestros trabajos (Sánchez-Medina, L. y González-Badillo, J.J.) nos permiten aportar las siguientes conclusiones:

- La fatiga ocasionada por una sesión de entrenamiento depende del porcentaje de pérdida de velocidad en la serie (al final de tres series), independientemente del número de repeticiones realizables en la propia serie
- Las pérdidas de velocidad con la carga que se puede desplazar a 1 m · $s^{-1}$ y en el CMJ son precisos estimadores de la fatiga ocasionada por una sesión de entrenamiento
- Las pérdidas de velocidad en la serie con la carga que se puede desplazar a 1 m · $s^{-1}$ y en el CMJ son precisos estimadores del estrés metabólico ocasionado por la sesión de entrenamiento

***Pérdida de velocidad en la serie y el efecto del entrenamiento***

En un estudio reciente hemos podido comprobar que una pérdida máxima de velocidad en la serie del 20% durante todo el ciclo de entrenamiento ofrece mejor efecto que una pérdida máxima próxima en la mayoría de las sesiones al 45-50%, que ha llevado a una situación próxima al fallo muscular. Por tanto, de los resultados de nuestros estudios se deduce que no parece necesario perder más del 20-25% de la velocidad de la primera repetición de una serie para conseguir el mejor rendimiento de la carga (kg) utilizada en cada sesión de entrenamiento, aunque esto no signifique que en todos los casos sea necesario llegar a esa pérdida de velocidad. La pérdida del 20% de la velocidad alcanzada en la primera repetición en la serie significa que el número de repeticiones realizadas en la serie sería aproximadamente la mitad de las repeticiones posibles.

***Efecto de realizar cada repetición a la máxima velocidad posible o al 50% de dicha velocidad***

En un estudio reciente hemos podido comprobar que utilizando la misma carga relativa (misma velocidad en la primera repetición) y el mismo número de repeticiones en la serie, realizar el desplazamiento de la carga de entrenamiento a la máxima velocidad posible ofrece mejor resultado que desplazar de manera intencionada la misma carga al 50% de la máxima velocidad posible.

***El control de la carga de entrenamiento a través de la pérdida de velocidad (altura en CMJ) cuando se realizan carreras cortas a la máxima velocidad posible***

En una estudio llevado a cabo con nueve corredores especialistas en velocidad, que realizaron series (repeticiones) de 40, 60 y 80m a la máxima velocidad posible hasta perder un 3% de la velocidad de la primera serie, hemos podido comprobar que la pérdida de altura de salto medida inmediatamente después de cada serie presentó una correlación media igual o superior a 0,97 con los valores de concentración de lactato y de amonio en cada una de las tres distancia recorridas. Estos resultados indican que el control de la pérdida de salto al final de cada serie es un buen estimador del grado de estrés o esfuerzo que está provocando la sesión de entrenamiento. Dado que lo que programamos en cada sesión es un *grado o carácter del esfuerzo*, dicho grado de esfuerzo podría ser cuantificado por la pérdida de altura del salto, que no es más que una pérdida de velocidad (velocidad de despegue). El problema que quedaría por

resolver sería decidir cuál es el grado de esfuerzo (pérdida de altura de salto) más adecuado para cada sesión de entrenamiento. Este problema ha de abordarlo, naturalmente, el técnico o entrenador, pero con la ventaja de que si trabaja basándose en este planteamiento, estará permanentemente en la vía de la mejora de su metodología de entrenamiento.

***Velocidad con la que se desplaza una carga y el rendimiento deportivo***

En un estudio con un grupo de 18 corredores, hemos podido comprobar que *la carga que se desplaza a una velocidad aproximada a 1 m · $s^{-1}$* en el ejercicio de sentadilla completa presenta una alta relación (de -0,7 a -0,84 a medida que aumenta la distancia) con el tiempo en recorrer distancias de 20, 30, 40 y 50m, y estas relaciones aumentan a medida que se aumenta la carga con la que los sujetos realizan las mismas distancias pero con cargas de arrastres añadidos. Estos resultados sugieren que el control de la carga que se puede desplazar a esa velocidad podría ser una buena referencia para comprobar el estado de rendimiento de un sujeto que pretende mejorar la velocidad de desplazamiento en distancias cortas. Este control sólo exige un esfuerzo ligero o muy moderado para el sujeto, ya que la carga que se desplaza aproximadamente a 1 m · $s^{-1}$ no representa más de un 56-57% de la RM del sujeto en el ejercicio de sentadilla completa.

***Medición del efecto del entrenamiento y la velocidad***

Cuando se trata de comprobar el efecto del entrenamiento sobre la "fuerza máxima" se suele utilizar la medida de la RM del sujeto antes y después del periodo de entrenamiento. Pero al menos por dos razones, ya indicadas anteriormente, este tipo de control debería descartarse. La primera razón está en lo que se indica en el segundo apartado de este resumen con respecto a la RM, y la segunda en que, como hemos indicado en el primer apartado, es razonable aceptar que el objetivo de cualquier entrenamiento es mejorar la velocidad ante la misma carga, que nunca debería ser la RM. Por tanto, la propuesta es que ni para comprobar el efecto del entrenamiento ni para dosificar la carga debería medirse la RM, sino los cambios de velocidad ante las mismas cargas pre-post entrenamiento, no estando entre estas cargas la RM. Quedaría pendiente una pregunta: ¿y si el sujeto llega a entrenar con una carga equivalente a la RM? La respuesta a esta pregunta queda fuera de los objetivos de esta conferencia, ya que la respuesta es que ningún sujeto necesita, ni debería, utilizar la RM como carga de entrenamiento.

En estos estudios han participado de manera muy directa y decisiva los siguientes investigadores: Luis Sánchez Medina, Fernando Pareja Blanco, David Rodríguez Rosell y Pedro Jiménez Reyes.

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# Fatiga: un poco de historia y algunos mecanismos moleculares

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La fatiga es el objetivo a entender, primero, y vencer después de disciplinas tan dispares como la Fisiología del ejercicio, la Neurología, la Psicología, la Medicina del trabajo, o la Medicina aeroespacial. Las observaciones relacionadas con la fatiga van desde la voluntad de realizar actos concretos a modificaciones en el comportamiento de proteínas intracelulares. En general, se podría conceptualizar como la incapacidad para proseguir una tarea a un nivel estipulado.

No obstante cuando se trata de estudiar la fatiga es necesario concretar la tarea y el mecanismo a enfocar. De otra manera sería, si no imposible, sí muy complejo, estudiar todos los elementos que pueden intervenir en la generación de fatiga de modo simultáneo. Por ejemplo, la rapidez y la extensión con que se produce la fatiga depende en gran manera del tipo e intensidad de la actividad física realizada (Fitts, 1994). Una actividad realizada a baja intensidad produce una fatiga por factores distintos a los que participan en una actividad de alta intensidad.

Actualmente, uno de los puntos de interés respecto a la fatiga es dilucidar los mecanismos que intervienen en la llamada fatiga de alta intensidad, esto es, brotes de ejercicio de corta duración y alta intensidad. Bajo estas condiciones la fatiga puede ocurrir a intensidades máximas y a intensidades submáximas. En cualquiera de estas dos circunstancias la fatiga sobreviene por una caída en la fuerza o en la velocidad de contracción de los músculos esqueléticos implicados.

Tanto la caída en la fuerza como en la velocidad de contracción de la maquinaria intracelular pueden ocurrir por muy distintos mecanismos, desde la disminución de la frecuencia de activación de las motoneuronas en la via final común del control muscular, hasta las interacciones moleculares a nivel de los puentes cruzados en las sarcómeras. La aparición de técnicas biofísicas como las trampas ópticas o la FRET (Förster resonance energy transfer**)** permiten medir lo que ocurre entre moléculas de un solo puente cruzado o varios eventos moleculares intracelulares en tiempo real.

La acumulación de metabolitos durante el trabajo muscular ha sido objeto de investigación desde la década de los setenta en el siglo pasado. La determinación por espectroscopía de resonancia magnética nuclear de metabolitos como los hidrogeniones ($H^+$), el fosfato inorgánico (Pi) o el difosfato de adenosina (ADP) pudo evidenciar su acumulación en situaciones de fatiga (Dawson y cols., 1978). A partir de ahí se utilizaron fibras musculares desprovistas de su membrana plasmática (*skinned fibers*) para comprobar que los aumentos de concentración de los metabolitos mencionados y otros eran capaces de inhibir la generación de fuerza isométrica y la velocidad de acortamiento (para una revisión reciente ver Fitts, 2008).

A pesar de los avances en la relación entre metabolitos intracelulares y la aparición de signos celulares de fatiga, aún se echaba en falta los mecanismos moleculares que participan en la pérdida de fuerza o velocidad a nivel de un solo puente cruzado de la sarcómera. Además, la pléyade de proteínas, distintas a la miosina y la actina, que modulan la función contráctil hace más difícil la disección molecular del fenómeno de

la fatiga. Afortunadamente el advenimiento de técnicas como las mencionadas más arriba (trampas ópticas de luz coherente y FRET) permiten observar directamente el comportamiento de un solo puente cruzado, lo que ha proporcionado una nueva perspectiva de los mecanismos moleculares implicados en la fatiga a nivel sarcomérico.

Tras la activación por calcio iónico libre y el subsiguiente contacto entre la molécula de miosina y la de actina, el Pi se libera del dominio S1 de la cabeza de miosina pesada y se produce la rotación del brazo de palanca de la miosina mientras que ésta está fuertemente unida a la actina, produciendo un desplazamiento de unos 10 nm en el filamento de actina (Holmes, 2005). A continuación la miosina libera ADP del sitio activo y la actomiosina entra en un estado de rigor, en el cual permanece hasta que otra molécula de ATP se une al sitio activo de la miosina y facilita la disociación de la actina (en realidad pasa a un estado de unión débil), entonces el ATP se hidroliza y el brazo de palanca de la miosina se recoloca para asegurar una unión útil en el próximo desplazamiento.

En relación al papel del Pi durante la fatiga, se sabe que altas concentraciones de Pi se correlacionan con estados de fatiga (Dawson et al, 1978 usando técnicas de espectroscopia de RMN); o que aumentando la concentración de Pi en fibras no fatigadas se reduce la fuerza de contracción isométrica (Pate y Coke, 1989; Debold u cols., 2004, 2006). El mecanismo por el cual el aumento de concentración de Pi deprime la fuerza parece estar basado en el mismo proceso molecular que genera la fuerza muscular. A nivel molecular la fuerza muscular depende de la fuerza unitaria de un puente cruzado (Fu) multiplicada por el número de puentes cruzados activados y del porcentaje de tiempo del ciclo de la ATPasa en que la miosina está fuertemente unida a la actina. Como dijimos más arriba, la liberación de Pi del puente cruzado produce la rotación del brazo de palanca del puente cruzado; sin embargo, una nueva unión de Pi al puente cruzado revierte la rotación de la cabeza del puente cruzado y lleva a un desenganche de la miosina con la actina, con lo cual se reduce el número de puentes cruzados enganchados en la unidad de tiempo, lo que se ha podido comprobar en preparaciones de *skinned fibers* (Pate y Cooke, 1989). Por lo tanto el aumento de Pi podría producir un descenso de la fuerza de contracción disponible, por una disminución de la energía disponible de la hidrólisis del ATP. La relación entre la caída en la fuerza isométrica máxima y el logaritmo de la concentración de Pi (pPi) es lineal.

El efecto del aumento de concentración de Pi sobre la velocidad de desplazamiento del filamento de actina es más discutible. Aún así, parece claro que el aumento de Pi puede aumentar la velocidad de desplazamiento del filamento de actina sobre todo en condiciones de bajo pH (6,5), mientras más alcalino el pH menos efecto del Pi sobre la velocidad de desplazamiento de actina (Debold y cols., 2011). Todo lo anterior lleva una hipótesis conclusiva en la que el Pi induce una aumento en la velocidad de acortamiento sarcomérico a expensas de una disminución de la generación de fuerza (Debold y cols., , 2005).

El papel de la acumulación de $H^+$ durante la situación de fatiga parece que empieza a aclararse. A diferencia del papel del Pi sobre la fuerza, la acidosis intracelular parece ser muy dependiente de la temperatura y no afectar a la producción de fuerza, sino a la velocidad de acortamiento sarcomérico. En realidad, a temperaturas fisiológicas el efecto de la acidosis sobre la fuerza isométrica es poco o casi nulo; incluso hay propuestas de un papel protector de la fuerza achacable a la acidosis (Pedersen y cols.,

2004). Por el contrario, el efecto de la acidosis sobre la velocidad de acortamiento parece estar bien establecido en experimentos *in vitro* (ensayos de motilidad, trampas de láser, transferencia de fluorescencia, etc). Este efecto estaría mediado por el efecto depresivo que la acidosis produce sobre las troponinas y la tropomiosina. En realidad, se ha podido medir un descenso de un 65% en la velocidad de acortamiento cuando el pH pasó de 7,4 a 6,4 y, mediante el uso de trampa óptica se ha podido medir un aumento de alrededor de tres veces en el tiempo de unión fuerte entre la actina y la miosina, lo que podría explicar por sí solo el descenso del 65% en la velocidad de acortamiento (Debold y cols., 2008). Estos datos suponen la primera evidencia directa de los efectos de la acidosis actuando sobre un solo puente cruzado en situación de fatiga.

La acidosis inducida durante la fatiga, además de su acción directa sobre los puentes cruzados, puede actuar sobre el mecanismo de activación de la contracción muscular, concretamente disminuyendo la sensibilidad al calcio iónico libre ($Ca^{2+}$) (Fabiato y Fabiato, 1978). Una caída de la sensibilidad al $Ca^{2+}$ supone una caída en la fuerza de contracción. Ciertamente cuando la $[Ca^{2+}]i$ se mantiene al máximo nivel, el efecto de la acidosis es escaso, sin embargo, en condiciones no saturantes de $[Ca^{2+}]i$, algo que ocurre en los estadios tardíos de la fatiga muscular, el efecto de la acidosis es más notable y por lo tanto su repercusión sobre la capacidad de producir fuerza del músculo esquelético.

Para entender mejor la interacción entre el aumento de concentración de hidrogeniones y la pérdida de fuerza en la fibra muscular durante las condiciones de fatiga, hay que profundizar un poco más en los mecanismo moleculares que intervienen en la activación mecánica de la fibra muscular. Actualmente se está ganado terreno la hipótesis de los tres estados de la tropomiosina ™ en relación a la actina durante la activación mecánina. La Tm oscilaría entre tres estados: "bloqueada", "cerrada" y "abierta". En el primer estado ("bloqueada") la Tm estaría en tal posición que el sitio activo para la miosina en la molécula de actina del filamento delgado estaría completamente oculto, sin posibilidad de unión entre actina y miosina. En el estado "cerrado" la posición de la Tm sería tal que permitiría una unión débil entre las moléculas de actina y miosina. En el estado "abierto" la Tm se colocaría en tal posición que permitiría la unión fuerte entre actina y miosina (Gordon y cols, 2000). La activación muscular completa requiere una adecuada $[Ca^{2+}]i$ y que la miosina esté fuertemente unida a la actina, esto es, en estado Tm "abierto". Este proceso de activación calcio-dependiente entre la miosina y la actina es altamente cooperativo, lo que quiere decir que cuando se produce una unión de una molécula de miosina con una de actina en presencia de calcio, la probabilidad de que la molécula vecina de miosina se una a otra de actina aumenta; la fatiga puede disminuir este proceso de cooperación intermolecular (Debold y cols., 2006). Más aún sí las concentraciones o la sensibilidad a las concentraciones de calcio caen. Las fibras rápidas son más sensibles a la caída en la sensibilidad al calcio en condiciones acidóticas; esto parce estar relacionado con la existencia de dos sitios activos de baja afinidad para el calcio de la troponina C en las fibras rápidas, mientras que en las fibras lentas la troponina C solo tiene un sitio de unión al calcio. La interacción entre la troponina C (troponina con afinidad por el calacio) y la troponina I (troponina con afinidad por el sitio activo de la actina para la molécula de miosina) es fundamental en el proceso de depresión a la sensibilidad al calcio inducida por la acidosis. La troponina T (subunidad de la troponina con un sitio de unión para la Tm) también interviene en el proceso de depresión de la activación inducida por acidosis, especialmente en las fibras rápidas (Nosek y cols., 2004).

Otro de los componentes a tener en cuenta durante las situaciones de fatiga es el difosfato de adenosina (ADP). Su concentración intracelular se mantiene baja (en torno a los 10 uM) debido a la reacción de la creatin quinasa que favorece intensamente la formación de adenosintrifosfato (ATP). Sin embargo, cuando la activación muscular llega a intensidades en las que se consume en gran manera la creatinfosfato, los niveles intracelulares de ADP puede alcanzar 1 mM (Nagesser y cols., 1993). En cualquier caso con concentraciones celulares de 0,5 mM de ADP la capacidad de producir fuerza y la velocidad de acortamiento de la miosina se afecta significativamente (Greenberg y cols., 2010). A concentraciones fisiológicas parece que el principal efecto de la acumulación de ADP es el aumento de tiempo que la miosina está fuertemente unida a la actina, con lo que disminuye la velocidad de acortamiento.

La misma acumulación de ADP no parece tener efecto sobre el desplazamiento de los puentes cruzados. No obstante, al aumentar el tiempo de enlace fuerte entre actina y miosina, aumenta la probabilidad de puentes cruzados ligados a la actina. Es decir, por un lado enlentece la velocidad de acortamiento pero por otro aumenta el número de puentes cruzados enganchados al filamento delgado y por lo tanto la fuerza que este puede ejercer. Este efecto coincide macroscópicamente con lo observado en fibras musculares fatigadas, en las que se mantiene la fuerza a expensas de una pérdida de velocidad. Por otro lado, experimentos de ensayo de motilidad han demostrado que a concentraciones elevadas de ADP hay poco efecto sobre la capacidad de generar potencia de la miosina en contra de una carga. En definitiva, el efecto directo de la acumulación de ADP sobre la fuerza o la velocidad ejercida por los puentes cruzados es de pequeña magnitud o incluso puede tener cierto papel protector durante la fatiga *in vivo* (Greenberg y cols., 2010). El efecto de altas concentraciones de ADP sobre la sensibilidad al calcio se ha evidenciado por una desviación a la izquierda de las curvas pCa-velocidad, lo que explicaría el papel protector del ADP sobre la fuerza mientras que disminuye la velocidad de desplazamiento de los filamentos delgados de actina.

Además de los efectos concretos descritos, existen otros muchos procesos susceptibles de fatigarse o de inducir a una situación de fatiga en el músculo. Entre los mejor conocidos están los que afectan a la pérdida de excitabilidad de neuronas motoras en toda la extensión del sistema nervioso central. Este tema es y sigue siendo motivo de debate o discusión respecto al origen central o periférico de la fatiga neuromuscular. Otros mecanismos que pueden inducir a la manifestación de la fatiga muscular están relacionado con metabolitos con inferencias en procesos fisiológicos como puede ser el ión potasio o el agotamiento de la capacidad de amortiguamiento de los sistemas tampones. Por ejemplo, el agotamiento de bicarbonato plasmático es una variable sistemáticamente asociada a la fatiga en ejercicios de resistencia. No cabe duda que su importante papel en el mantenimiento del estado ácido-base en el medio sanguíneo y, por extensión, en el espacio extracelular lo hace protagonista en actividades musculares que lleven a su rápido consumo por el sistema pulmonar.

En conclusión, hay que aceptar que la fatiga, en general, es un proceso multifactorial y complejo que no puede ser explicado en su totalidad. Sin embargo, la aparición y aplicación de nuevas técnicas de estudios nos revelan datos y propiedades a nivel molecular que ayudan a entender los fenómenos empíricos que podemos observar cuando se mide el rendimiento muscular o deportivo.

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ARTICLES

# Resveratrol improves health and survival of mice on a high-calorie diet

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**Resveratrol (3,5,4′-trihydroxystilbene) extends the lifespan of diverse species including *Saccharomyces cerevisiae*, *Caenorhabditis elegans* and *Drosophila melanogaster*. In these organisms, lifespan extension is dependent on Sir2, a conserved deacetylase proposed to underlie the beneficial effects of caloric restriction. Here we show that resveratrol shifts the physiology of middle-aged mice on a high-calorie diet towards that of mice on a standard diet and significantly increases their survival. Resveratrol produces changes associated with longer lifespan, including increased insulin sensitivity, reduced insulin-like growth factor-1 (IGF-I) levels, increased AMP-activated protein kinase (AMPK) and peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α) activity, increased mitochondrial number, and improved motor function. Parametric analysis of gene set enrichment revealed that resveratrol opposed the effects of the high-calorie diet in 144 out of 153 significantly altered pathways. These data show that improving general health in mammals using small molecules is an attainable goal, and point to new approaches for treating obesity-related disorders and diseases of ageing.**

The number of overweight individuals worldwide has reached 2.1 billion, leading to an explosion of obesity-related health problems associated with increased morbidity and mortality[1,2]. Although the association of obesity with increased risk of cardiovascular disease and diabetes is well known, it is often under-appreciated that the risks of other age-related diseases, such as cancer and inflammatory disorders, are also increased. At the other end of the spectrum, reducing caloric intake by ~40% below that of *ad libitum*-fed animals (caloric restriction) is the most robust and reproducible way to delay age-related diseases and extend lifespan in mammals[3,4].

Experiments with *Saccharomyces cerevisiae* and *Drosophila melanogaster* have implicated the sirtuin/Sir2 family of $NAD^+$-dependent deacetylases and mono-ADP-ribosyltransferases as mediators of the physiological effects of caloric restriction[5]. In mammals, seven sirtuin genes have been identified (*SIRT1–7*). SIRT1 regulates such processes as glucose and insulin production, fat metabolism, and cell survival, leading to speculation that sirtuins might mediate effects of caloric restriction in mammals[5]. We previously screened over 20,000 molecules to identify ~25 that enhance SIRT1 activity *in vitro*[6]. Resveratrol, a molecule produced by a variety of plants in response to stress, emerged as the most potent.

Resveratrol has since been shown to extend the lifespan of evolutionarily distant species including *S. cerevisiae*, *C. elegans* and *D. melanogaster* in a Sir2-dependent manner[6–9]. A recent study found that resveratrol improves health and extends maximum lifespan by 59% in a vertebrate fish[10]. In mammalian cells, resveratrol produces SIRT1-dependent effects that are consistent with improved cellular function and organismal health[11–15]. Whether resveratrol acts directly or indirectly through Sir2 *in vivo* is currently a subject of debate[16].

On the basis of the unprecedented ability of resveratrol to improve health and extend lifespan in simple organisms, we have asked whether it has similar effects in mice. We hypothesized that resveratrol might shift the physiology of mice on a high-calorie diet towards that of mice on a standard diet and provide the associated health benefits without the mice having to reduce calorie intake. Cohorts of middle-aged (one-year-old) male C57BL/6NIA mice were provided with either a standard diet (SD) or an otherwise equivalent high-calorie diet (60% of calories from fat, HC) for the remainder of their lives. To each of the diets, we added resveratrol at two concentrations that provided an average of $5.2 \pm 0.1$ and $22.4 \pm 0.4\ mg\ kg^{-1}\ day^{-1}$, which are feasible daily doses for humans. After 6 months of treatment, there was a clear trend towards increased survival and insulin sensitivity. Because the effects were more prominent in the higher dose ($22.4 \pm 0.4\ mg\ kg^{-1}\ day^{-1}$, HCR), we initially focused our resources on this group and present the results of those analyses herein. Analyses of the other groups will be presented at a later date.

## Increased survival

Mice on the HC diet steadily gained weight until ~75 weeks of age, after which average weight slowly declined (Fig. 1a). Although mice on the HCR diet were slightly lighter than the HC mice during the initial months, there was no significant weight difference between the

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groups from 18–24 months, when most of our analyses were performed. There was also no difference in body temperature (Table 1), food consumption (Supplementary Fig. 1a, b), total faecal output or lipid content (Supplementary Fig. 1c, d), or post-mortem body fat distribution (Supplementary Fig. 2).

At 60 weeks of age, the survival curves of the HC and HCR groups began to diverge and have remained separated by a 3–4-month interval (Fig. 1b). A similar effect on survival was observed in a previous study of one-year-old C57BL/6 mice on caloric restriction, ultimately resulting in a 20% extension of mean lifespan[17]. With the present age of the colony at 114 weeks, 58% of the HC control animals have died (median lifespan 108 weeks), as compared to 42% of the HCR group and 42% of the SD controls. Although we cannot yet confidently predict the ultimate mean lifespan extension, Cox proportional hazards regression shows that resveratrol reduced the risk of death from the HC diet by 31% (hazard ratio = 0.69, $P = 0.020$), to a point where it was not significantly different from the SD group (hazard ratio = 1.03, $P = 0.88$).

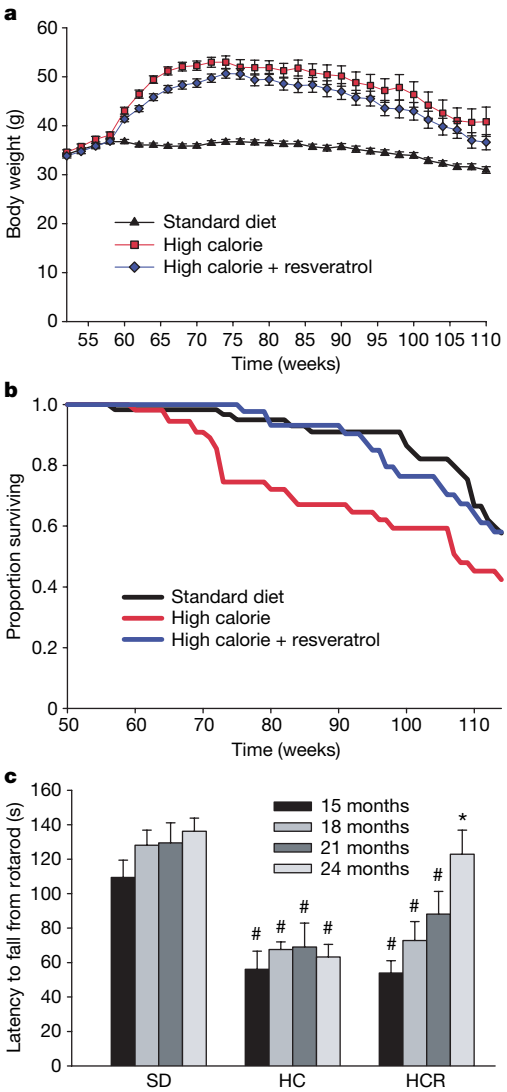


**Figure 1 | Resveratrol increases survival and improves rotarod performance.** **a**, Body weights of mice fed a standard diet (SD), high-calorie diet (HC), or high-calorie diet plus resveratrol (HCR). **b**, Kaplan–Meier survival curves. Hazard ratio for HCR is 0.69 ($\chi^2 = 5.39$, $P = 0.020$) versus HC, and 1.03 ($\chi^2 = 0.022$, $P = 0.88$) versus SD. The hazard ratio for HC versus SD is 1.43 ($\chi^2 = 5.75$, $P = 0.016$). **c**, Time to fall from an accelerating rotarod was measured every 3 months for all survivors from a pre-designated subset of each group; $n = 15$ (SD), 6 (HC) and 9 (HCR). Asterisk, $P < 0.05$ versus HC; hash, $P < 0.05$ versus SD. Error bars indicate s.e.m.

Although resveratrol increased survival, it was important to ascertain whether quality of life was maintained. One way to assess this was to measure balance and motor coordination, which we did by examining the ability to perform on a rotarod. Surprisingly, the resveratrol-fed HC mice steadily improved their motor skills as they aged, to the point where they were indistinguishable from the SD group (Fig. 1c). It is possible that the improved rotarod performance might have been due to minor differences in body weight but we view this as unlikely because we found no correlation between body weight and performance within groups and rotarod performance was also improved for resveratrol-treated SD mice (R.deC. and K.P., unpublished data). These data are reminiscent of the resveratrol-mediated increase in motor activity in older individuals of the vertebrate fish species *Nothobranchius furzeri*[10].

## Increased insulin sensitivity

In humans, high-calorie diets cause numerous pathological conditions including increased glucose and insulin levels leading to diabetes, cardiovascular disease and non-alcoholic fatty liver disease, a condition for which there is no effective treatment[18]. The HC-fed mice had alterations in plasma levels of markers that predict the onset of diabetes and a shorter lifespan, including increased levels of insulin, glucose and IGF-1 (Table 1). The HCR group had significantly lower levels of these markers, paralleling the SD group. An oral glucose tolerance test indicated that the insulin sensitivity of the resveratrol-treated mice was considerably higher than controls (Fig. 2a–d). Homeostatic model assessment, which is used to quantify insulin resistance, gave scores of 2.5 for SD, 8.8 for HC and 3.5 for HCR, confirming improved sensitivity (HCR versus HC, $P = 0.01$). Although the persistence of high glucose levels for more than 60 min following an oral dose is unusual for young mice, it is typical for older animals[19]. Compared to the HC controls, the areas under the curves for both glucose and insulin levels were significantly decreased in the resveratrol-fed HC group and were not significantly different from mice in the SD group (Fig. 2b, d).

Next, we investigated possible mechanisms behind these metabolic effects. AMPK is a metabolic regulator that promotes insulin sensitivity and fatty acid oxidation. Its activity correlates tightly with phosphorylation at Thr 172 (p-AMPK). Chronic activation of AMPK occurs on a calorically restriction diet and has been proposed as a longevity strategy for mammals[20]. Consistent with this idea, additional copies of the AMPK gene are sufficient to extend lifespan in *C. elegans*[21]. Because we and others[22] have observed that resveratrol can activate AMPK in cultured cells through an indirect mechanism (Fig. 2e; see also Supplementary 3a–d), we examined whether AMPK activation occurred in the livers of the resveratrol-fed group. Resveratrol showed a strong tendency towards inducing phosphorylation of AMPK (Fig. 2f), as well as two downstream indicators of activity, namely phosphorylation of acetyl-coA carboxylase at Ser 79 and decreased expression of fatty acid synthase (Supplementary Fig. 3e, f).

## Decreased organ pathology

At 18 months of age it was apparent that the high-calorie diet greatly increased the size and weight of livers and that resveratrol prevented these changes (Fig. 3a–c; see also Supplementary Fig. 4a, b) without altering plasma lipid levels (Table 1). Histological examination of liver sections by staining with haematoxylin and eosin or oil red O revealed a loss of cellular integrity and the accumulation of large lipid droplets in the livers of the HC but not the HCR group. Blinded scoring of the liver sections for overall pathology on a scale of 0–4 (with 4 being the most severe) gave mean values of 1.3 for the SD group, 2.8 for the HC group and 0.8 for the HCR group (Fig. 3b). Plasma amylase, which can indicate pancreatic damage, was elevated in the HC group and was significantly reduced by resveratrol (Table 1). The reasons for the elevation of plasma amylase levels in the HC group are unclear given that pancreatic sections of all animals

revealed no damage to the pancreas or decrease in islet area (data not shown). Differences in the weights of other organs did not reach statistical significance.

The ability of resveratrol to improve motor function and increase insulin sensitivity indicated that its effects were not confined to the liver. To test directly whether other organs also benefited, we examined heart tissue of the SD, HC and HCR mice. Blinded scoring of overall pathology—taking into account subtle changes in the abundance of fatty lesions, cardiac muscle vacuolization, degeneration and inflammation—on a relative scale of 0–4 (with 4 being the most severe) gave mean values of 1.6 for the SD group, 3.2 for the HC group and 1.2 for HCR group (Fig. 3d; see also Supplementary Fig. 4c). Improvements in the morphology of the aortic elastic lamina were also apparent (Supplementary Fig. 4d).

### Increased mitochondria

Exercise and reduced caloric intake increase hepatic mitochondrial number[23,24] and we wondered whether resveratrol might produce the same effect. The livers of the resveratrol-treated mice had considerably more mitochondria than those of HC controls and were not significantly different compared to those of the SD group (Fig. 3e, f). There was also a trend towards higher citrate synthase activity in the resveratrol-fed mice (an indicator of increased mitochondrial content) although the effect was not significant (Table 1). Culturing FaO hepatoma or HeLa cells in the presence of resveratrol increased mitochondrial number (Fig. 3g, h), similar to the previously reported effect of culturing cells in serum from calorically restricted rats[24].

Mitochondrial biogenesis in liver and muscle is controlled, in large part, by the transcriptional coactivator PGC-1α[25,26], the activity of which, in turn, is positively regulated by SIRT1-mediated deacetylation[27,28]. Hence, the acetylation status of PGC-1α is considered a marker of SIRT1 activity *in vivo*[27]. Because this assay required more tissue than was available, we examined a separate cohort of one-year-old mice on the HC diet that had been treated with resveratrol for 6 weeks at 186 mg kg$^{-1}$ d$^{-1}$. The acetylation status of PGC-1α in the resveratrol-fed mice was threefold lower than the diet-matched controls (Fig. 3i, j). There was no detectable increase in SIRT1 protein levels in resveratrol-treated mice (data not shown), suggesting that SIRT1 enzymatic activity was enhanced by resveratrol.

### Microarrays and pathway analysis

These data demonstrate that resveratrol can alleviate the negative impact of a high-calorie diet on overall health and lifespan. To determine to what extent resveratrol had shifted the physiology of the high-calorie group towards the lower calorie group, we performed whole-genome microarrays and pathway analysis on liver samples. Z ratios were calculated as described previously[29] and a subset of expression changes was verified by polymerase chain reaction with reverse transcription (RT–PCR) (Supplementary Fig. 5). In the HCR group, expression patterns for 782 out of 41,534 (<2%) individual genes changed significantly relative to the diet-matched controls (Fig. 4a, b). Notably, within the top 12 most highly elevated transcripts were serum amyloid proteins (*Saa1–3*), major urinary proteins (*Mup1* and *Mup3*), and both forms of hydroxysteroid dehydrogenase that degrade testosterone (*Hsd3b4*, *Hsd3b5*). The list of 12 most highly downregulated transcripts included three cytochrome p450 enzymes (*Cyp2a4*, *Cyp2a5* and *Cyp2b9*) that are known to activate pro-carcinogens[30]. The complete data set is available at http://www.grc.nia.nih.gov/branches/rrb/dna/index/dnapubs.htm#2.

We next performed parametric analysis of gene set enrichment (PAGE), a computational method that determines differences between pathways using *a priori* defined gene sets[31,32]. PAGE analysis indicated that resveratrol caused a significant alteration in 127 pathways, including the TCA cycle, glycolysis, the classic and alternative complement pathways, butanoate and propanoate metabolism, sterol biosynthesis and Stat3 signalling (Supplementary Fig. 6; for a complete list see Supplementary Fig. 7). Some of the most highly downregulated pathways in the resveratrol-fed group are known to extend lifespan in model organisms when attenuated, including insulin signalling, IGF-1 and mTOR signalling, oxidative phosphorylation and electron transport[33–36]. Downregulation of glycolysis is a well known marker of caloric restriction[37] and has been proposed as a mechanism by which caloric restriction works[38]. The increase in Stat3, a transcription factor involved in cell survival and liver

**Table 1 | Effects of a high-fat diet and resveratrol on various biomarkers in plasma**

| Parameter | Standard diet | High calorie | High calorie + resveratrol | Fed or Fasted |
|---|---|---|---|---|
| Free fatty acids (mequiv.) | 0.27 (0.04) | 0.59 (0.06)† | 0.53 (0.03)† | Fed |
| | 0.83 (0.10) | 0.45 (0.20) | 0.54 (0.05) | Fasted |
| Triglycerides (mg dl$^{-1}$) | 76.6 (6.8) | 81.4 (6.6) | 88.2 (10.8) | Fasted |
| Cholesterol (mg dl$^{-1}$) | 135 (7) | 183 (20)† | 204 (16)† | Fasted |
| Insulin (ng ml$^{-1}$) | 1.77 (0.64) | 9.21 (1.95)† | 2.46 (0.47)* | Fed |
| | 0.73 (0.14) | 2.70 (0.36)† | 1.06 (0.30)* | Fasted |
| Glucose (mg dl$^{-1}$) | 129.0 (5.4) | 118.3 (4.7) | 114.8 (6.3) | Fed |
| | 94.5 (3.3) | 125.3 (11.6)† | 85.6 (10.3)* | Fasted |
| IGF-I (ng ml$^{-1}$) | 346 (40) | 534 (12)† | 482 (21)†‡ | Fed |
| | 625 (33) | 999 (102)† | 929 (81)† | Fasted |
| IGFBP-1 (AU) | 1.0 (0.3) | 1.7 (0.3) | 1.7 (1.0) | Fed |
| | 1.0 (0.2) | 0.5 (0.3) | 0.3 (0.1)† | Fasted |
| IGFBP-2 (AU) | 1.0 (0.2) | 0.7 (0.04) | 0.9 (0.1) | Fasted |
| Leptin (ng ml$^{-1}$) | 2.0 (1.1) | 21.6 (7.2) | 11.6 (6.5) | Fasted |
| Adiponectin (μg ml$^{-1}$) | 12.1 (1.6) | 9.5 (0.5) | 9.0 (0.8) | Fed |
| Amylase (U l$^{-1}$) | 2,060 (150) | 2,960 (320)† | 2,190 (230)* | Fasted |
| Ala aminotransferase (U l$^{-1}$) | 347 (119) | 390 (61) | 446 (88) | Fasted |
| Asp aminotransferase (U l$^{-1}$) | 448 (85) | 425 (90) | 512 (46) | Fasted |
| Creatine phosphokinase (U l$^{-1}$) | 4,260 (1820) | 2,010 (810) | 2,520 (680) | Fasted |
| Lactate dehydrogenase (U l$^{-1}$) | 1,530 (240) | 1,610 (170) | 2,020 (180) | Fasted |
| Alkaline phosphatase (U l$^{-1}$) | 43.8 (3.4) | 44.6 (6.0) | 34.2 (1.4) | Fasted |
| Bilirubin (mg dl$^{-1}$) | 0.16 (0.02) | 0.10 (0.03) | 0.16 (0.02) | Fasted |
| Albumin (g dl$^{-1}$) | 2.78 (0.16) | 2.88 (0.19) | 2.66 (0.14) | Fasted |
| Creatinine (mg dl$^{-1}$) | 0.54 (0.02) | 0.48 (0.04) | 0.46 (0.04) | Fasted |
| Cyclo-oxygenase (liver, AU mg$^{-1}$) | 1.00 (0.14) | 0.80 (0.11) | 0.83 (0.11) | Fed |
| Citrate synthase (liver, AU mg$^{-1}$) | 141 (14) | 128 (21) | 138 (11) | Fed |
| Body temperature (°C) | 34.71 (0.14) | 35.52 (0.17)† | 35.57 (0.15)† | Fed |

Values shown are mean (±s.e.m.). AU, arbitrary units; U l$^{-1}$, units per litre.
* $P < 0.05$ versus high calorie.
† $P < 0.05$ versus standard diet.
‡ $P < 0.05$ versus high calorie by one-tailed Student's t-test.

regeneration[39], is of note because its activity is known to be suppressed in the liver by high caloric diets and shows an age-related decline in activity that is attenuated by caloric restriction[40,41].

A few of the pathway changes were unanticipated. Although we had observed an increase in mitochondrial number in the HCR group, there was a decrease in the transcription of numerous mitochondrial genes, suggesting that the turnover of mitochondrial proteins was reduced. This result was unexpected, but is consistent with a previous report showing that SIRT1-dependent activation of PGC-1α does not enhance transcription of mitochondrial genes[27]. Upregulation of complement, which occurs in obese and aged mice, was also observed in the HCR group for reasons that are currently unclear.

It is notable that resveratrol opposed the effects of high caloric intake in 144 out of 153 significantly altered pathways (Fig. 4c). In fact, the PAGE signature of the HCR group was considerably more similar to that of the SD group than the HC controls. Principal component analysis yielded values of −1.82 (SD), −1.41 (HCR) and 3.22 (HC), with 88.4% of the variability assigned to the first principal component, making the HC group the clear outlier (Fig. 4d).

We next compared our PAGE results to a pre-existing caloric restriction data set for C57BL/6 mice known as AGEMAP, hypothesizing that the comparison of changes induced by these two paradigms might reveal pathways common to the enhancement of health and longevity. Of the 36 different pathways identified by AGEMAP as being significantly altered by caloric restriction, there was sufficient overlap to compare 19 of them to our data (Fig. 4e). Pathways altered in the same direction by caloric restriction and resveratrol included the downregulation of IGF-1 and mTOR signalling, downregulation of glycolysis, and upregulation of Stat3 signalling. One interesting difference was that cell cycle checkpoint and apoptotic pathways were elevated in the caloric restriction group but downregulated by resveratrol. We do not favour the interpretation that the resveratrol-treated livers were undergoing less apoptosis because levels of AST and ALT, two indicators of hepatic apoptosis, were unchanged (see Table 1). Perhaps the downregulation of cell cycle checkpoints is linked to the recent discovery that inhibition of checkpoint function in *C. elegans* increases stress resistance and lifespan[42]. Although the statistical power of this analysis is limited by the overlap in data sets, the results suggest that more comprehensive comparisons of the effects of resveratrol and caloric restriction are warranted.

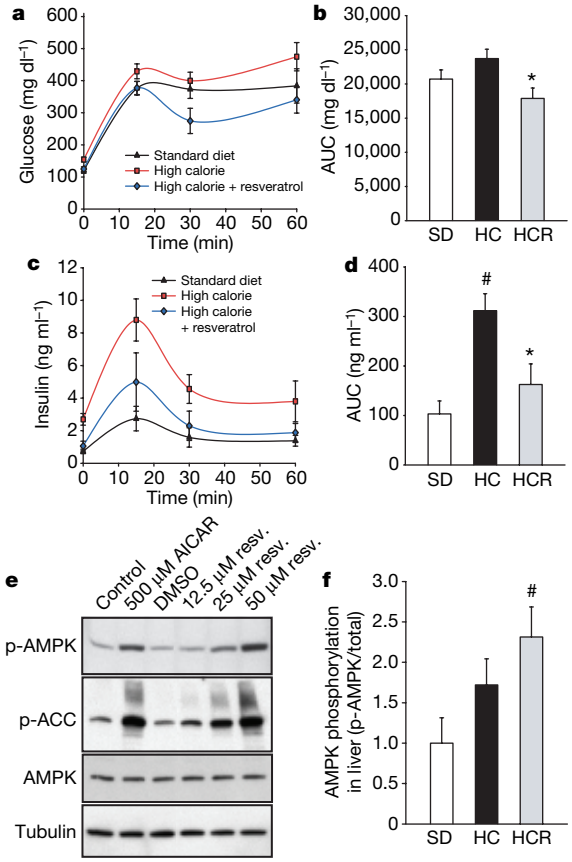


**Figure 2 | Resveratrol improves insulin sensitivity and activates AMPK.** **a**–**d**, Plasma levels of glucose (**a**, **b**) and insulin (**c**, **d**) were measured after a 2 g kg$^{-1}$ oral glucose dose. Areas under the curves (AUC) were significantly reduced by resveratrol treatment. **e**, Activation of AMPK by resveratrol in CHO cells. In the presence of resveratrol or 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR) as a positive control, phosphorylation of AMPK and its downstream target, acetyl-coA carboxylase (ACC), are increased. **f**, AMPK activity in liver. Phosphorylation of AMPK (**f**), acetyl-coA carboxylase (Supplementary Fig. 3e) and decreased expression of fatty acid synthase (Supplementary Fig. 3f) are indicative of enhanced AMPK activity. Asterisk, $P < 0.05$ versus HC; hash, $P < 0.05$ versus SD. $n = 5$ for all groups. Error bars indicate s.e.m.

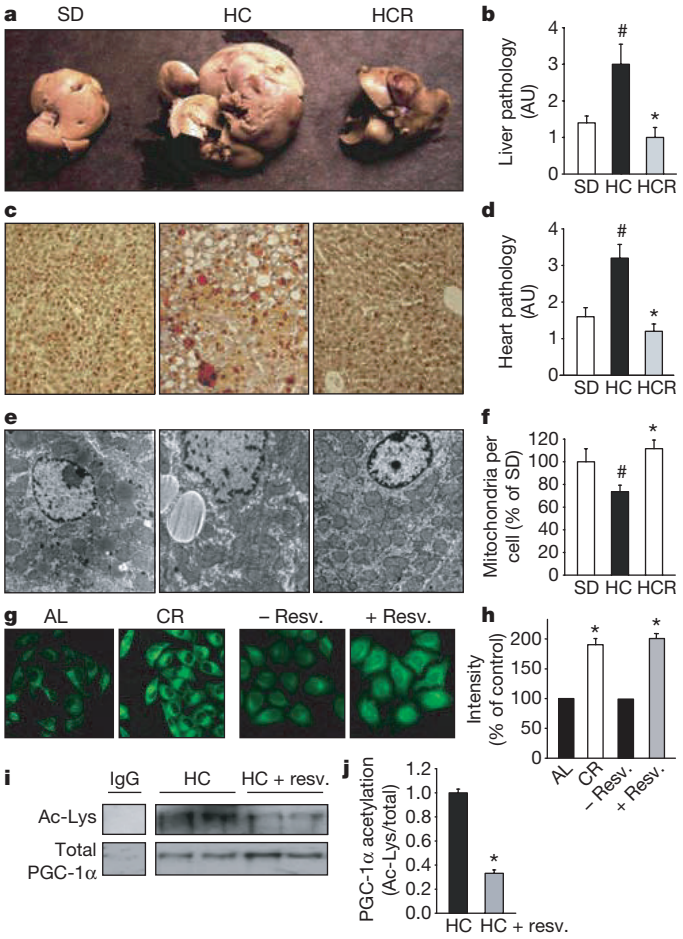


**Figure 3 | Resveratrol improves liver histology, increases mitochondrial number and decreases acetylation of PGC-1α.** **a**–**c**, Resveratrol prevents the development of fatty liver, as assessed by organ size (**a**), overall pathology (**b**) and decreased fat accumulation as measured by oil red O staining (**c**). AU, arbitrary units. **d**, Pathology of heart sections. Additional histology of liver, heart and aorta is shown in Supplementary Fig. 4. **e**, **f**, Transmission electron microscopy of liver sections (**e**) and mitochondrial counts (**f**). **g**, **h**, Mitochondrial number in HeLa cells treated with serum from *ad libitum*-fed (AL) or calorically restricted (CR) rats, or resveratrol, and stained with Mitotracker green FM. **i**, **j**, Resveratrol reduces the acetylation of PGC-1α, a known SIRT1 target and regulator of mitochondrial biogenesis, *in vivo*. PGC-1α was immunoprecipitated from liver extracts then blotted for acetyl lysine (**i**) and quantified (**j**). Asterisk, $P < 0.05$ versus HC; hash, $P < 0.05$ versus SD. $n = 5$ for **b** and **d**; $n = 3$ for **f** and **j**. Error bars indicate s.e.m.

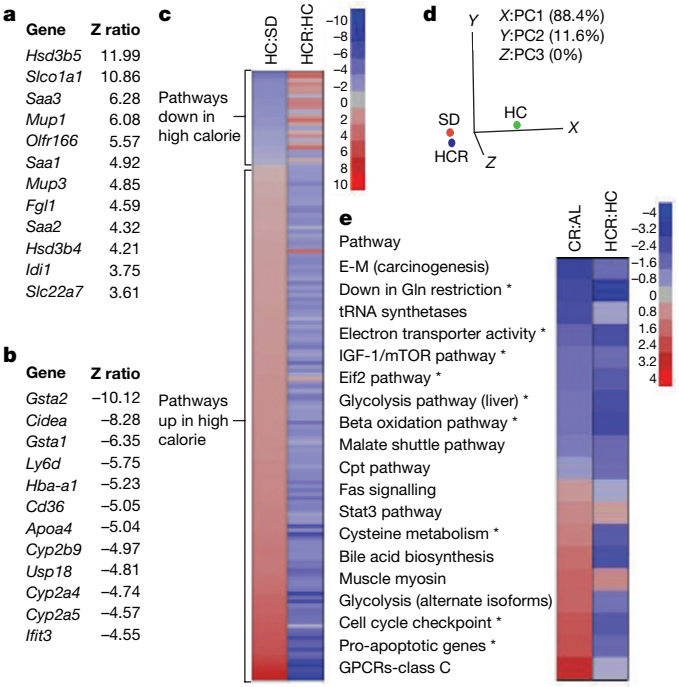


**Figure 4 | Resveratrol shifts expression patterns of mice on a high-calorie diet towards those on a standard diet.** **a**, **b**, The most highly significant upregulated (**a**) and downregulated (**b**) genes in livers of HC and HCR groups are shown. **c**, Parametric analysis of gene-set enrichment (PAGE) comparing every pathway significantly upregulated (red) or downregulated (blue) by either the HC diet or resveratrol (153 in total, with 144 showing opposing effects). **d**, Principal component analysis of PAGE data. The first principal component (PC1) is dominant, with 88.4% variability, and shows HCR to be more similar to SD than HC. **e**, Comparison of pathways significantly altered by resveratrol treatment and caloric restriction using data from the AGEMAP caloric restriction study. Pathways with significant differences between HC and HCR are indicated by an asterisk. Complete pathway listings are in Supplementary Fig. 7. Asterisk, $P < 0.05$ versus HC. $n = 5$ for SD and HC; $n = 4$ for HCR.

## Discussion

The ability of resveratrol to prevent the deleterious effects of excess caloric intake and modulate known longevity pathways suggests that resveratrol and molecules with similar properties might be valuable tools in the search for key regulators of energy balance, health and longevity. As a case in point, the most highly upregulated gene in the HC group and second most highly downregulated gene in the HCR group was *Cidea*, which regulates energy balance in brown fat and provides resistance to obesity and diabetes when knocked out[43].

Taken together, the findings in this study show that resveratrol shifts the physiology of mice consuming excess calories towards that of mice on a standard diet, modulates known longevity pathways, and improves health, as indicated by a variety of measures including survival, motor function, insulin sensitivity, organ pathology, PGC-1α activity, and mitochondrial number. Notably, all these changes occurred without a significant reduction in body weight. Whether these effects are due to resveratrol working primarily through SIRT1, which is the case for simpler metazoans, or through a combination of interactions, as predicted by the xenohormesis hypothesis[6,44], remains to be determined. In either case, this study shows that an orally available small molecule at doses achievable in humans can safely reduce many of the negative consequences of excess caloric intake, with an overall improvement in health and survival.

## METHODS

**Animals and diets.** Animal housing and diets are described in Supplementary Information. Briefly, one-year-old male C57BL/6NIA mice were maintained on AIN-93G standard diet (SD), AIN-93G modified to provide 60% of calories from fat (HC), or HC diet with the addition of 0.04% resveratrol (HCR).

**Electron microscopy.** Tissues were processed as previously reported, imaged at ×1,500 on a Jeol 1210 transmission microscope, and photographed using a Gatan US 4000 MP Digital camera. Mitochondria per cell and hepatocyte size were quantified using grid counting by three blinded raters.

**PAGE analysis.** RNA extracted from the livers of five 18-month-old mice per group was hybridized to Agilent 44k whole-genome microarrays following protocols listed on the Gene Expression and Genomics Unit website at the National Institute on Aging (http://www.grc.nia.nih.gov/branches/rrb/dna/index/protocols.htm). One array from the HCR group was discarded owing to RNA degradation. Raw data were subjected to Z normalization and tested for significant changes as previously described[29]. For parametric analysis of gene set enrichment (PAGE), a complete set of 522 pathways in the cell was obtained from http://www.broad.mit.edu/gsea/msigdb/msigdb_index.html. Details of the method are described in Supplementary Information and elsewhere[31]. Principal component analysis was performed on the replicate average for the three groups. These tools are part of DIANE 1.0, a program developed by V.V.P and available at http://www.grc.nia.nih.gov/branches/rrb/dna/diane_software.pdf. Caloric restriction data were extracted from the AGEMAP project.

**Cell-based mitochondrial assays.** Mitochondrial mass was determined using the mitochondria-specific fluorescent dye Mitotracker green FM after fixation of cells, with minor modifications. Staining after fixation permits determination of the incorporation of the dye due to mitochondrial mass independently of the mitochondrial membrane potential ($\Delta\psi m$).

**Serum markers and hormones.** Details of biochemical assays are described in Supplementary Information.

**AMPK and PGC-1α analysis.** Extraction of tissues and cells for western blotting were performed using standard techniques. Details and antibodies are outlined in Supplementary Information.

**Histology.** Eighteen-month-old mice were fasted overnight and fixed using Streck fixative (Streck). Organs were sectioned and stained with haematoxylin and eosin or frozen and stained with oil red O lipid stain, then scored blindly for overall pathology.

**Rotarod.** Results shown are the average of three trials per mouse, measuring time to fall from an accelerating rotarod (4–40 r.p.m. over 5 min). Data from animals that survived to 24 months are shown.

**Statistical analyses.** Single factor analysis of variance followed by Fisher's post-hoc tests were used for all comparisons, except where noted. Details are provided in Supplementary Information.

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**Supplementary Information** is linked to the online version of the paper at www.nature.com/nature.

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# Muscle Physiology Changes Induced by Every Other Day Feeding and Endurance Exercise in Mice: Effects on Physical Performance

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## Abstract

Every other day feeding (EOD) and exercise induce changes in cell metabolism. The aim of the present work was to know if both EOD and exercise produce similar effects on physical capacity, studying their physiological, biochemical and metabolic effects on muscle. Male OF-1 mice were fed either *ad libitum* (AL) or under EOD. After 18 weeks under EOD, animals were also trained by using a treadmill for another 6 weeks and then analyzed for physical activity. Both, EOD and endurance exercise increased the resistance of animals to extenuating activity and improved motor coordination. Among the groups that showed the highest performance, AL and EOD trained animals, ALT and EODT respectively, only the EODT group was able to increase glucose and triglycerides levels in plasma after extenuating exercise. No high effects on mitochondrial respiratory chain activities or protein levels neither on coenzyme Q levels were found in gastrocnemius muscle. However, exercise and EOD did increase β-oxidation activity in this muscle accompanied by increased CD36 levels in animals fed under EOD and by changes in shape and localization of mitochondria in muscle fibers. Furthermore, EOD and training decreased muscle damage after strenuous exercise. EOD also reduced the levels of lipid peroxidation in muscle. Our results indicate that EOD improves muscle performance and resistance by increasing lipid catabolism in muscle mitochondria at the same time that prevents lipid peroxidation and muscle damage.

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## Introduction

Every other day feeding (EOD) is a procedure that resembles many of the effects of caloric restriction (CR). EOD increases life-span, decrease cancer incidence and protects against age-associated diseases and neurodegeneration or endogenous damage of DNA [1–4]. EOD is considered to produce a mild CR inducing around a 15% of reduction of the total ingestion of calories. On the other hand, exercise increases energy expenditure and affect morbidity and lifespan in a similar way than CR on morbidity and lifespan [5,6]. Regular physical activity induces analogous cellular and molecular changes in cardiovascular and nervous systems to those observed in animals fed under CR conditions [4]. Furthermore, endurance exercise and CR delay the progression of immunosenescence [7] and induce a general anti-inflammatory effect preventing the age-dependent increase of plasmatic C-reactive protein levels in rats [8].

The similar effects induced by CR and exercise suggest that both interventions share some mechanisms that modify cell physiology. *Ex vivo* experiments on rat gastrocnemius muscle exposed to electrical stimulation have suggested synergy between CR and aerobic exercise in muscle bioenergetics [9]. In humans, the practice of aerobic exercise together with CR improves insulin sensitivity and reduces plasmatic LDL levels [10] and also seems to improve neurocognitive function [11]. However, in other cases, CR and exercise induce contrary effects such as in the decrease of diet-induced weight loss muscle mass in cases of intentional weight loss in old individuals [12].

Both, CR and exercise modify significantly the bioenergetics of muscle. Long-term CR delays the decline of the skeletal muscle aerobic capacity that occurs during aging [13]. Endurance exercise and CR promote changes in muscle fibers and mitochondrial activity by increasing the activity of PPAR-δ [14–16]. Interestingly, during aging, the activity of complexes involved in aerobic energy production decreases in muscle whereas both CR and exercise maintain them [17]. It is known that CR induces changes in mitochondrial biogenesis and activity through activation of SIRT-1 and PGC1α-dependent mechanisms [18]. Moreover, resveratrol, a known polyphenol considered a mimetic of CR, also induces changes in muscle mitochondrial activity and improves physical performance in mice [19,20] and could be considered as an ergogenic factor able to modify muscle physiology [21].

Most of the studies reported about dietary restriction and exercise have been performed by comparing the effect of CR

versus exercise [22,23]. There are few data available about the effect combined effect of CR and exercise in metabolism and muscle performance. Thus, the aim of this work is to study the effect of the combination of endurance training and EOD on physiological, molecular and metabolic aspects affecting muscle activity. We hypothesize that some of the modifications induced by EOD mimic the effect of aerobic exercise and improve physical performance. It is possible that EOD and exercise could show additive effects in some capacities. In fact, the results shown here suggest that EOD by itself or together with moderate physical activity positively affects muscle performance by increasing the metabolism of lipids by β-oxidation of fatty acids, increasing fuel mobilization during exercise and preventing oxidative stress and also muscle damage.

## Results

### Effects of EOD and training on physical activity

The scope of our research includes the study of different physical activities such as: spontaneous locomotion, grip strength, startle response, motor coordination and resistance to strenuous exercise in mice submitted EOD and/or endurance exercise. All these parameters might be influenced by difference in mice's weight since they were fed in different conditions. However along our experiment we did not find any difference in weight between groups under either AL or EOD (Fig. 1A). Furthermore, endurance exercise did not produce any significant modification on the weight of animals. Determination of animal's locomotion by measuring the movement of each animal on an open field did not show differences between the sedentary groups (Figs. 1B–C). However, training significantly decreased both free activity of animals and peripheral exploration probably by a higher habituation capacity of these animals.

To determine the effect of EOD and/or exercise on muscle capacity we determined grip strength, startle assay, rotarod coordination and strenuous exercise tests. Grip strength was used to determine the improvement for the press force after the 6 weeks of training. We found that exercise significantly improved press force in four limbs (86.88±12.17% of improvement in ALT+EODT animals, n = 32, *vs.* 66.05±10.41% in AL+EOD animals, n = 32, $P = 0.002$). Further, EOD also produced significant improvement in this test (82.77±10.51% in EOD+EODT animals, n = 32, *vs.* 69.93±12.49% in AL+ALT animals, n = 32, $P = 0.019$). When we compared the four groups, the higher and more significant improvement was found in the EODT group (Fig 1D) affecting both two and four limbs tests. Muscle potency, determined by using the startle assay showed no differences between groups (Fig. 1E). Motor coordination and resistance was determined carrying out a specific test designed by using a Rotarod. Animals were forced to resist onto an accelerating Rotarod until 100 rpm, once reached this speed, the time each animal remained on the device was quantified. Training increased motor coordination being significantly higher in EODT animals in comparison with sedentary EOD animals ($P = 0.001$), (Fig. 1F).

Finally, we carried out an extenuating endurance test. Eight animals of each group ran until extenuation. Among all the groups, EODT was the group that ran longer and covered more distance (Fig. 1G). Among non-trained animals, EOD group showed a significant higher resistance in comparison with AL group (1167±57 *vs.* 840±108 s, $P = 0.015$) and covered more distance (603.7±69.4 *vs.* 285.6±47.6 m, $P = 0.003$). Remarkably, EOD group showed almost the same capacity than trained groups (Fig. 1G), indicating that EOD itself was able to increase muscle resistance mimicking endurance exercise training.

### EOD and training affect nutrients mobilization during exercise

The two EOD groups were able to maintain or even increase (in the case of EODT) glucose levels after extenuating exercise (Fig. 2). A mean of 37% increase of glucose level was found in the EODT group after extenuating exercise ($P = 0.014$). In contrast, plasma glucose levels decreased in the ALT group after exercise although showing similar performance in the extenuating test (Fig. 2A). In the AL group, glucose levels did not changed but we have to remark that the resistance of this group was considerably less than in both EOD groups. In fact, when we analyzed the levels of glucose of both EOD groups in comparison with AL groups, a significant increase of glucose in plasma after extenuating exercise was found (140.0±11.8 mg/dl in AL+ALT animals, n = 32, *vs.* 185.4±14.2 in EOD+EODT animals, n = 32) ($P = 0.012$).

In the case of lactate, levels of witness animals showed a tendency to decrease in trained animals (Fig. 2B) and especially in the EODT group. After exercise, all the groups, except EOD, showed a significant increase in plasmatic lactate, even in the AL group besides the poor performance.

In the case of lipids, levels of circulating triglycerides (TGs) in witness animals were significantly lower in animals fed under EOD conditions (86.7±3.4 mg/dl in EOD+EODT animals, n = 32) *vs.* AL animals (125.3±6.7 mg/dl in AL+ALT animals, n = 32) independently of training ($P = 0.001$). After extenuating exercise, EOD and EODT groups were able to maintain or even increase TGs levels while they decreased in AL group (Fig. 2C). As in the case of glucose, the EODT group showed an increase of around 42.5% in circulating TGs after exercise. In the case of cholesterol, training induced higher plasmatic levels before extenuating exercise independently of nutrition (97.1±4.1 mg/dl in ALT+EODT animals, n = 32 *vs.* 80.0±5.2 mg/dl in AL+EOD animals, n = 32, $P = 0.02$). After extenuating exercise, both AL and ALT groups showed increases in plasmatic cholesterol while EOD and EODT groups did not present any changes respecting witness animals (Fig. 3D). This increase was significantly different ($P = 0.017$) when all AL fed animals (AL+ALT: 111.0±6.0 mg/dl, n = 32) were compared with all animals fed under EOD conditions (EOD+EODT: 89.2±5.9 mg/dl, n = 32).

Finally, in the case of urea lower basal levels were found in trained animals (ALT+EODT: 56.3±2.7 mg/dl, n = 32) respecting to sedentary animals (AL+EOD: 73.2±5.7 mg/dl, n = 32) ($P = 0.011$). The effect of exercise on initial levels of urea was higher in EOD groups being significant between EOD and EODT groups ($P = 0.042$). Extenuating exercise did not produce any remarkable change in the levels of plasmatic urea except in the case of EODT group (Fig. 3E). In the case of uric acid, no significant differences of levels between groups were found although we did find a tendency to increase after extenuating exercise. Similar small modifications were also found in the levels of albumin in plasma (data not shown).

### EOD and training modify in different degree mitochondrial muscle activities

A higher physical resistance can be explained by changes in muscle metabolism. Thus, we proceeded to determine mitochondrial activities in gastrocnemius muscle (Fig. 3). In the case of citrate synthase (CS) activity, a marker of mitochondrial mass, we did not find any substantial differences in whole muscle homogenates between mice groups in CS activity (Fig. 3A).

Mitochondrial function was determined by quantification of respiratory complexes activities. Training and EOD induced a slight although not significant increase of complex I activity

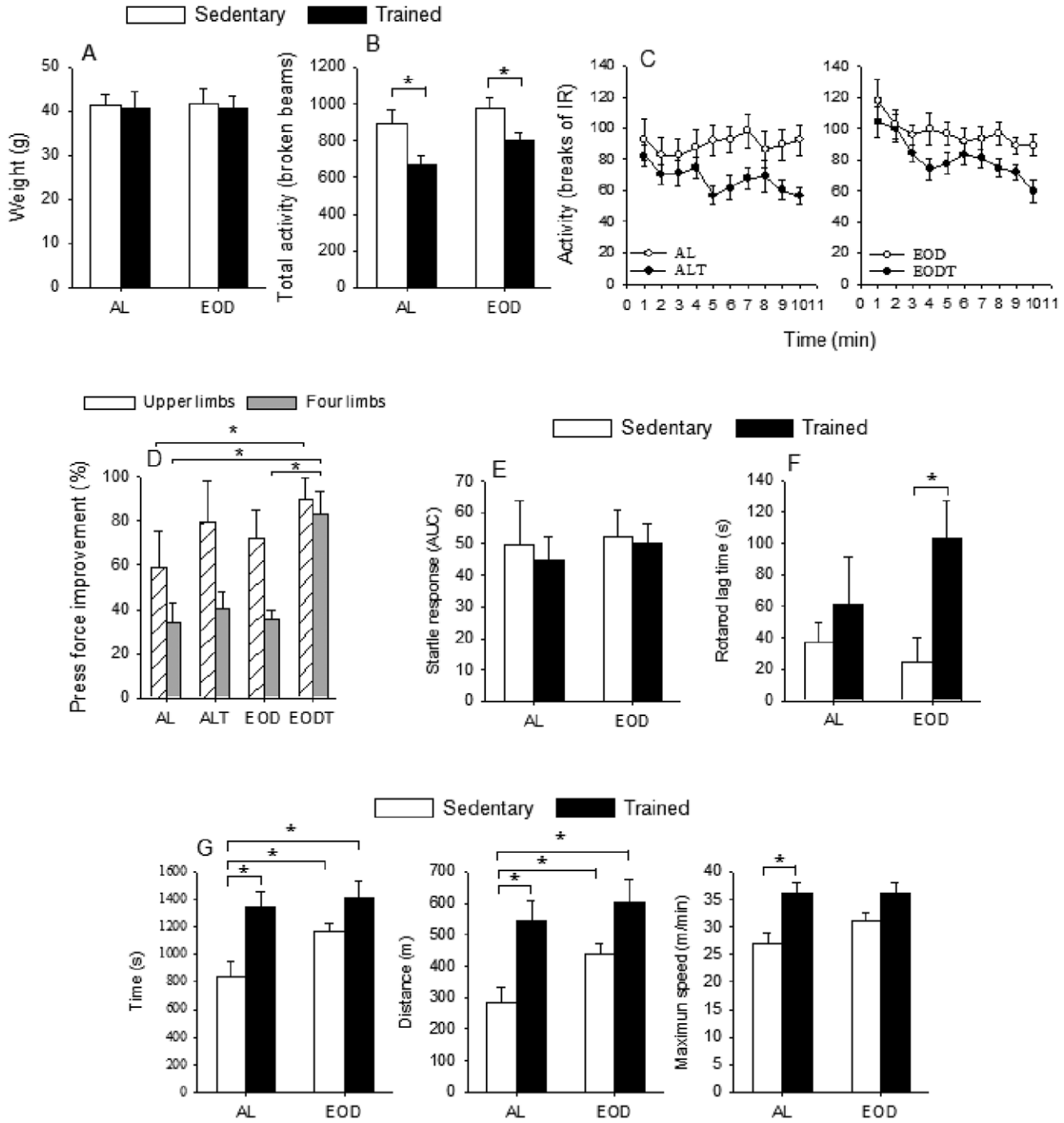


**Figure 1. Physical performance analysis.** A) Weight of animals at the end of the experiment (n = 16). B) Exploratory locomotion activity in broken beans after 10 min. C) Time course of periphery exploration of animals (n = 16). D) Grip strength of upper limbs (left) and four limbs (right). E) Startle response measured as area under curve. F) Rotarod lag time at 100 rpm. G) Time after exhaustion on treadmill (left), distance (middle) and maximum speed reached (right). X axis in each figure indicates the groups AL or EOD. In figures A, B and E–G open bars indicate sedentary animals whereas closed bars indicate trained animals in each group. * Significant differences *vs.* indicated group, p≤0.05; ** Significant differences *vs.* indicated group, p≤0.005.

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(Fig. 3B). Complexes II and III activity of the EOD group increased significantly respect to AL group ($P = 0.014$ and $P = 0.045$ respectively). On the other hand, training slightly but not significantly increased activity in AL fed animals ($P = 0.148$, ALT vs. AL in complex II and $P = 0.868$ in complex III). However, in EODT animals a decrease of activity vs. EOD animals was found being significant in the case of complex II ($P = 0.017$) but not in the case of complex III ($P = 0.379$) (Figs. 3C–D). No changes were found in complex IV (Fig. 3E). In the case of the activity of complex V (Fig. 3F) we found a non-significant increase in EOD group when compared with AL group ($P = 0.183$). Training induced a slight decreased of this activity

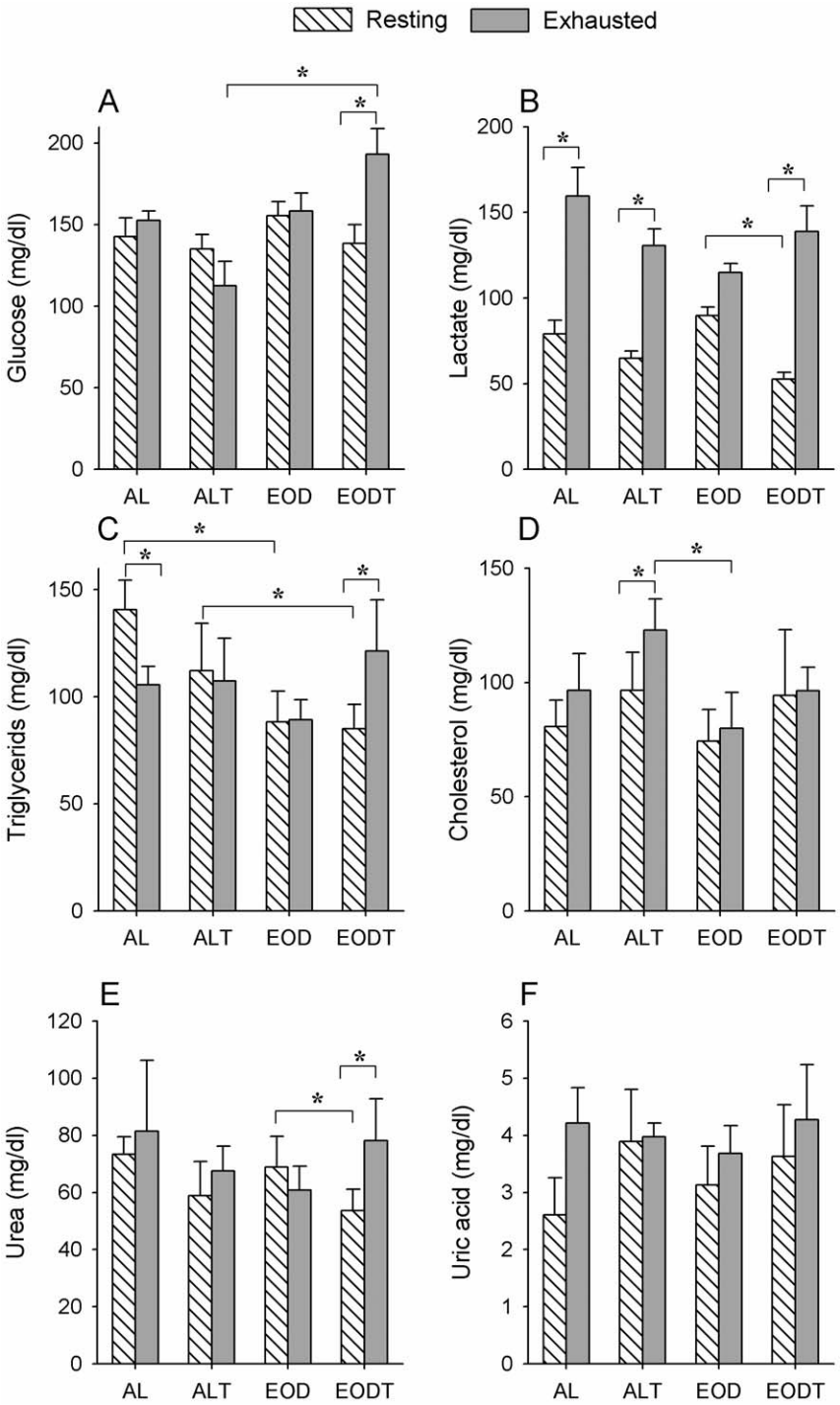


**Figure 2. Metabolites in plasma of animals.** Levels of metabolites in plasma of witness animals are indicated in white and plasma levels of animals just after strenuous exercises are indicated in black. A) Glucose, B) Lactate, C) Triglycerides, D) Cholesterol, E) Urea, F) Uric acid. * Significant differences vs. indicated group, $p \leq 0.005$.
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in AL fed animals ($P = 0.402$) being higher and significant in EOD fed animals ($P = 0.035$). We also determined the coupled activity of complexes I+III and II+III that depend on coenzyme Q (Q) amount in mitochondrial membrane. No changes among groups were found in both activities (Figs. 3G–H).

When we analyzed the amount of protein complexes of mitochondria no significant changes among groups were found in any of the four respiratory complexes and ATPase (Fig. 4). Then, our results suggest that the small changes found in the activity of mitochondrial complexes may be due to post-translational changes. We also proceeded to quantify Q in whole muscle homogenate. $Q_9$ is the predominant form in mice being around 10 times more abundant than $Q_{10}$. No significant differences among the different mice groups were found

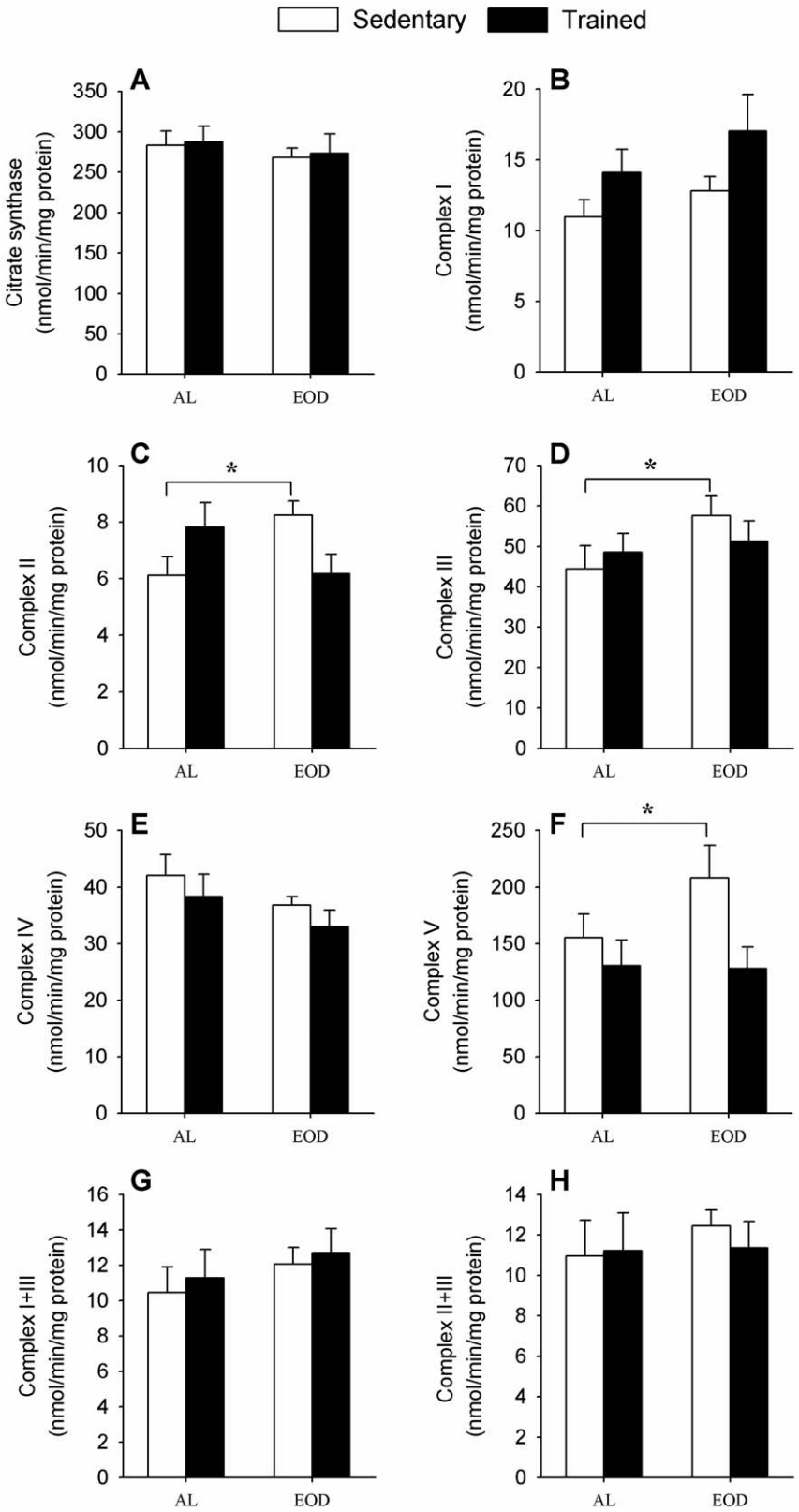


**Figure 3. Mitochondrial activities in whole muscle homogenate.** Indicated activities were determined as nmol/min/mg protein. A) Citrate synthase, B) Complex I (NADH:ubiquinone oxidoreductase), C) Complex II (succinate dehydrogenase), D) Complex III (ubiquinol:cytochrome c oxidoreductase), E) Complex IV (cytochrome c oxidase), F) Complex V (ATP synthase), G) Complex I+III (NADH:cytochrome c oxidoreductase), H) Complex II+III (succinate:cytochrome c oxidoreductase). * Significant differences vs. indicated group, $p \leq 0.05$.
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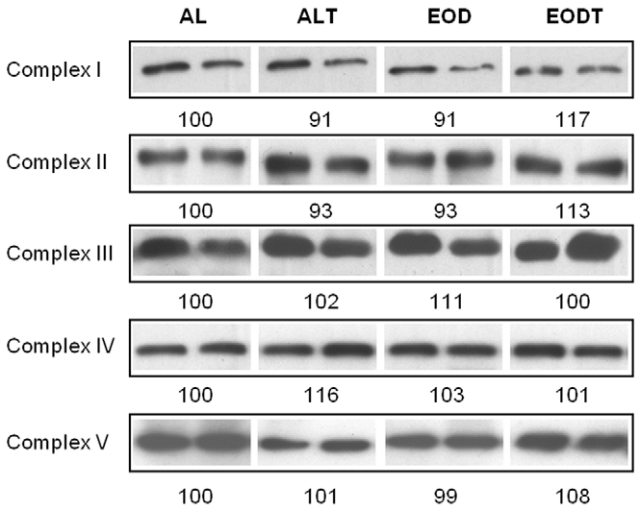


**Figure 4. Levels of proteins markers of each mitochondrial complex.** Proteins from whole homogenate were resolved by SDS-PAGE electrophoresis and the presence of the markers for each complex was determined by immunoblotting as indicated in Material and Methods section. Blots were quantified by densitometry and normalized vs. protein loading determined by membrane staining with red Ponceau. Numbers indicate the mean (n = 5) in arbitrary units normalized vs. AL group as 100. No significant differences were found between groups.
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(Table 1). However, EOD animals showed a non-significant increase of the amount of $Q_9$ and total Q in comparison with AL group similarly to the effect found with training. On the other hand, $Q_{10}$ increases in trained animals but not in dietary restricted animals. This different effect affected the ratio between $Q_9$ and $Q_{10}$ that increased in EOD groups whereas decreased in trained animals.

We also analyzed the activity of β-oxidation in muscle (Fig. 5A). Training significantly increased β-oxidation activity in gastrocnemius muscle ($P = 0.050$), whereas EOD only induced a non-significant increase ($P = 0.305$). However, when combined, EOD and exercise highly increased β-oxidation in comparison with the levels found in the AL group ($P = 0.027$). Further, we determined the level of the fatty acid translocase (CD36), a protein involved in the incorporation of fatty acids from plasma to muscle and in mitochondrial fatty acid oxidation. Surprisingly, EOD significantly increased CD36 levels in comparison with AL animals ($P = 0.0009$) whereas in EODT animals the levels of this protein decreased to the levels of AL group (Fig. 5B).

Electron micrographs of gastrocnemius muscle were also analyzed (Fig. 6A). Quantification of intermyofibrillar mitochondria in muscle demonstrated that, in general, the surface occupied by mitochondria did not show significant differences between the four groups (Fig. 6B). However, when we determined the number of mitochondrial structures per surface unit, we found that EOD induced a significant increase being higher in the case of the EODT group (Fig. 6C). Training increased the average area per mitochondria in both, AL and EOD animals, whereas EOD induced a clear decrease in this area (Fig. 6D). Differences in shape were determined by measuring the roundness of mitochondria. EOD significantly decreased this parameter indicating more longitudinal mitochondria (Fig. 6E). These parameters confirmed the observations of micrographs indicating that training induced hypertrophy by increasing the size of mitochondria in muscle fibers whereas EOD modified their shape and localization increasing the amount of intermyofibrillar mitochondria and modifying the shape of these mitochondria that appeared longer and sinusoid around the myofibrils.

**Table 1.** Coenzyme Q levels in gastrocnemius muscle.

| | $Q_9$ | $Q_{10}$ | Q total | Ratio $Q_9/Q_{10}$ |
|---|---|---|---|---|
| **AL** | 1245±154 | 122±19 | 1367±171 | 10.8±0.7 |
| **ALT** | 1298±121 | 139±18 | 1384±150 | 9.2±0.5 |
| **EOD** | 1352±65 | 116±5 | 1464±69 | 11.8±0.5 |
| **EODT** | 1293±123 | 127±16 | 1420±139 | 10.9±0.6 |

Data represent the average (pmol/mg protein from whole homogenate) ± SE, n = 16.
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## EOD prevents exercise-induced muscle damage

Accumulation of oxidative damage can be one of the causes of muscle fatigue. For this reason we determined the levels of lipid peroxidation in gastrocnemius muscle. No differences were found between witness and extenuated animals probably by the short time passed between extenuation and sacrifice. Analysis of the lipid peroxidation in the whole population of each group indicated that ALT animals showed the highest levels of MDA (Fig. 7). On the other hand, both groups of EOD animals showed significant lower levels of MDA indicating that EOD protected muscle against lipid peroxidation in muscle independently of training.

Levels of muscle damage after extenuating exercise were also determined by the presence of creatine kinase activity in plasma. Besides the lower physical resistance, AL animals showed the higher muscle damage of all groups (Fig. 7). On the other hand, non-trained EOD animals showed similar levels of muscle damage than trained animals (ALT and EODT) indicating a similar degree of resistance of muscle to damage.

## Discussion

Many studies about CR are based on a decrease of 40–50% calories in the daily uptake of food. In our study we have used the every-other day feeding model (EOD), which is considered to produce only a mean of 15% deficit in the calorie input due to the fact that animals eat more when they have access to food [24]. Although EOD did not produce a high caloric deficit, it has been demonstrated that EOD mimics many of the beneficial aspects of classical CR. However, the effect of this model on longevity is currently under discussion [25] including higher longevity and higher resistance to stress [1,4,26–29]. The advantage of the EOD model in our study is that it did not affect the weight of animals during the study and thus, weight, size and nutrients availability were not factors that could influence the physical capacity or muscle performance of the animals.

Edge exploration and elevated plus maze tests indicate that the differences in physical activity were not due to a different degree of anxiety between groups. However, an interesting finding was that, with independence of the feeding procedure, trained animals decreased their total activity in an open field after few minutes probably indicating a higher capacity to recognize new environments. Our outcomes have also shown that EOD and aerobic training increase motor coordination and resistance to exhausting exercise. In the case of running on rolling carpet, EOD slightly increased physical resistance in trained animals whereas it did significantly increase resistance in the sedentary group when

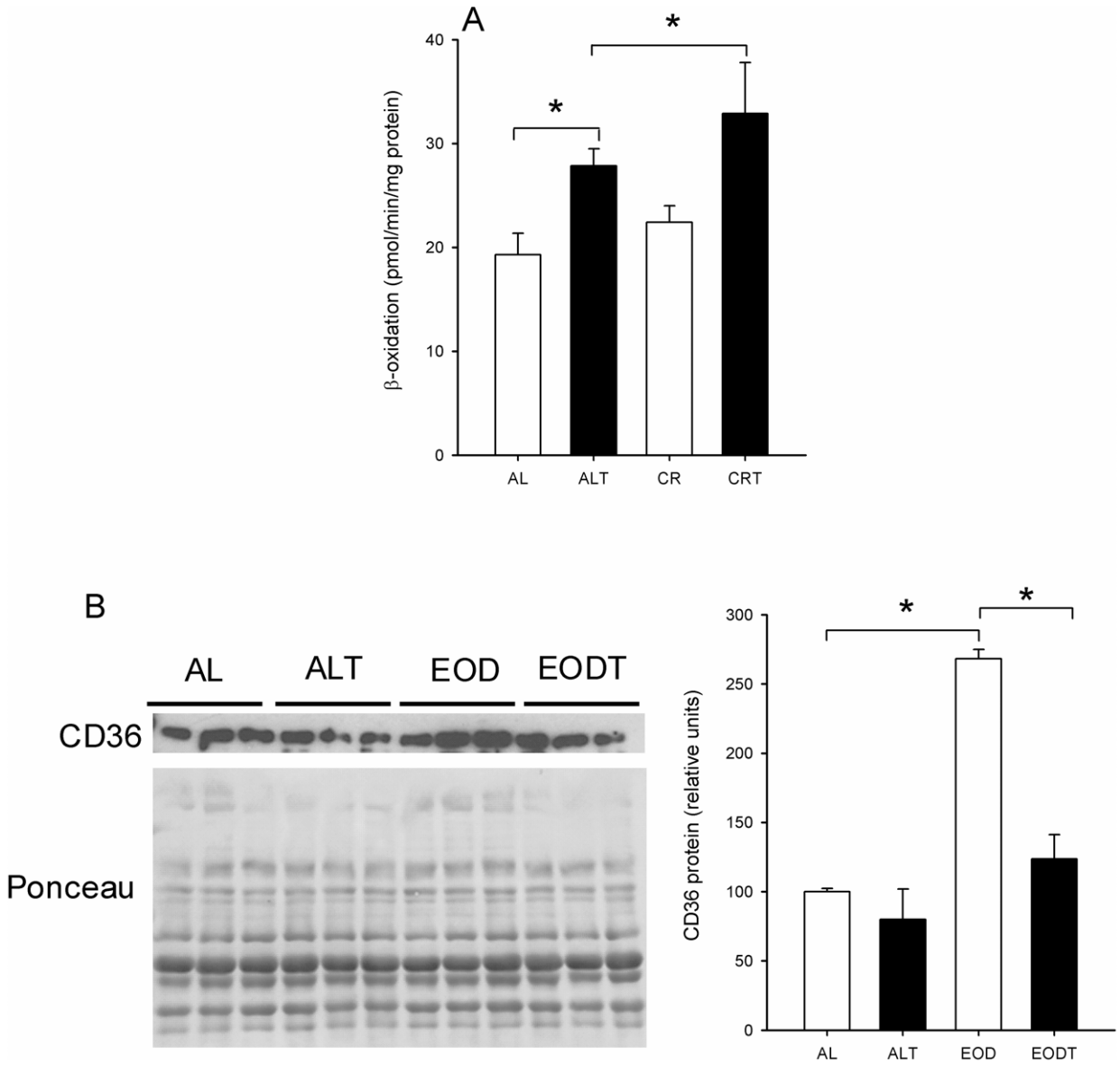


**Figure 5. Lipid catabolism in muscle.** A) β-oxidation in whole muscle homogenate (pmol/min/mg protein). B) Levels of CD36 measured from whole muscle homogenate. Left, CD36 levels measured by immunoblotting and respective Ponceau staining of proteins transferred to membrane. Right, densitometry analysis of CD36 protein levels/protein loading. * Significant differences vs. indicated group, $p \leq 0.05$.
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compared with the AL group. Interestingly, one of the documented changes in rodents fed under CR is the increase of non-forced physical activity when compared with AL fed animals [30,31].

Another interesting issue was the effect of EOD on the capacity of mobilization of nutrients such as sugars and fats. Our study differs from others mainly in the fact that metabolite analysis of blood was performed in animals that were not fasted overnight. Our aim was to determine the physical capacity and associated parameters, thus, we could not submit the animals to strenuous exercise after fasting. Our data indicate that EOD improves the capacity of mobilization of glucose and lipids maintaining the levels of these metabolites during a strenuous exercise. These results suggest that animals under EOD were more efficient in mobilizing nutrients necessary for physical activity. In agreement with our results, a study made on body builders has proved that restriction in calories during a brief period of time (7 days) increases the capacity to mobilize lipids during running [32].

Induction of mitochondrial biogenesis by CR or exercise has been shown in different organisms and tissues [33–35]. We already demonstrated that CR increases mitochondrial biogenesis and modifies activity of mitochondria in liver and cell culture models [36]. However, in the present model of CR we have not found changes in mitochondrial mass determined by CS activity neither notable changes in mitochondrial respiratory chain proteins. This is not due to the EOD model of CR used in our project since, in agreement with our results, no significant changes in CS activity have been also reported in rats fed under classical CR [37]. Further, it has been suggested that only two weeks of CR are able to significantly decrease CS activity and other mitochondrial activities in whole muscle homogenate of Sprague Dawley rats [38]. Furthermore, 40% CR reduces CS activity in rat

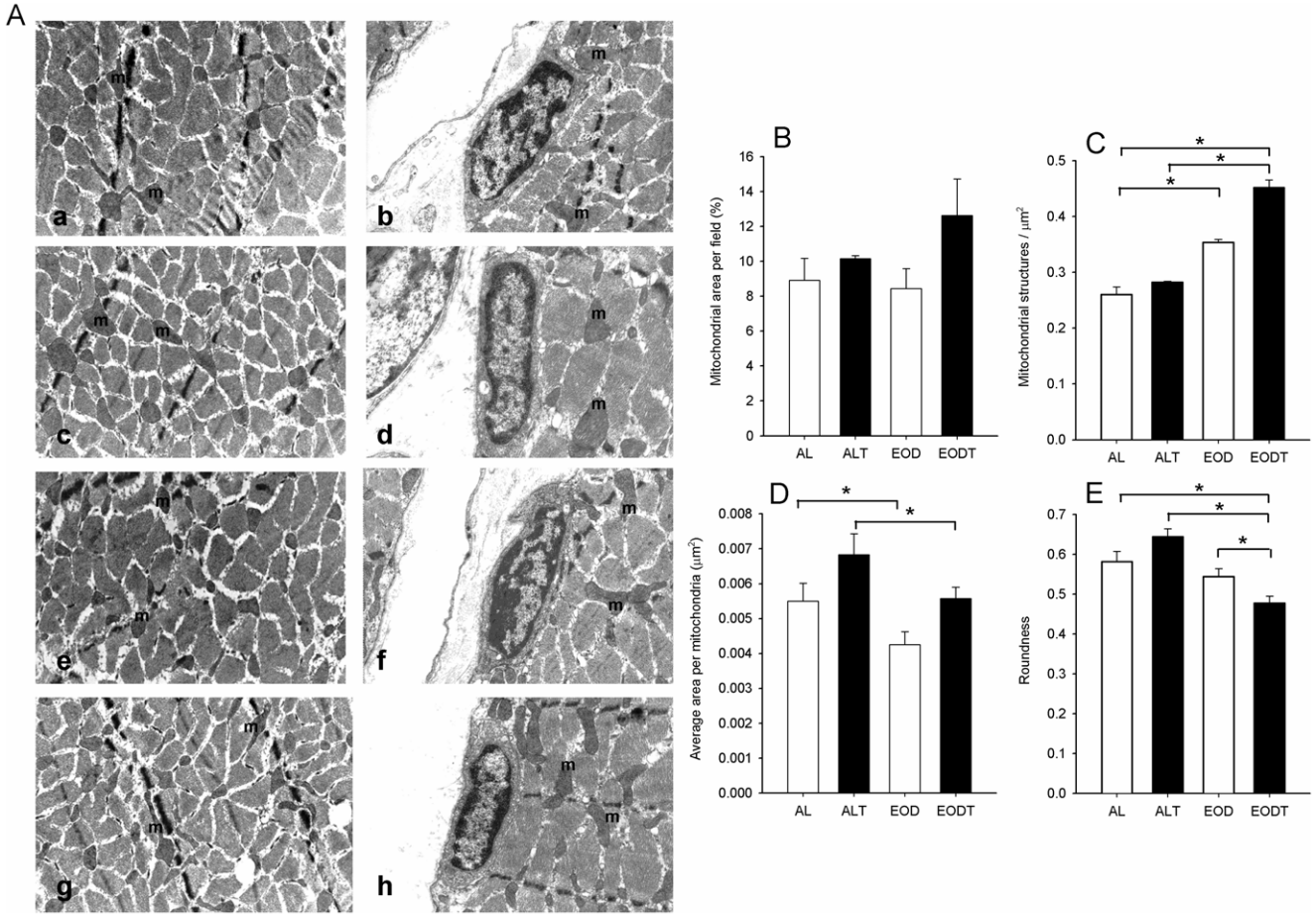


**Figure 6. Mitochondrial structures in gastrocnemius muscle.** A) Electron micrographs of gastrocnemius muscle. Left (a,c,e,g), ×5,200; right (b,d,f,h), a higher detail of mitochondria in gastrocnemius muscle and nucleus of satellite cell, ×8,900; a,b: ad libitum (AL) group, c,d: trained ad libitum group (ALT), e,f: every-other day fed group (EOD), g,h: trained every-other day fed group (EODT); m: indicates the presence of mitochondria. B) Mitochondrial area per field in muscle from each group (n = 10 per group). C) Number of mitochondrial structures per µm2 (n = 10 per group). D) Average area per mitochondria (n>100). E) Roundness of mitochondria (n>100). Error bars indicate SE. * Indicates significant difference vs. indicated group p≤0.05.

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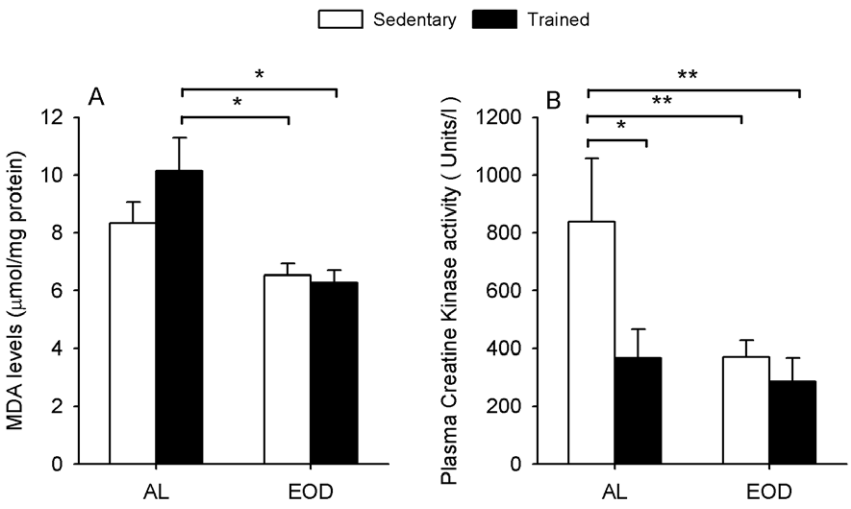


**Figure 7. Levels of damage in muscle.** A) Levels of the marker of oxidative damage MDA in whole muscle membranes. B) Plasma creatine kinase activity as marker of muscle damage after strenuous activity. * Significant differences vs. indicated group, p≤0.05; ** Significant differences vs. indicated group, p≤0.005.

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gastrocnemius muscle [39]. CR also induces the decrease of the activities of complexes I, III and IV especially in young mice [40].

The increase in mitochondrial content is considered a well-established adaptation of muscle to exercise, and is mainly referred as mitochondrial biogenesis. In our hands, neither EOD nor exercise induced changes in the activity and amount of proteins involved in respiratory chain. Similar results have been recently shown in other models of CR and exercise where no changes in porin, a mitochondrial mass indicator, have been reported [41]. In agreement with our results, it has been reported that similar protocols of endurance exercise does not produce changes in β-oxidation in gastrocnemius muscle of mice [42] neither in other activities of the respiratory chain in rat gastrocnemius muscle [43]. These discrepancies can be explained because in most cases mitochondrial biogenesis in muscle is only suggested by the increase of PGC1α mRNA levels. However, recently Civitarese et al. [44], have described that the expression of genes encoding proteins involved in muscle mitochondrial biogenesis is induced by CR and CR plus exercise in healthy humans but at the same time neither CS activity nor β-oxidation and activity of electron transport chain are affected. Furthermore, no effects of CR on mitochondrial activity have been reported in rats besides the increase of the transcription of genes involved in energy metabolism [37]. These results indicate that induction of mitochondrial biogenesis factors are not necessarily accompanied by significant changes in mitochondrial mass or activities. In our experiments, EOD did induced small significant changes in the activity of mitochondrial complexes II and III indicating that the effect of EOD on mitochondrial activity may be due to modifications of the activity of mitochondrial complexes but not by changes in the amount of these complexes. We have previously shown that CR improves the efficiency of mitochondria in cells [36]. In agreement with this suggestion, other reports have suggested that CR preserves the oxidative capacity of muscle by protecting mitochondrial function rather than content [39]. Taken together, small and fine modifications of mitochondrial activities, rather than significant increases of mitochondrial mass, may be enough to maintain a more equilibrated and effective mitochondrial activity in muscle and to enhance their efficiency.

Our results also indicate that both EOD and exercise increase β-oxidation in muscle. Electron microscopy analysis of the mitochondria in gastrocnemius muscle demonstrated that the size, localization and morphology of mitochondria were different in EOD animals. EOD and EODT animals showed more mitochondrial structures in the intermyofibrillar area and around the myofibers. Intermyofibrillar location of mitochondria has been associated with lipid droplets. Thus, these changes in the mitochondria in an intermyofibrillar location in muscle suggest a more efficient activity producing ATP near the sarcomera by using lipid catabolism [45–47]. In agreement with our results, recent works have suggested that changes in mitochondrial morphology affect intracellular energy levels and also mitochondrial activity [48,49]. Furthermore, the levels of the fatty acid translocase (CD36) increased in EOD group indicating a higher capacity to import lipids from plasma and also a higher mitochondrial fatty acid metabolism [50,51]. Further, higher amounts of glycogen and TGs have been also found in muscles of rats after EOD conditions [52] indicating a higher capacity to use internal fuel sources during exercise.

In agreement with previous results, levels of Q were also slightly affected by EOD and exercise [53]. Very interestingly, EOD and training seem to modify the ratio between $Q_9$ and $Q_{10}$ in a different way. Exercise decreased the ratio whereas EOD increased it. These changes probably respond to a more bioenergetic function of $Q_9$ whereas $Q_{10}$ mainly plays an antioxidant role in mice. CR decreases the levels of reactive oxygen species (ROS) in cells [36] and increases Q-dependent reductases activity protecting cell membranes against oxidative damage [54,55]. On the other hand, depending on the intensity, exercise increases free radical production and induces oxidative stress [56,57]. Therefore, under CR conditions, the role of Q must be displaced to benefit bioenergetics instead of antioxidant aspects that are covered by the increase of cellular antioxidant capacity of the cell and then, $Q_9/Q_{10}$ ratio increases [54,55]. On the other hand, the increase of ROS induced by exercise will need more Q-dependent antioxidant protection and then, the ratio decreases. It seems that the equilibrium between Q levels and Q-dependent activities must be important to maintain a balanced muscle activity [58].

EOD protected muscle fibers against oxidative stress even in animals that suffered endurance activity. This higher protection may be related to lower muscle damage after extenuating activity in non-trained EOD animals determined by the levels of creatine kinase in plasma. Our results agree with several other reports that suggest that EOD protects muscle against oxidative stress and even avoids muscle loss during aging [59,60]. This protection is based on lower ROS production by mitochondria probably by the metabolic changes based on the catabolism of lipids [61], by more balanced activity of mitochondria [36] and higher level of Q-dependent antioxidant protection of membranes [55].

In summary, our work indicate that a nutritional stress induced by the EOD model of CR together with a moderate increase of energy expenditure through physical exercise produces metabolic changes that increase the efficiency of mitochondrial activity in muscle, reduces oxidative damage and improves physical performance. Subtle modifications at the cellular and biochemical levels in response to dietary stress seem to be the basis for a higher mitochondrial efficiency. Taking into consideration that the decline in physical activity during age is a common factor in many species, the results shown here suggest that the combination of the reduction of calorie intake and the practice of aerobic exercise would also increase physical performance in humans and then, improve their quality of life.

## Materials and Methods

### Animals

A cohort of 64 non-consanguineous swiss-OF1 male mice aged 6 weeks was used (Animal Services, University of Granada, Granada, Spain). Animals were housed into enriched environmental conditions in groups of 6 animals per polycarbonate cage in a colony room under a 12 h light/dark cycle (8:00 AM–8:00 PM) under temperature (22±3°C) and humidity controlled. Animals were maintained accordingly to a protocol approved by the Pablo de Olavide University Ethical Committee and following the international rules for animal research.

### Caloric restriction and endurance training

Animals were first randomly assigned to two initial groups: half of the animals were fed *ad libitum* (AL) and the other half under CR using the every-other-day feeding model (EOD) [1]. Water was available *ad libitum* for all the groups. After four months under these conditions, each group was randomly subdivided again in a sedentary group and a trained group following a mild forced aerobic exercise protocol. During the first two weeks, a training protocol was performed by a routine increasing both speed and time on a treadmill (Treadmill Columbus 1055M-E50, Cibertec SA) until reaching 20 meters/min and 20 min. This protocol

consisted in the 70–80% of average maximum speed reached in the initial tests. After that, animals were trained at this speed for 20 min/5 days a week during the following 6 weeks similarly to already published endurance exercise protocols [42]. The final animals groups were as follows: AL, *ad libitum* and sedentary group; ALT, *ad libitum* and trained group; EOD, caloric restricted and sedentary group and EODT, caloric restricted and trained group. Weight was determined every 15 days.

## Physical and behavioral activity analysis

All physical activity analysis was carried out between 9–11 hours in the morning and just after a feeding day for the EOD groups. Spontaneous activity was determined by using an open field box (26×39 cm) (Cibertec S.A., Madrid, Spain) determined by the number of broken light beams during a period of 10 min per animal. Peripheral exploration was determined directly by eye in sessions of 5 min as anxiety measure. Also, elevated plus maze test was used to quantify anxiety levels during 5 min per animal. Press force tests were performed by using a Grip Strength (Columbus, Cibertec SA, Spain). For startle response, animals were placed individually inside a startle chamber (Cibertec SA, Spain). The startle response was determined by using a piezoelectric accelerometer controlled by homemade software. Startle stimulus was 100 ms at 125 db and the response is indicated as the average from 20 to 30 recordings. Motor activity and coordination tests were performed on Rotarod (Hugo Bassile, Italy). After an adaptation period, a test was performed to combine coordination and resistance by using Rotarod speed acceleration up to 100 rpm and determining the maximum riding time of animals at this speed.

In the case of extenuating activity, all groups of animals performed a week of habituation before test. Half of the animals from each group (n = 8) were exposed to extenuating physical exercise on treadmill associated with electric stimuli, without inclination and fastening speed by 5 meters/min every 5 min. We established the end of the experiment at the moment the animal stopped for more than 5 seconds under electric stimuli without trying to move back to the treadmill. Animals were sacrificed by cervical dislocation and dissection was performed just after the test was finished. The rest of the animals of each group remained as witnesses without running and were sacrificed at the same time than the runners. These witness animals were considered as controls of the plasmatic metabolic situation of animals before exercise. To avoid any effect of nutrient uptake on extenuating activity performance, all groups received food during the day before the exercise test.

## Analysis of plasmatic compounds

Blood was collected by cardiac puncture just after cervical dislocation. Plasma was obtained by centrifugation in Vacuette Z serum Sep. Clot activator tubes for 10 min at 3000×*g* and stored in small aliquots kept at −80°C until the determination of different blood metabolites. Metabolites were analyzed by using commercial kits for triglycerides (Randox TR210), cholesterol (Randox CH201), urea (Randox UR457), uric acid (Randox UA233), L-lactate (Randox, LC2389), glucose (Randox GL2623), total protein (Randox TP245) and albumin (Randox, AB388). Creatine kinase (CK) activity was analyzed by the CK-NAC (Randox, CK113) test. In all cases, samples were always processed in parallel with the respective quality controls provided by the supplier.

## Muscle mitochondrial activities

Gastrocnemius muscles were dissected immediately after sacrifice and frozen in liquid nitrogen. After thawing and clearing from connective tissue, muscle was homogenized by using lysis buffer (2 mM Tris-HCl, 20 mM Hepes, 1 mM EDTA, 70 mM sucrose and 220 mM Mannitol) supplemented with 1 mM PMSF and protease inhibitor cocktail 1:50 (Sigma) in a 1:9 volume-to-weight ratio, followed by centrifugation for 10 min at 700×*g* to eliminate debris and nuclei. Protein was determined by Stoschek-modified Bradford's method [62]. Activities are indicated as nmol/min/mg protein.

Mitochondria chain complexes activities were measured in a spectrophotometer (Thermo Spectronic Unicam UV 500). Complex I was measured in 20 mM phosphate buffer pH 8.0 by kinetic quantification of 0.2 mM NADH consumption at 340 nm for 2–3 min at 30°C after adding 1 mM $CoQ_1$ as electron acceptor ($\varepsilon_{340}$: 6.81 $mM^{-1}$ $cm^{-1}$). Complex II was measured in 50 mM phosphate buffer pH 7.0 by kinetic quantification of the reduction of 0.1 mM diclorophenol-indophenol (DCPIP) at 600 nm for 2 min at 30°C after adding 32 mM succinic acid to the reaction ($\varepsilon_{600}$: 19 $mM^{-1}$ $cm^{-1}$). Complex III was determined in 50 mM phosphate buffer pH 7.5 by kinetic quantification of the reduction of the cytochrome C (0.05 mM) at 550 nm for 2 min at 30°C after adding decylubiquinol (0.05 mM) as electron supplier to the reaction ($\varepsilon_{550}$: 21 $mM^{-1}$ $cm^{-1}$). Complex IV was measured in 10 mM phosphate buffer pH 7.0 by kinetic quantification of oxidation of reduced cytochrome C as electron donor at 550 nm for 2 min at 38°C using oxygen as electron acceptor ($\varepsilon_{550}$: 21 $mM^{-1}$ $cm^{-1}$). The combined activities of complex I+III and II+III were determined in 50 mM phosphate buffer pH 7.5 by determining the kinetics of the reduction of cytochrome C for 2 min at 30°C by adding NADH (0.1 mM) (complex I+III) or succinic acid (3 mM) (complex II+III) as electrons donors ($\varepsilon_{550}$: 21 $mM^{-1}$ $cm^{-1}$). For each determination, specific inhibitors such as rotenone (Complex I, 5 µM), actimycin A (Complex III, 1 µg/ml), sodium azide (Complex IV, 1–2 mM) and ferric cyanide (Complex IV, 1.5 mM), were used as previously indicated [63].

Complex V/ATPase activity was determined by the measurement of its ATP phosphatase activity. Assay was performed in Hepes-Mg pH 8.0 (50 mM) by adding NADH (0.2 mM), phosphoenol pyruvate (2.5 mM), pyruvate kinase (10 mg/ml), lactate dehydrogenase (5 mg/ml) and antimycin A (0.2 mg/ml). After 2 min of incubation, ATP (25 mM) pH 7.0 was added ($\varepsilon_{340}$: 6.22 $mM^{-1}$ $cm^{-1}$). Inhibition with oligomycin (0.2 mg/ml) was used to specifically determine mitochondrial ATPase activity.

Citrate synthase (CS) activity was determined in 75 mM Tris-HCl buffer (pH 8.0) by quantification of ditio-bis-nitrobenzoate (0.1 mM) reduction in the presence of Triton X-100 1%, acetyl CoA (7 mg/ml) and oxalacetate (5 mM) at 412 nm for 2 min at 30°C ($\varepsilon_{412}$: 13.6 $mM^{-1}$ $cm^{-1}$).

## Measurement of β-oxidation Activity

Palmitate oxidation was measured as an indicator of β-oxidation [64]. Briefly, the reaction mixture contained 50 ml Tris/HCl, pH 8.0, 40 mM NaCl, 2 mM KCl, 2 mM $MgCl_2$, 1 mM DTT, 5 mM ATP, 0.2 mM L-carnitine, 0.2 mM NAD, 0.6 mM FAD, 0.12 mM CoA, 0.1 µCi [$^{14}$C]palmitic acid, and 100 µg of whole muscle homogenate in a final volume of 200 µl. The reaction was initiated by adding substrate and incubated at 37°C for 60 min. Reaction was terminated by adding 200 µl of 0.6 N perchloric acid followed by centrifugation. The resulting supernatant was extracted three times with 800 µl of N-hexane to remove any remaining palmitate and radioactivity of the aqueous phase was measured.

## Coenzyme Q determination

One to 1.5 mg of protein of whole muscle homogenates were resuspended in 500 µl of PBS 1x and incubated with SDS (1%

final concentration) for 10 min followed by a vigorous shake with vortex. Q was extracted with hexane as indicated [63]. Briefly, 2 volumes of ethanol:isopropyl alcohol (95:5) were added to SDS solution and mixed with vortex for 1 minute. The organic phase was recovered by the addition of 4 volumes of hexane, shaking for 1 min in vortex and centrifugation at 1,000×*g* for 5 min at 4°C. The higher phase containing Q was recovered and extraction was repeated. Hexane phase was dried by using a Rotavapor and residue resuspended in 1 ml of ethanol HPLC grade. Ethanol was dried again by using a Speed Vac and residue was kept at −20°C until determination. After dissolving again in ethanol, Q levels were determined by using a HPLC equipped with a Spherisorb C-18 column at a flow of 1 ml/min and a UV/Vis Beckman detector and an Electrochemical ESA Coulochem III. $Q_6$ and decylubiquinone were used as internal controls. Q concentrations are indicated as pmol/mg protein.

### Western blotting

Whole muscle homogenate was obtained after disruption in lysis buffer as above indicated by using a mechanical driven homogenizer and further centrifugation at 700×g to remove debris. Supernatant was mixed with two times concentrated Laemmli Buffer (Santa Cruz Biotechnology, USA) and proteins separated by SDS-PAGE. Proteins were transferred to Hybond ECL nitrocelullose membranes (GE-Healthcare, USA) and visualized by using the mouse monoclonal antibodies against the 39 kDa subunit of NADH:ubiquinone oxidoreductase (clone 20C11, complex I), 70 kDa subunit of Succinate:ubiquinone oxidoreductase (clone 2E3, complex II), core 2 subunit Ubiquinol:cytochrome c oxidoreductase (clone 13G12, complex III), subunit Vb of the Cytochrome c oxidase (clone 16H12, Complex IV) and α-subunit of ATP synthase (clone 7H10, complex V) (Invitrogen-Molecular Probes, USA) at 1:1000 dilution and HRP-labeled anti-mouse IgG at 1:1000. Blots were visualized by ECL technique and quantified by using the Quantity One 1-D analysis software (Biorad). Blots were digitalized and quantified by using Quantity One software (Biorad). Protein expression levels were corrected for whole protein loading determined by staining membrane with Red Ponceau.

### Measurement of Malondialdehyde levels (MDA)

MDA levels were measured according to the method of Gérard-Monnier et al. with some modifications [65]. Briefly, the reaction mixture contained 6.6 mM N-methyl-2-phenyl-indol (MPI), 0.015 mg/ml butylated hydroxytoluene and 5.55% HCl. The assay was initiated by the addition of sample (1–1.5 mg) and incubated at 45°C for 45 min. To determine the amount of MDA, known concentrations of 1,1,3,3-tetra-ethoxypropane (malondialdehyde bis(diethyl-acetal) (0–20 nmol) were used.

### Electron microscopy

Freshly isolated muscle samples were fixed with 2.5% Glutaraldehyde in PBS and further with 1% Osmium Tetroxide, dehydrated with acetone and embedded in araldite resin. Sections (50 nm) were obtained by using an ultramicrotome (LKB 8800 Ultrotome III). After placing onto a cooper grid, slices were stained for 5 min with uranyl acetate and lead citrate followed by 2 min incubation with lead citrate. Samples were visualized by using a transmission electron microscope (Philips CM10). Ten random images (×5,200) were analyzed to measure the area occupied by mitochondria, the number of mitochondrial structures per unit of area, the area per mitochondria (at least 10 mitochondria per image) and the roundness of each mitochondrial structure by using the ImageJ software version 1.42i (National Institutes of Health, United States).

### Statistics

SigmaStat 3.5 program was used for the statistical analysis and figures were performed by using SigmaPlot 10.0 program (Systat Software Inc). All data are expressed as means ± S.E. For all experiments, 16 mice per group were analyzed. The information obtained from each group was statistically processed pursuant to the most suitable technique for each case. Student t-test or two ways Analysis of Variance (ANOVA) followed by Post-Hoc pairwise multiple comparison procedures (Bonferroni t-test) were performed. The critical significance level α was = 0.050 and, then, statistical significance was defined as $P<0.05$.

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## Author Contributions

Conceived and designed the experiments: GLL. Performed the experiments: ERB SSCC FL JPA FJBdlR MC PN GLL. Analyzed the data: ERB SSCC FL JPA FJBdlR MC GLL. Contributed reagents/materials/analysis tools: ERB SSCC FL JPA FJBdlR PN GLL. Wrote the paper: ERB MC PN GLL.

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