Supplementary file 1

Table S1. Inclusion criteria for participants

Sex	Male
Age	18-35 years
Performance level	Well trained; with $VO2_{max}$ 55-64,9 ml/kg/min; performance level 3 or more (1)
BMI	BMI 19-25 kg/m^2; in normal range for adult males) and body fat 5-20 %
Body Fat Percentage	5-20 %
Health status	1. No acute disease or chronic disease in relapse (allowing only for chronic diseases that are stable and not affecting performance) 2. At the time of procedures be free of injuries and no injuries in previous three months that could affect performance
Additional criteria	Stable body mass for the last 12 months Not undertaking and specific diet regime At the time of procedures will refrain from alcohol consumption and any drug or other substance use Complete all procedures and report any factors that could influence changes in blood values or performance (lack of motivation due to psychological factors, factors in between measurements that could influence results etc.)

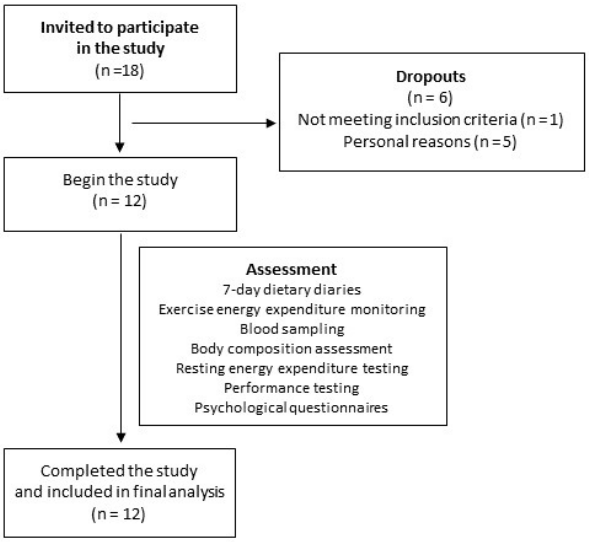

Figure S1: Flowchart of enrolment

1. De Pauw K, Roelands B, Cheung SS, de Geus B, Rietjens G, Meeusen R. Guidelines to classify subject groups in sport-science research. Int J Sports Physiol Perform [Internet]. 2013 Mar [cited 2018 Nov 6];8(2):111–22. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23428482

Supplementary file 2

Performance tests

To test performance, three different tests were chosen to assess vertical jump height (explosive power of lower extremities), motor task execution time (agility) and maximal aerobic capacity (aerobic endurance). The details of warm-up protocol, countermovement jump (CMJ), agility t-test and the incremental test can be found in the supplementary file.

Performance tests were performed in this exact order for each participant following the general warm up consisting of 2 min of cycling on a stationary bike at a 50 revolutions per minute at approximately 1.5 W/kg. Then they performed arm, hip, knee and ankle mobility exercises (10 reps each); dynamic stretches of hip flexors, knee extensors, knee flexors and ankle extensors (10 reps each); and heel raise, squat, crunch resistance exercises (10 reps each).

Countermovement jump (CMJ) test was performed using a bilateral force plate system (Type 9260AA, Kistler Instrumente AG, Winterthur, Switzerland) with Kistler MARS software (S2P Ltd., Ljubljana, Slovenia) to acquire ground reaction force. Each subject performed three to five maximal counter movement jumps before the testing. For CMJ data were sampled at 1000 Hz, filtered using a moving average filter with 50-ms window and analyzed using the built-in module for CMJ. Test execution was supervised from the experienced researcher to improve proficiency in jumping technique [1]. Before each jump, participants were instructed to stand up straight and still on the center of the force plate with their hands akimbo. This hand position remained the same during the entire movement. From this position, participants initiated a fast downward movement until a crouching position with a knee angle of about 90°, followed by a jump for maximal height as quickly and explosively as possible. Three valid trials were performed with one-minute recovery period. The main outcome measure was CMJ height in centimeters that was calculated from the maximum velocity [2].

To asses motor task execution time, validated modified agility t-test was used, as described by Haj-Sassi, et al. (2011). The T-test was performed after 5 minutes rest from vertical jump testing without additional warm up. Each participant had three trials (each trail consisting of two sprints separated by 30 seconds rest). The rest between trials was 90 seconds. Cones were placed in a T-shape layout, 5 and 2.5 m apart, respectively. Subjects were instructed to sprint and change direction as fast as possible. They began with both feet 0.3 m behind the starting line (A). At their own discretion subject sprinted forward to cone B and touched the base of it with the right hand. Facing forward and without crossing feet, they shuffled to the left to cone C and touched its base with the left hand. Subjects then shuffled to the right to cone D and touched its base with the right hand. They shuffled back to the left to cone B and touched its base. Finally, subjects ran backward as quickly as possible to cross the finish line (cone A) ending the first sprint. The second sprint started after 30 seconds rest. Three such trials separated by 90 seconds rest were performed, all with verbal encouragement. The subject who crossed one foot in to each direction (firstly starting with shuffling to the left) front of the other, failed to touch the base of the cone or failed to face forward throughout, had to repeat the test. The time to complete each repetition was measured using one pair of the electronic timing system sensors (Witty Timing System, Microgate, Bolzano) mounted on tripods. They were set approximately 0.75 m above the floor positioned 2 m apart facing each other on either side of the starting line (A). The time of best repetition (seconds) and three repetition average were used in further analysis.

After 1 hour of rest, endurance was measured with the incremental test to exhaustion. Heart rate, ventilatory, and gas data were collected during the incremental test with metabolic cart (K5, Cosmed, Italy). All measurements were performed in the physiological laboratory with ambient temperature of 21°C. For measuring VO_{2max}, the following procedure was performed. After a 15-minute warm-up on bicycle set up on a cycle ergometer (Cyclus 2, Leipzig, Germany), workload constantly increased until volitional exhaustion (100 W + 20 W every minute). Lactate was analyzed in capillary blood drop from the earlobe. Samples were obtained at rest before any physical activities, at the end of the test and 5 minutes after the test. Lactate was analyzed with the blood lactate analyzer Biosen C_Line (EKF Diagnostics, Germany).

Blood samples

All blood samples were drawn in the morning at 9am in a fasted state *to assess* complete blood count, ferritin, serum iron (Fe), triiodothyronine (T3), thyroid stimulating hormone (TSH), morning testosterone, fasting insulin, insulin like growth factor 1 (IGF-1) and 9am cortisol. Blood was collected using standard clinical procedures. Haemoglobin was analysed with Sysmex XN-550 (photometric detection, EDTA tubes), iron with Cobas c501 (colorimetric analysis, serum tubes), ZSH, T3, testosterone, cortisol and ferritin with Cobas e411 (electrochemiluminescence immunoassay, serum tubes). Serum insulin level was analyzed with a double antibody RIA (serum tubes) and for IGF-1 the RIA kit (serum tubes) was used.

Body composition assessment

Body composition was assessed using tetra polar eight-point tactile bioelectrical impedance device InBody 720 (Biospace, Seul, South Korea). Participants were instructed how to be adequately hydrated prior measurement.

Resting energy expenditure assessment

REE was measured with indirect calorimetry (V2 mask (Hans Rudolph, USA), K5 (Cosmed, Albano Laziale, Rome, Italy) with Quark 8.1. PC software support) based on the Weir equation [4,5], for 30 minutes in a thermoneutral environment, in silence, between 6.00 and 9.00 a.m. The final 20 min were used for REE analysis [6]. For predicted REE (pREE) calculation, the Harris-Benedict equation was used [7]. The mREE/pREE ratio was then calculated to assess energy conservation.

Psychological assessment

The TFEQ-R18 and Well-being questionnaire were used [8,9]. TFEQ-R18 and its subscale, cognitive restriction, were used to look for changes in eating behaviours as used in similar research setting before. General well-being was assessed by a simple questionnaire as recommended by Hooper and Mackinnon [10] including six subjective ratings (fatigue, sleep, stress, muscle soreness, mood and morning erections) on a 1-5 scale. The last item about morning erection was to the original set as proposed by study on professional rugby players to assess functional testosterone effect [11] (Table S2).

Table S2: Well-being subjective questionnaire with scoring system

	5	**4**	**3**	**2**	**1**
FATIGUE	very fresh	fresh	normal	more tired than normal	always tired
SLEEP QUALITY	very restful	good	difficulty falling asleep	restless sleep	insomnia
GENERAL MUSCLE SORENESS	feeling great	feeling good	normal	increase in soreness/tightness	very sore
STRESS LEVELS	very relaxed	relaxed	normal	feeling stressed	highly stressed
MOOD	very positive mood	a generally good mood	less interested in others &/or activities than usual	snappiness at teammates, family and co-workers	highly annoyed/ irritable/ down
MORNING ERECTIONS	>5 per week			<2 per week	almost never/not present

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3. Haj-Sassi, R.; Dardouri, W.; Gharbi, Z.; Chaouachi, A.; Mansour, H.; Rabhi, A.; Mahfoudhi, M.-E. Reliability and Validity of a New Repeated Agility Test as a Measure of Anaerobic and Explosive Power. *J. Strength Cond. Res.* **2011**, *25*, 472–480, doi:10.1519/JSC.0b013e3182018186.

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Supplementary file 3

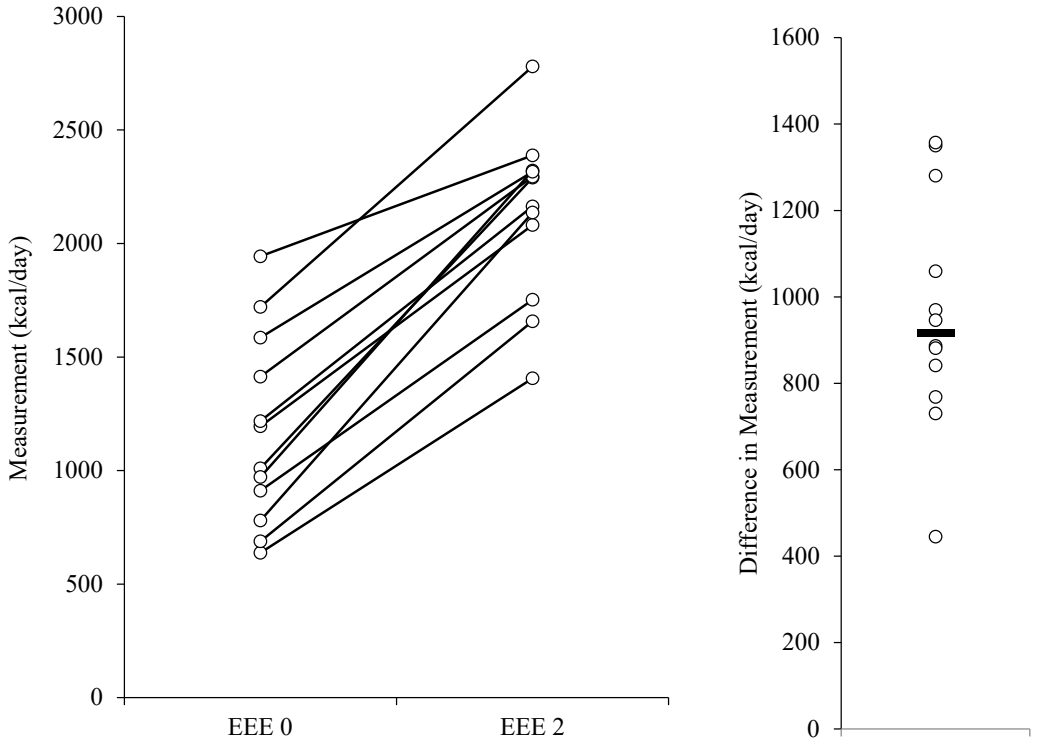

Figure S2: Differences in EEE before intervention and during the 50% EA reduction state

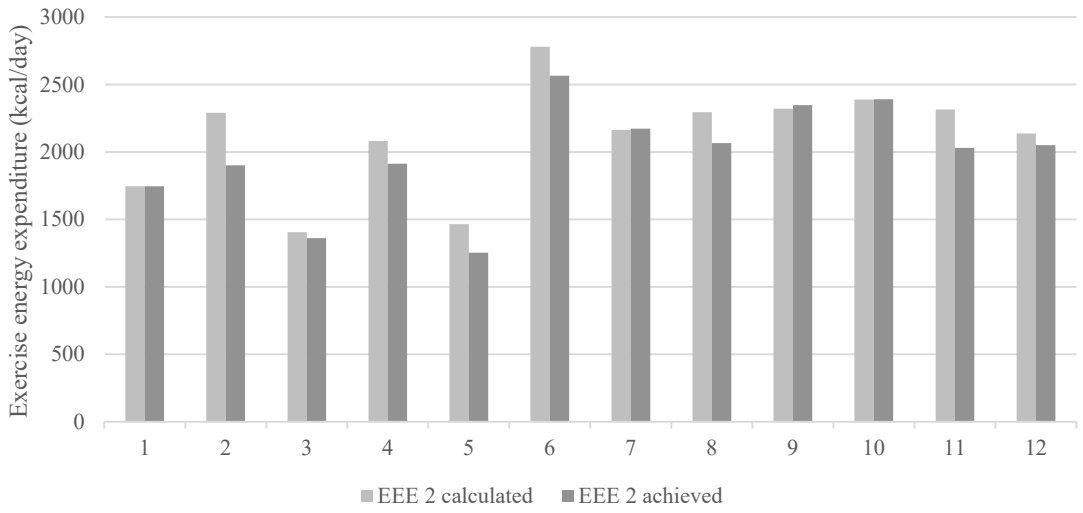

Figure S3: The calculated EEE for 50% EA reduction compared to the achieved EEE for each of the 12 subjects

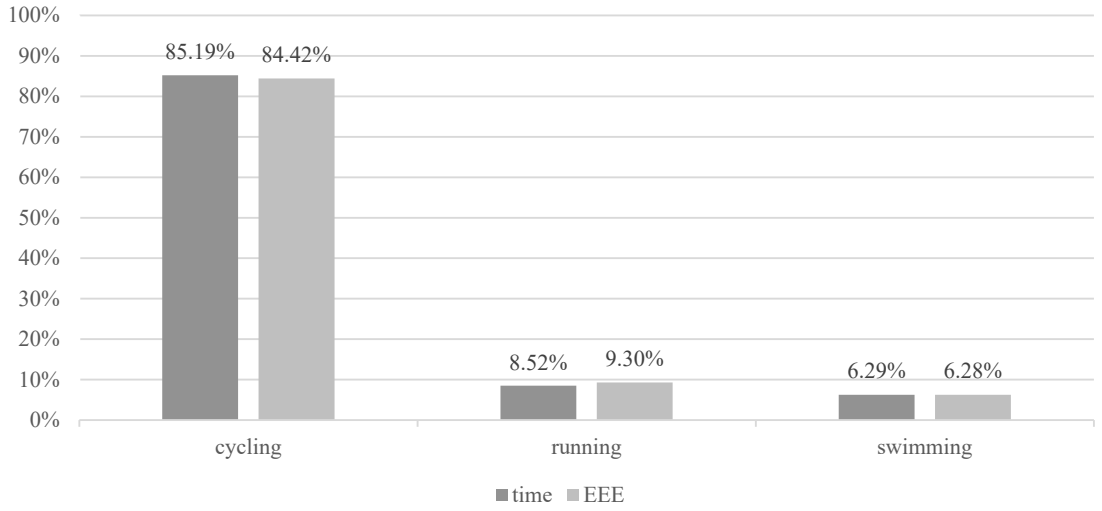

Figure S4: Time and EEE divided into training modalities as measured in 50% EA reduction intervention

Table S3: Subjective assessment of 50% EA reduction from all 12 participants

1	too tired to finish, hungry
2	in the second half he felt he was going to be ill, got a migraine, did not have a fever, did not exhibit any other signs. He missed 3 days of trainings due to fatigue.
3	no problems
4	fatigue, hard legs, empty energy levels, inability to raise HR
5	pain in shoulder (at the site of previously healed injury), inability to achieve EEE levels entirely
6	headache, migraine, inability to raise HR
7	managed to achieve all training load requirements but felt hungry, with low energy storage, noticed reduced HR
8	sore throat, felt he is about to get sick, tired, hunger slightly present
9	very hungry
10	very fatigued, got sick with high fever. Had to abort the protocol and restart after another washout period; very hungry, irritable
11	very fatigued, back pain started in the middle of running at the beginning of week 2, was not so hungry, but was unmotivated, his mental preparation was "not right"
12	training was hard especially in the second week, he had difficulties because sugar was not allowed during training in unlimited quantities, noticed difficulties to reach EEE because of lower HR