Manipulation of Muscle Creatine and Glycogen Changes Dual X-ray Absorptiometry Estimates of Body Composition

Manipulation of muscle creatine & glycogen changes DXA estimates of body composition

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Abstract

Standardising a dual x-ray absorptiometry (DXA) protocol is thought to provide a reliable measurement of body composition.

Purpose: We investigated the effects of manipulating muscle glycogen and creatine content independently and additively on DXA estimates of lean mass.

Method: Eighteen well-trained male cyclists undertook a parallel group application of creatine loading (n=9) (20 g/d for 5 d loading; 3 g/d maintenance) or placebo (n=9) with crossover application of glycogen loading (12 v 6 g/kg BM/d for 48 h) as part of a larger study involving a glycogen-depleting exercise protocol. Body composition, total body water, muscle glycogen and creatine content were assessed via DXA, bioelectrical impedance spectroscopy and standard biopsy techniques. Changes in the mean were assessed using the following effect-size scale: >0.2 small, >0.6, moderate, >1.2 large and compared with the threshold for the smallest worthwhile effect of the treatment.

Results: Glycogen loading, both with and without creatine loading, resulted in substantial increases in estimates of lean body mass (mean ± SD; 3.0 ± 0.7 % and 2.0 ± 0.9 %) and leg lean mass (3.1 ± 1.8 %and 2.6 ± 1.0 %) respectively. A substantial decrease in leg lean mass was observed following the glycogen depleting condition (-1.4 ± 1.6 %). Total body water showed substantial increases following glycogen loading (2.3 ± 2.3 %), creatine loading (1.4 ± 1.9 %) and the combined treatment (2.3 ± 1.1 %).

Conclusions: Changes in muscle metabolites and water content alter DXA estimates of lean mass during periods in which minimal change in muscle protein mass is likely. This information needs to be considered in interpreting the results of DXA-derived estimates of body composition in athletes.

Introduction

Dual x-ray absorptiometry (DXA) is recognised as a criterion technique for the measurement of body composition and has become a routine part of the preparation and monitoring of athletes (29). Strategies which improve the precision of measurement can have real-life importance in sports nutrition; we have previously shown that the use of a standardised protocol which allowed the detection of small but worthwhile changes in total lean body mass and body fat that would have otherwise been missed if measured under non-standardised conditions (28). Although the current recommendations for standardizing DXA scanning protocols aim to reduce the error/variability associated with gastrointestinal content from recent meals, general hydration status and fluid shifts associated with exercise (25, 27), we have proposed that alteration of intramuscular solutes (e.g., glycogen, creatine, carnosine) and their associated water binding may cause another source of biological variation. Indeed, even with the implementation of a Best Practice Protocol, we sometimes observe within-athlete differences in lean body mass estimates of up to 2 kg over an acute time frame, which are unlikely to be explained by real changes in muscle mass.

It has previously been shown that changes in cellular substrates achieved by common practices in sports nutrition can cause detectable changes in muscle size and mass. For example, an investigation using Magnetic Resonance Imaging (MRI) showed increases in muscle cross-sectional area following a carbohydrate loading diet (30). Similarly, a 10-day creatine loading protocol in untrained individuals was shown to increase body mass and DXA estimates of lean body mass (35). A recent study reported an increase in the DXA estimate of lean mass in healthy males following the intake of a high carbohydrate in the three days prior to a DXA scan (34). However, how the variety of changes in muscle solutes and water content commonly experienced by athletes interact to alter estimates of muscle mass has not been considered. Therefore, it is of interest to undertake a systematic investigation of the

variability in DXA measurements of body composition that can be attributed to acute changes in muscle creatine, glycogen and their effect on total body water. We undertook such an investigation, within a larger study of creatine and glycogen loading, with the aim of further refining Best Practice Protocols for body composition assessment by DXA and/or allowing better interpretation of the results. We hypothesized that activities that increased muscle solutes and water would create an artefact in measurement of body composition by increasing the estimate of lean body mass, while depletion would be associated with a decrease in the estimate of lean mass.

Methods

Participants:

Eighteen competitive male cyclists (age 31.4 ± 5.6 yr; body mass 78.2 ± 8.8 kg; height 182.7 ± 7.2 cm; VO_2max 65.2 ± 7.1 ml/kg/min) participated in this study which was approved by the human research ethics committees of the Australian Institute of Sport (20140612) and the Australian Catholic University (2014 254N). Participants were informed of protocols and risks of the study before providing written informed consent.

Study Design:

This study, which was part of a larger investigation of creatine and glycogen loading on cycling performance, employed a parallel group design to investigate the effect of creatine loading, followed by a within-group cross-over application of carbohydrate loading.

All participants underwent baseline measurements on day 0, followed by tests in the Glycogen Depleted state on day 1. Following Day 1 measurements, participants were

randomized into either the creatine loading or placebo group and returned for two subsequent testing days one week apart (day 7 and day 14) (see fig 1).

Creatine and Glycogen Loading:

Creatine loading was achieved by intake of 20 g/d of creatine monohydrate (Musashi Creatine Monohydrate, Vitaco, NSW, Australia) for five days using a split dose regimen (4 x 5 g/d, consumed at the same time as a carbohydrate-containing meal or snack) followed by creatine maintenance (3 g/d) (13). Normalised glycogen stores were achieved by consuming a pre-packaged diet providing a carbohydrate intake (6 g/kg/d) for 48 hr as well as imposing a standardised training protocol including a rest day prior to the DXA scan. Meanwhile, glycogen loading was achieved by providing a pre-packaged diet providing 12 g/kg/d of carbohydrate for the same standardised time period (5). Hydration status was standardised by implementing a standardised fluid intake for the 24 h period prior to the DXA scans. Glycogen depletion was achieved by undertaking a cycling protocol in the laboratory lasting ~ 3 h 30 min, with consumption of a pre-packaged low carbohydrate diet following completion of the protocol until the next morning's DXA scan.

The achievement of these protocols provided scenarios to reflect normal-creatine normal-glycogen (Baseline; n = 18), normal-creatine glycogen-depleted (n = 18; Glycogen Depleted), creatine-loaded glycogen-loaded (n=9; Creatine-Glycogen Loaded), normal-creatine glycogen-loaded (n = 9; Glycogen Loaded), and creatine-loaded normal-glycogen (n = 9; Creatine Loaded).

Dietary Standardisation:

An individualised two day menu was constructed for each participant using FoodWorks Professional Edition, Version 7.0 (Xyris Software, Brisbane, Australia) based on their body

mass and food preferences. Prior to the baseline trial, subjects received a moderate-carbohydrate diet providing 6 g.kg-1BM/d carbohydrate; 1.5 g/kg/BM/d protein; 1.5 g/kg-/BM/d fat, with a total energy goal of ~215 kJ/kg/BM per day. The participants were then randomised to receive either a repeat of the moderate-carbohydrate diet (6g.kg-1BM/d) or a carbohydrate-loading diet (12 g/kg/BM/d) in the two days prior to the Glycogen Loaded and Glycogen Normal trials (Day 7 and Day14) in a cross-over allocation. These dietary treatments were implemented using a placebo-controlled design, whereby the overall menu for the day was kept constant, but key items were provided either as a low-kilojoule/low carbohydrate option or an indistinguishable carbohydrate-enriched/high kilojoule. Protein and fat intake each remained constant at 1.5 g/kg/BM/d in these diets, but energy intake was increased in the carbohydrate-loading diet (~320 kJ/kg/d). Participants refrained from any intake of alcohol during the dietary standardisation period. Caffeine and fluid intake was allowed ad lib two days prior to the baseline trial and up to two standard serves (e.g. 1 cup of coffee or 1 can caffeinated soft drink) the day before the experimental trial. Participants recorded their caffeine and fluid intake and this was repeated during the dietary standardisation period of subsequent trials. Following the glycogen depleting exercise (Day 0), participants were fed a pre-packaged standardised low carbohydrate diet (<1 g/kg/BM) for the reminder of the day to minimise resynthesis of muscle glycogen stores. Subjects were provided with all foods and most of their fluids in a standardised menu in portion controlled packages, and were given verbal and written instructions on how to follow the diet. Checklists were used to record each menu item as it was consumed, and to note any deviations from the menu. An analysis of all the actual diets consumed by participants was undertaken on completion of the study using the same software.

Muscle Biopsy:

Each participant underwent 4 biopsies over the course of the study, with each being collected from the same leg from an incision that was as least 2 cm from the previously biopsied site. All biopsies were conducted by medical practitioners using a 5-mm Bergstrom needle modified with suction (9). The site was anesthetised using 1% xylocaine prior to an incision being made through the dermal layer and facia on the quadriceps. Muscle tissue was immediately frozen in liquid nitrogen and stored at -80°C for later analysis.

Biochemical Analysis:

Muscle creatine and glycogen concentrations were measured as described previously (6, 12). Glycogen concentrations were determined via enzymatic analysis with fluorometric detection (Jasco FP-750 spectrofluorometer, Easton, MD) at excitation 365 nm/emission 455 nm. Concentrations were expressed as millimoles of glycogen per kilograms of dry weight (mmol/kg dw). Muscle tissue was analyzed in duplicate for free creatine, creatine phosphate, and adenosine triphosphate (ATP) using fluorometric techniques. Total creatine was measured as a sum of free creatine and creatine phosphate (13).

DXA and Total Body Water Protocol:

For each of the four different conditions, participants reported to the laboratory in the morning after an overnight fast and undertook a total body DXA scan as per a standardised protocol (29). Body composition was assessed using a whole body scan on a narrowed fan-beam DXA (Lunar Prodigy, GE Healthcare, Madison, WI) with analysis performed using GE Encore 12.30 software (GE, Madison, WI). The DXA technical error of measurement (TEM) was ~ 0.1% for total mass, 0.4% for total lean, 1.9% for total fat and 0.7% for total bone mineral content (25). Following 15 min of rest, total body water and fluid compartments were assessed using Bioelectrical Impedance Spectroscopy (BIS) (IMP SFB7, ImpediMed Limited, Queensland, Australia) and analysed using BioImp Analysis 5.4.0 Software

(ImpediMed Limited, Queensland, Australia) according to the protocol described by Moon et al. (24). The BIS has a TEM of 0.81L. Hydration status was monitored by measurement of urine specific gravity (UG-a, Atago Refractomer, Japan) from a sample collected upon waking.

Statistical Analysis:

We used a mixed linear model (Proc Mixed in version 9.4 of the statistical Analysis System; SAS Institute, Cary, NC) to estimate the effect of the treatments on muscle glycogen concentration, muscle creatine concentration, the mass of each component of body and leg composition, and the mass of intracellular, extracellular and total body fluids. Treatment was a fixed effect in the model (nominal, with six levels), while random effects were the athlete identity and its interaction with dummy variables to estimate error additional to the residual (individual responses) to glycogen depletion, glycogen loading, creatine loading, and combined glycogen and creatine loading. All dependent variables were log transformed for analysis. The smallest important change was determined as per Nana et al. (26). by using the default approach of standardization with an appropriate between-subject standard deviation, here the baseline standard deviation. The magnitudes of changes the resulting effects were assessed using the following scale:, <0.2 trivial, >0.2 small, >0.6 moderate, >1.2 large (14). Small or larger changes were considered substantial when the threshold for the small effect was reached (≥0.2). Uncertainty in the changes is shown as expressed by 90% confidence limits when the upper and lower confidence limits represented substantial increases and decreases, respectively. Owing to the considerable number of effects investigated, the effects were assessed as clear or unclear using 99% confidence limits. All other effects were deemed clear, and shown with the probabilities that the true effect was a substantial decrease, a trivial change, or a substantial increase.

Results

Baseline values and percentage changes with the different treatments are presented in a series of tables: total body composition (Table 1), leg regional body composition (Table 2), body water (Table 3) and muscle glycogen concentrations (Table 4).

Body mass (Table 1): There was a substantial increase in body mass in the combined Creatine–Glycogen Loaded treatment compared to baseline, the observed effect being small. Changes in the separate Glycogen Loaded and Creatine Loaded treatments on body mass were clearly not substantial. Additionally, there was no substantial change in body mass following the Glycogen Depleted condition.

Lean Mass (Table 1 and 2): There were substantial increases in lean body mass following the Creatine-Glycogen Loaded and the sole Glycogen Loaded treatments compared with baseline measurements, with the observed effects being small. Similar results were observed for leg lean mass with a small but substantial increase with both treatments. The was no substantial decrease in lean body mass following the Glycogen Depleted condition but there was a substantial decrease in leg lean mass. The effects of the Creatine Loading condition on lean body mass and leg lean mass were likely trivial.

Fat mass and Bone mass (Table 1 and 2): Compared to baseline measurements, changes in total fat mass and leg fat mass in Glycogen Depleted and Glycogen Loaded conditions were not substantial and produced trivial effect sizes relative to the smallest important effect. The effects of Creatine-Loading and the combined Creatine-Glycogen Loading conditions on total body fat mass and leg fat mass were also not-substantial. Changes in total bone mass and leg bone mass for all treatment conditions were not substantial.

Body water (Table 3): There were likely substantial effects of Glycogen Depletion and Glycogen Loading treatments on total body water. There was a likely decrease in extracellular fluid in the Glycogen Depletion treatment. An increase in total body water and intracellular fluid with the combined Creatine-Glycogen Loaded condition was very likely, with a possible increase in extracellular fluid. The Creatine Loading condition was associated with a possible likely increase in total body water and intracellular fluid, but no clear effect on extracellular fluid.

Muscle glycogen (Table 4): The effects of Glycogen Depletion, Glycogen Loading and the combined Creatine-Glycogen loading treatments on muscle glycogen concentration were clear. There was no clear effect of the Creatine Loading treatment on muscle glycogen concentrations.

Discussion

This study is the first to systematically investigate the effect of glycogen loading, creatine loading and their interaction on DXA estimates of body composition. Estimates of lean body mass were substantially higher with glycogen loading translating to a mean 1.3 kg increase in lean body mass and 1.7 kg increase in leg lean mass following our glycogen loading treatment and a 1.9 kg increase in lean body mass and 2.0 kg increase in leg lean mass following a combined creatine glycogen loading treatment. On the other hand, glycogen depleting exercise resulted in a mean decrease of 1.0 kg and 0.8kg of lean body mass and leg lean mass which was deemed very likely trivial. The changes in the DXA estimates of lean body mass and leg lean mass were reflected by the changes in total body water and intracellular fluid. Our findings of increased total body water, and more specifically intracellular fluid, with glycogen loading were expected. However, we have demonstrated,

for the first time that this creates an artefact in DXA-derived measurements of body composition in well trained athletes.

It is well accepted that water is bound to the glycogen molecule in the cellular environment. Indeed, a ratio of three grams of water to one gram of muscle glycogen is commonly stated, based primarily on a single rat study from the 1940s which determined that 1 g of liver glycogen was associated with 2.7 g of water over a range of concentrations (23). Olsson and colleagues assessed body water by tritium trace in dilution in males before and after glycogen loading, reporting that each gram of glycogen was stored with 3-4 g of water (31). They observed a mean increase in body mass of 2.4 kg, of which 2.2 L was attributed to the increase in total body water. (31). However, Sherman et al. completed studies of rat skeletal muscle and failed to find a consistent relationship between glycogen and water content over a range of glycogen concentrations (36). More recently, Fernández-Elías and colleagues reported different ratios of muscle glycogen to water following post-exercise glycogen repletion under different fluid intakes. A ratio of 1:3 was found when only 400 ml of water was consumed, while a ratio of 1:17 was determined when participants replaced the fluid lost during exercise (10). Although anecdotes and studies have noted that glycogen loading is associated with a gain in total body mass (4), and that changes in glycogen can confound the results of weight loss programs in the general community (19), few studies have investigated how changes in glycogen loading (and consequently body water) would affect interpretations of body composition in athlete populations.

An increase in body mass is considered a direct side effect of the creatine supplementation during the initial loading phase (15, 16, 38). The mass increase is often attributed to water retention, as five days is considered too short a period to detect real changes in myofibrillar protein content (15, 16, 32, 38). Since creatine is an osmotic particle, increases in its

concentration in muscle could induce cellular swelling leading to fluid retention (2, 11). Indeed, acute decreases in urinary output (15) and increases in total body and intracellular water have both been reported following creatine loading protocols. Furthermore, creatine supplementation of 20-25 g per day for 5-7 days has been associated with increases of 1.0 to 2.0 kg (18, 37, 38) in body mass and 1.3 to 2.3 L in total body water (7, 32, 35). However, not all studies have found a concurrent increase in the intracellular water compartment (32).

To our knowledge, only a handful of studies has have investigated the effect of carbohydrate loading or creatine loading on body composition or muscle size (1, 30, 34), and we are the first to investigate the interaction of these two strategies. Another novel aspect of our study was the assessment of total body water as an adjunct to the measurement of body composition; to our knowledge, no other study has reported on the effect of glycogen loading on lean body mass and total body water. Our findings support those of Nygren et al. (30) and Rouillier et al. (34), with substantial increases in muscle glycogen, lean body mass and total body water observed following 48 h of glycogen loading. Nygren et al. (30) reported an increase in the vastus lateralis cross sectional area by MRI following four days of carbohydrate loading in healthy males. The increase in muscle cross sectional area was attributed to the increase in glycogen (281 to 634 mmol/kg/dw) along with the binding of the water (30). However, neither body water nor measures of body composition were assessed in this investigation. Meanwhile Balon and colleagues found no increase in muscle girth following a three day high carbohydrate diet (80% carbohydrate) compared with a low carbohydrate diet (10% carbohydrate) with concurrent resistance training (1).

A recent study investigating three days of increased carbohydrate intake on DXA estimates of body composition reported a mean 0.9 kg increase in lean body mass and 1.4 kg increase in appendicular lean mass (arms and legs) (34). Although the authors attributed the increase in appendicular lean mass to increased glycogen storage, no biopsies were conducted to verify changes in muscle glycogen content (34). Furthermore, dietary intake was not prescribed and although carbohydrate intake achieved the stated goal of exceeding 75% of total energy intake, this amounted to a total daily intake of 8 g/kg, compared to 12g/kg in our study. Some concerns regarding the standardization of the methodology of the DXA scans are also noted: although not clearly stated, the DXA scans were conducted following an overnight rest and fast (3) but it is unknown whether carbohydrate intake was standardized prior to the baseline scan.

Several studies have investigated the effect of creatine supplementation on body composition, however they are often for longer supplementation periods and taken concurrently with resistance training (2, 8, 11, 20, 39). Currently only one other study has assessed the sole effect of short term creatine supplementation on body water and body composition (35). Safdar et al. reported increases in lean body mass by DXA following a 10 day creatine supplementation period in untrained individuals (35). Furthermore, measurement of total body water by BIS revealed an increase in intracellular fluid compartment, although the magnitude of this increase was not provided (35). Our creatine loading treatment resulted in only trivial changes in muscle creatine content and lean mass, and showed only possible increases in total body water and intracellular fluid. Due to our study design, the assessment of all these parameters occurred on either Day 7 or Day 14 of the supplementation protocol, where participants had changed to a reduced creatine dosage (3 g/d), believed to maintain elevated creatine stores (33), for two or nine days respectively. However, since van Loon et

al. (37) recently reported that this “maintenance” dose is not always sufficient for maintaining creatine levels, it is possible that a reduction in creatine content occurred over the longer maintenance period, obscuring any earlier effects.

We note some other real and apparent limitations of this study. Due to the requirements of the larger study, we were unable to add further measurements such as an assessment of body composition and body water under a creatine loaded-glycogen depleted condition. Furthermore, we anticipate the criticism that the glycogen depleted condition was monitored 15-18 h after the completion of the glycogen-depleting task. However, as we conducted our scans using a standardised protocol based on Nana et al. (28) which require fasted and rested conditions to standardize gut contents and hydration status, we needed to undertake these measurements on the morning following the exercise session. However, we attempted to minimize glycogen resynthesis during the recovery period by providing participants with a diet providing < 1 g/kg carbohydrate. This was successful in maintaining glycogen content below pre-exercise levels, and indeed may mirror the real-life practices of athletes who “sleep low” (restrict carbohydrate intake) after quality training sessions to prolong the adaptive response to exercise by delaying muscle glycogen storage (21). We acknowledge that BIS is an indirect measurement of total body water, however, the use of the criterion dilution methods did not fit within the constraints of the larger study. Additionally, BIS has been recently validated against criterion methods in athletes and was considered appropriate in this setting (17, 22).

In summary, the results from this study provide further evidence of daily variability in the DXA assessments of body composition of athletes due to factors frequently experienced in

sport. Recent work by our centre has developed techniques to standardize DXA assessment protocols (25, 27, 28), showing that the implementation of overnight fasted and rested conditions can reduce variability to allow greater sensitivity in the detection of real and interesting changes in body composition (28). The present study expands on this work and indicates that when DXA is used for longitudinal monitoring of physique, scans should be undertaken with consideration of recent practices of training and diet that might be expected to manipulate muscle glycogen stores. Where standardization of these practices is impractical, the interpretation of the results of DXA assessments of body composition should take into account the likely artefacts with respect to lean mass. Future studies should also investigate the effect of other sources of changes in intramuscular fluid and substrate such as muscle damage or carnitine supplementation, alongside those caused by exercise or dietary manipulation.

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352 References:

353 1. Balon TW, Horowitz JF, Fitzsimmons KM. Effects of carbohydrate loading and
354 weight-lifting on muscle girth. *Int J Sport Nutr*. 1992;2(4):328-34.
355 2. Bemben MG, Bemben DA, Loftiss DD, Knehans AW. Creatine supplementation
356 during resistance training in college football athletes. *Med Sci Sports Exerc*.
357 2001;33(10):1667-73.
358 3. Bone J, Burke LM. DXA Estimates of Body Composition and Carbohydrate Loading.
359 *Ann Nutr Metab*. 2016;68(3):228-9.
360 4. Brotherhood JR, Swanson M. Nutrient intakes and body weight changes of distance
361 runners using the glycogen loading procedure. *Aust J Sports Med*. 1979;11:45-7.
362 5. Burke LM, Hawley JA, Wong SH, Jeukendrup AE. Carbohydrates for training and
363 competition. *J Sports Sci*. 2011;29 Suppl 1:S17-27.
364 6. Churchley EG, Coffey VG, Pedersen DJ et al. Influence of preexercise muscle
365 glycogen content on transcriptional activity of metabolic and myogenic genes in well-
366 trained humans. *J Appl Physiol (1985)*. 2007;102(4):1604-11.
367 7. Deminice R, Rosa FT, Pfrimer K, Ferrioli E, Jordao AA, Freitas E. Creatine
368 Supplementation Increases Total Body Water in Soccer Players: a Deuterium Oxide
369 Dilution Study. *Int J Sports Med*. 2016;37(2):149-53.
370 8. Earnest CP, Snell PG, Rodriguez R, Almada AL, Mitchell TL. The effect of creatine
371 monohydrate ingestion on anaerobic power indices, muscular strength and body
372 composition. *Acta Physiol Scand*. 1995;153(2):207-9.
373 9. Evans WJ, Phinney SD, Young VR. Suction applied to a muscle biopsy maximizes
374 sample size. *Med Sci Sports Exerc*. 1982;14(1):101-2.

10. Fernandez-Elias VE, Ortega JF, Nelson RK, Mora-Rodriguez R. Relationship between muscle water and glycogen recovery after prolonged exercise in the heat in humans. *Eur J Appl Physiol*. 2015;115(9):1919-26.
11. Francaux M, Poortmans JR. Effects of training and creatine supplement on muscle strength and body mass. *Eur J Appl Physiol Occup Physiol*. 1999;80(2):165-8.
12. Harris RC, Hultman E, Nordesjö LO. Glycogen, glycolytic intermediates and high-energy phosphates determined in biopsy samples of musculus quadriceps femoris of man at rest. Methods and variance of values. *Scand J Clin Lab Invest*. 1974;33(2):109-20.
13. Harris RC, Soderlund K, Hultman E. Elevation of creatine in resting and exercised muscle of normal subjects by creatine supplementation. *Clin Sci (Lond)*. 1992;83(3):367-74.
14. Hopkins WG, Marshall SW, Batterham AM, Hanin J. Progressive statistics for studies in sports medicine and exercise science. *Med Sci Sports Exerc*. 2009;41(1):3-13.
15. Hultman E, Soderlund K, Timmons JA, Cederblad G, Greenhaff PL. Muscle creatine loading in men. *J Appl Physiol*. 1996;81(1):232-7.
16. Juhn MS, Tarnopolsky M. Potential side effects of oral creatine supplementation: a critical review. *Clin J Sport Med*. 1998;8(4):298-304.
17. Kerr A, Slater G, Byrne N, Chaseling J. Validation of Bioelectrical Impedance Spectroscopy to Measure Total Body Water in Resistance-Trained Males. *Int J Sport Nutr Exerc Metab*. 2015;25(5):494-503.
18. Kinugasa R, Akima H, Ota A, Ohta A, Sugiura K, Kuno SY. Short-term creatine supplementation does not improve muscle activation or sprint performance in humans. *Eur J Appl Physiol*. 2004;91(2-3):230-7.

19. Kreitzman SN, Coxon AY, Szaz KF. Glycogen storage: illusions of easy weight loss, excessive weight regain, and distortions in estimates of body composition. *Am J Clin Nutr*. 1992;56(1 Suppl):292S-3S.

20. Kutz MR, Gunter MJ. Creatine monohydrate supplementation on body weight and percent body fat. *J Strength Cond Res*. 2003;17(4):817-21.

21. Marquet LA, Brisswalter J, Louis J et al. Enhanced Endurance Performance by Periodization of Carbohydrate Intake: "Sleep Low" Strategy. *Med Sci Sports Exerc*. 2016;48(4):663-72.

22. Matias CN, Santos DA, Judice PB et al. Estimation of total body water and extracellular water with bioimpedance in athletes: A need for athlete-specific prediction models. *Clin Nutr*. 2016;35(2):468-74.

23. McBride JJ, Guest MM, Scott EL. The storage of the major liver components; emphasizing the relationship of glycogen to water in the liver and the hydration of glycogen. *J Biol Chem*. 1941;139(2):943-52.

24. Moon JR, Tobkin SE, Roberts MD et al. Total body water estimations in healthy men and women using bioimpedance spectroscopy: a deuterium oxide comparison. *Nutr Metab (Lond)*. 2008;5:7.

25. Nana A, Slater GJ, Hopkins WG, Burke LM. Effects of daily activities on DXA measurements of body composition in active people. *Med Sci Sports Exerc*. 2012;44(1):180-9.

26. Nana A, Slater GJ, Hopkins WG, Burke LM. Techniques for undertaking dual-energy X-ray absorptiometry whole-body scans to estimate body composition in tall and/or broad subjects. *Int J Sport Nutr Exerc Metab*. 2012;22(5):313-22.

27. Nana A, Slater GJ, Hopkins WG, Burke LM. Effects of exercise sessions on DXA measurements of body composition in active people. *Med Sci Sports Exerc*. 2013;45(1):178-85.

28. Nana A, Slater GJ, Hopkins WG et al. Importance of Standardized DXA Protocol for Assessing Physique Changes in Athletes. *Int J Sport Nutr Exerc Metab*. 2016;26(3):259-67.

29. Nana A, Slater GJ, Stewart AD, Burke LM. Methodology review: using dual-energy X-ray absorptiometry (DXA) for the assessment of body composition in athletes and active people. *Int J Sport Nutr Exerc Metab*. 2015;25(2):198-215.

30. Nygren AT, Karlsson M, Norman B, Kaijser L. Effect of glycogen loading on skeletal muscle cross-sectional area and T2 relaxation time. *Acta Physiol Scand*. 2001;173(4):385-90.

31. Olsson KE, Saltin B. Variation in total body water with muscle glycogen changes in man. *Acta Physiol Scand*. 1970;80(1):11-8.

32. Powers ME, Arnold BL, Weltman AL et al. Creatine Supplementation Increases Total Body Water Without Altering Fluid Distribution. *J Athl Train*. 2003;38(1):44-50.

33. Preen D, Dawson B, Goodman C, Beilby J, Ching S. Creatine supplementation: a comparison of loading and maintenance protocols on creatine uptake by human skeletal muscle. *Int J Sport Nutr Exerc Metab*. 2003;13(1):97-111.

34. Rouillier MA, David-Riel S, Brazeau AS, St-Pierre DH, Karelis AD. Effect of an Acute High Carbohydrate Diet on Body Composition Using DXA in Young Men. *Ann Nutr Metab*. 2015;66(4):233-6.

35. Safdar A, Yardley NJ, Snow R, Melov S, Tarnopolsky MA. Global and targeted gene expression and protein content in skeletal muscle of young men following short-term creatine monohydrate supplementation. *Physiol Genomics*. 2008;32(2):219-28.

36. Sherman WM, Plyley MJ, Sharp RL et al. Muscle glycogen storage and its relationship with water. *Int J Sports Med*. 1982;3(1):22-4.

37. van Loon LJ, Oosterlaar AM, Hartgens F, Hesselink MK, Snow RJ, Wagenmakers AJ. Effects of creatine loading and prolonged creatine supplementation on body composition, fuel selection, sprint and endurance performance in humans. *Clin Sci (Lond)*. 2003;104(2):153-62.

38. Volek JS, Kraemer WJ. Creatine Supplementation: Its Effect on Human Muscular Performance and Body Composition. *J Strength Condit Res*. 1996;10(3):200-10.

39. Ziegenfuss TN, Rogers M, Lowery L et al. Effect of creatine loading on anaerobic performance and skeletal muscle volume in NCAA division I athletes. *Nutrition*. 2002;18(5):397-402.

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Table 1. Baseline values, smallest important change, and percent changes from baseline following various treatments for total body composition. Also shown are magnitude-based inferences for the mean changes with each treatment.

		Change (%) from baseline (mean; ±CL)				
Measure	Baseline (mean ± SD)	SIE	Glycogen Depleted	Glycogen Loaded	Creatine Loaded	Creatine-Glycogen Loaded
Body mass	77 ± 9 kg	2.3	-1.3; ±0.3	2.1; ±0.7	1.2; ±0.5	2.8; ±0.5 ↑**
DXA whole body mass						
Total	78 ± 8 kg	2.2	-1.3; ±0.3	2.3; ±0.6 ↑*	1.3; ±0.6	3.0; ±0.5 ↑***
Lean	84 ± 6 %BM	1.5	-1.3; ±0.3	2.1; ±0.5 ↑**	1.3; ±0.5	3.0; ±0.4 ↑***
Fat	12 ± 6 %BM	8.6	-2.0; ±1.5	4.5; ±3.4	3.3; ±5.7	5.2; ±3.9
Bone	4.2 ± 0.3 %BM	1.8	-0.2; ±0.5	0.4; ±1.1	0.0; ±0.5	-0.2; ±0.5

%BM, percent of baseline body mass; CL, 90% confidence limits;

SIE, smallest important effect. This is 0.2 of the between subject SD and the percent change a variable has to meet to be considered a substantial change; ↑ indicates substantial increase; ↓, indicates substantial decrease;

Asterisk/s indicates how clear the change is at the 99% confidence level, *possible clear change, **likely clear change, ***very or likely clear change.

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Table 2. Baseline values, smallest important change, and percent changes from baseline following various treatments for regional leg composition. Also shown are magnitude-based inferences for the mean changes with each treatment.

			Change (%) from baseline (mean; ±CL)			
Measure	Baseline (mean ± SD)	SIE	Glycogen Depleted	Glycogen Loaded	Creatine Loaded	Creatine-Glycogen Loaded
Total	35.3 ± 2.0 %BM	1.1	-1.4; ±0.6 ↓**	2.9; ±0.8 ↑***	1.1; ±1.2 ↑*	2.9; ±1.3 ↑***
Lean	29.4 ± 2.3 %BM	1.5	-1.4; ±0.7 ↓*	2.6; ±0.8 ↑***	1.2; ±1.0	3.1; ±1.2 ↑***
Fat	4.3 ± 2.5 %BM	8.5	-2.5; ±1.7	6.2; ±1.8	2.4; ±6.1	3.2; ±4.1
Bone	1.65 ± 0.15 %BM	2.0	0.0; ±0.3	0.7; ±0.5	-0.2; ±0.7	-0.2; ±0.5

%BM, percent of baseline body mass; CL, 90% confidence limits;

SIE, smallest important effect. This is 0.2 of the between subject SD and the percent change a variable has to meet to be considered a substantial change; ↑ indicates substantial increase; ↓, indicates substantial decrease;

Asterisk/s indicates how clear the change is at the 99% confidence level, *possible clear change, **likely clear change, ***very or likely clear change.

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Table 3. Baseline values, smallest important change, and percent changes from baseline following various treatments for total body water and water compartments. Also shown are magnitude-based inferences for the mean changes with each treatment.

			Change (%) from baseline (mean; ±CL)			
Measure	Baseline (mean ± SD)	SIE	Glycogen Depleted	Glycogen Loaded	Creatine Loaded	Creatine-Glycogen Loaded
Total body	61.2 ± 3.8 %BM	1.3	-2.0; ±1.1 ↓**	2.3; ±1.3 ↑**	1.3; ±1.7 ↑*	2.5; ±1.0 ↑***
Intra-cellular	36.1 ± 3.0 %BM	1.4	-1.3; ±1.6 ↓*	2.2; ±1.9 ↑*	1.4; ±2.0 ↑*	6.8; ±4.5 ↑***
Extra-cellular	25.3 ± 1.4 %BM	1.0	-3.5; ±1.2 ↓***	2.2; ±1.5 ↑**	0.3; ±1.8	1.4; ±1.9 ↑*

%BM, percent of baseline body mass; CL, 90% confidence limits;

SIE, smallest important effect. This is 0.2 of the between subject SD and the percent change a variable has to meet to be considered a substantial change; ↑ indicates substantial increase; ↓, indicates substantial decrease;

Asterisk/s indicates how clear the change is at the 99% confidence level, *possible clear change, **likely clear change, ***very or likely clear change.

Table 4. Baseline values, smallest important change, and percent changes from baseline following various treatments for muscle glycogen and total creatine. Also shown are magnitude-based inferences for the mean changes with each treatment.

			Change from baseline (mean; ± CL)			
Measure	Baseline (mean ± SD)	SIE	Glycogen Depleted	Glycogen Loaded	Creatine Loaded	Creatine-Glycogen Loaded
Muscle Glycogen	580 ± 140 mmol/kg dw	1.9	-57; ±7.3 ↓***	22; ±12.6 ↑***	-2; ±15.4	20; ±15.9 ↑***
Muscle Creatine	136 ± 17 µmol/g	2.2	0; ±6.2	-2; ±8.5	6; ±10.1	6; ±4.6 ↑**

%BM, percent of baseline body mass; CL, 90% confidence limits;

SIE, smallest important effect. This is 0.2 of the between subject SD and the percent change a variable has to meet to be considered a substantial change; ↑ indicates substantial increase; ↓, indicates substantial decrease;

Asterisk/s indicates how clear the change is at the 99% confidence level, *possible clear change, **likely clear change, ***very or likely clear change.

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466 Figure 1: Overivew of study design. Pla: Placebo, Cr: Creatine, CHO: Carbohydrate, TT:

467 time trial.

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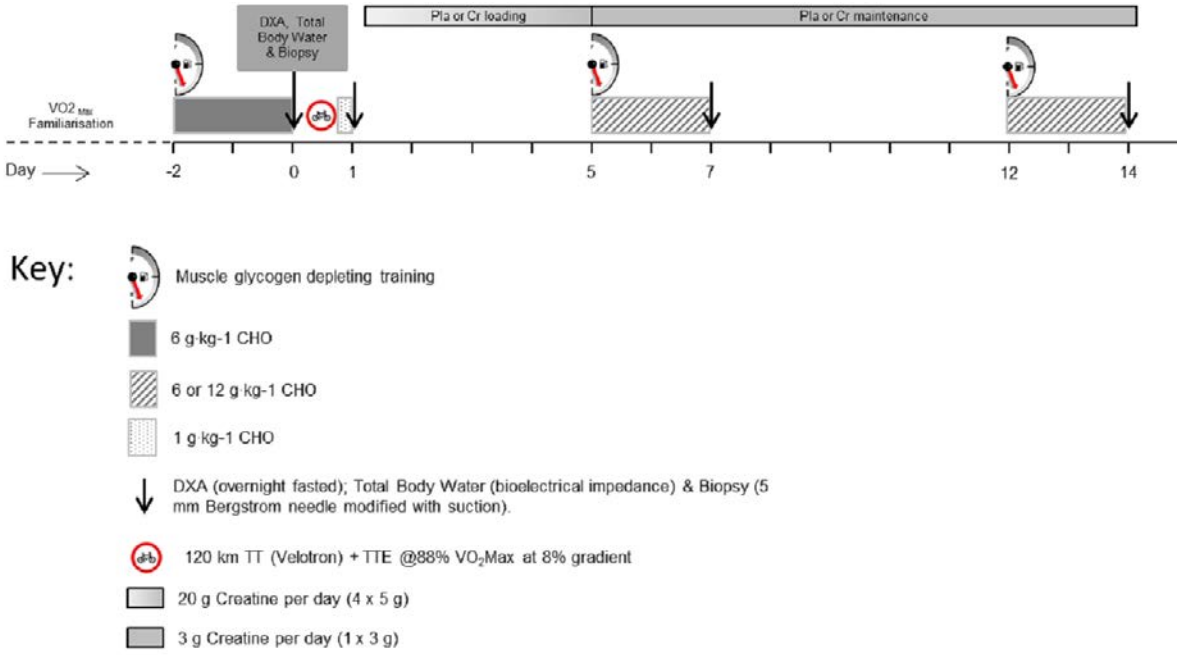

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