

# The effect of hydration status on the measurement of lean tissue mass by dual-energy X-ray absorptiometry

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## Abstract

**Purpose** Athletes cycle between exercise and recovery. Exercise invokes changes in total body water from thermal sweating, muscle and hepatic glycogen depletion and metabolic water loss. Recovery from exercise results in rehydration, substrate repletion, and possible glycogen supercompensation. Such changes may corrupt the measurement of hydrated tissues, such as lean tissue mass (LTM), by dual-energy X-ray absorptiometry (DXA). The purpose of this study was to determine the effect of exercise and thermal dehydration and subsequent glycogen supercompensation on DXA-based measurement of body composition.

**Methods** Twelve active adult (18–29 years) males exercised at 70%  $VO_{2max}$  on a cycle ergometer in a thermal environment (30 °C) to induce a 2.5% reduction in body mass. Participants subsequently underwent a glycogen supercompensation phase, whereby a high carbohydrate diet (8–12 g/kg body mass/day) was consumed for a 48-h period. Whole-body DXA measurement was performed at baseline, following exercise and supercompensation.

**Results** Following exercise, mean body mass decreased by –1.93 kg (95% CI –2.3, –1.5), while total LTM decreased by –1.69 kg (–2.4, –1.0). Supercompensation induced a mean body mass increase of 2.53 kg (2.0, 3.1)

and a total LTM increase of 2.36 kg (1.8, 2.9). No change in total fat mass or bone mineral content was observed at any timepoint.

**Conclusions** Training regimens that typically induce dehydration and nutrition regimens that involve carbohydrate loading can result in apparent changes to LTM measurement by DXA. Accurate measurement of LTM in athletes requires strict observation of hydration and glycogen status to prevent manipulation of results.

**Keywords** DXA · Hydration · Lean tissue mass · Measurement · Glycogen · Athletes

## Abbreviations

ANOVA	Analysis of variance
BM	Body mass
BMI	Body mass index
BMC	Bone mineral content
BMD	Bone mineral density
CI	Confidence interval
DXA	Dual-energy X-ray absorptiometry
FFM	Fat-free mass
FM	Fat mass
HR	Heart rate
LTM	Lean tissue mass
LSC	Least significant change
PAR-Q	Physical activity readiness questionnaire
ROI	Region of interest
TBW	Total body water
$VO_{2max}$	Maximal oxygen uptake

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## Introduction

With the evolution of several technological advances in soft-tissue imaging, dual-energy X-ray absorptiometry (DXA) has become an accepted criterion measure of body composition. Originally developed for clinical measurement of areal bone mineral density (BMD) to diagnose skeletal conditions, such as osteoporosis, the application of DXA to the assessment of soft-tissue composition, i.e., fat and lean tissue mass, has gained prominence. This method has reported accurate and precise measurement of composition in the most advanced DXA models (Toombs et al. 2012) and has become a criterion method for monitoring body composition change in the context of athletic health and performance (Meyer et al. 2013). However, athletes in training habitually cycle between exercise and recovery and the error introduced by this cyclical process on DXA-based measurement of body composition is relatively unknown.

Potential error or variability in DXA estimates of body composition can be divided into two types: technical error and biological variation (Lohman et al. 2000; Nana et al. 2015). Technical or precision error is the variation generated by the instrument following calibration or by failure to standardise the preparation, positioning or regional analysis of the subject. Biological variation is the variation of the composition of the individual, which includes changes in hydration status of the tissues arising from the short-term effects of exercise and the effects of food and fluid intake in the hours before measurement. Standardised DXA protocols are recommended to reduce measurement error (Hangartner et al. 2013; Nana et al. 2012, 2015; Toomey et al. 2015). However, limited information exists as to the extent by which habitual cycling of hydration status, tissue substrate, and metabolic water that occurs during exercise and recovery can alter the measurement of lean tissue mass (LTM) measurement in athletes. Knowledge of potential errors in compositional measurement in athletes is an imperative given the extreme challenge to body and tissue specific hydration that can potentially occur, e.g., ultra-endurance events (Hew-Butler et al. 2015; Mueller et al. 2013) and weight division sports (Clark et al. 2007; Santos et al. 2010).

Euhydration or 'normal' total body water (TBW) content is required for accurate DXA body composition measurement, and is thought to maintain relative stability throughout the adult lifespan (Lohman et al. 2000; Schoeller 1989). DXA-based measurement of fat-free mass (FFM) assumes a constant water content of FFM (TBW/FFM) at  $0.73 \pm 0.03$  (Moore and Boyden 1963; Wang et al. 1999). With standardised ranges established for the adult population (0.71–0.75), values outside these ranges would be defined as overhydrated or dehydrated (Lohman et al. 2000). Water gain occurs from consumption (liquids and

food) and production (metabolic water), while water losses occur from respiratory, gastrointestinal, renal, and sweat losses (Sawka et al. 2007). Sweating provides the primary avenue of thermoregulatory water loss, particularly during exercise in a hot environment. A variety of factors will influence sweat loss rates in individuals, including exercise duration and intensity, temperature, humidity, clothing, body mass, genetic predisposition, and metabolic efficiency (Sawka 1992). As a result, large ranges in sweat rates and total sweat losses of individuals are observed during and between activities (0.5–2.0 L/h) (Sawka et al. 2007). Of relevance to DXA measurement, any state of abnormal hydration can alter the LTM attenuation coefficient, which in turn leads to an error in the amount of lean tissue attributed to each pixel (Roubenoff et al. 1993). Therefore, close regulation of hydration status in athletes is required prior to the measurement of body composition by DXA.

To investigate dehydration-induced changes in body composition measurement by DXA, Going et al. (1993) recruited 17 male and female participants to undergo a 24-h period with no fluid intake and no exercise, including a complete fast for the final 12-h period. A mean body mass change of  $-1.5 \pm 0.8$  kg ( $-2.1\%$ ) was reflected in the LTM change of  $-1.47 \pm 1.0$  kg ( $-2.8\%$ ), with no alteration to fat or bone estimates. Rehydration involved water consumption ( $=75\%$  of mass lost) over the first 30-min period and  $=120\%$  of the remaining mass difference over the next hour. Following rehydration, body mass ( $+1.21 \pm 0.7$  kg;  $+1.7\%$ ) and LTM ( $+1.30 \pm 0.7$  kg;  $+2.5\%$ ) returned to baseline. Nana et al. (2013) observed that exercise resulted in decreases in total ( $-0.4 \pm 0.3\%$ ) and trunk ( $-1.5 \pm 0.7\%$ ) LTM in males, following a  $110 \pm 42$  min outdoor cycling session. However, the full effect of exercise dehydration was not established due to the permission of fluid and food ingestion as part of the exercise protocol. Although no quantifiable method of hydration measurement was used, both studies highlight that the importance of a pre-measurement protocol to ensure the DXA-based measurement of body composition is not corrupted by non-standardised pre-measurement control of subjects' exercise and dietary behaviour.

Cycling of skeletal muscle and hepatic glycogen concentration is a likely contributor to error in DXA-based measurement of LTM in athletes. Each gram of glycogen within the muscle is estimated to be stored with 3 g of water (Olsson and Saltin 1970), with more recent studies estimating a ratio closer to 4:1 (Shiose et al. 2016). Therefore, variation of muscle glycogen content could lead to 'apparent' changes in the true level of LTM measurement by DXA. Manipulation of the carbohydrate content of an athlete's diet prior to and in the recovery from exercise can lead to up to three-fold difference in muscle glycogen concentration (Bergstrom et al. 1967;

Rauch et al. 1995). Glycogen supercompensation strategies (achieving supraphysiological glycogen levels due to carbohydrate depletion followed by loading) are a popular protocol amongst endurance athletes to maximise glycogen storage pre-competition and thus improve fuel utilization and performance (Ahlborg et al. 1967; Bergstrom et al. 1967). Varying carbohydrate-loading protocols following exercise has been shown to achieve high glycogen levels following 1–6 days of high carbohydrate intake (Fairchild et al. 2002). Similar strategies have been employed by bodybuilders to increase the volume and definition of muscle (Hackett et al. 2013). How these strategies influence that the monitoring of athlete body composition is not well understood. To the authors' knowledge, only one study has measured the 'apparent' change in LTM measurement by DXA in active males resulting from a dietary intervention, demonstrating increased LTM (+0.9 kg) following a 3-day high carbohydrate diet (>75% total calorie intake) (Rouillier et al. 2015). An initial depletion period preceding the carbohydrate-loading protocol is likely to incur greater manipulation of the muscle glycogen and thus LTM component measurement. However, this paradigm has not yet been investigated.

The training and dietary practices of athletes often command that they are in a dynamic state of cycling of hydration and muscle glycogen that may in cases also present at their extremes (Hawley and Burke 2010). This study set out to manipulate hydration and glycogen status through thermal- and exercise-induced dehydration and nutritional supercompensation to mimic the physiological ranges that athletes may present prior to the measurement body composition by DXA. We hypothesised that an endurance-based exercise protocol would induce an apparent reduction in total body LTM, while a subsequent 48-h high carbohydrate diet would result in an apparent increase in total body LTM as measured by DXA.

## Methods

### Participants

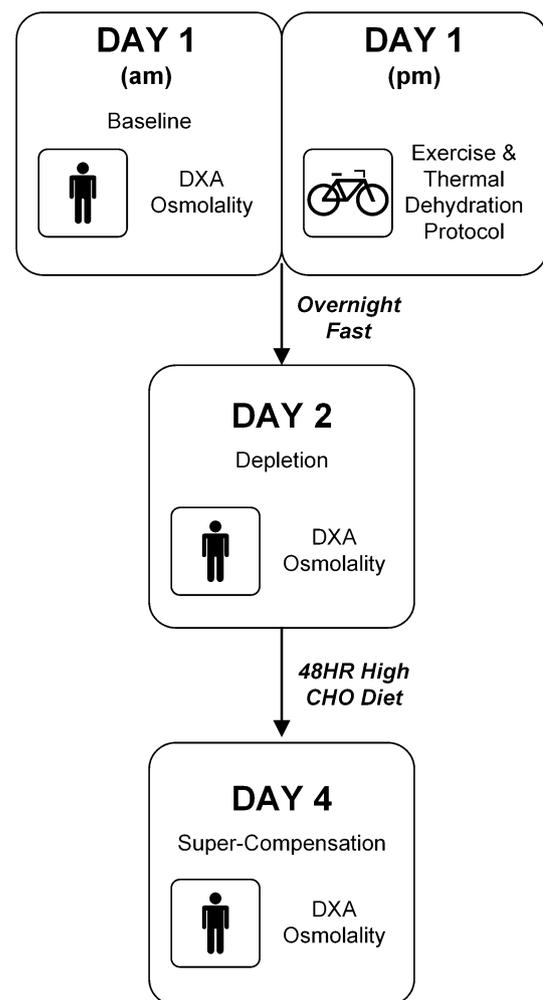
Twelve male participants were recruited via a convenience sample of students in the University of Limerick, Ireland. Inclusion required participants to be male, aged 18–35 years, physically active ( $\geq 150$  min per week) and with no contraindications to exercise as assessed by a standard PAR-Q. Written, informed consent was obtained from all participants. The study was approved by the University of Limerick Research Ethics Committee (EHSREC 2012/10/18).

### Study design

The experimental overview is shown in Fig. 1. Each participant completed a 4-day protocol, including a baseline measurement, followed by an endurance exercise protocol and a period of supercompensation. Body composition measurement, utilizing DXA, and urine osmolality were measured at each timepoint.

#### Baseline

On Day 1, participants arrived at 0700 h for baseline body composition measurement after an overnight fast. Height was measured to the nearest 0.1 cm using a stadiometer (Seca, Birmingham, UK) and body mass to the nearest 0.1 kg (Tanita MC-180MA Body Composition Analyser, Tanita UK Ltd.). DXA methods were employed, and a mid-stream urine sample was collected.



**Fig. 1** Experimental overview

## Depletion

Following baseline measurement, participants were permitted to eat and drink ad libitum up to 3 h prior to engaging in an exercise depletion protocol. To standardise fluid intake, participants consumed 500 ml of water 1 h before commencement of the thermal- and exercise-induced dehydration. The exercise bout took place at 1900 h on Day 1 in a climatic chamber at an ambient temperature of 30 °C and relative humidity 40%. Participants exercised at 70% (of max HR or  $\dot{V}O_2$ ) on Monark cycle ergometers (814 E; Monark Exercise AB, Vansbro, Sweden) using a Polar heart rate monitor (RS200X) until a 2.5% reduction in body mass was observed. Nude body mass after towelling was measured every 20–30 min until target body mass was achieved (~1 to 2.5 h). Any voided volume in this time period was recorded and added to the required body mass reduction. Participants were asked to refrain from food or fluid intake for the remainder of the day and present for a repeat body composition assessment in a fasted, dehydrated state on Day 2 at 0700 h. This delay allowed fluid distributions to re-equilibrate following the exercise dehydration bout and to allow the comparison to the baseline condition.

## Supercompensation

Participants were subsequently prescribed a diet high in carbohydrate (8–12 g/kgBM/day) by a registered dietitian, which was consumed for a 48-h period. A 48-h loading protocol was deemed to be an adequate time frame to achieve supra-normal levels of glycogen based on previous research (Fairchild et al. 2002), while not implicating participant adherence. Participants were able to monitor their individual carbohydrate intake using the personalised web application “<http://MyFitnessPal.com>”. On Day 4 at 0700 h, participants presented for a body composition assessment in a supercompensated state under standardised conditions following an overnight fast.

## Dual-energy X-ray absorptiometry

A Lunar iDXA™ scanner (GE Healthcare, Chalfont St Giles, Bucks., UK) with the enCORE™ v.14.1 software was used to capture whole-body scans. The standardised test condition required participants to refrain from strenuous exercise in the 12-h period before testing, to attend after an overnight fast and to consume 500 mL of water 1 h before the scan. At the depletion timepoint, 500 mL was not ingested prior to scanning for observational purposes. Participants were required to empty their bladder immediately prior to measurement and defecate if required. The enCORE software provided segmentation of the total body into regions of interest (ROIs) for arms, legs, and trunk,

with manual adjustments made where necessary. Root mean square coefficient of variance and least significant change (LSC) for repeat measurement ( $n=78$ ) at the 95% confidence interval was 0.6% and 0.72 kg, respectively, for total lean tissue mass, and 1.2% and 0.58 kg, respectively, for total fat mass.

## Urine osmolality

Participants were asked to provide a mid-stream urine sample immediately prior to each DXA scan. An osmometer (Advance® Model 3320 Micro-Osmometer) was used to measure urine osmolality. The equipment was calibrated according to manufacturer instructions prior to commencing each test day.

## Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics 22.0 for Windows (SPSS, Inc., Chicago, IL). Data were found to be normally distributed using a Shapiro–Wilks test ( $p < 0.05$ ) allowing for parametric analysis. Mean difference in body composition between conditions is presented as mean (95% confidence interval) with confidence intervals not containing the null value inferring statistical significance. Change in body composition due to depletion and supercompensation was analysed using a two-way repeated measures ANOVA design and Bonferroni adjustment for multiple comparisons. The assumption of sphericity was tested using Mauchly’s test. Pearson correlation was used to determine a relationship between change in LTM and change in urine osmolality. Level of significance was set at  $p < 0.05$  for all analyses.

## Results

Twelve male participants with a mean age of 23.6 years (range 18–34), baseline body mass of 75.5 kg (range 56.9–94.1 kg), and height of 178.3 cm (range 162.3–189.6 cm) completed the study. Body composition characteristics for each experimental timepoint (baseline, depletion, supercompensation) are presented in Table 1 with repeated measures ANOVA results. Figure 2 shows the individual changes in LTM throughout the testing period.

## Depletion

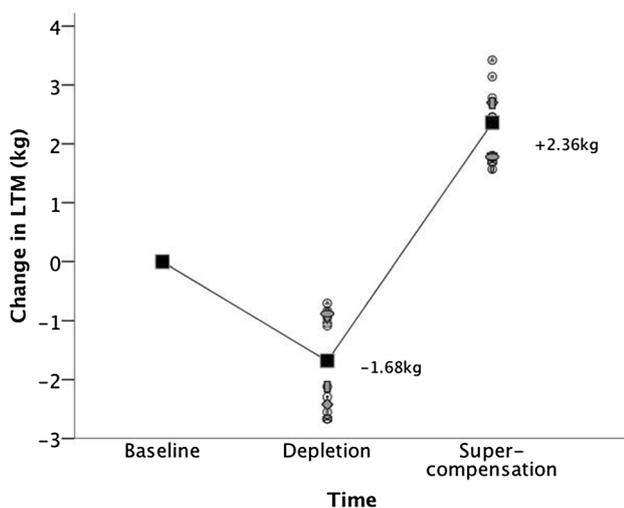
The target 2.5% reduction in body mass was achieved following the exercise and depletion protocol. Mean body mass decreased by  $-1.93$  kg (95% CI  $-2.3, -1.5$ ). Total fat mass decreased by  $-0.19$  kg ( $-0.4, 0.03$ ) which did not

**Table 1** Body composition results for participants ( $n = 12$ ) at baseline, depletion, and supercompensated timepoints

	Baseline Mean (95% CI)	Depletion	Supercomp.	$p$ value <sup>a</sup>
Body mass (kg)	75.5 (68.2, 82.7)	73.6 (66.5, 80.7)	76.1 (68.8, 83.4)	$\leq 0.001$
DXA mass (kg)	75.9 (68.6, 83.2)	74.0 (66.9, 81.8)	76.6 (69.3, 84.0)	$\leq 0.001$
BMI (kg/m <sup>2</sup> )	23.6 (22.1, 25.2)	23.0 (21.5, 24.5)	23.8 (22.3, 25.3)	$\leq 0.001$
Total LTM (kg)	60.0 (54.1, 65.8)	58.3 (52.6, 63.9)	60.6 (54.8, 66.5)	$\leq 0.001$
LTM arms (kg)	7.7 (6.9, 8.5)	7.6 (6.8, 8.3)	7.7 (6.9, 8.6)	0.142
LTM legs (kg)	21.5 (19.2, 23.8)	21.1 (18.9, 23.3)	21.7 (19.3, 24.0)	$\leq 0.001$
LTM trunk (kg)	27.3 (24.6, 30.1)	26.3 (23.6, 28.9)	27.8 (25.2, 30.5)	$\leq 0.001$
Total FM (kg)	12.6 (10.1, 15.1)	12.5 (9.9, 15.0)	12.7 (10.1, 15.2)	0.018
BMC (kg)	3.3 (3.0, 3.6)	3.3 (3.0, 3.6)	3.3 (3.0, 3.6)	0.078
Osmolality (mOsm/kg)	775 (661, 889)	1035 (970, 1100)	601 (430, 771)	$\leq 0.001$

*Supercomp.* supercompensated, *CI* confidence interval, *DXA* dual-energy X-ray absorptiometry, *BMI* body mass index, *FM* fat mass, *FMI* fat mass index, *BMC*, bone mineral content, *LTM* lean tissue mass

<sup>a</sup>Repeated measures ANOVA



**Fig. 2** Mean and individual change in LTM compared to the previous time-point. Depletion (depletion baseline) and supercompensation (supercompensation depletion)

exceed the LSC of 0.58 kg. No change in bone mineral content was observed [ $-0.007$  kg ( $-0.02, 0.005$ )]. Total LTM decreased by  $-1.69$  kg ( $-2.4, -1.0$ ), which surpassed the LSC of 0.72 kg. The greatest change in the lean component was observed in the trunk [ $-1.1$  kg ( $0.7, 1.5$ )], followed by the legs ( $-0.4$  kg ( $-0.8, -0.08$ )), with no change observed in the arms [ $-0.15$  kg ( $-0.4, 0.09$ )]. Urine osmolality increased by  $+260$  mOsm/kg ( $147, 372$ ).

### Supercompensation

Following the high carbohydrate diet, mean body mass increased by 2.53 kg ( $2.0, 3.1$ ) compared to the depletion timepoint. No apparent change in total fat mass [ $+0.22$  kg

( $0.01, 0.4$ )] or bone mineral content [ $+0.01$  kg ( $-0.004, 0.03$ )] was observed. Total lean tissue mass increased by 2.36 kg ( $1.8, 2.9$ ), which surpassed the LSC of 0.72 kg. From baseline, this increase was 0.67 kg ( $0.2, 1.2$ ). Lean mass change in the trunk was  $+1.60$  kg ( $1.0, 2.2$ ); change in the legs was  $+0.60$  kg ( $-0.8, -0.4$ ), with no change observed in the arms [ $+0.15$  kg ( $-0.7, 0.4$ )]. Urine osmolality decreased by  $-434$  mOsm/kg ( $-639, -230$ ).

Across timepoints, the total change in LTM was negatively correlated with a change in hydration status as assessed by urine osmolality ( $r = -0.85$ ).

### Discussion

The objective of this study was to monitor DXA estimates of body composition measurement over a short time-span, where actual changes in body composition are unlikely to occur. Alterations to hydration status were deliberately elicited to determine their effect on DXA measurement. It was hypothesised that a change in soft-tissue hydration would specifically alter the detection of lean tissue, since this component is comprised of  $\sim 73\%$  water, thus disrupting the LTM attenuation coefficient leading to an error in measurement (Roubenoff et al. 1993) and an apparent change in LTM.

The exercise and thermal dehydration protocol devised was successful in achieving a  $\sim 2\%$  decrease in body mass. The change in hydration status is reflected in the increase in urine osmolality ( $+260$  mOsm/kg), with a mean osmolality of 1035 mOsm/kg corresponding to a “very dehydrated” category (Armstrong et al. 2010). Repeat DXA measurement revealed a significant decrease of 1.7 kg of LTM in a 24-h time period, which exceeded the least significant change at the 95% confidence limit of 0.72 kg.

One previous study monitored the effect of a single exercise bout on DXA LTM measurement and observed a decrease of 0.4 kg in men after a cycling session (Nana et al. 2013). No difference was observed in women or after a 1-h strength session. This difference may be attributable to the fact that men have greater total LTM and undertook longer endurance cycling sessions (men,  $110 \pm 42$  min, vs. women,  $79 \pm 53$  min), thereby losing more body water. However, the small magnitude of effect in this study will have been compensated by permitted fluid and food intake during the exercise bout. Furthermore, the cycling session described produced fluid re-compartmentalisation, where there was shunting of volume from the trunk to the periphery. In the current study, the principal lean mass loss was observed in the trunk ( $-4\%$  trunk LTM), which may be partly due to the depletion of liver glycogen and loss of gastrointestinal fluid. However, there was also an apparent loss from legs ( $-2\%$  leg LTM) suggesting further glycogen and extracellular fluid loss through increased sweating. Due to the nature of the exercise, it is logical that the effect of the depletion protocol would be localized to the muscles that have worked (Bergstrom et al. 1967). The addition of an overnight fast and resting period following the exercise bout may have restored the potential for fluid shunting from the trunk to the appendices. In addition, liver glycogen content is primarily affected by fasting while skeletal glycogen is not (Jensen et al. 2011).

The high carbohydrate supercompensation protocol induced apparent increases in LTM (+2.4 kg) that surpassed the baseline measurement of LTM by 0.6 kg. Increasing hydration in this manner is possible due to increased storage of water per gram of glycogen in muscle (Olsson and Saltin 1970). This manipulation was reflected in the observed decrease in urine osmolality, placing participants into a “slightly hyperhydrated” category [601 mOsm/kg; (Armstrong et al. 2010)]. LTM gain occurred primarily in the trunk region (+6% trunk LTM), likely repleting the region most affected during the depletion protocol. However, a significant increase was also observed in the legs (+2.8% leg LTM). One previous study has investigated the influence of a high carbohydrate diet on DXA measurement, observing a 0.9 kg increase in total LTM in young active males following a 3-day diet with  $\geq 75\%$  carbohydrate intake (Rouillier et al. 2015). In contrast to the current study, the apparent changes occurred in the appendicular regions and not the trunk, possibly due to the lack of an initial depletion protocol. A period of carbohydrate deprivation may have further stimulated glycogen resynthesis in the trunk region when carbohydrates were given after exercise. In addition, since the greatest LTM loss was in the trunk following depletion, glycogen will be restored in this region. Further research into regional body

composition change due to the short-term alteration of hydration status and diet is required.

A total FM decrease ( $-0.2$  kg) and increase ( $+0.2$  kg) during depletion and supercompensation, respectively, did not exceed the least significant change (0.58 kg), confirming the results from the previous studies that fat mass is less likely to be affected by changes in body water and hydration status (Going et al. 1993; Nana et al. 2013). However, interpretation of the metric used to monitor serial measurement of adiposity may be confounded by the hydration status of soft tissues. Since body mass has been shown to decrease in a depletion phase, body fat percentage values will subsequently increase, despite no actual change in fat mass. Therefore, caution is warranted in the interpretation of this metric in athletes over time if stringent scanning protocols are not in place. To monitor change in adiposity over time while adjusting for body size, use of a fat mass index (total fat/height<sup>2</sup>) is recommended, since it is measured independent to changes in total and fat-free mass (Toomey et al. 2015).

It is important to note that the observed apparent changes in lean mass are not true changes; rather an error in measurement elicited through manipulation of hydration status. This is reflected in the correlation between change in LTM and change in urine osmolality. This study highlights the importance of employing a pre-scan protocol to standardise hydration status and reduce error in DXA measurement (Nana et al. 2015). The current standardised practice dictates that the participant is not to perform structured or strenuous training for 12 h prior to scanning. This condition is in place to minimise varying glycogen concentration in the muscle and fluid shifts in the body. Since DXA is reported to be widely used with athletes in weight-sensitive sports (Meyer et al. 2013), care is warranted in the assessment of athletes who often undergo drastic weight changes preceding competition as a result of change to hydration status (Santos et al. 2010). Despite carbohydrate-loading regimens being widely employed by athletes (Hawley and Burke 2010) and glycogen depleted training becoming a more recognised nutritional strategy for athletes (Bartlett et al. 2015), dietary measures in standardisation are greatly overlooked. Athletes typically have greater glycogen stores as an adaptation to training, and depending on their current phase in training may present in varying states of glycogen and hydration status. This study has highlighted scenarios, where manipulation of these states can drastically alter DXA results.

Extrapolation of the findings from this study is limited to young active non-obese men. It is unknown if similar alterations to hydration and LTM measurement would be observed in women with less relative LTM per body mass or elderly adults with slower restoration of body fluid homeostasis in response to water deprivation and exercise

than younger adults (Sawka et al. 2007). In comparison with plasma osmolality, urine indices of hydration status have limitations in the identification of changes in hydration status during periods of rapid body fluid turnover (Shirreffs 2003). However, to allow for acute body water fluctuations to stabilize, DXA and urine osmolality measures were taken the morning after the depletion protocol. Participants self-reported full adherence to the 48-h high carbohydrate diet. However, the authors did not have access to the personalised web accounts to validate this report, which is a limitation of the study.

This study has highlighted the extent to which DXA LTM results can be manipulated through short-term changes in hydration status and carbohydrate intake. Depletion and supercompensation protocols altered LTM measurement from baseline by 1.7 and 0.7 kg, respectively, far surpassing typical measurement error. This rapid loss and gain of LTM were reflected in changes in hydration status, warranting a strict measurement protocol, where serial measurements are carried out throughout periods of training. The importance of accurate body composition measurement should be highlighted to athletes and coaches to ensure that only rigorous data are used as a basis for decision making in training, nutrition, and performance.

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#### Compliance with ethical standards

**Ethical approval** All procedures performed involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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