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Errors in dual energy x-ray absorptiometry estimation of body composition induced

by hypohydration

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ABSTRACT

Dual energy X-ray absorptiometry (DXA) is a popular tool to determine body composition (BC) in athletes, and is used for analysis of fat-free soft tissue mass (FFST) or fat mass (FM) gain/loss in response to exercise or nutritional interventions. The aim of the present study was to assess the effect of exercise-heat stress induced hypohydration (HYP, >2% of body mass (BM) loss) vs. maintenance of euhydration (EUH) on DXA estimates of BC, sum of skinfolds (SF), and impedance [\(Berk et al.\)](#page-13-0) measurements in athletes. Competitive athletes (23 males and 15 females) recorded morning nude BM for 7 days prior to the first main trial. Measurements on the first trial day were conducted in a EUH condition, and again after exercise-heat stress induced HYP. On the second trial day, fluid and electrolyte losses were replaced during exercise using a sports drink. A reduction in total BM (1.6±0.4 kg; 2.3±0.4% HYP) and total LM (1.3±0.4 kg), mainly from trunk (1.1±0.5 kg), was observed using DXA when participants were HYP, reflecting the sweat loss. Estimated fat percent increased $(0.3±0.3%)$, however, total FM did not change $(0.1±0.2$ kg). SF and IMP declined with HYP (losses of $1.5\pm2.9\%$ and $1.6\pm3\%$ respectively) suggesting FM loss. When EUH was maintained there were no significant changes in BM, DXA estimates, or SF values pre to post exercise, but IMP still declined. We conclude that use of DXA for LM assessment in athletes must ensure a EUH state, particularly when considering changes associated with nutritional or exercise interventions.

Keywords: Fat-free soft tissue mass, fat mass, hydration, exercise, DXA,

INTRODUCTION

Body composition assessment is an important part of athlete monitoring; and is widely used to assess changes following exercise or nutritional interventions. Athletes competing in gravitational, weight class and aesthetic sports often reduce their body mass / fat mass, or maintain it as low as possible to gain a competitive advantage. In extreme cases, athletes could develop severe medical problems sometimes with fatal consequences [\(Nattiv et al., 2007\)](#page-14-0). Considering these practices, the International Olympic Committee Medical and Scientific Commission set up a Working Group on Body Composition Health and Performance to determine whether optimum body composition and/or minimum values for body fat content and body water content could be established [\(Sundgot-Borgen et al.,](#page-15-0) [2013\)](#page-15-0). Publications arising from the IOC working group highlight that greater understanding of factors influencing all aspects of body composition estimation is important [\(Ackland et al.,](#page-13-1) [2012;](#page-13-1) [Meyer et al., 2013;](#page-14-1) [Sundgot-Borgen et al., 2013\)](#page-15-0).

Dual energy X-ray absorptiometry (DXA) was originally designed to measure specific bone regions (bone mineral density of hip and spine) of older adults, has been used for over two decades, and is considered the gold standard technique for these assessments [\(Blake &](#page-13-2) [Fogelman, 2009\)](#page-13-2). More recently DXA has become a popular and accessible tool to determine fat and lean tissue composition. Many factors affect body composition estimation by DXA, one of which is soft tissue hydration. DXA scanning assumes soft tissues are normally hydrated for accurate partitioning into fat and lean fractions [\(Plank, 2005\)](#page-14-2) and that there is a constant hydration status of fat-free soft tissue mass (73%) ([\(Pietrobelli, Wang,](#page-14-3) [Formica, & Heymsfield, 1998\)](#page-14-3). However, hydration of fat-free soft tissue mass can range from 67-85% [\(Moore & Boyden, 1963\)](#page-14-4). Acute changes in hydration status can therefore alter fat-free soft tissue mass DXA estimates [\(Lohman, Harris, Teixeira, & Weiss, 2000\)](#page-14-5). Several clinical studies suggest DXA is able to detect small individual changes in total mass, soft and lean tissue mass in healthy adults and patients [\(Going et al., 1993;](#page-13-3) [Kohrt, 1995,](#page-13-4) [1998;](#page-13-5) [Pietrobelli et al., 1998\)](#page-14-3). To date, the effect of hypohydration followed by rehydration on DXA

estimates of fat-free soft tissue mass and fat mass has only been analysed in a non-athletic group using a 24h fluid restriction protocol [\(Going et al., 1993\)](#page-13-3) and has not used the most recent scanning technology. Therefore, analysing and understanding the effects of hypohydration on body composition estimation in an athlete population could be very important for sports nutrition practitioners and researchers. This is particularly true when assessing minimum body fat criteria and/or fat-free soft tissue mass changes in response to nutritional or exercise interventions.

Studies on the influence of daily activities, meal ingestion, and acute exercise on body composition estimates have already been performed in healthy controls [\(Horber,](#page-13-5) [Thomi, Casez, Fonteille, & Jaeger, 1992\)](#page-13-5) and more recently on athletes [\(Nana, Slater,](#page-14-6) [Hopkins, & Burke, 2013;](#page-14-6) [Nana, Slater, Hopkins, & Burke, 2011\)](#page-14-7). Furthermore, previous work has not compared DXA estimates with skinfolds/impedance analysis outcomes following an acute fluid deficit. We hypothesize that DXA estimates of fat-free soft tissue mass will track fluid balance deficits incurred during exercise-heat stress and that control of hydration status is a crucial part in assessment of body composition in athletes.

METHODS

We recruited 38 participants (23 males, 15 females) from different athletic clubs representing the range of physiques found among athletic populations. The study was approved by the University of Stirling Research Ethics Committee and the NHS East of Scotland Research Ethics Committee. Participants were excluded from the study if they were older than 40 years (older than typical athletic population on whom our research is focused) or not currently training / competing in their sport. Participants were involved in a range of sports (running, cycling, rowing, rugby, boxing, football, gymnastics, triathlon, martial arts, rock climbing, and tennis). Subject characteristics were: age 28.1±5.5 years, height 172.6±9.3 cm, stable baseline body mass 69.5±10.6 kg. Females were asked to complete all laboratory visits during the same menstrual phase to avoid potential changes in

body fluid and body mass. To achieve this we obtained menstrual cycle phase history information from them prior to, and during participation in the study.

Early morning body mass measurement

Participants were provided with a set of scales (Seca Quadra 808, Birmingham, UK) to record body mass for 7 days before the first test in their own homes. The scales were individually calibrated against known mass (range: 0-90 kg) prior to use. Calibration correlation coefficients were 0.99-1.00. Morning, fasted, nude body mass was recorded after emptying bladder and bowels to establish stability of body mass over the period before starting the trials. To reduce potential variance all participants used the same set of scales throughout the entire study period. No correction was applied to mass recordings to account for the slight differences between sets of scales.

Study design overview

Participants attended the laboratory on three occasions. The first visit was for prescreening, signing consent and issuing of scales for daily body mass recording. The second visit was 1 week later and was the first main trial day (Day 1) involving anthropometric measurements, impedance analysis and then DXA scanning. These measurements were conducted on entering the laboratory in a euhydrated condition, and again after a period of exercise-heat stress aimed at producing a fluid deficit of ≥2% of the initial body mass. A further week later participants attended for a final visit (Day 2) in which we repeated all of the measurements and the same exercise-heat stress work period as Day 1, but fluid losses and estimated energy/glycogen usage were replaced using a carbohydrate-electrolyte sports drink (Gatorade®) to maintain body mass (Figure 1A).

Standardized baseline conditions

For both days of testing, participants were asked to attend to the laboratory in the morning after fasting for at least eight hours, without doing strenuous exercise or ingesting alcoholic beverages the previous day. Participants were instructed to drink 500ml of water

2h before entering the laboratory to ensure euhydration. On arrival at the laboratory, participants emptied their bladder and bowels and provided a urine sample for initial hydration status assessment. Urine samples were measured for osmolality using a freezing point depression osmometer (Roebling, Camlab, UK). Initial nude body mass and anthropometric measures including stature and 8 skinfolds (triceps, subscapular, biceps, iliac crest, supraspinale, abdominal, front thigh and medial calf) using a Harpenden caliper were recorded. Land marking and measurements were done by the same International Society for the Advancement of Kinanthropometry (I.S.A.K.) level 3 trained anthropometrist following the International Standards for Anthropometric Assessment [\(Stewart, 2011\)](#page-14-8). Impedance was then estimated using a single frequency (50 kHz) bioelectrical impedance analysis device (Bodystat 1500) with participants in a supine position. All readings were obtained within 1 minute of adopting the supine posture. This procedure was to avoid erroneous impedance readings through fluid shifts that occur with prolonged periods in this position [\(Shirreffs & Maughan, 1994\)](#page-14-9) .Following these initial measurements participants were carefully positioned for one whole body DXA scan.

DXA scan

Body composition was measured from a whole body scan using a narrowed fanbeam DXA (iDXA GE Healthcare) with analysis performed using GE Encore 13.40.038 Software (GE Healthcare). All scans were performed and analysed by the same trained technician. iDXA was calibrated with phantoms as per manufacturer guidelines each day before measurement. All scans were undertaken using the standard thickness mode; automatically chosen by the software. Subjects wore minimal clothing (underwear) and removed jewellery and metallic objects for scans. We established a protocol for undertaking whole body scans which emphasized consistency in the positioning of subjects on the scanning area of the DXA instrument (Figure 1B).

Exercise protocol

Following scanning, participants undertook an exercise-heat stress protocol. On Day 1, exercise was performed without any food/fluid ingestion. Exercise was conducted on a stationary cycle ergometer (Monark 874E) in a warm environment (26.4±0.9°C) with participants wearing a plastic bin bag and warm clothing to enhance heat stress. The protocol consisted of 30 minutes of cycling at a predetermined fixed load (160±25W (males), 94±16W (females)) and pedal cadence (70 rpm) followed by subsequent 10 minute bouts. Between exercise bouts participants were asked to dry themselves off before undertaking a nude body mass measurement. Nude body mass measurement was required to ensure sweat in clothing did not influence mass loss assessment, and all measures were made with participants behind a privacy screen. Repeated bouts of cycling were performed until a 2% body mass loss was achieved. Activity duration, heart rate, power and pedal cadence were recorded during exercise. Following a 30 minute rest period to cool down, shower, and empty bladder we obtained final nude body mass, skinfold, impedance, and DXA measures.

On Day 2 participants replicated the exact intensity and duration of exercise performed on Day 1, with ingestion of a known volume of sports drink (Gatorade®) to replace the fluid losses experienced on Day 1. On Day 2, the aim was to maintain initial body mass and euhydration status.

Statistical analysis

Statistical analyses were conducted using Minitab, version 16.1.0. For tabulated and graphical data, mean and standard deviation (SD) values were used. Differences related to pre- or post-exercise scanning, gender or hydration status were tested using repeated measures analysis of variance and general linear model. P values < 0.05 were considered significant. Reliability measures for pre- and post-exercise anthropometric measurements, impedance and DXA scans also were conducted. Paired t-tests were used to assess whether absolute differences existed between pre-exercise measurements or measurements

pre- and post-exercise on Days 1 and 2 also to analyse pre exercise characteristics on both days to analyse if there was any difference in baseline conditions.

We also compared the results from repeat DXA scans and calculated the coefficient of variation (CV); defined by the SD of difference in duplicate measurements expressed as a percentage of the overall mean data [\(Hopkins, 2000\)](#page-13-6).

Results

Participants were of 8 different nationalities. Analysis of menstrual cycle phase history (female participants) revealed 40% (n=6) were in follicular phase, 53% (n=8) in luteal phase and 7% (n=1) presented amenorrhea confirmed by a sports medicine physician.

Reliability of baseline measures and conditions prior to and during each trial day

Participant body mass was not different across the 7 days preceding the trials and on trial days (Figure 2A). Urine osmolality (Day 1, 268 mOsm/kg (min: 98, max: 1203); Day 2, 290 mOsm/kg (min: 94, max: 1196)) demonstrated that most participants were generally well hydrated based on ACSM euhydration criteria [\(American College of Sports Medicine, 2007\)](#page-13-7) . However, urine osmolality values were sometimes variable within individuals between trials (Figure 2B) despite following the pre-trial water ingestion criteria.

All other pre-exercise data, including DXA measurements, were consistent between trial days. When analysing CV for the DXA body composition estimates between the preexercise scans on Day 1 and on Day 2, data were considered reliable. We found trivial CV of bone mineral density, fat expressed as a percentage, whole body tissue mass, whole body fat mass, whole body fat-free soft tissue mass and estimated body mass demonstrating minimal variability between Day 1 and Day 2 (Table 1). Room temperature (Day 1; 26.4 \pm 0.9°C, Day 2; 26.7 \pm 0.9°C) and relative humidity (Day 1; 37 \pm 5%, Day 2; 38 \pm 6%) were similar between trials. Average exercise duration for 2% body mass reduction was 60.9±12.1 min (males: 55.7±11.2; females: 69.0±8.5 min). Average exercising heart rate was 155±13

(Day 1) and $150±14$ beats per minute (Day 2) representing $(81±7$ and $78±8\%$ of age predicted maximum heart rate, respectively (Table 2).

Effects of exercise induced hypohydration on body mass and estimates of body composition

Exercise on Day 1 led to a mean body mass reduction of 1.6 ± 0.4 kg (2.3 ±0.4 %) hypohydration). Gender differences in losses were 1.8±0.3kg (males) and 1.3±0.3kg (females); representing $2.4\pm0.4\%$ and $2.2\pm0.3\%$ hypohydration, respectively. On Day 2 the mean fluid intake to match sweat losses was 1.5±0.4L (1.6±0.3L (males) and 1.2±0.3L (females)) and body mass was maintained during the exercise period.

DXA body composition values (including bone mineral density (BMD) and fat, lean and total mass), sum of skinfolds, and impedance are summarized in Table 3. On Day 1 there was a statistically significant reduction of 1.5 ± 0.4 kg (2.2%) in total tissue mass and 1.3±0.4kg (2.5%) in fat-free soft tissue mass from pre- to post-exercise (Figure 3). However, significant increases in fat mass percentage were observed following hypohydration (0.3±0.3%); no change in absolute fat mass (kg). With fluid replacement on Day 2 there were no significant changes in any DXA estimates.

Differences in body segment composition (arms, legs and trunk) were also assessed pre- and post–exercise using DXA data (Figure 4). On Day 1, trunk (tissue fat percentage increased (0.5 \pm 0.7%) while total tissue mass (1.2 \pm 0.5kg) and lean tissue mass (1.1 \pm 0.6kg) decreased, with no changes in segment composition pre- post-exercise noted on Day 2. Sum of skinfolds was significantly lower on Day 1 (Table 3) following hypohydration $(1.5\pm2.9\%)$; all participants). Analysed by gender the reduction in males was 1.4 \pm 3.5% and in females 1.6 \pm 2.0%. On Day 2, sum of skinfolds decreased by 0.3 \pm 2.5% (full group) and $0.1\pm2.7\%$ and $0.8\pm2.1\%$, respectively for males and females, but there was no significant interaction effect. Impedance was significantly reduced by hypohydration on Day 1 (Table 3) from 504±66 to 495±64Ω. On Day 2 there was also a significant reduction in impedance from 507±69 to 498±61 Ω.

Discussion

This is the first study in a trained athlete population to examine the effects of combined exercise-heat stress and accompanying hypohydration on DXA estimates of whole and regional body composition. This type of intervention related to hydration status (hypo- and hyper-hydration) has only been analysed previously in non-athletic groups using 24h fluid restriction or dialysis [\(Going et al., 1993;](#page-13-3) [Horber, Thomi, Casez, Fonteille, &](#page-13-8) [Jaeger, 1992\)](#page-13-8). In the present study, baseline measures (initial body mass, initial hydration status, sum of skinfolds, impedance and body composition), and environmental conditions were all consistent and demonstrated that under these experimentally controlled conditions estimates of body composition are reliable. In our study, exercise-induced hypohydration reduced total mass, total tissue mass and fat-free soft tissue mass estimates from DXA. With maintenance of euhydration we did not observe any significant differences in estimations and measurements from pre- to post-exercise.

A recent study investigated the effects of exercise and *ad libitum* meal/fluid intake on DXA estimates of body composition in cyclists [\(Nana et al., 2013\)](#page-14-6). The authors observed these factors are associated with changes in mean estimates of total and regional body composition that range from trivial to small but substantial [\(Nana et al., 2013\)](#page-14-6). The loss of body mass examined in the present study represents a common level of hypohydration that could be presented as a non-optimal hydration strategy or as part of an intentional dehydration to "make the weight" in category classified sports [\(Ackland et al., 2012;](#page-13-1) [Klungland Torstveit & Sundgot-Borgen, 2012;](#page-13-9) [Sundgot-Borgen & Garthe, 2011\)](#page-15-1). With hypohydration we observed a significant reduction in total tissue and fat-free soft tissue mass determined by DXA from pre- to post-exercise that was not evident when euhydration was maintained. Sum of skinfolds and impedance data demonstrated reductions from pre- to post-exercise on the hypohydration trial suggesting a loss in fat mass that was not evidenced in DXA scan data. Skinfold results correspond with previous studies which have shown hydration affects elasticity and compressibility of tissues modifying the measurement

of skinfolds [\(Ward, Rempel, & Anderson, 1999\)](#page-15-2). The output from impedance matches with previous findings which demonstrated small fluid changes (gains or losses) could be misinterpreted as changes in fat content of an athlete [\(Saunders, Blevins, & Broeder, 1998\)](#page-14-10).

DXA changes in body composition by region in the pre- to post-exercise scans on the hypohydration trial revealed changes were mainly localised to the trunk region. The localisation of effects to the trunk could be explained by losses in specific body fluid compartments, particularly blood volume. A reduction in blood volume would lead to reduced central blood volume when lying in a supine position for scanning, as the flow from splanchnic and renal circulations is redistributed [\(Kenney, 2008;](#page-13-10) [Rowland, 2001;](#page-14-11) [Rowland &](#page-14-12) [Roti, 2004\)](#page-14-12). Although previous studies have found an effect of gender [\(Buehring et al.,](#page-13-11) [2013\)](#page-13-11), the current research observed the significant differences with hypohydration were consistent between genders. When euhydration was maintained there was no significant change in body mass, whole body or regional DXA scan indices, or sum of skinfolds from pre- to post-exercise. A previous study in non-athletes observed, following intake of 0.8–2.4 L of water, that the determination of bone mineral content and of fat mass by DXA were not affected, while estimation of fat-free soft tissue mass in the trunk region was considerably increased [\(Horber et al., 1992\)](#page-13-8). The present work adds to this literature by demonstrating the magnitude of change in fat-free soft tissue mass estimates with a 2% hypohydration in an athlete population.

Participants were asked to carefully control baseline conditions prior to arrival in the laboratory to achieve reliable measurements for body composition from DXA, skinfolds, and impedance analysis. The values obtained were clearly consistent between pre-exercise assessments on the two trial days. This suggests our attempts to avoid variation in baseline body mass and composition, by controlling hydration status, dietary intake, and prior exercise, were effective. In female participants we ensured they completed the trials during the same phase of their menstrual cycle as body mass may fluctuate throughout the menstrual cycle. Findings from a previous study on 41 females demonstrated average body

mass increased by 0.3% between follicular and luteal phases [\(Pliner & Fleming, 1983\)](#page-14-13). Research suggests an increase in body mass during luteal phase is not attributable to fluid retention; but rather an alteration in energy intake [\(Chihal, 1990;](#page-13-12) [Pliner & Fleming, 1983;](#page-14-13) [Tomazo-Ravnik T, 2006\)](#page-15-3). By considering menstrual cycle phase we could track the unique differences between trials with changes in hydration status from pre- to post-exercise alone.

In conclusion, this research provides additional guidance for future use of DXA in athletes, such as ensuring athletes are euhydrated before scanning. Assessment of hydration status should be considered optimal practice for test-retest scans, and consideration should be given to menstrual cycle phase in females. Thus, by controlling hydration status prior to scanning practitioners can more accurately evaluate fat-free soft tissue mass changes in athletes as part of nutritional or exercise interventions.

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REFERENCES

- Ackland, T. R., Lohman, T. G., Sundgot-Borgen, J., Maughan, R. J., Meyer, N. L., Stewart, A. D., & Muller, W. (2012). Current status of body composition assessment in sport: review and position statement on behalf of the ad hoc research working group on body composition health and performance, under the auspices of the I.O.C. Medical Commission. *Sports Med, 42*(3), 227-249. doi: 10.2165/11597140-000000000-00000
- American College of Sports Medicine, S., M. N., Burke, L. M., Eichner, E. R., Maughan, R. J., Montain, S. J., Stachenfeld, N. S. (2007). American College of Sports Medicine Position Stand. Exercise and fluid replacement. *Med Sci Sports Exerc, 39*(2), 377-390. doi: 10.1249/mss.0b013e31802ca597
- Berk, L. S., Nieman, D. C., Youngberg, W. S., Arabatzis, K., Simpson-Westerberg, M., Lee, J. W., . . . Eby, W. C. (1990). The effect of long endurance running on natural killer cells in marathoners. *Med Sci Sports Exerc, 22*(2), 207-212.
- Blake, G. M., & Fogelman, I. (2009). The clinical role of dual energy X-ray absorptiometry. *Eur J Radiol, 71*(3), 406-414. doi: 10.1016/j.ejrad.2008.04.062
- Buehring, B., Krueger, D., Libber, J., Heiderscheit, B., Sanfilippo, J., Johnson, B., . . . Binkley, N. (2013). Dual-Energy X-Ray Absorptiometry Measured Regional Body Composition Least Significant Change: Effect of Region of Interest and Gender in Athletes. *J Clin Densitom*. doi: 10.1016/j.jocd.2013.02.012
- Chihal, H. J. (1990). Premenstrual syndrome: an update for the clinician. *Obstet Gynecol Clin North Am, 17*(2), 457-479.
- Going, S. B., Massett, M. P., Hall, M. C., Bare, L. A., Root, P. A., Williams, D. P., & Lohman, T. G. (1993). Detection of small changes in body composition by dual-energy x-ray absorptiometry. *Am J Clin Nutr, 57*(6), 845-850.
- Hopkins, W. G. (2000). Measures of reliability in sports medicine and science. *Sports Med, 30*(1), 1- 15.
- Horber, F. F., Thomi, F., Casez, J. P., Fonteille, J., & Jaeger, P. (1992). Impact of hydration status on body composition as measured by dual energy X-ray absorptiometry in normal volunteers and patients on haemodialysis. *Br J Radiol, 65*(778), 895-900.
- Kenney, W. L. (2008). Human cardiovascular responses to passive heat stress. *J Physiol, 586*(1), 3. doi: 10.1113/jphysiol.2007.147215
- Klungland Torstveit, M., & Sundgot-Borgen, J. (2012). Are under- and overweight female elite athletes thin and fat? A controlled study. *Med Sci Sports Exerc, 44*(5), 949-957. doi: 10.1249/MSS.0b013e31823fe4ef
- Kohrt, W. M. (1995). Body composition by DXA: tried and true? *Med Sci Sports Exerc, 27*(10), 1349- 1353.
- Kohrt, W. M. (1998). Preliminary evidence that DEXA provides an accurate assessment of body composition. *J Appl Physiol (1985), 84*(1), 372-377.

- Lohman, T. G., Harris, M., Teixeira, P. J., & Weiss, L. (2000). Assessing body composition and changes in body composition. Another look at dual-energy X-ray absorptiometry. *Ann N Y Acad Sci, 904*, 45-54.
- Meyer, N. L., Sundgot-Borgen, J., Lohman, T. G., Ackland, T. R., Stewart, A. D., Maughan, R. J., . . . Muller, W. (2013). Body composition for health and performance: a survey of body composition assessment practice carried out by the Ad Hoc Research Working Group on Body Composition, Health and Performance under the auspices of the IOC Medical Commission. *Br J Sports Med, 47*(16), 1044-1053. doi: 10.1136/bjsports-2013-092561
- Moore, F. D., & Boyden, C. M. (1963). Body Cell Mass and Limits of Hydration of the Fat-Free Body: Their Relation to Estimated Skeletal Weight. *Ann N Y Acad Sci, 110*, 62-71.
- Nana, A., Slater, G. J., Hopkins, W. G., & Burke, L. M. (2013). Effects of exercise sessions on DXA measurements of body composition in active people. *Med Sci Sports Exerc, 45*(1), 178-185. doi: 10.1249/MSS.0b013e31826c9cfd
- Nana, A., Slater, G. R., Hopkins, W. G., & Burke, L. M. (2011). Effects of daily activities on DXA measurements of body composition in active people. *Med Sci Sports Exerc*. doi: 10.1249/MSS.0b013e318228b60e
- Nattiv, A., Loucks, A. B., Manore, M. M., Sanborn, C. F., Sundgot-Borgen, J., Warren, M. P., & American College of Sports, M. (2007). American College of Sports Medicine position stand. The female athlete triad. *Med Sci Sports Exerc, 39*(10), 1867-1882. doi: 10.1249/mss.0b013e318149f111
- Pietrobelli, A., Wang, Z., Formica, C., & Heymsfield, S. B. (1998). Dual-energy X-ray absorptiometry: fat estimation errors due to variation in soft tissue hydration. *Am J Physiol, 274*(5 Pt 1), E808-816.
- Plank, L. D. (2005). Dual-energy X-ray absorptiometry and body composition. *Curr Opin Clin Nutr Metab Care, 8*(3), 305-309.
- Pliner, P., & Fleming, A. S. (1983). Food intake, body weight, and sweetness preferences over the menstrual cycle in humans. *Physiol Behav, 30*(4), 663-666.
- Rowland, T. W. (2001). The circulatory response to exercise: role of the peripheral pump. *Int J Sports Med, 22*(8), 558-565. doi: 10.1055/s-2001-18526
- Rowland, T. W., & Roti, M. W. (2004). Cardiac responses to progressive upright exercise in adult male cyclists. *J Sports Med Phys Fitness, 44*(2), 178-185.
- Saunders, M. J., Blevins, J. E., & Broeder, C. E. (1998). Effects of hydration changes on bioelectrical impedance in endurance trained individuals. *Med Sci Sports Exerc, 30*(6), 885-892.
- Shirreffs, S. M., & Maughan, R. J. (1994). The effect of posture change on blood volume, serum potassium and whole body electrical impedance. *Eur J Appl Physiol Occup Physiol, 69*(5), 461-463.
- Stewart, A. D., Marfell-Jones, M., Olds, T., de Ridder, H. (2011). International Standards for Anthropometric Assessment.

- Sundgot-Borgen, J., & Garthe, I. (2011). Elite athletes in aesthetic and Olympic weight-class sports and the challenge of body weight and body compositions. *J Sports Sci, 29 Suppl 1*, S101-114. doi: 10.1080/02640414.2011.565783
- Sundgot-Borgen, J., Meyer, N. L., Lohman, T. G., Ackland, T. R., Maughan, R. J., Stewart, A. D., & Muller, W. (2013). How to minimise the health risks to athletes who compete in weightsensitive sports review and position statement on behalf of the Ad Hoc Research Working Group on Body Composition, Health and Performance, under the auspices of the IOC Medical Commission. *Br J Sports Med, 47*(16), 1012-1022. doi: 10.1136/bjsports-2013- 092966
- Tomazo-Ravnik T, J. V. (2006). Changes in total body water and body fat in young women in a course of menstrual cycle. . *Int Jnl Anthropology*(21), 55-60.
- Ward, R., Rempel, R., & Anderson, G. S. (1999). Modeling dynamic skinfold compression. *Am J Hum Biol, 11*(4), 531-537. doi: 10.1002/(SICI)1520-6300(1999)11:4<531::AID-AJHB13>3.0.CO;2-6

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Figure 1: Study design (A). The exercise time varied depending on each subject and positioning protocol (B) for dual energy X ray absorptiometry (DXA): Subjects were centrally aligned in the scanning area of the DXA instrument, we measured 3 cm from the distance of the top line drawn on the surface of the bed to the vertex of the head of the participants, the hands were in a prone position and we ensured that the distance between the thumbs and the legs was 3 cm, we placed a foam block between their feet which was transparent under the DXA scan to maintain a constant distance between the feet of 28 cm in each scan. All the distances were measured with a metric ruler in each scan. The scans were analysed automatically by the software, with regions of interest subsequently confirmed by the technician prior to data analysis.

IBM= initial body mass.

Figure 2: Mean (SD) nude body mass (A) of the participants over 7 days prior to beginning the trials and also the body mass before exercise on both trial days (D-1 and D-2). No differences in nude body mass were observed across days. Urine osmolality (B) determined on attending the laboratory on Day 1 and Day 2 for initial hydration status assessment. Values are shown as the median (range) for n=38. Individual data are also plotted in the figure.

Figure 3: Absolute changes from pre to post exercise induced hypohydration on Day 1 and on Day 2: change in fat percentage (tissue %fat), change in tissue mass (tissue, kg) and change in fat mass (Fat, kg) and fat-free soft tissue mass (FFST, kg) estimated by DXA.

(*indicates significant difference)

Figure 4: Changes in DXA body composition in different body segments. Part A shows absolute changes between pre and post exercise on Day 1. Part B presents the differences between pre and post exercise on Day 2. (*indicates significant difference)

Table 2. Exercise and environmental characteristics recorded on each trial day. Day 1 refers to the hypohydration trial; Day 2 refers to the euhydration trial.

Table 3: Body composition data analysed on each trial day. Pre and post exercise (Pre-ex, Post-ex) values, percentage change pre to post exercise (% \triangle), and mean difference (95% confidence interval) between pre and post-exercise values are shown. Day 1 refers to the hypohydration trial; Day 2 refers to the euhydration trial.

*** indicates significant difference from pre-exercise