



LEEDS
BECKETT
UNIVERSITY

Citation:

Harley, JA and Hind, K and O'Hara, J (2011) Three-compartment body composition changes in elite rugby league players during a super league season, measured by dual-energy X-ray absorptiometry. *Journal of strength and conditioning research / National Strength & Conditioning Association*, 25 (4). 1024 - 1029. ISSN 1064-8011 DOI: <https://doi.org/10.1519/JSC.0b013e3181cc21fb>

Link to Leeds Beckett Repository record:

<https://eprints.leedsbeckett.ac.uk/id/eprint/313/>

Document Version:

Article

The aim of the Leeds Beckett Repository is to provide open access to our research, as required by funder policies and permitted by publishers and copyright law.

The Leeds Beckett repository holds a wide range of publications, each of which has been checked for copyright and the relevant embargo period has been applied by the Research Services team.

We operate on a standard take-down policy. If you are the author or publisher of an output and you would like it removed from the repository, please [contact us](#) and we will investigate on a case-by-case basis.

Each thesis in the repository has been cleared where necessary by the author for third party copyright. If you would like a thesis to be removed from the repository or believe there is an issue with copyright, please contact us on openaccess@leedsbeckett.ac.uk and we will investigate on a case-by-case basis.

Three-compartment body composition changes in elite rugby league players during a *Super League* season, measured by dual-energy X-ray absorptiometry.

J.A.Harley^{1,2}

Karen Hind³

John O'Hara³

¹ *Teesside University, Teesside, UK.*

² *Middlesbrough Football Club, UK.*

³ *Carnegie Research Institute, Leeds Metropolitan University, Leeds, UK.*

Correspondence to:

Jamie Harley

Middlesbrough Football Club Training Headquarters,

Rockliffe Park,

Hurworth Place,

Nr Darlington,

DL2 2DU.

UK.

07841 697504

jamie.harley@mfc.co.uk

Abstract

This study investigated the acute changes in body composition that occur over the course of a competitive season in elite rugby league players. Twenty elite senior players from an English *Super League* rugby league team underwent a total-body dual-energy x-ray absorptiometry scan at three phases of a competitive season: pre-season (February), mid-season (June) and post-season (September). Body mass, fat mass, lean mass, percentage body fat and bone mineral content were reported at each phase. Between the start and mid-point of the season, body mass, lean mass, fat mass and body fat percentage showed no significant change ($p>0.05$), however bone mineral content was significantly increased ($+0.71\%$; $30.70 \pm 38.00\text{g}$; $p<0.05$). Between the mid-season and post-season phase, body mass and bone mineral content showed no significant change ($p>0.05$), however significant changes were observed in lean mass (-1.54% ; $1.19 \pm 1.43\text{kg}$), fat mass ($+4.09\%$; $0.57 \pm 1.10\text{kg}$) and body fat percentage ($+4.98\%$; $0.78 \pm 1.09\%$; $p<0.05$). The significant changes in body composition seen over the latter stages of the competitive season may have implications for performance capabilities at this important stage of competition. An increase in fat mass and decrease in lean mass may have a negative effect on the power/body mass ratio, and therefore may be a cause for concern for playing, coaching and medical staff.

Keywords: Body composition, elite sport, rugby league, DXA.

Introduction

Body composition analysis is frequently carried out in professional sport in order to monitor acute changes in physiological status, using a range of techniques including skinfold analysis, body density and volume measurements, bioelectrical impedance methods and dual energy x-ray absorptiometry (Ellis, 2000), to assess individual levels of body mass (BM), fat mass (FM), and lean tissue mass (LM).

Professional rugby league players require high muscular power and strength (Meir, 2001), and consequently the LM to FM ratio that will provide optimal performance is often targeted and measured at regular intervals throughout the training and competition period. Due to the highly intermittent nature of rugby league, it is difficult to describe an 'optimal' body composition which encompasses all elite players, because of the inherent individual differences that exist between players and teams, and somatotopic requirements for the different playing positions (Gabbett, 2005a). To provide more valuable information, the body composition of rugby league players can be monitored throughout a competition cycle in order to create an anthropometric profile of each individual player that can be monitored throughout the season. This can also help avoid any changes in body composition that may be detrimental to performance, as well as providing reference body composition values that can be targeted after a period of detraining or injury, and to act as an indicator of physiological status and training adaptations.

The seasonal changes in anthropometric profile have previously been considered in both amateur (Gabbett, 2005b) and junior (Pyne et al., 2003) rugby league players using skinfold analysis. Egan *et al.* (2005) assessed the seasonal variations in body composition of elite soccer players using five testing phases over two seasons, but were not able to collect data at the pre-season, mid-season and end-of season phases because of the international playing commitments of players post-season. This study, in accordance with Gabbett (2005b) and Holymard & Hazeldine (1993), utilised testing phases which encompassed an entire competitive season, in order to build a detailed picture of the anthropometric changes that occur as a result of playing and training status. Measurements were taken at the pre-season, mid-season and post-season phases.

The physiological and anthropometric characteristics of rugby league players have previously been reported with junior (Gabbett, 2002), amateur (Gabbett, 2000), sub-elite (Gabbett, 2006) and senior (Gabbett, 2002) rugby league players, but have not previously been reported for any professional male players from the English *Super League*. Furthermore, studies have been limited in that the body fat percentage (BF%) of players have commonly been measured using a four-site skinfold (biceps, triceps, subscapular, suprailiac) and the Durnin & Womersley (1974) equation. Skinfold analysis, which is an example of a two-compartment (2-C) model of body composition, has previously been shown to significantly overestimate BF% in elite rugby league players when using the Durnin & Womersley (1974) four-site and Jackson & Pollock (1978) seven-site equation in comparison to DXA, a three-compartment model (3-C) (Harley et al., 2009). Other 2-C models, such as the Jackson & Pollock (1978) three-site skinfold equation, when using either the Siri (1961) or Brozek (1963) equations to convert body density to BF%, have been shown to significantly underestimate BF% in this population (Harley et al., 2009). The proportions of FM and LM of the human body can be measured more accurately by increasing the level of compartments considered as part of the whole body from a 2-C to a 3-C model, using dual energy x-ray absorptiometry (DXA).

DXA uses a three-compartment model (3-C) to assess body composition (bone mineral content (BMC), LM and FM). DXA can provide highly precise measurements of soft tissue composition (Laskey, 1996) and can provide details of the body composition of various body segments. DXA has been reported to provide accurate body composition estimates when compared with the four-compartment (4-C) model in young adults who vary in gender, race athletic status, body size, musculoskeletal development and body fatness (Prior et al., 1997). Conversely, the 4-C method can be very time consuming and expensive, and subsequently DXA has rapidly gained acceptance as a reference method for body composition analysis (Van der Ploeg et al., 2003). In addition to the accurate assessment of FM and LM, the use of DXA in this study enabled the quantification of BMC in elite rugby league players, and the changes that occur in BMC across a competitive season, which has not previously been reported in the literature.

It may be suggested that physiological performance capabilities should peak by the end of the pre-season phase and be maintained throughout the entire competition period. However, previous studies have shown that fitness levels in rugby league deteriorate as a season progresses, perhaps due to increased playing commitments (Gabbett, 2005b; Pyne et al., 2003). Traditional periodisation of strength training programmes is difficult in many team sports as players are often required to play between 1-3 competitive matches per week. Therefore, maintenance of body composition throughout the competition phase can be a good indicator of physiological status. The aim of this study was to assess the extent to which body composition values are maintained throughout a competitive season, by the measurement of changes in BM, FM, LM and BMC between the pre-season, mid-season, and post-season phases, in elite male rugby league players.

Methods

Twenty (20) male participants from an English *Super League* rugby league team agreed to take part in the study (mean \pm SD age 25.48 ± 3.36 years; stature 182.41 ± 6.53 cm; body mass 95.28 ± 11.33 kg). The ethnicity of the study group consisted of 16 Caucasian, 2 black British and 2 Pacific Island participants. Written informed consent to participate was received from all players prior to testing, and the project was approved by a radiation protection advisor and the University's Faculty Research-Ethics Sub Committee.

Testing Phases

All participants were tested on three separate occasions during the 2008 *Super League* season (Fig. 1). The first testing phase (T1) occurred at the end of the pre-season period (February 2008). The second testing phase (T2) occurred at the mid-point of the competitive season (June 2008). The third and final testing phase (T3) occurred the week following the conclusion of the competitive season, before many of the players had International commitments (September 2008).

Body Composition Assessment

Participants underwent a total-body DXA scan (iDXA, GE Medical Systems, Lunar, UK), from which estimates of FM, LM, BF% and BMC was derived based on an extrapolation of fatness from the ratio of soft tissue attenuation of two x-ray energies in pixels, not containing bone (Mazess et al., 1990). Participants lay horizontally on the scanning table with all metal artefacts removed (so as not to interfere with the results). The scan is conducted as the scanning arm travels over the participant's body from head to toe, and the standard mode scans took 6.5 minutes. Scans for heavier participants (those weighing over 100kg) were conducted using the thick mode, and these scans took 15 minutes. All scanning and subsequent analysis was made by the same trained operator, on the same day of the week at each testing phase, and prior to daily training. Machine calibration checks were carried out on a daily basis, and showed no significant machine drift during the study. Data were excluded from the study should a player receive an injury that resulted in exclusion from training and match-play for a period of longer than two weeks.

Statistical Analysis

Statistical analyses were conducted using SPSS Version 16.0 (SPSS Inc, Chicago, Ill). Changes in LM, FM, BF%, BM, and BMC between testing phases were made using t-tests for matched samples. Comparisons of anthropometric profiles between playing position were assessed using t-tests for independent samples. Effect size was reported to assess the magnitude of the observed differences, along with 90% Confidence Intervals (CI). Where presented in the text, data are given as mean \pm standard deviation (significance value (p); effect size (d) [90% CI lower:upper]). Statistical significance was set at $P < 0.05$.

Results

Group mean \pm SD body composition values at the three testing phases are shown in Table 1.

Table 1. Mean \pm SD body composition values at each testing phase.

	Body Mass (kg)	Fat Mass (kg)	Body Fat (%)	Lean Mass (kg)	BMC (g)
T1	95.28 \pm 11.33	13.59 \pm 3.66	15.21 \pm 3.55	77.38 \pm 9.36	4292 \pm 595
T2	95.55 \pm 11.99	13.92 \pm 4.06	15.47 \pm 3.66	77.40 \pm 9.56	4338 \pm 613 ^a
T3	95.05 \pm 11.81	14.49 \pm 4.05 ^{a, b}	16.24 \pm 3.79 ^{a, b}	76.21 \pm 9.44 ^{a, b}	4326 \pm 594 ^a

^a significantly different to T1

^b significantly different to T2; ($p < 0.05$).

Mean group BM was not significantly different between testing phases ($p > 0.05$), with a similar range of BM values being observed across phases, indicated by consistent standard deviations of the data (Tab. 1). There was an increase in mean FM of 0.57 \pm 1.10kg ($p = 0.002$; $d = 0.4$ [0.21:0.59]) between phases T2 and T3, a mean

change of 4.09%. Over the course of the competitive season, between T1 and T3, fat mass increased by $0.90 \pm 1.14\text{kg}$ ($p=0.031$; $d=0.22$ [0.06:0.38]), a mean increase of 6.62%. FM measured between T1 and T2 showed a mean group increase of 2.42%, however this result did not show statistical significance ($p=0.273$; $d=0.06$ [-0.032:0.15]). In line with the significant increase in group FM between the mid-point and end of the competitive season, mean group LM decreased significantly between T2 and T3 by $1.19 \pm 1.43\text{kg}$ ($p=0.001$; $d=0.42$ [0.23:0.61]), a decrease of 1.54%. LM also decreased over the entire season by $1.17 \pm 1.33\text{kg}$ ($p=0.001$; $d=0.45$ [0.25:0.65]), a seasonal decrease of 1.51%. LM measured between T1 and T2 was not significantly different ($p=0.95$; $d=0.001$ [-0.026:0.02]). Changes in BF% are directly affected by changes in FM, but are also indirectly affected by changes in LM. Therefore the observed increase in FM and decrease in LM between the mid-point and end of the season would suggest a mean increase in BF%. In absolute terms, mean BF% increased by $0.78 \pm 1.09\%$ ($p=0.005$; $d=0.35$ [0.16:0.54]) between T2 and T3, representing a significant change of 4.98%. BF% also increased significantly by $1.03 \pm 1.14\%$ ($p=0.001$; $d=0.46$ [0.26:0.66]) over the course of the entire season, a relative increase of 6.77%. Mean BF% measured between T1 and T2, although increased, was not significantly different ($p>0.359$; $d=0.04$ [-0.034:0.11]).

BMC showed a significant increase between the start and mid-point of the season ($30.70 \pm 38.00\text{g}$ ($p=0.002$; $d=0.41$ [0.21:0.61]), representing an increase of 0.71%. Over the seasonal period, BMC increased by $31.55 \pm 33.64\text{g}$ ($p<0.001$; $d=0.48$ [0.31:0.65]). BMC measured between T2 and T3 was not significantly different ($p>0.05$).

Significant differences in baseline anthropometric properties were found between playing position for BM (Forwards $102.72 \pm 8.22\text{kg}$; Backs $87.84 \pm 8.96\text{kg}$; $p=0.01$), FM (Forwards $15.28 \pm 3.29\text{kg}$; Backs $11.90 \pm 3.33\text{kg}$; $p=0.035$), LM (Forwards $82.81 \pm 7.36\text{kg}$; Backs $71.94 \pm 8.05\text{kg}$; $p=0.005$) and BMC (Forwards $4601.40 \pm 547.05\text{g}$; Backs $3988.10 \pm 489.58\text{g}$; $p=0.17$). BF% was not significantly different between playing positions (Forwards $16.25 \pm 3.28\%$; Backs $14.18 \pm 3.68\%$; $p>0.05$). However, no significant between groups differences were observed in changes in BM, FM, LM, BF% or BMC over the course of the season between forwards and backs, with both groups of players displaying similar trends at each testing phase.

Discussion

Mean group BM showed no significant change over the course of the competitive season. Although acute changes in BM can be a useful indicator of a change in physiological status of an individual (e.g. increased fat mass), the absence of a significant change in BM does not indicate that the internal composition of FM and LM has not altered.

In this study, LM showed no significant change between the beginning and mid-point of the season. The reduction in LM (-1.54%) across the group which was found between mid point and the end of season, may have been due to various factors. The increase in the number of games played changes the training load towards the conclusion of the season. This often results in a shift of the focus of training from the development of fitness levels to the maintenance of fitness levels (Gabbett, 2005b). The prevalence of injuries towards the end of the season mean that certain players are not able to maintain the weekly training load that has brought about the

positive physiological changes in body composition, and results in the effects of detraining including muscular atrophy.

It may be suggested that in many sports, especially aerobically-based team sports, carrying excess body fat may be detrimental to performance, as it is extra weight that has to be lifted against gravity, which will only act to slow a performer (Meir, 2001). Some studies have suggested that rugby league players may benefit from higher subcutaneous fat levels as a means of protection from injury (Brewer, 1995; Meir, 1993). However, the majority of evidence suggests that carrying excess body fat has a negative effect on performance (eg. power/body mass ratio, thermoregulation, aerobic capacity) (Gabbett, 2005a). Therefore, decreasing fat mass is usually advantageous to athletic performance. The increase in FM (4.09%) in the latter stages of the season coincided with the reduction in LM at the same stage of competition, and it is possible that these outcomes were related. However, although a decrease in LM is most likely caused by a change in training status, the cause of a significant increase in FM, whilst also possibly due to a change in training practices, could also be caused by dietary and nutritional factors. In particular, maintaining the same weekly energy intake over the course of a season, whilst decreasing weekly energy expenditure (decrease in training load) would be likely to result in a state of positive energy balance, leading to an increase in the stores of both visceral and subcutaneous body fat levels (Manore & Thompson, 2000).

The observed decrease in LM and increase in FM over the season resulted in a net increase in BF% of 6.7% over the competition period. This may have significant implications in terms of the speed, agility, power and functional strength of individual players, as decreased LM, and therefore increased BF%, may contribute to inferior speed and muscular power due to the reduced power to body mass ratio and performance in match specific tasks (Gabbett, 2005a). It may be suggested that the aim for strength and conditioning professionals should be to maintain a favourable body composition throughout the season in all players, especially in a sport where excess FM and insufficient LM could have a negative implication for performance. It is also important for players to be in suitable physical condition towards the end of the season where outcomes may decide final league positions (Egan et al., 2005).

In addition to changes in FM and LM, an important benefit of physical training is its ability to increase bone mass. An exposure to a variety of strain types, magnitudes, rates and frequencies offers ideal conditions for bone mineralisation (Lanyon, 1984). In particular, the demands of rugby league require frequent and significant impacts, quick accelerations and decelerations, jumping, kicking and scrummaging, all of which will offer the stimuli for bone growth according to the Mechanostat theory (Wolff, 1892; Schoenau & Fricke, 2006). The use of DXA in this study allowed for the measurement of BMC, which has not previously been reported in *Super League* rugby league players, and also for the assessment of changes in BMC over the course of the competitive season. Maximizing peak bone mass, maintaining bone mass thereafter, and minimizing bone loss over a competition period, are three factors that could reduce the risk of osteoporosis and subsequent fractures (Nordstrom et al., 2005). The ability of bone to adapt to mechanical loading is increased during the growth period (puberty) (Schoenau, 2006), which is the time when most players in this study began to play rugby league, undertaking training sessions to develop muscular strength and power, and subsequent skeletal loading.

Each bone remodelling cycle typically takes three months (Watts, 1999); the duration of this study was seven months. The significant increase in BMC (0.71%) from the start to the mid-point of the season in this study, over four months, may reflect change in training methods between phases. The hypertrophy phase of

training towards the beginning of the season may stimulate the greatest rate of bone accrual as a result of the amount of axial loading that occurs through this type of training. The lack of change in BMC between the mid-point and end of the season (0.02%), may reflect the focus of training which changed from muscular development to the maintenance of general fitness levels. This results in less skeletal load being applied during training, and therefore decreasing the rate of bone accrual. Future studies to investigate this further would benefit from the investigation of bone turnover through biochemical markers measured at each phase of training, rather than DXA, as bone turnover markers can identify acute changes more effectively.

The findings of this study over the seven month competition period have important implications for sports-science, coaching and conditioning staff. FM and LM should be measured independently of total body mass measures, in order to maintain a favourable physiological status over the entire competition period. Information on acute changes in body composition may be used to direct training and weight-control practices, to enable performance to be maximised until the conclusion of the competitive season.

Acknowledgements

Thanks to Aleks Gross for his help in organising and enabling this study, and to the players for their participation.

Authors' Affiliations

This study was funded by the Carnegie Research Institute, Leeds Metropolitan University, UK. At the time of data collection, all authors were affiliated with Leeds Metropolitan University, UK. At the time of publication, J.A.Harley was affiliated with Teesside University, UK, and Middlesbrough Football Club, UK.

References

- 1 Astorino, T.A., Tam, P.A., Reitschel, J.C., Johnson, S.M., Freedman, T.P. (2004) Changes in physical fitness parameters during a competitive field hockey season. *Journal of Strength and Conditioning Research*. 18, pp.850-54.
- 2 Brewer, J., Davis, J., Kear, J. (1994). A comparison of the physiological characteristics of rugby league forwards and backs. *Journal of Sports Science*. 12, pp.158.
- 3 Brewer, J., Davis, J. (1995). Applied physiology of Rugby League. *Sports Medicine*. 20(3), pp.129-135.
- 4 Durnin, J.V., Womersley, J. (1974). Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. *British Journal of Nutrition*. 32(1), pp.77-97.

- 5 Egan, E., Wallace, J., Reilly, T., Chantler, P., Lawlor, J. (2005). Body composition and bone mineral density during a premier league season as measured by dual-energy X-ray absorptiometry. *International Journal of Body Composition Research*. 4(2), pp.61-66.
- 6 Ellis, K.J. (2000). Human body composition: in vivo methods. *Physiological Reviews*. 80, pp. 649-680.
- 7 Gabbett, T.J. (2000). Physiological and anthropometric characteristics of amateur rugby league players. *British Journal of Sports Medicine*. 34, pp.303–307.
- 8 Gabbett, T.J. (2002). Physiological characteristics of junior and senior rugby league players. *British Journal of Sports Medicine*. 36, pp.334–339.
- 9 Gabbett, T.J. (2005a). Science of rugby league football: a review. *Journal of Sports Sciences*. 23(9), pp.961-976.
- 10 Gabbett, T.J. (2005b). Changes in physiological and anthropometric characteristics of rugby league players during a competitive season. *Journal of strength and conditioning research*. 19, 400-408.
- 11 Gabbett, T.J. (2006). A comparison of physiological and anthropometric characteristics among playing positions in sub-elite rugby league players. *Journal of Sports Sciences*. 24(12), pp.1273–1280.
- 12 Harley, J.A., Hind, K., O’Hara, J., & Gross, A. (2009). Validation of the skin-fold thickness method and air displacement plethysmography with dual energy X-ray absorptiometry, for the estimation of % body fat in professional male rugby football league players. *International Journal of Body Composition Research*. 7(1), pp.7-14.
- 13 Holymard, D.J., & Hazeldine, R.J. (1993). Seasonal variation in the anthropometric and physiological characteristics of international Rugby Union players. In: *Science and Football II: Proceedings of the Second World Congress of Science and Football*. T. Reilly, J. Clarys, A. Stibbe eds. London: E and FN Spon, pp21-26.
- 14 Johnson, G.O., Nebelsick-Gullett, L.J., Thorland, W.G., Housh, T.J. (1989). The effect of a competitive season on the body composition of university female athletes. *Journal of Sports Medicine and Physical Fitness*. 29, pp.314-20.
- 15 Lanyon, L.E., Rubin, C.T. (1984). Static vs. dynamic loads as an influence on bone remodelling. *Journal of biomechanics*. 17(12), pp.897-905.
- 16 Laskey, M.A. (1996). Dual-energy X-ray absorptiometry and body composition. *Nutrition*.12, pp.45–51.
- 17 Manore, M., & Thompson, J. (2000). *Sport nutrition for health and performance*. Human Kinetics, Leeds, UK.
- 18 Mazess, R.B., Barden, H.S., Bisek, J.P., Hanson, J. (1990). Dual-energy x-ray absorptiometry for total-body and regional bone mineral and soft-tissue composition. *American Journal of Clinical Nutrition*. 51, pp.1106-1112.
- 19 Meir, R., Newton, R., Curtis, E., Fardell, M., Butler, B. (2001). Physical fitness qualities of professional rugby league football players: determination of positional differences. *Journal of Strength and Conditioning Research*. 15, pp.450– 458.
- 20 Meir, R. (1993). Evaluating players’ fitness in professional rugby league: reducing subjectivity. *Strength and Conditioning Coach*. 1, pp.11–17.
- 21 Nordstrom, A., Karlsson, C., Nyquist, F., Olsson, T., Nordstrom, P., Karlsson, M. (2005). Bone loss and fracture risk after reduced physical activity. *Journal of bone and mineral research*. 20, pp.202-207.

- 22 Prior, B.M., Cureton, K.J., Modlesky, C.M., Evans, E.M., Sloniger, M.A., Saunders, M., Lewis, R.D. (1997). In vivo validation of whole body composition estimates from dual-energy X-ray absorptiometry. *Journal of Applied Physiology*. 83(2), pp.623-630.
- 23 Schoneau, E. (2006). Bone mass increase in puberty: what makes it happen? *Hormone Research*. 65 (suppl.2), pp.2-10.
- 24 Schoenau, E., Fricke, O. (2006). Interaction between muscle and bone. *Hormone Research*. 66 (suppl.1), pp.73-78.
- 25 Shlers, W.A., Bolonchuk, W.W., Lukasi, H.C. (1991). Effects of participation in a collegiate sport season on body composition. *Journal of Sports Medicine and Physical Fitness*. 31, pp.571-6.
- 26 Van der Ploeg, G.E., Withers, R.T., Laforgia, J. (2003). Percent body fat via DEXA: comparison with a four-compartment model. *Journal of Applied Physiology*. 94, pp.499-506.
- 27 Watts, N.B. (1999). Clinical utility of biochemical markers of bone remodelling. *Clinical Chemistry*. 45, pp.1359-1368.
- 28 Wolff, J. (1892). *Das Gesetz der Transformation der Knochen*. Berlin A. Hirschwild. Translated by Maquet, P., & Furlong, R. (1986). *The Law of Bone Remodelling*. Springer-Verlag, Berlin.

Figures

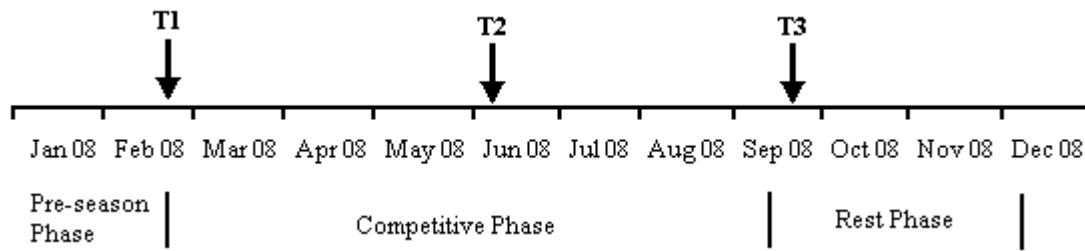


Figure 1 Timeline for anthropometric assessments

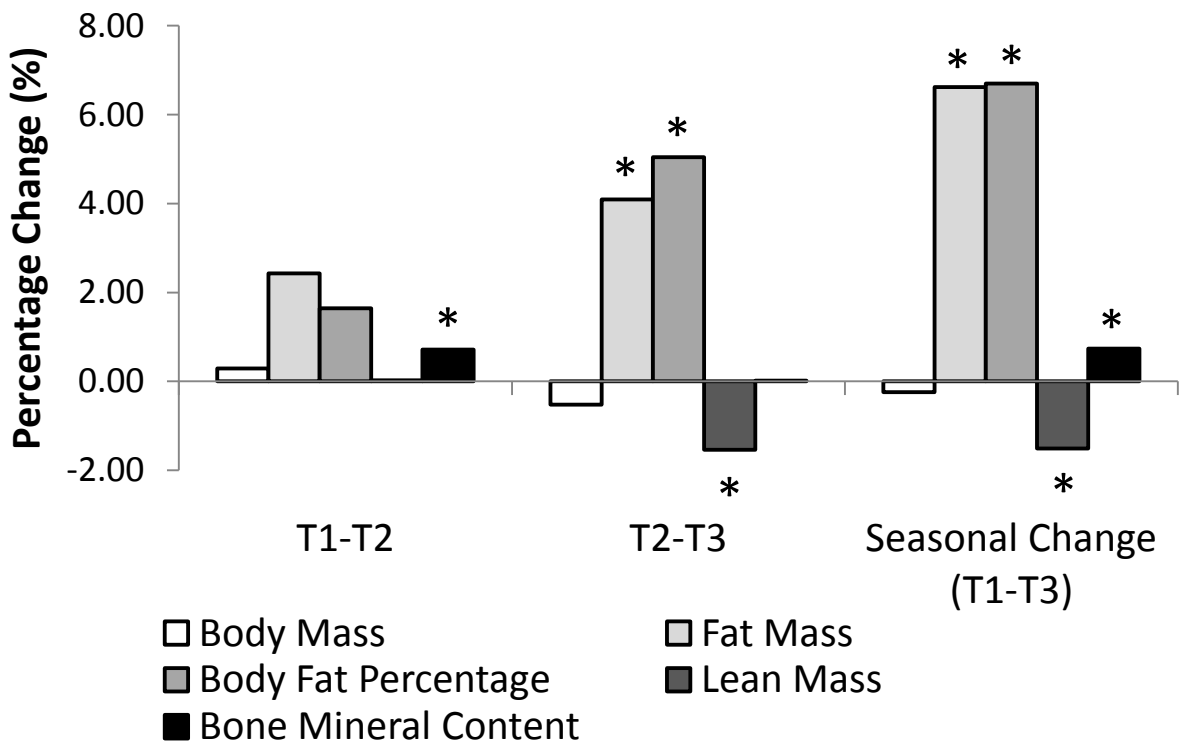


Figure 2. Percentage change in BM, FM, BF%, LM and BMC between testing phases. *=significant change ($p < 0.05$)